Supplementary Material (ESI) for Chemical Communications

Supporting Information:

Microarray-guided discovery of two-photon (2P) small molecule probes for live-cell imaging of cysteinyl cathepsin activities

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1. General information.

All chemicals were purchased from commercial vendors and used without further purification. Tetrahydrofuran (THF) was distilled over sodium benzophenone and used immediately. All reactions requiring anhydrous conditions were carried out under an argon or nitrogen atmosphere using oven-dried glassware. HPLC grade solvents are used for all other solvents. Reaction progress was monitored by TLC on precoated silica plates (Merck 60 F^{254}, 0.25 μm) and spots were visualized by UV, iodine or $KMnO_4$ stain. Flash column chromatography was carried out using Merck 60 F^{254} 0.040-0.063 μm silica gel. ^1H and ^{13}C NMR spectra were recorded on Bruker Avance ACF300 or AMX500 spectrometers. Chemical shifts are reported in parts per million relative to internal standard trimethylsilane ($Si(CH_3)_4$ = 0.00 ppm) or residual solvent peaks ($CDCl_3$ = 7.26 ppm, MeOD = 3.31 ppm, DMSO = 2.50 ppm). UV-vis absorption/extinction and fluorescence spectra were measured by using a Shimadzu UV-vis spectrophotometer and a Perkin Elmer LS50 spectrofluorometer, respectively. The two-photon excitation fluorescence measurements were performed by using a Spectra Physics femtosecond Ti: sapphire oscillator (Tsunami) as the excitation source. The output laser pulses have a tunable center wavelength from 750 nm to 860 nm with pulse duration of 40 fs and a repetition rate of 76 MHz. The laser beam was focused onto the samples that were contained in a cuvette with path length of 1 cm. The emission from the samples was collected at 90° angle by a pair of lenses and an optical fiber that was connected to a monochromator (Acton, Spectra Pro 2300i) coupled with CCD (Princeton Instruments, Pixis 100B) system. A short pass filter with cut-off wavelength at 700 nm was placed before the spectrometer to minimize the scattering from the pump beam. All the measurements were performed at room temperature.

2. Procedures for the synthesis of 105 aldehyde library.

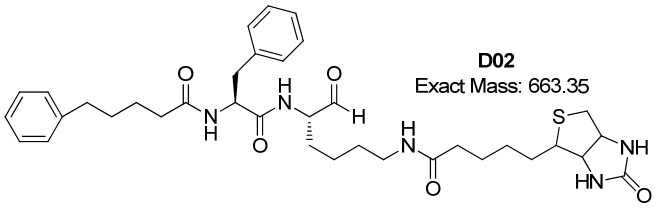
The Synthesis of Fmoc-Lys(Mtt)-CHO was based on previously published

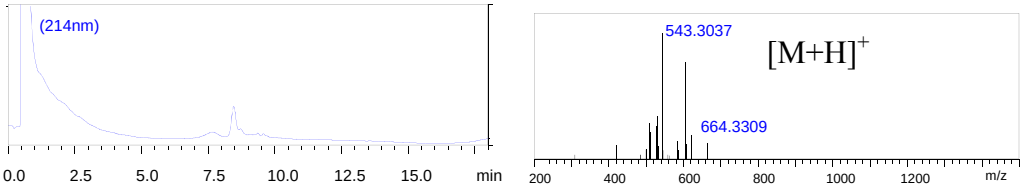
procedures.[1] AM-Resin (15.00 g, 7.50 mmol) was swelled in DMF for approximately 30 min and then filtered. Fmoc-Gly-OH (4.0 eq., 8.90 g, 30 mmol), and standard coupling reagents, HOBT (4.0 eq., 4.05 g, 30 mmol), HBTU (4.0 eq., 11.93 g, 30 mmol) and DIEA (8.0 eq., 10.20 mL, 30 mmol) were dissolved in 150 mL of DMF. The mixture was added to the resin and the reaction was stirred overnight. The resin was washed nine times, alternating between DCM/DMF and then dried *in vacuo*. Fmoc was deprotected using a 20% piperidine solution (in DMF) for 2 h and the resin was washed and dried as before. A mixture of Fmoc-Thr(OtBu)-OH (4.0 eq., 11.93 g, 30 mmol), HOBT (4.0 eq., 4.05 g), HBTU (4.0 eq., 11.93 g) and DIEA (8.0 eq., 10.20 mL) dissolved in 150 mL of DMF was added to the Gly-Resin, and the reaction was stirred overnight. The resin was washed and dried as before. Fmoc deprotection was next carried out, followed by DCM/DMF washes. Subsequently, *t*-butyl deprotection was carried out with a 1:1 solution of TFA in DCM for 1 h. Upon further washes with DCM (3×), the resin was neutralized with 10% DIEA in DCM, followed by repeated washing with DCM (5×), DMF (5×) and MeOH (1×). Next, Fmoc-Lys(Mtt)-CHO (2.0 g) dissolved in 1% DIEA/MeOH was added to the resin in a 60 ^{o}C oil bath. After about 4 h, the resin was filtered, and washed 9 times with DCM/DMF, then dried *in vacuo*. A ninhydrin test for free amines tested negative and a chlorinyl test for a secondary amine tested positive to confirm the loading of the aldehyde.

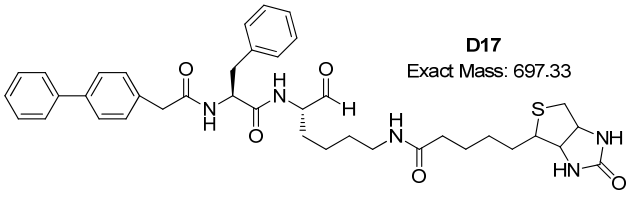
Boc_2O (10.0 eq.) was dissolved in DMF and DIEA (20.0 eq.) and combined with the resin, and allowed to stir for 2 h, and then washed 9 times with DCM/DMF. Mtt deprotection was carried out with 1% TFA in DCM for 30 min. Deprotection was confirmed with a positive ninhydrin test. The resin was washed as usual with DCM and DMF. The deprotected resin was added to D-biotin (4.0 eq.), HOBt (4.0 eq.), HBTU (4.0 eq.) and DIEA (8.0 eq.) in DMF. The reaction was stirred overnight and then washed with DCM/DMF. A negative ninhydrin test confirmed that the free primary amine group was no longer present. The resin was split into three different flasks and a different P_2 group (Figure1B in maintext) was coupled overnight onto the resin in each of the flasks. In each flask, the standard couple reagents HOBT (4.0 eq.), HBTU (4.0 eq.) and DIEA (8.0 eq.) were dissolved in DMF in addition to Fmoc-AA-OH (4.0 eq.). The three different P_2 groups used were Leucine, Valine and Phenylalanine. Again, the resin was washed and dried as before. Finally, the resin from each flask was further divided into 45 equal portions and placed into separate microreactors, with each holding ~40 mg of the resin, and coded with an R_f tag. Again, standard coupling reagents were used, with HOBT (4.0 eq.), HBTU (4.0 eq.) and DIEA (8.0 eq.) dissolved in DMF, and each of the 35 different acid building blocks. After the coupling completed, the resins were washed with DCM and DMF, and dried *in vacuo*.

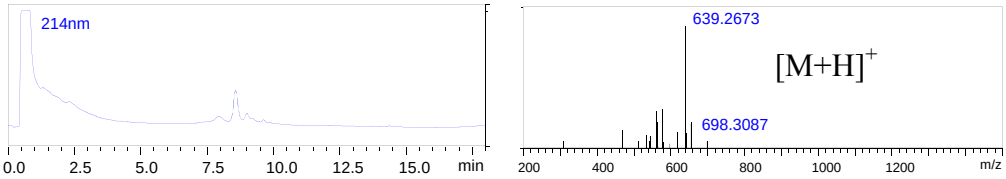
In total, 105 compounds were synthesized on the resin. The resin-bound compounds were first separated into their own blue capped containers. Each container was added a ~1 mL of a deprotection cocktail containing 95% TFA and 5% TIS and the incubation was continued for 30 min. After a quick wash with DCM, 1 mL of a cleavage cocktail (acetonitrile/water/TFA = 60:40:0.1) was applied and the reaction was continued for 30 min at 60 ^{o}C. The microreactors were removed from the blue

cap containers and the product was concentrated to give the crude products. All compounds were characterized by LCMS and all were shown to be of correct molecular weight and most (> 95%) with sufficient purity (> 80%). They were used directly for subsequent microarray experiments. Below are representative LCMS profiles of selected compounds.









3. Gateway cloning and transfection.

The entry clone containing the Cathepsin L gene was from Human ORFenome Release 1 in the donor vector pDONR223. The LR reaction was performed using LR Clonase II using two different destination vectors (pDEST47 and pDEST53) following protocols provided by the vendor. The final constructs were named pDEST47-CTSL and pDEST53-CTSL, which contain C- and N-terminal GFP fusion, respectively. Gene size and sequence were confirmed again using PCR and DNA sequencing with attB1 and attB2 primers. Both the pDEST47-CTSL and pDEST53-CTSL were transiently transfected into HEK293T cells. Successful expression of the GFP-fused Cathepsin was confirmed by fluorescence microscopy and western blotting (Figure S1) using anti-GFP antibody (Santa Cruz, USA).

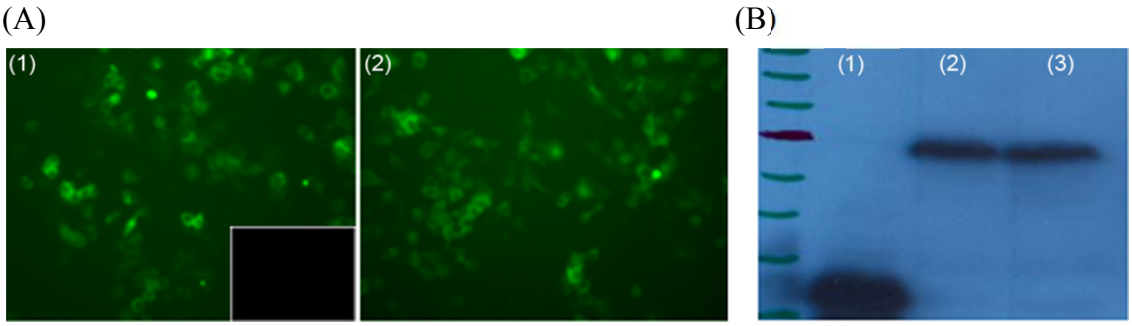


Figure S1. (A) One-photon excited fluorescence images of transfected HEK293T cells. (1) pDEST47-CTSL (C-terminal GFP fusion). Inset: negative control (no plasmid tranfected); (2) pDEST53-CTSL (N-terminal GFP fusion). (B) Western blotting analysis of cell lysates from experiments in (A). Lane 1: 10 μg lysates of HEK293T cells transfected with control GFP plasmid (expected protein MW: 27KD); Lane 2: 10 μg lysates of HEK293T cells transfected with pDEST47-CTSL (expected protein MW: 68KD); Lane 3: 10 μg lysates of HEK293T cells transfected with pDEST53-CTSL (expected protein MW: 68KD).

4. Microarray preparation.

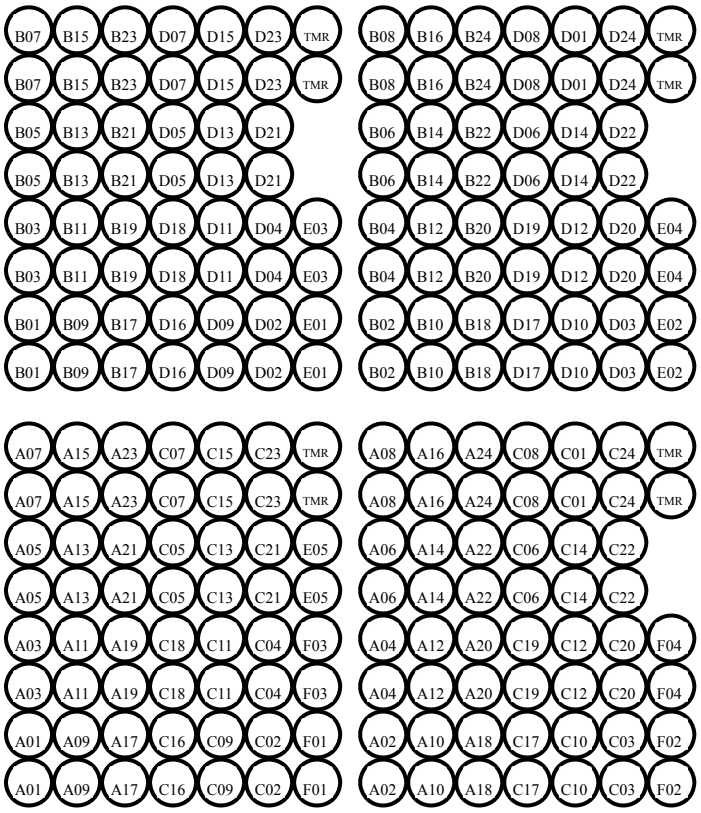


Figure S2. Spotting pattern of the small molecule library.

The microarray fabrication and compound immobilization were carried out following previously published protocols.[1] The spotting pattern and compound ID were shown in Table S1 and Figure S2. To screen the compound-immobilized SMM,

15 μL of the above cell lysate (pDEST47-CTSL), containing over-expressed Cathepsin L, was added to 60 μL of a NaOAc buffer (0.1 mol sodium acetate, *p*H 5.5, 1 mM EDTA and 4 mM dithiothreitol) to activate the enzyme. After 30 min, 15 μL of 0.1% Tween 20 in NaOAc buffer and 60 μL NaOAc buffer was added. Using a micropipette, approximately 40 μL of this mixture was spread overtop the slide, in the sections were the compound had been printed. After 1.5 h, the mixture was washed off with deionized water and the slides were scanned under the Typhoon 9410 fluorescence scanner (GE Amersham) under the microarray setting.

5. Procedure for IC_{50} evaluation against recombinant Cathepsin L.

IC_{50} measurements were performed to determine the potency of two identified hits **D02** and **D17** against commercially available recombinant Cathepsin L. The dose-dependent inhibition assays were performed by varying the concentration of inhibitors under fixed enzyme and fluorogenic substrate Z-FR-AMC (Biotium) concentrations of 5 nM and 10 μM, respectively. The fluorometric assays were performed in 384-well Greiner black plates, and the fluorescence readouts were monitored over 1.5 h assay runs using BioTek Multi-Mode Microplate Reader for fluorescence background at $\lambda_{ex}/\lambda_{em}$ = 360/460 nm. Kinetic endpoints were obtained and analyzed using GraphPad Prism software to plot out the IC_{50} graphs (Figure S3).

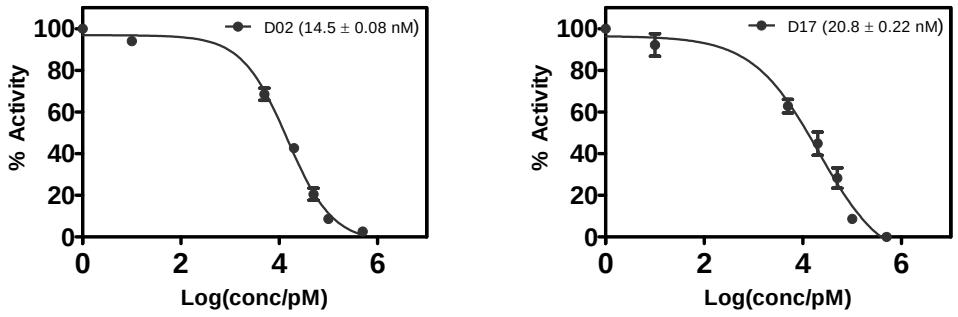


Figure S3. IC_{50} evaluation of **D02** and **D17** against Cathepsin L**.**

6. Synthesis of 2P probes (ZK-1) & (ZK-2).

*(S,E)-tert-butyl(4-azido-1-((2-(ethyl(4-((4-nitrophenyl)diazenyl)phenyl)amino)ethyl)amino)-1-oxobutan-2-yl)carbamate (**2**).* To a solution of **1** (100 mg, 0.32 mmol)[2] and Boc-Homoserine(N_3)-OH (100 mg, 0.4 mmol) in DCM (10 mL) was added EDC (153 mg, 0.8 mmol). The resulting mixture was stirred at room temperature overnight. The reaction was quenched by addition of water (20 mL) which was extracted with ethyl acetate (20 mL ×2). The collected organic layer washed with water (20 mL ×2), brine (20 mL ×2), dried over anhydrous Na_2SO_4 and concentrated *in vacuo.* The residue was purified by chromatography with hexane/ethyl acetate (4:1) to give the red powder (140 mg, 77% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.29 (d, *J* = 9.0 Hz, 2H), 7.87 (m, 4H), 6.80 (d, *J* = 9.0 Hz, 2H), 6.67 (s, 1H) 5.20 (s, 1H), 4.22 (s, 1H)3.54 (m, 5H),2.83 (s,1H) 2.07 (m, 1H), 1.90 (m, 1H), 1.44 (s, 9H), 1.23 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.1, 156.3, 151.4, 147.5, 143.1, 126.1, 124.6, 122.6,

111.4, 48.8, 46.3, 45.4, 37.3, 31.3, 28.2, 12.2; LC-MS(IT-TOF): calcd for $[M+H]^+$ = 540.29, Found = 540.30.

tert-butyl((S)-1-(((S)-4-azido-1-((2-(ethyl(4-((E)-(4-nitrophenyl)diazenyl)phenyl)amino)ethyl)amino)-1-oxobutan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate ***(3).*** To a solution of **2** (140 mg, 0.26 mmol) in 10 mL DCM was added 3 mL TFA. The mixture was stirred at 0 ^oC for 1 h, then evaporated to dryness. Subsequently, 40 mL ethyl acetate was added, and the solution was washed with saturated aqueous $NaHCO_3$ solution (20 mL ×3) and Brine (20 mL ×3), dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Further adding 20 mL DCM, Boc-Phe-OH (80 mg, 0.3 mmol), and EDC (76 mg, 0.4 mmol), the resulting mixture was stirred at room temperature overnight. After quenched by water (20 mL), the solution was extracted with ethyl acetate (40 mL ×2). The collected organic layer was washed with water (20 mL ×2), brine (20 mL ×2), dried over anhydrous Na_2SO_4 and concentrated *in vacuo.* The residue was purified by chromatography with hexane/ethyl acetate (4:1) to give the red powder (153 mg, 86% yield). ^1H NMR (300 MHz, DMSO-d_6): δ 8.33 (d, J = 9.0 Hz, 2H), 8.16 (s, 1H), 8.13 (s, 1H), 7.93 (d, J = 9.0 Hz 2H), 7.82 (d, J = 9.0 Hz 2H), 7.27 (d, J = 8.0 Hz, 2H), 7.19 (m, 1H), 7.06 (d, J = 8.0 Hz, 2H), 6.95 (d, J = 8.0 Hz, 2H), 4.32 (m, 1H), 4.16 (m, 1H), 3.49 (m, 4H), 3.30 (m, 2H), 2.97 (m, 1H), 1.87 (m, 1H), 1.76 (m, 1H), 1.44 (s, 9H), 1.23 (t, J = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.2, 171.1, 156.3, 155.4, 152.4, 147.5, 143.1, 138.3, 129.6, 128.4, 126.5, 125.3, 122.6, 111.4, 78.53, 56.28, 48.8, 47.3, 45.4, 37.3, 36.9, 31.3, 28.2, 12.2; LC-MS(IT-TOF): calcd for $[M+H]^+$ = 687.30, Found = 687.31.

N-((S)-1-(((S)-4-azido-1-((2-(ethyl(4-((E)-(4-nitrophenyl)diazenyl)phenyl)amino)ethyl)amino)-1-oxobutan-2-yl)amino)-1-oxo-3-phenylpropan-2-yl)-5-phenylpentanamide ***(4).*** To a solution of **3** (153 mg, 0.22 mmol) in 10 mL DCM was added 3 mL TFA. The mixture was stirred at 0 ^oC for 1 h, then evaporated the solvent and TFA , added 40 mL ethyl acetate, washed with saturated aqueous $NaHCO_3$ solution (20 mL ×3) and Brine (20 mL ×3), dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Further adding 20 mL DCM, 5-phenylpentanoic acid (55 mg, 0.3 mmol), and EDC (76 mg, 0.4 mmol), the mixture was stirred at room temperature overnight. After being quenched by water (20 mL), the solution was extracted with ethyl acetate (40 mL ×2). The collected organic layer was washed with water (20 mL ×2), brine (20 mL ×2), dried over anhydrous Na_2SO_4 and concentrated *in vacuo.* The residue was purified by chromatography with hexane/ethyl acetate (1:1) to give the red powder (130 mg, 84% yield). ^1H NMR (300 MHz, DMSO-d_6): δ 8.34 (d, J = 9.0 Hz, 2H), 8.16 (d, J = 9.0 Hz, 1H), 8.13 (d, J = 9.0 Hz, 1H), 7.93 (d, J = 9.0 Hz 2H), 7.83 (d, J = 9.0 Hz 2H), 7.24 (m, 5H), 7.13 (m, 5H), 6.95 (d, J = 8.0 Hz, 2H), 4.49 (m, 1H), 4.26 (m, 1H), 3.46 (m, 4H), 3.30 (m, 2H), 2.99 (m, 1H), 2.87 (s, 1H), 2.83 (m, 1H), 2.76 (s, 1H), 2.30 (s, 1H), 2.17 (s, 1H), 1.89 (m, 1H), 1.78 (m, 1H), 1.36 (m, 5H), 1.27 (m, 2H), 1.23 (t, J = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.2, 171.6, 171.1, 156.3, 152.4, 147.5, 142.1, 129.6, 128.6, 128.4, 128.3, 126.5, 125.9, 125.4, 122.8, 111.8, 54.28, 50.1, 48.8, 47.3, 45.4, 37.3, 35.9, 31.3, 30.4, 29.2, 25.5, 22.5, 12.2;

LC-MS(IT-TOF): calcd for $[M+H]^+$ = 747.37, Found = 747.38.

(S)-2-((S)-2-(2-([1,1'-biphenyl]-4-yl)acetamido)-3-phenylpropanamido)-4-azido-N-(2-(ethyl(4-((E)-(4-nitrophenyl)diazenyl)phenyl)amino)ethyl)butanamide ***(5).*** Compound **5** was synthesized based on the same procedure as **4** by using 2-((1,1'-biphenyl)-4-yl)acetic acid to afford red powder (137 mg, 85% yield). ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.43 (d, *J* = 9.0 Hz, 2H), 8.34 (d, *J* = 9.0 Hz, 2H), 8.25 (d, *J* = 9.0 Hz, 2H), 8.06 (m, 1H), 7.93 (d, *J* = 9.0 Hz 2H), 7.86 (d, *J* = 9.0 Hz 2H), 7.58 (d, *J* = 9.0 Hz, 2H), 7.48 (d, *J* = 9.0 Hz, 2H), 7.43(d, *J* = 9.0 Hz, 2H), 7.36 (m, 1H), 7.24 (m, 4H), 7.21 (m, 3H), 6.95 (d, *J* = 8.0 Hz, 2H), 4.55 (m, 1H), 4.27 (m, 1H), 3.46 (m, 4H), 3.30 (m, 2H), 2.99 (m, 1H), 2.87 (s, 1H), 2.83 (m, 1H), 2.76 (s, 1H), 2.30 (s, 1H), 2.17 (s, 1H), 1.89 (m, 1H), 1.78 (m, 1H), 1.24 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.6, 171.4, 170.3, 156.3, 152.4, 147.5, 142.1, 140.3, 138.6, 138.1, 135.8, 129.8, 129.6, 129.2, 128.6, 127.4, 126.5, 125.9, 122.8, 111.8, 55.28, 50.1, 48.8, 47.3, 45.4, 42.4, 37.3, 35.9, 31.3, 12.2; LC-MS(IT-TOF): calcd for $[M+H]^+$ = 781.35, Found = 781.36.

ZK-1. Compound **4** (100 mg, 13 mmol) and the corresponding alkyne-containing dye **DL-1** (67 mg, 14 mmol) were suspended in a mixture of DMSO/acetonitrile (2:1) (9 mL). To the suspension was added CuI (25 mg, 14 mmol) and DIEA (50 μL). The resulting mixture was stirred at room temperature for 48 h. Then the product was directly purified by preparative HPLC with elution gradients using acetonitrile (0.1% TFA) and water (0.1% TFA) to give the final product 13 mg. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.33 (m, 3H), 8.15 (m, 3H), 7.99 (m, 2H), 7.84 (d, 3H), 7.76 (m, 2H), 7.57 (d, 3H), 7.25(m, 12H), 7.05 (m, 3H), 6.9 (m, 5H), 5.6 (m, 2H), 4.51 (m, 1H), 4.35 (m, 2H), 4.20 (m, 2H), 3.46 (m, 11H), 3.27 (m, 2H), 2.99 (m, 1H), 2.87 (s, 1H), 2.83 (m,2H), 2.18 (m, 1H), 2.08 (m, 3H), 1.40 (m, 4H), 1.08 (m, 16H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 173.6, 172.16, 171.3, 164.67, 156.3, 151.4, 147.5, 142.3, 138.25, 135.8, 128.54, 128.37, 126.5, 125.24, 122.79, 111.8, 110.05, 60.35, 54.32, 50.56, 46.5, 45.21, 37.3, 35.3, 35.18, 32.64, 30.69, 25.07, 12.4; LC-MS(IT-TOF): calcd for $[M+H]^+$ = 1227.65, Found = 1227.68.

ZK-2 was synthesized using the same procedure as ***ZK-1*** by using compound **5** to afford the final product 10 mg. ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.43 (m, 2H), 8.34 (m, 2H), 8.15 (m, 3H), 8.05 (m, 2H), 7.81 (d, 5H), 7.47 (m, 10H), 7.25 (m, 10H), 6.90 (m, 6H), 5.60 (m, 2H), 4.51 (m, 1H), 4.35 (m, 2H), 4.20 (m, 2H), 3.46 (m, 7H), 3.27 (m, 2H), 2.99 (m, 1H), 2.87 (s, 1H), 2.83 (m,2H), 2.49 (m, 4H), 1.08 (m, 16H) ; ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.9, 171.2, 165.5, 164.6, 156.7, 154.7, 151.9, 147.3, 143.1, 142.3, 138.25, 137.9, 135.8, 129.2, 129.4, 128.54, 128.37, 126.8, 126.5, 125.28, 122.79, 111.8, 111.6, 66.35, 65.21, 63.77, 54.32, 54.32, 45.14, 35.18, 34.13, 29.51, 12.4; LC-MS(IT-TOF): calcd for $[M+H]^+$ = 1161.64, Found = 1161.65.

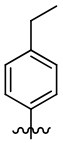
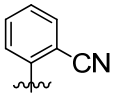
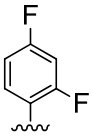
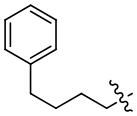
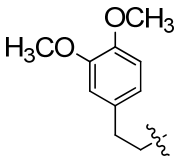
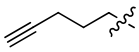
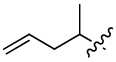
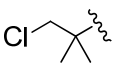
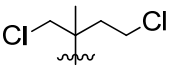
7. Cell culture and imaging.

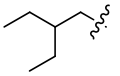
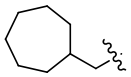
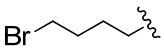
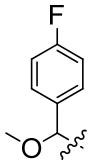
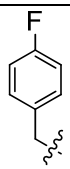
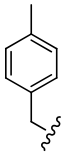
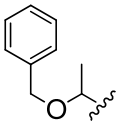
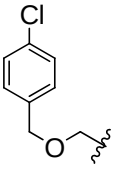
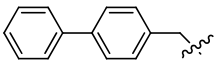
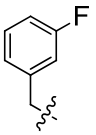
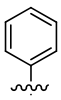
HepG2 cells were seeded in glass-bottom dishes (Mattek) and grown till 70~80% confluency. Subsequently, the cells were incubated with **ZK-1** or **ZK-2** (2 μM prepared in fresh media) for 2 h, then further incubated with 0.25 μg/mL of the lysosomal tracker (LysoTracker® Red DND-99). Cells were next washed with PBS three times, then imaged with the Leica TCS SP5X Confocal Microscope System equipped with Leica HCX PL APO 63x/1.20 W COPP CS, 405 nm Diode laser, White laser (470 nm to 670 nm, with 1 nm increments, with 8 channels AOT for simultaneous control of 8 laser lines, each excitation wavelength provides 1.5 mV), and Ti-Sappire laser (~4 W at 800 nm) with PMT detector ranging from 420 nm to 700 nm for one-photon excited fluorescence. All images were processed with Leica Application Suite Advanced Fluorescence (LAS AF, Figure 3 in the maintext).

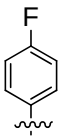
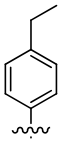
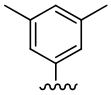
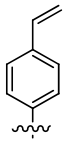
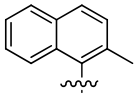
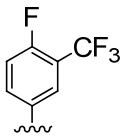
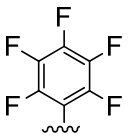
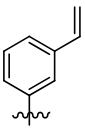
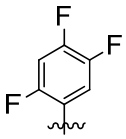
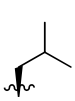
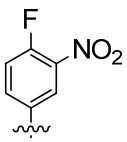
8. References

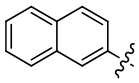
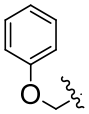
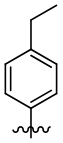
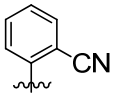
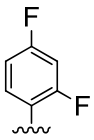
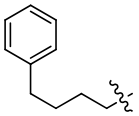
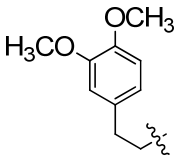
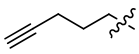
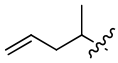
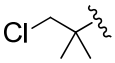
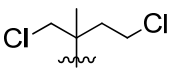
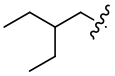
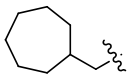
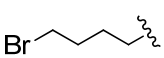
1. H. Wu, J. Ge, P. Yang, M. Uttamchandani.and S. Q. Yao, *J. Am. Chem. Soc*., **2011**, *133*, 1946-1954.
2. A. Michaela, H. Markus, B. Klaus and M. Ronald, *Chembiochem*. **2011**, *12*, 47-51.

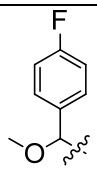
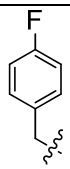
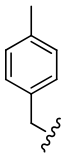
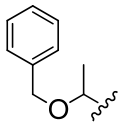
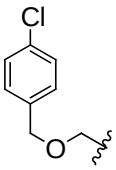
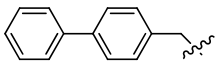
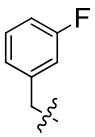
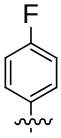
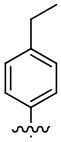
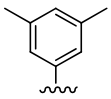
Table S1. Compound ID and the corresponding printing pattern.

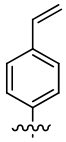
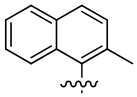
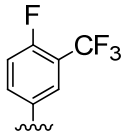
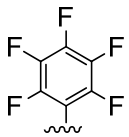
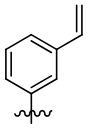
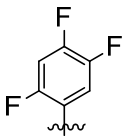
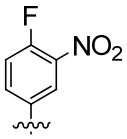
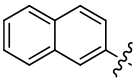
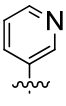
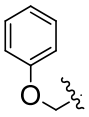
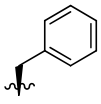
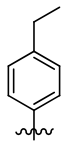
Printing Pattern	Structure		MW Exp	MW Obs
	P_2	P_3		
A01			601.33	602.34
A02			598.29	599.26
A03			609.28	610.26
A04			629.39	630.37
A05			661.35	662.32
A06			563.31	564.45
A07			565.31	566.47
A08			587.29	588.45
A09			635.80	636.67
A10			536.28	537.31

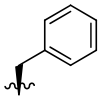
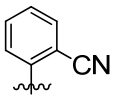
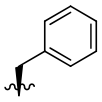
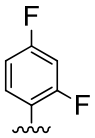
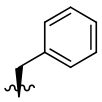
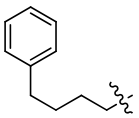
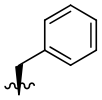
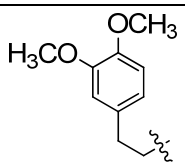
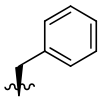
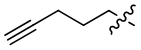
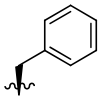
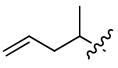
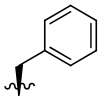
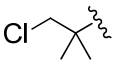
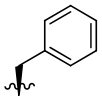
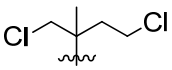
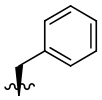
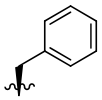
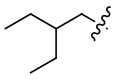
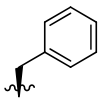
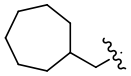
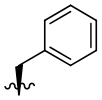
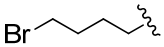
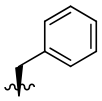
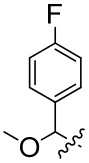
A11			567.35	568.56
A12			607.38	608.74
A13			631.24	632.21
A14			635.32	636.76
A15			605.30	606.31
A16			601.33	602.45
A17			631.34	632.67
A18			651.29	652.18
A19			663.35	664.21
A20			605.30	606.46
A21			573.30	574.65

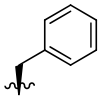
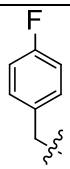
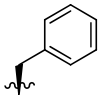
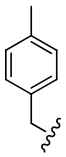
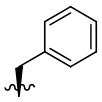
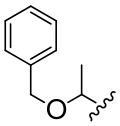
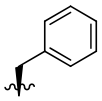
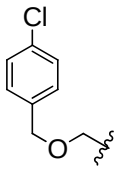
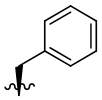
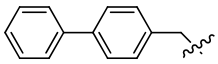
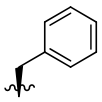
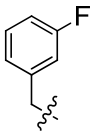
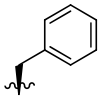
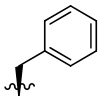
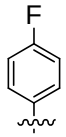
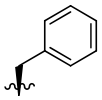
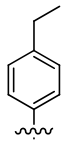
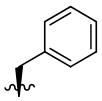
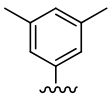
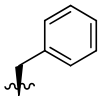
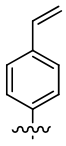
A22			591.29	592.51
A23			601.33	602.56
A24			601.33	602.36
B01			599.31	600.56
B02			637.33	638.56
B03			659.28	660.84
B04			663.25	664.67
B05			591.29	592.67
B06			599.31	600.57
B07			627.27	628.65
B08			636.27	636.78

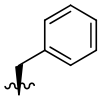
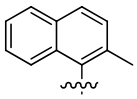
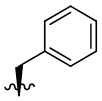
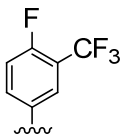
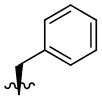
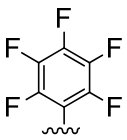
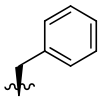
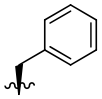
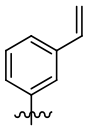
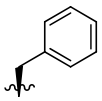
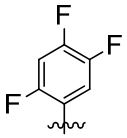
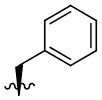
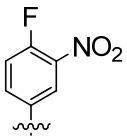
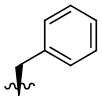
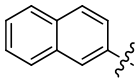
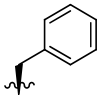
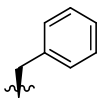
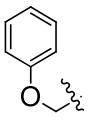
B09			623.31	624.72
B10			574.29	575.41
B11			603.31	604.41
B12			587.31	588.42
B13			584.28	585.27
B14			595.20	596.26
B15			615.35	616.38
B16			647.38	648.41
B17			549.30	550.45
B18			551.31	552.47
B19			573.28	574.71
B20			621.25	622.64
B21			522.26	523.43
B22			553.33	554.43
B23			593.36	594.37
B24			617.22	618.32

C01			621.30	622.31
C02			591.21	591.23
C03			587.31	587.53
C04			617.32	618.39
C05			637.27	638.45
C06			649.33	650.43
C07			591.29	592.41
C08			559.28	560.41
C09			577.27	578.37
C10			587.31	588.34
C11			587.31	588.56

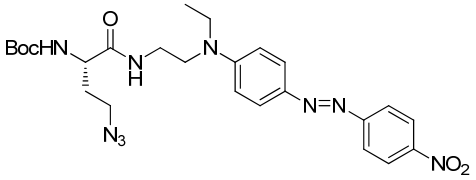
C12			585.30	586.45
C13			623.31	624.34
C14			645.26	646.36
C15			649.24	650.43
C16			577.27	578.56
C17			585.30	586.41
C18			613.25	614.36
C19			622.26	623.46
C20			609.30	610.41
C21			560.28	561.18
C22			589.29	590.41
C23			635.31	636.45

C24			632.28	633.34
D01			643.26	644.29
D02			663.35	664.33
D03			695.35	696.41
D04			597.30	598.41
D05			599.31	600.45
D06			621.28	622.31
D07			669.25	670.71
D08			570.26	571.17
D09			601.33	602.31
D10			641.36	642.54
D11			665.12	666.09
D12			669.30	670.41

D13			639.29	640.34
D14			635.31	636.36
D15			665.32	666.20
D16			685.27	686.31
D17			697.33	698.38
D18			639.29	640.28
D19			607.28	608.34
D20			625.27	627.19
D21			635.31	636.29
D22			635.31	636.45
D23			633.30	634.61

D24			671.31	672.40
E01			693.26	694.15
E02			697.24	698.25
E03			625.27	626.21
E04			633.30	634.45
E05			661.25	662.27
F01			670.26	671.35
F02			657.30	658.31
F03			608.28	609.23
F04			637.29	638.41

9. ^{1}H and ^{13}C NMR.



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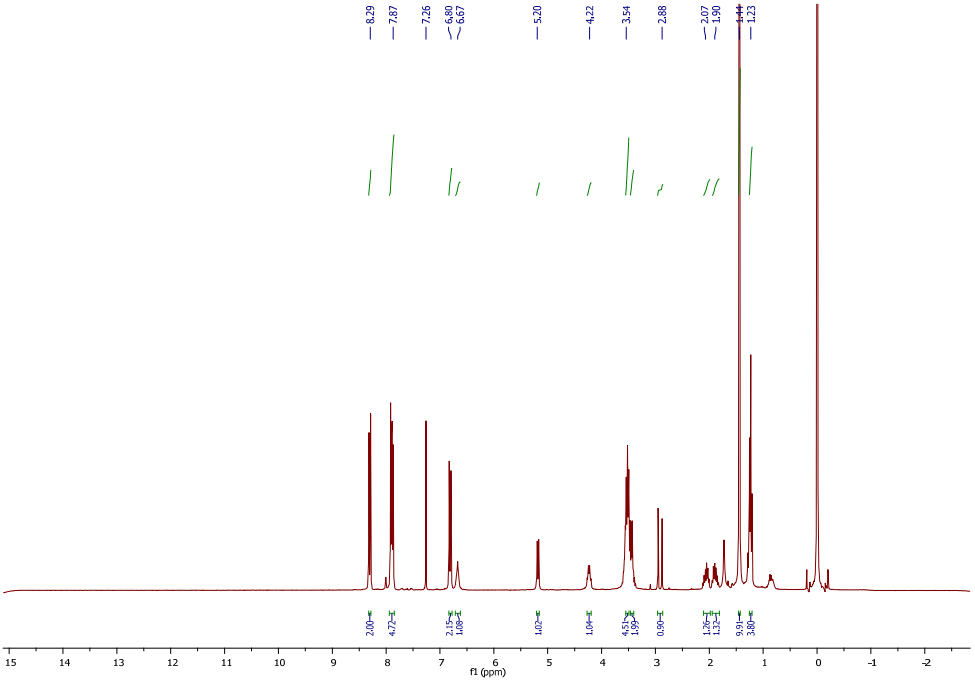

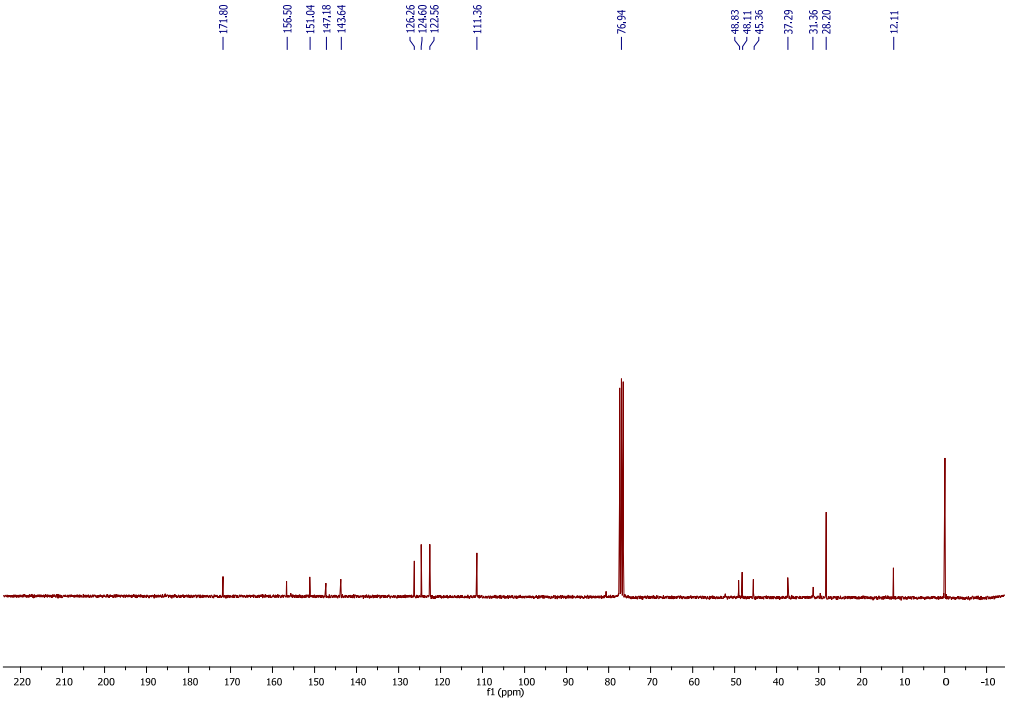

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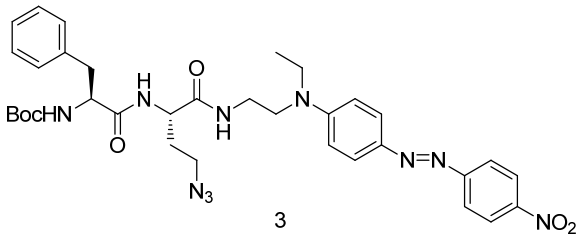

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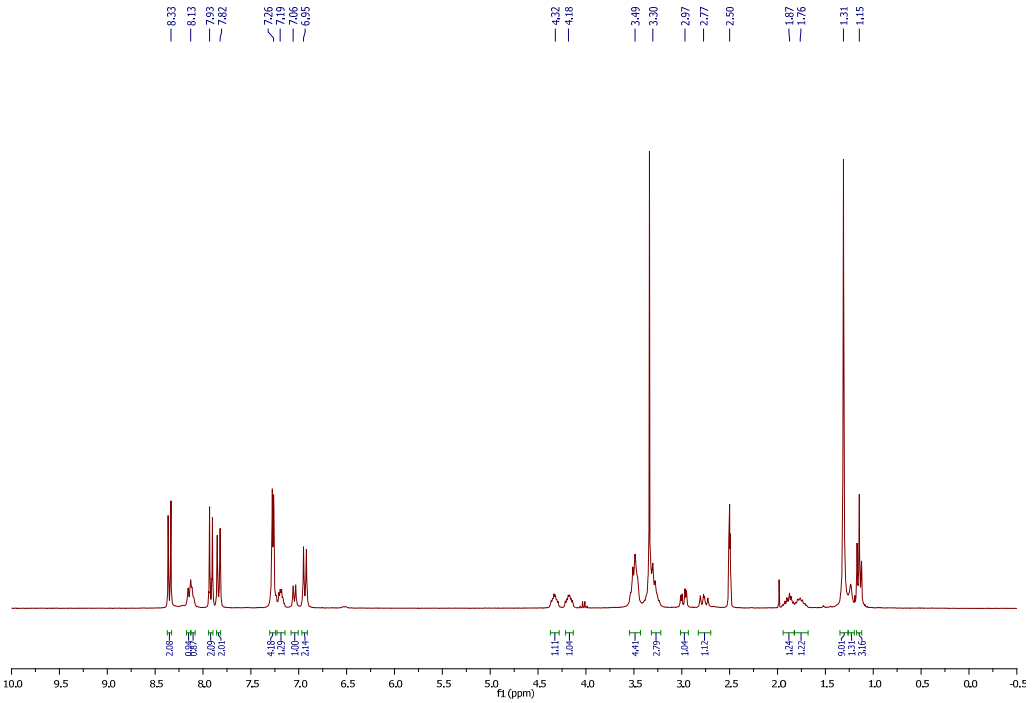

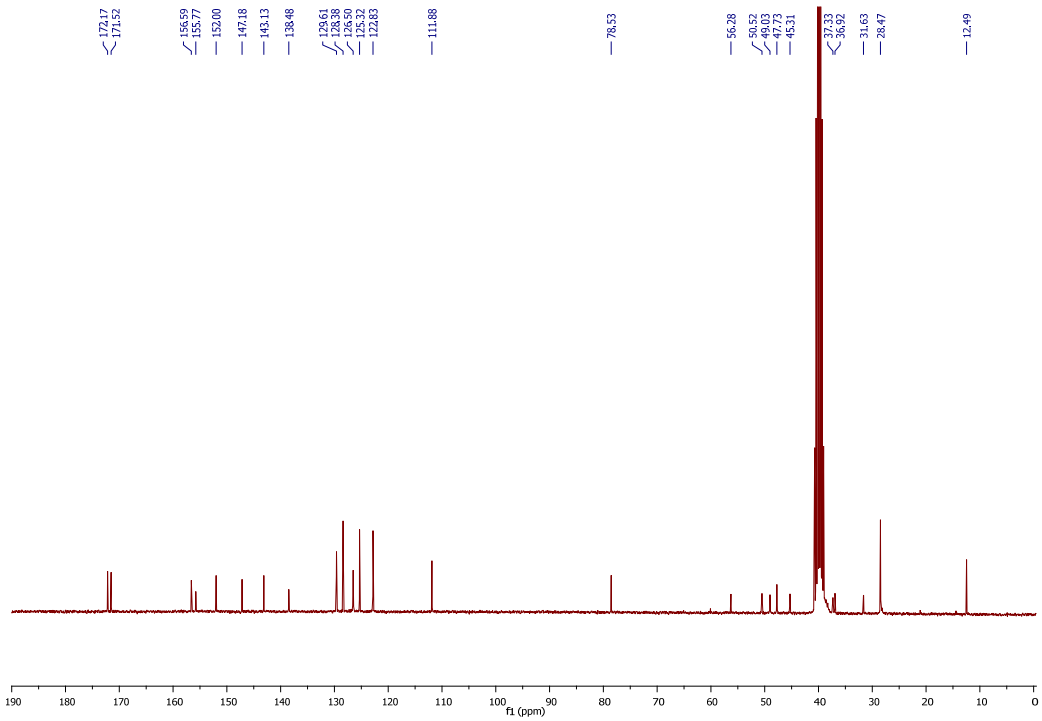

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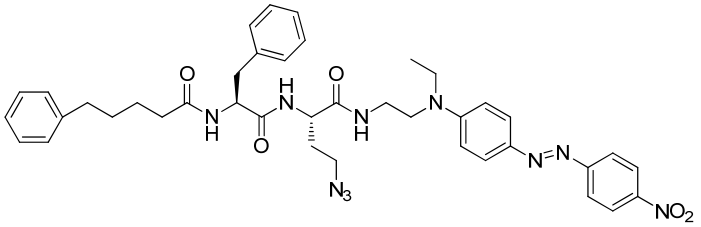

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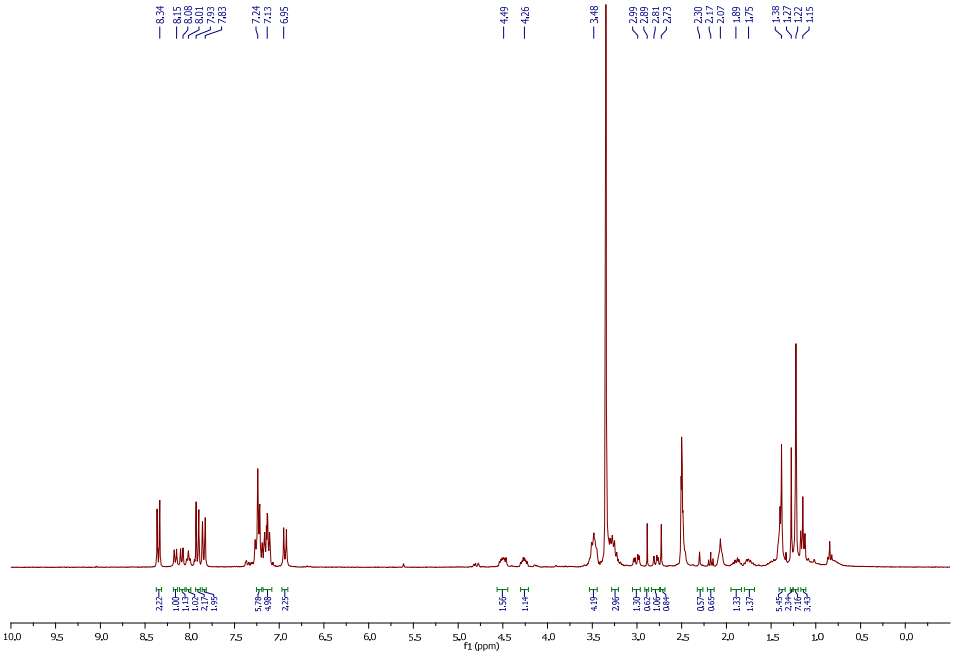

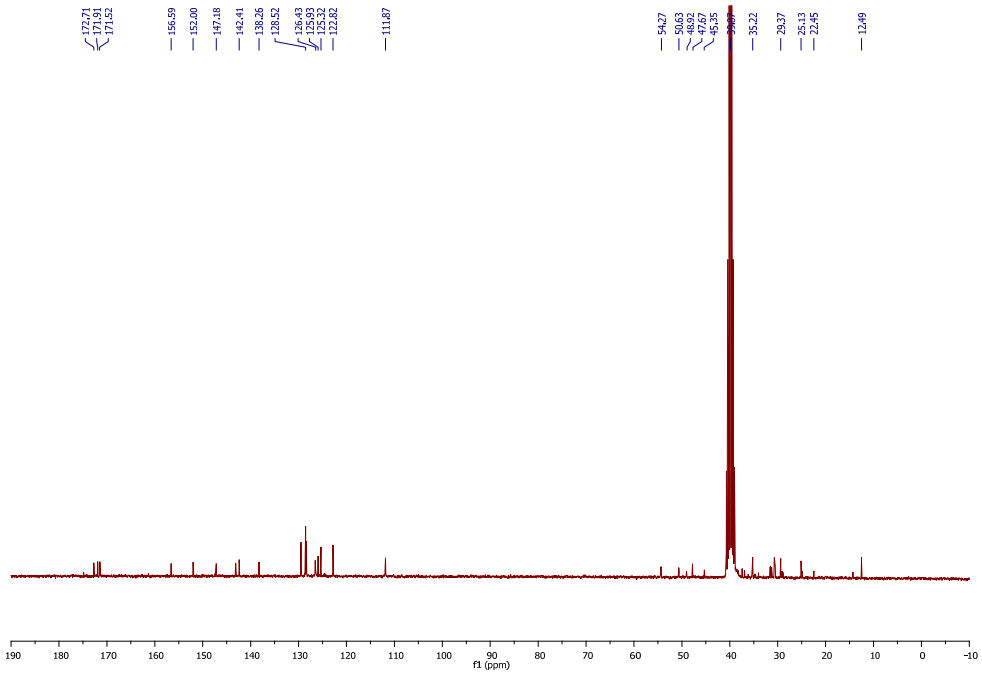

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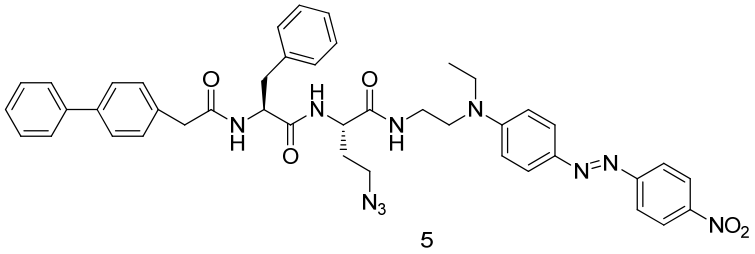

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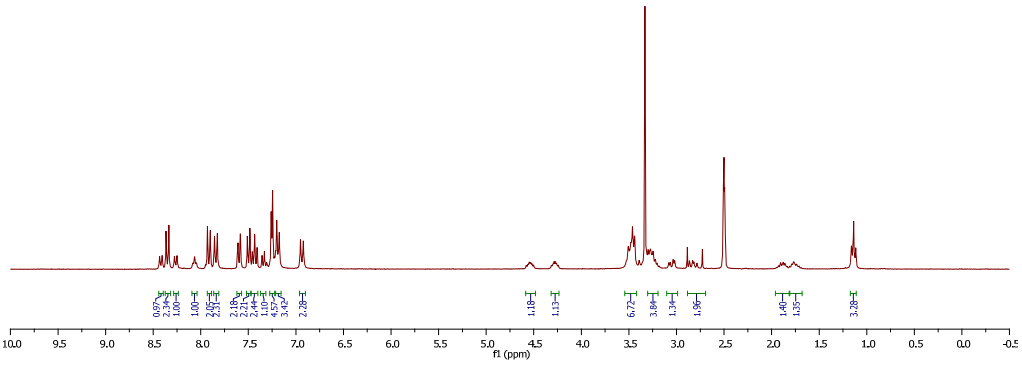
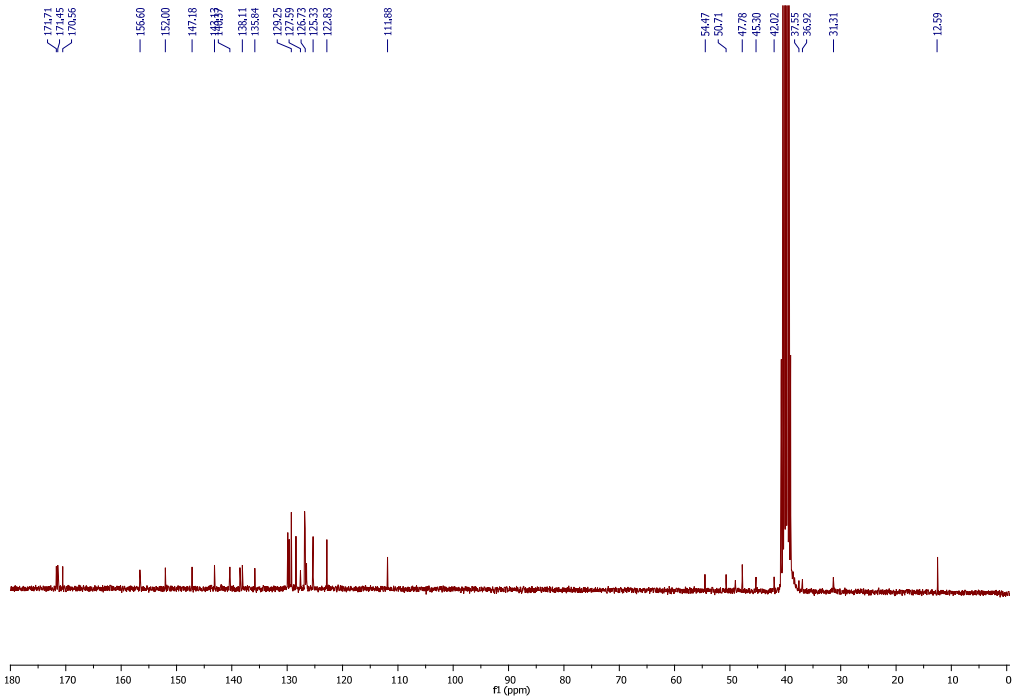
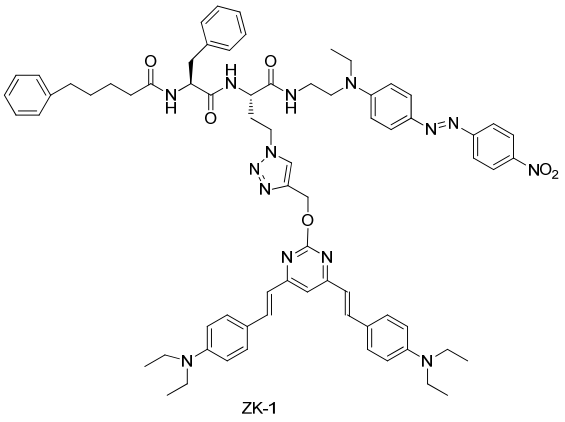
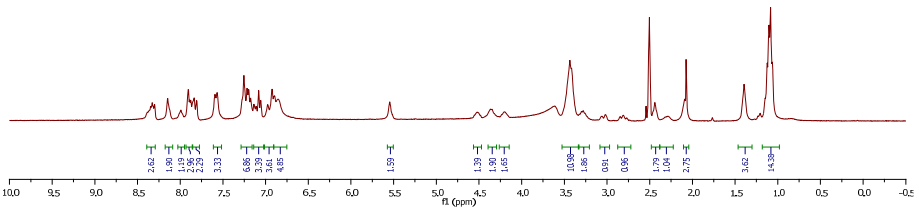
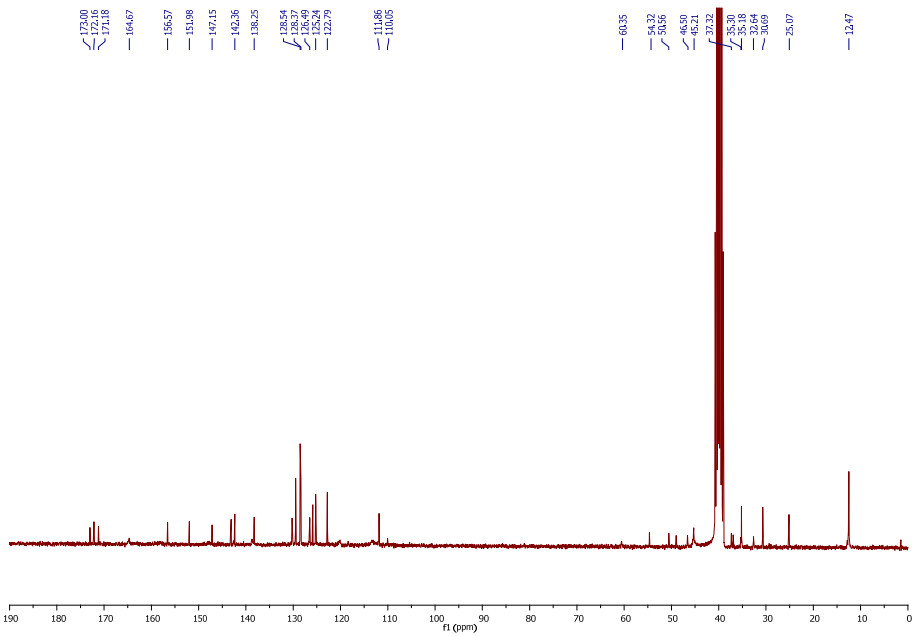






ZK-1





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