Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Information

Dual aggregation-induced emission for enhanced fluorescence sensing of furin activity in vitro and in living cells

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Contents:

1. General methods

All the starting materials were obtained from Sigma or Sangon Biotech. Commercially available reagents were used without further purification, unless noted otherwise. All chemicals were reagent grade or better. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker AV 300. Matrix-assisted laser desorption (MALDI) ionization-time of flight (TOF)/TOF and ESI mass spectra were obtained on a time-of-flight Ultrflex II mass spectrometer (Bruker Daltonics) and on a Finnigan LCQ Advantage ion trap mass spectrometer (ThermoFisher Corporation) equipped with a standard ESI source, respectively. High performance liquid chromatography (HPLC) purification was performed on a Shimazu UFLC system equipped with two LC-20AP pumps and an SPD-20A UV/vis detector using a Shimazu PRC-ODS column. HPLC analyses were performed on an Agilent 1200 system equipped with a G1322A pump and in-line diode array UV detector an Agilent Zorbax 300SD-C_{18} RP column, with CH_3CN (0.1% of TFA) and water (0.1% of TFA) as the eluent. Fluorescence spectra were recorded on an F-4600 fluorescence spectrophotometer (Hitachi High-Techonologies Corporation, Japan) with excitation wavelength set to 320 nm. UV-vis absorption spectra were recorded on a Pekin-Elmer lambda 25 spectrophotometer. Dynamic light scattering (DLS) was measured on a Zeta Sizer Nano Series (Malvern Instruments). Transmission electron micrograph (TEM) images were obtained on a JEM-2100F field emission transmission electron microscope operated at an acceleration voltage of 200 kV. Cell images were obtained on the IX71 fluorescence microscope (Olympus, Japan).

General produce for enzymatic assay

DMSO stock solutions of **1, 1-Scr**, or **1-Ctrl** were diluted with furin working buffer (100 mM HEPES, 1 mM $CaCl_2$, pH = 7.4) to a final concentration of 100 μM working solutions, respectively. Next, 1 mM of tris(2-carboxyethyl)-phosphine (TCEP) and 1 nmol U^{-1} furin were added. The reaction mixtures were incubated at room temperature (RT) for 4 h and then their fluorescence spectra were measured. Each solution was excited at 320 nm, and the emission was collected from 340 to 620 nm.

Cell culture

MDA-MB-468 human breast adenocarcinoma epithelial cells were cultured in Dulbecco's modified eagle medium (DMEM, GIBCO) supplemented with 10% fetal bovine serum (FBS, GIBCO) and streptomycin (100 μg/mL). The cells were expanded in tissue culture dishes and kept in a humid atmosphere of 5% CO_2 at 37 °C. The medium was changed every other day.

MTT assay

The cytotoxicity of MDA-MB-468 cells was measured with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells growing in log phase were seeded into 96-well cell culture plate at 3×10^3/well. The cells were incubated for 12 h at 37 °C under 5% CO_2. The solutions of **1**, **1-Scr**, or **1-Ctrl** (100 μL/well) at concentrations of 5, 10, 20, or 40 μM in 100 μL of medium were added to the wells, respectively. The cells were incubated for 2, 4, or, 8 h at 37 °C under 5% CO_2. 10 μL solution of 5 mg/mL MTT dissolved in PBS buffer (pH 7.4) was added to each well of the 96-well plate. Then 100 μL solution DMSO was added to each well to dissolve the formazan after an additional 4 h of incubation. The data were obtained using an enzyme-linked

immunosorbent assay (ELISA) reader (VARIOSKAN FLASH) to detect its absorption at 490 nm. The following formula was used to calculate the viability of cell growth: viability (%) = (mean of absorbance value of treatment group/mean of absorbance value of control) × 100.

Time course cell imaging

The MDA-MB-468 human breast adenocarcinoma epithelial cells were plated on 3.5 cm cell culture dish at 50% cell density in the next day. Then the MDA-MB-468 cells were washed for three times with PBS and incubated with 5 μM **1** and 50 μM **C** in serum-free medium at 37 °C in a CO_2 incubator. The fluorescence images at DAPI channel were acquired every 10 min on the IX71 fluorescence microscope.

Sensing furin activity in living cells

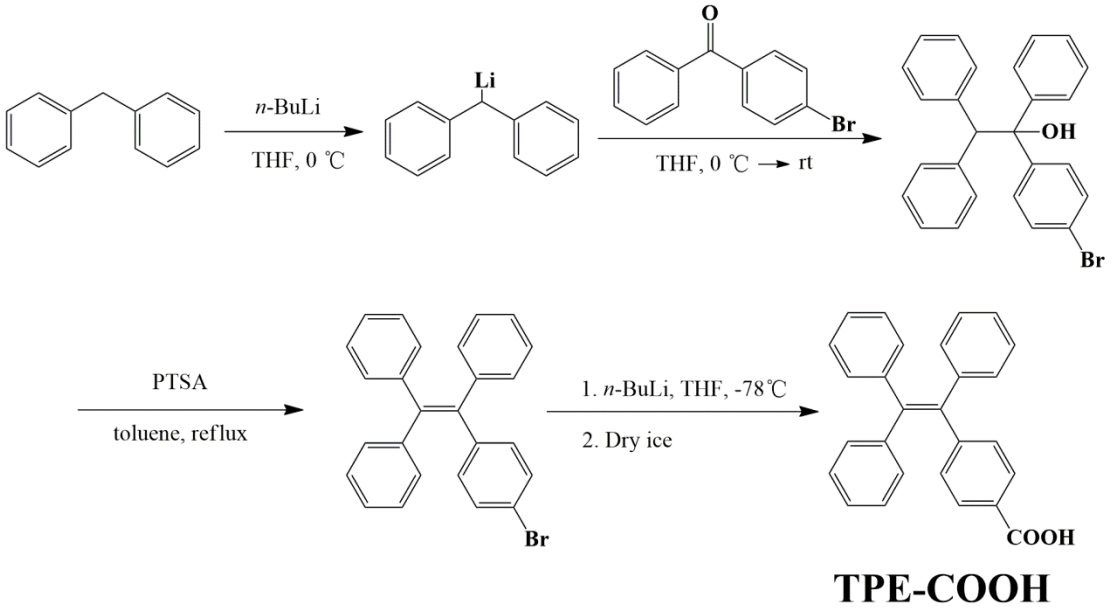
The MDA-MB-468 human breast adenocarcinoma epithelial cells were plated on 3.5 cm cell culture dish at 50% cell density in the next day. Then the MDA-MB-468 cells were washed for three times with PBS and co-incubated 5 μM **1, 1-Scr**, or **1-Ctrl** with 50 μM **C** in serum-free medium at 37 °C for 1 h in a CO_2 incubator, respectively. Then, the cells were washed with PBS for another three times prior to microscopic imaging. For the furin-inhibiting group, MDA-MB-468 cells were pretreated with furin inhibitor II (H-(D)Arg-Arg-Arg-Arg-Arg-Arg-NH_2, 500 μM) for 30 min in serum-free medium at 37 °C, washed with PBS for three times, then incubated with 5 μM **1** (co-incubated with 50 μM **C**) in serum-free medium at 37 °C for 1 h, and washed with PBS for another three times prior to microscopic imaging. Fluorescence intensities of the images from DAPI channel were analyzed with ImageJ (Universal Imaging Corp.).

2. Chemical syntheses and characterizations of TPE-COOH, 1, 1-Scr and 1-Ctrl

Preparation of TPE-COOH.

4-(1,2,2-Triphenylvinyl)benzoic acid (TPE-COOH) was synthesized following the literature method.[1]

Scheme S1. The synthetic route for TPE-COOH.



Synthesis of 1-(4-Bromophenyl)-1,2,2-triphenylethene (TPE-Br):

TPE-Br was synthesized according to the synthetic route shown in *Scheme S1*. The procedures for its synthesis are shown as follows. To a solution of diphenylmethane (2.9 g; 17.5 mmol) in dry tetrahydrofuran (50 mL) was added dropwise 11 mL of a 1.6 M solution of *n*-butyllithium in hexane (17.5 mmol) at 0 °C under nitrogen. The resulting orange-red solution was further stirred for 1 h. Then 4-bromobenzophenone (3.8 g, 14.6 mmol) was added into this solution. Afterwards, the reaction mixture was allowed to warm to RT and stirred overnight. The reaction was quenched with the addition of an aqueous solution of ammonium chloride. The organic layer was then extracted with dichloromethane (3 ×

50 mL). The organic layers were combined, washed with saturated brine solution and dried over anhydrous $NaSO_4$. After solvent evaporation, the resulting crude alcohol (containing excess diphenylmethane) was dissolved in about 80 mL of toluene in a 100 mL Schlenk flask with a Dean-Stark trap. A catalytic amount of *p*-toluenesulfonic acid (555.4 mg; 2.92 mmol) was added and the mixture was refluxed for 4 h. After the reaction mixture was cooled to RT, the toluene layer was washed with 10% aqueous $NaHCO_3$ solution (2 × 30 mL) and evaporation of the toluene solvent. The solution was then extracted with dichloromethane (3 × 50 mL). The organic layers were combined, washed with saturated brine solution and dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure afforded the crude tetraphenylethene derivative, which was further purified by silica gel column chromatography using PE : DCM = 10 : 1 as eluent. Yield: 70%. ^{1}H NMR (d_6-DMSO, 300 MHz) δ (TMS, ppm): 6.88−7.26 (m, 19 H, Ar−H).

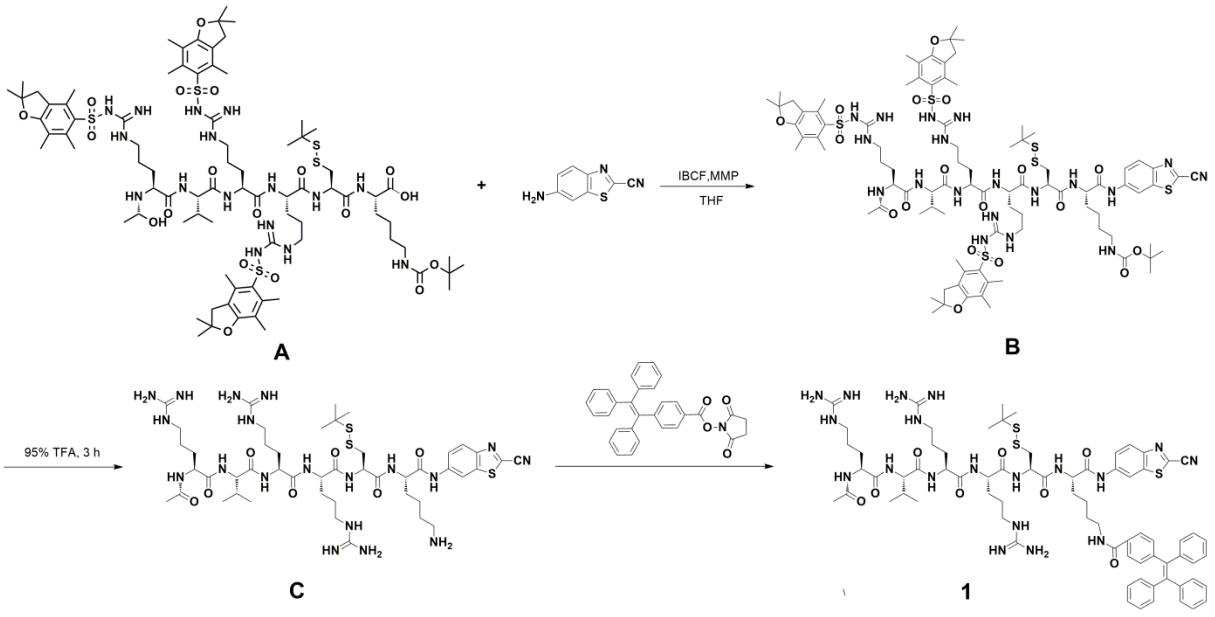
Synthesis of 4-(1,2,2-Triphenylvinyl)benzoic acid (TPE-COOH):

To a solution of TPE-Br (2.2 g, 5.35 mmol) in 30 mL dry THF was added dropwise 4 mL (6.42 mmol) of *n*-butyllithium (1.6 M in *n*-hexane) at -78 °C under stirring. The reaction mixture was stirred for 2 h to get a dark brown solution. Then dry ice pieces in small portions were slowly added to the obtained solution at -78 °C under stirring. The solution was allowed to warm to RT and stir overnight. The resulting solution was acidified with an excess amount of 1 M hydrochloric acid and extracted with DCM several times. The organic layer was combined and dried over anhydrous Na_2SO_4. The organic solvent was removed under reduced pressure. The crude product was obtained by recrystallization. TPE-COOH was further purified with HPLC using water-acetonitrile added with 0.1% TFA as the eluent. Yield: 68%. ^{1}H NMR (d_6-DMSO,

400 MHz, Figure S1) δ (TMS, ppm): 6.96−7.16 (m, 17 H, Ar−H), 7.69 (d, 2H, Ar−H). MS: calculated for TPE-COOH $[(M+K)]^+$ = 415.11, obsvd. ESI-MS: *m*/*z* 415.10. (Figure S2).

*Preparation of Ac-Arg-Val-Arg-Arg-Cys(StBu)-Lys(TPE)-CBT (**1**)*:

Scheme S2. The synthetic route for **1**.



*Synthesis of **B***:

Compound **A** was synthesized with solid phase peptide synthesis (SPPS). Isobutyl chloroformate (IBCF, 48 mg, 0.35 mmol) was added to a mixture of Compound **A** (600 mg, 0.33 mmol) and 4-methylmorpholine (MMP, 54 mg, 0.53 mmol) in THF (8.0 mL) at 0 °C under N_2. The reaction mixture was stirred for 30 min. The solution of 2-cyano-6-aminobenzothiazole (CBT, 58 mg, 0.33 mmol) was added to the reaction mixture and further stirred for 1 h at 0 °C. Then the mixture was stirred overnight at RT. Compound **B** (420 mg, yield: 65%) was purified with HPLC using water-methanol added with 0.1% TFA as the eluent. MS: calculated for **B** $[(M+H)^+]$: 1960.85; obsvd. ESI-MS: *m*/*z* 1962.10.

*Synthesis of **C***:

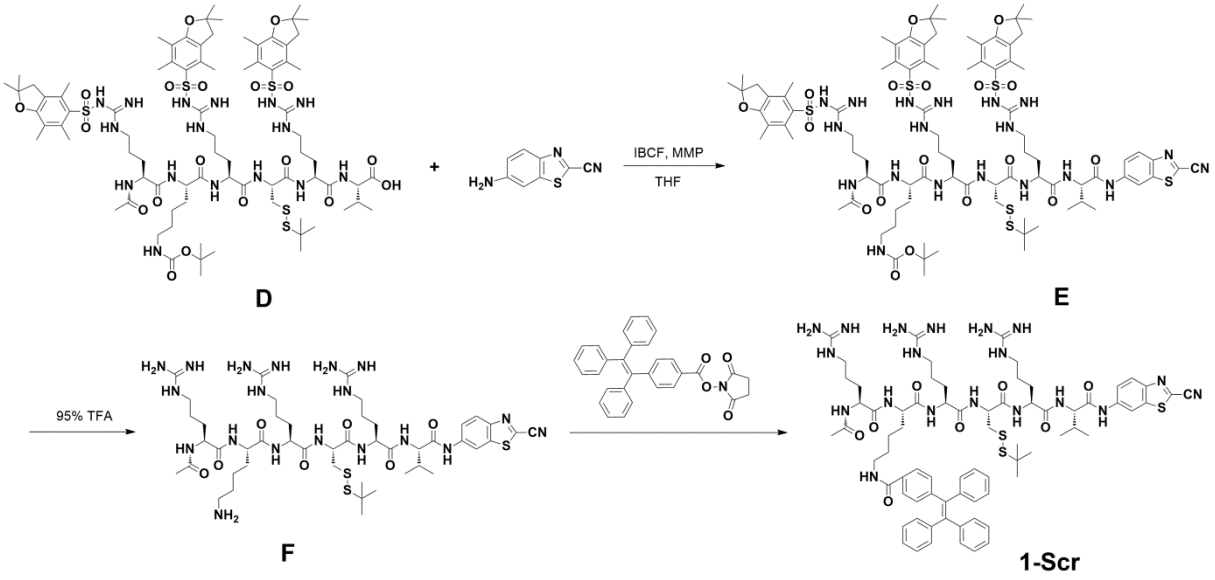
The Boc and Pbf protecting groups of compound **B** were removed with dichloromethane (DCM, 300 μL) and triisopropylsilane (TIPS, 200 μL) in TFA (9.5 mL) for 3 h. Compound **C** (145 mg, yield: 89%) was obtained after HPLC purification using water-methanol added with 0.1% TFA as the eluent. MS: calculated for **C** [$(M-H)^-$]: 1102.53; obsvd. ESI/MS: *m*/*z* 1102.44.

*Synthesis of **1***:

TPE-COOH (37.6 mg, 0.1 mmol), N-hydroxysuccinimide (13.8 mg, 0.12 mmol), EDC • HCl (23 mg, 0.12 mmol) were dissolved in 1 mL DMF and stirred overnight at RT to yield TPE-ester, then Compound **C** (110 mg, 0.1 mmol) and N,N-Diisopropylethylamine (DIPEA, 74.2 mg, 0.57 mmol) was added into the mixture and further stirred for 12 h at RT to yield **1**. Compound **1** (103 mg, yield: 71%) was purified with HPLC using water-acetonitrile added with 0.1% TFA as the eluent. ^{1}H NMR of **1** (d_6-DMSO, 300 MHz, Figure S3) δ (ppm): 8.74 (s, 1 H), 8.39-8.03 (m, 7 H, NH), 7.76 (dd, 1 H), 7.69 (dd, 1 H), 7.14-6.93 (m, 19 H, Ar-H), 4.55 (t, 1 H), 4.39 (t, 1 H), 4.28 (t, 3 H), 4.18 (t, 1 H), 3.21 (t, 2 H), 3.10 (m, 6 H), 2.96 (d, 2 H), 1.97 (m, 2 H), 1.87 (s, 3 H), 1.69 (m, 6 H), 1.51 (m, 10 H), 1.41 (m, 2 H), 1.26 (s, 9 H), 0.81 (m, 6 H). ^{13}C NMR of **2** (d_6-DMSO, 75 MHz, Figure S4) δ (ppm): 171.58, 171.35, 170.96, 169.70, 169.57, 165.78, 159.06, 158.60, 156.77, 147.65, 145.94, 142.83, 142.72, 141.34, 139.73, 139.31, 136.63, 134.97, 132.39, 130.56, 130.39, 127.85, 127.79, 126.73, 126.65, 120.77, 118.07, 114.17, 113.51, 57.36, 53.89, 52.41, 52.30, 47.67, 42.50, 40.37, 40.24, 38.58, 31.53, 30.64, 29.44, 28.76, 25.09, 22.82, 22.38, 19.11, 17.92. MS: calculated for **1** $C_{73}H_{96}N_{19}O_8S_3$ [$(M+H)^+$]: 1462.6851; obsvd. MALDI-TOF/MS: *m*/*z* 1462.6825 (Figure S5).

*Preparation of Ac-Arg-Lys(TPE)-Arg-Cys(StBu)-Arg-Val-CBT (**1-Scr**)*:

Scheme S3. The synthetic route for **1-Scr**.



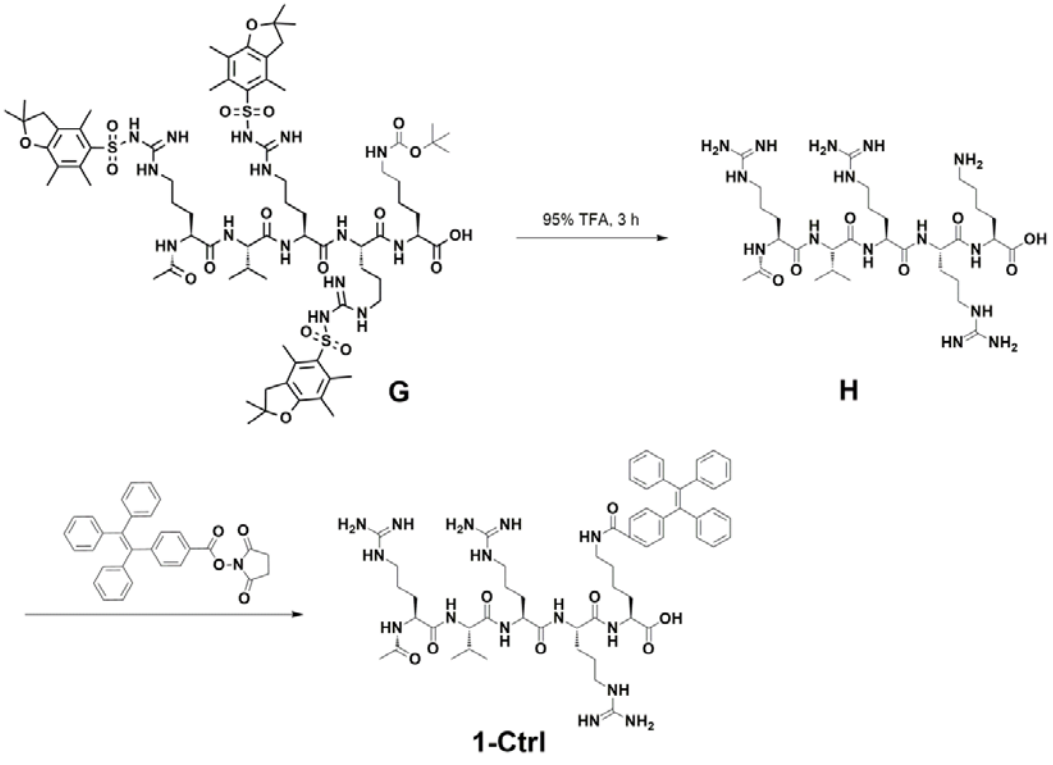
*Synthesis of **1-Scr:***

Compound **D** was synthesized with solid phase peptide synthesis (SPPS). Then coupled to CBT with the same method as above, followed by labeling with TPE-NHS ester in DMF overnight at RT. Compound **1-Scr** (20 mg, yield 60%) was obtained after HPLC purification using water-acetonitrile added with 0.1% TFA as the eluent. ^{1}H NMR of **1-Scr** (d_6-DMSO, 300 MHz, Figure S6) δ (ppm): 8.58 (d, J=3.0 Hz, 1 H), 7.46 (s, 1 H), 7.44 (s, 1 H), 6.99-6.81 (m, 19 H, Ar-H), 4.37 (d, J = 6.0 Hz, 1 H), 4.20 (t, J = 6.0 Hz, 2 H), 4.09 (d, J = 6.0 Hz, 3 H), 3.03 (m, 2 H), 2.94 (m, 6 H), 2.83 (m, 2 H), 2.35 (s, 3 H, NH), 1.91 (m, 1 H), 1.70 (s, 3 H), 1.54 (m, 4 H), 1.34 (m, 12 H), 1.10 (s, 9 H), 0.76 (d, J = 6.0 Hz, 6 H). ^{13}C NMR of **1-Scr** (d_6-DMSO, 75 MHz, Figure S7) δ (ppm): 171.82, 171.68, 171.37, 170.60, 170.50, 169.78, 169.48, 165.70, 159.11, 158.69, 156.78, 156.75, 147.68, 145.99, 142.84, 142.73, 141.36, 139.78, 139.19, 136.65, 135.01, 132.41, 130.57, 130.53, 130.43, 127.86, 127.81, 126.70, 120.76, 118.82, 113.52, 111.29, 58.67, 52.67,

52.35, 52.21, 47.64, 42.85, 40.28, 38.61, 31.54, 30.65, 29.48, 28.95, 24.96, 23.00, 22.44, 19.09, 18.14. MS: calculated for **1-Scr** $C_{73}H_{96}N_{19}O_8S_3$ $[(M+H)^+]$: 1462.68514; obsvd. MALDI-TOF/MS: *m*/*z* 1462.68510 (Figure S8).

*Preparation of Ac-Arg-Val-Arg-Arg-Lys(TPE)-OH (**1-Ctrl**):*

Scheme S4. The synthetic route for **1-Ctrl**.



*Synthesis of **1-Ctrl:***

Compound **G** was synthesized with solid phase peptide synthesis (SPPS). Then the Boc and Pbf protecting groups of compound **G** were removed with dichloromethane (DCM, 300 μL) and triisopropylsilane (TIPS, 200 μL) in TFA (9.5 mL) for 3 h. Finally, compound **H** reacted with TPE-NHS ester in DMF overnight at RT. Compound **1-Ctrl** was obtained after HPLC purification using water-acetonitrile added with 0.1%

TFA as the eluent. ^{1}H NMR of **1-Ctrl** (d_6-DMSO, 300 MHz, Figure S9) δ (ppm): 8.39-7.57 (NH, 11 H), 7.11-6.96 (m, 19 H, Ar-H), 4.27-4.11 (m, 5 H), 3.16 (m, 2 H), 3.07 (m, 6 H), 1.94 (m, 1 H), 1.85 (s, 3 H), 1.64 (m, 4 H), 1.47 (m, 12 H), 1.22 (m, 2 H), 0.80 (t, 6 H). ^{13}C NMR of **1-Ctrl** (d_6-DMSO, 75 MHz, Figure S10) δ (ppm): 173.35, 171.46, 171.23, 171.04, 170.79, 169.59, 166.71, 158.95, 158.51, 156.75, 146.01, 142.84, 142.73, 141.37, 139.78, 132.39, 130.58, 130.45, 127.88, 127.81, 126.69, 57.24, 52.31, 52.06, 51.90, 40.30, 40.02, 30.71, 29.50, 28.77, 25.09, 22.88, 22.40, 19.11, 17.87. MS: calculated for **1-Ctrl** $C_{58}H_{80}N_{15}O_8$ [$(M+H)^+$]: 1113.6236; obsvd. MALDI-TOF/MS: *m*/*z* 1114.6314 (Figure S11).

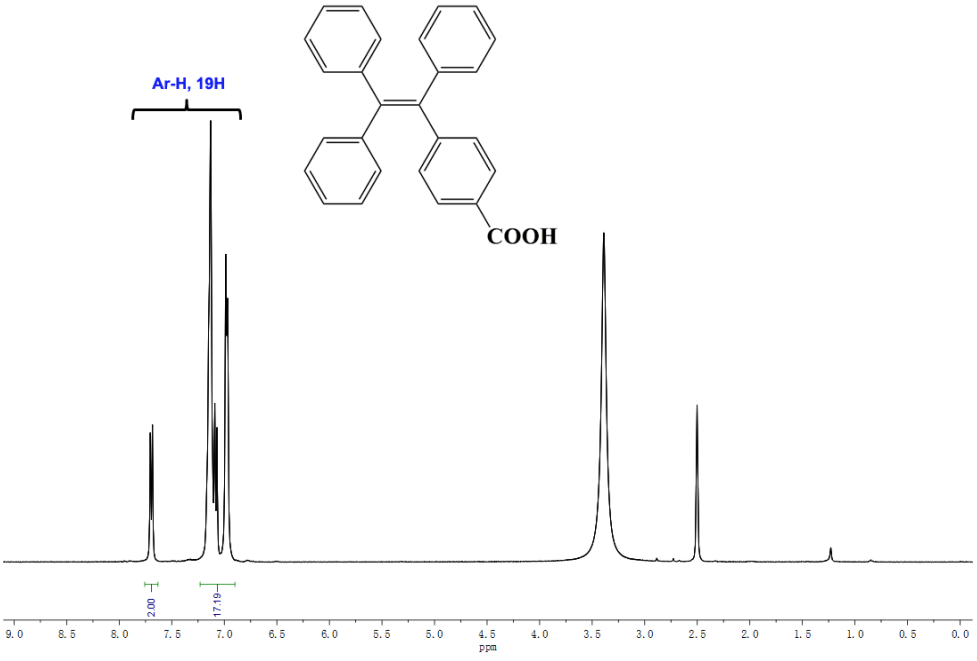


Figure S1. ^{1}H NMR spectrum of TPE-COOH.

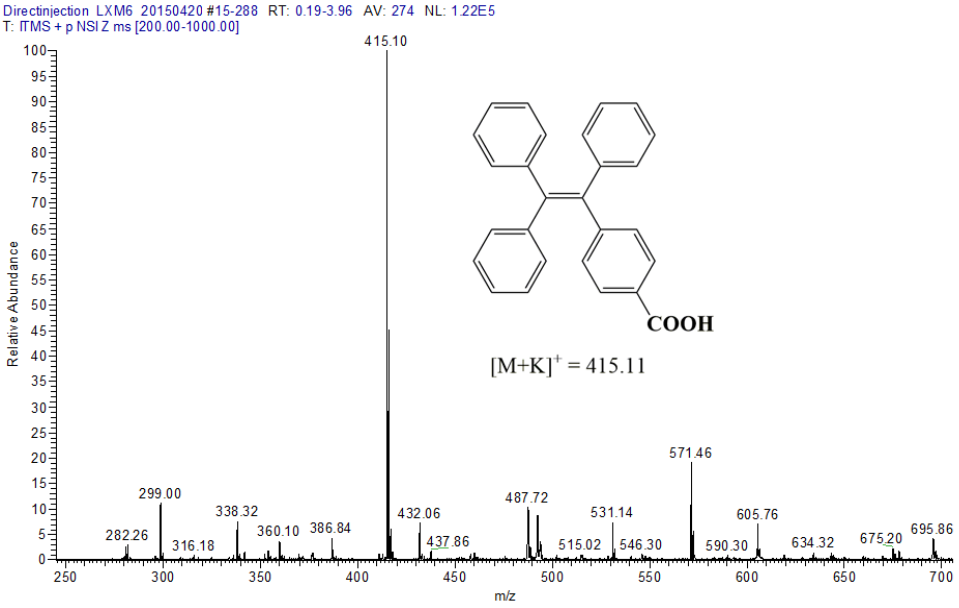


Figure S2. ESI/MS spectrum of TPE-COOH.

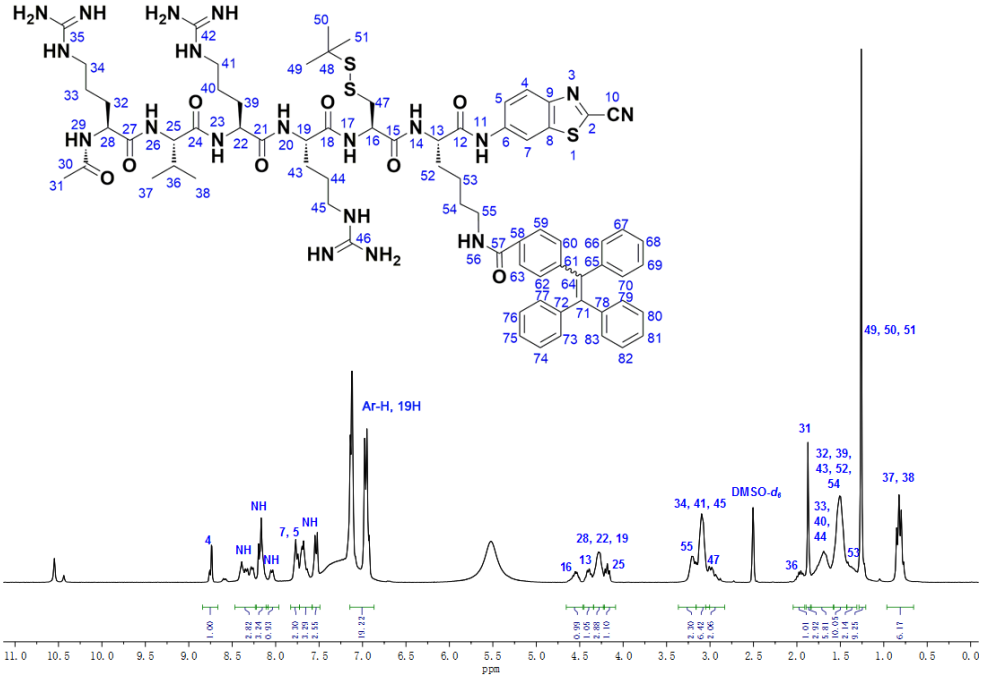


Figure S3. ^{1}H NMR spectrum of **1** in d_6-DMSO.

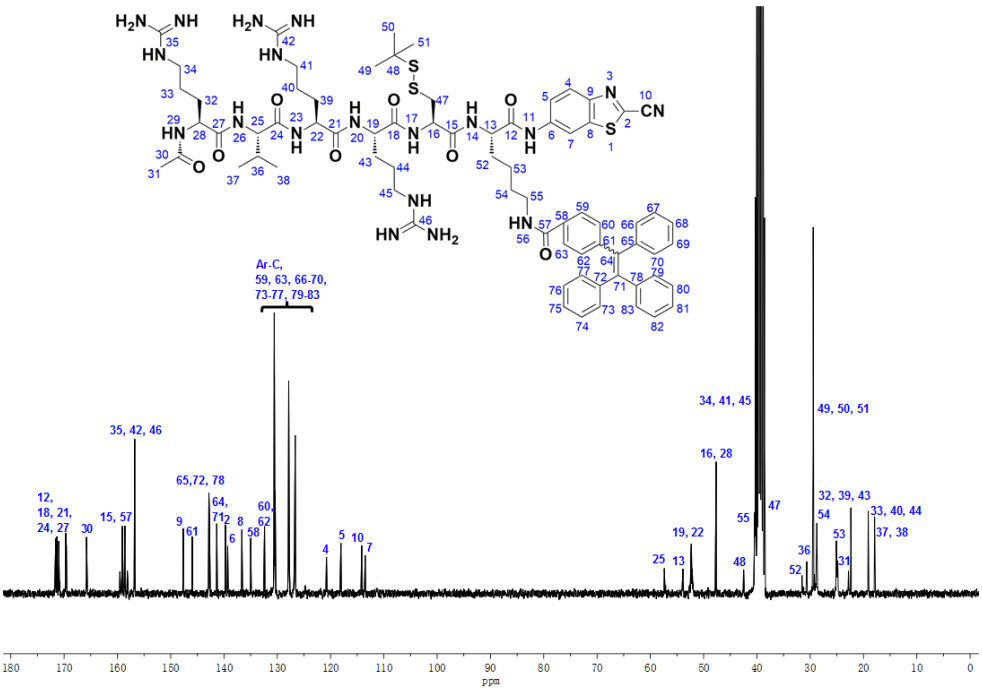


Figure S4. ^{13}C NMR spectrum of **1** in d_6-DMSO.

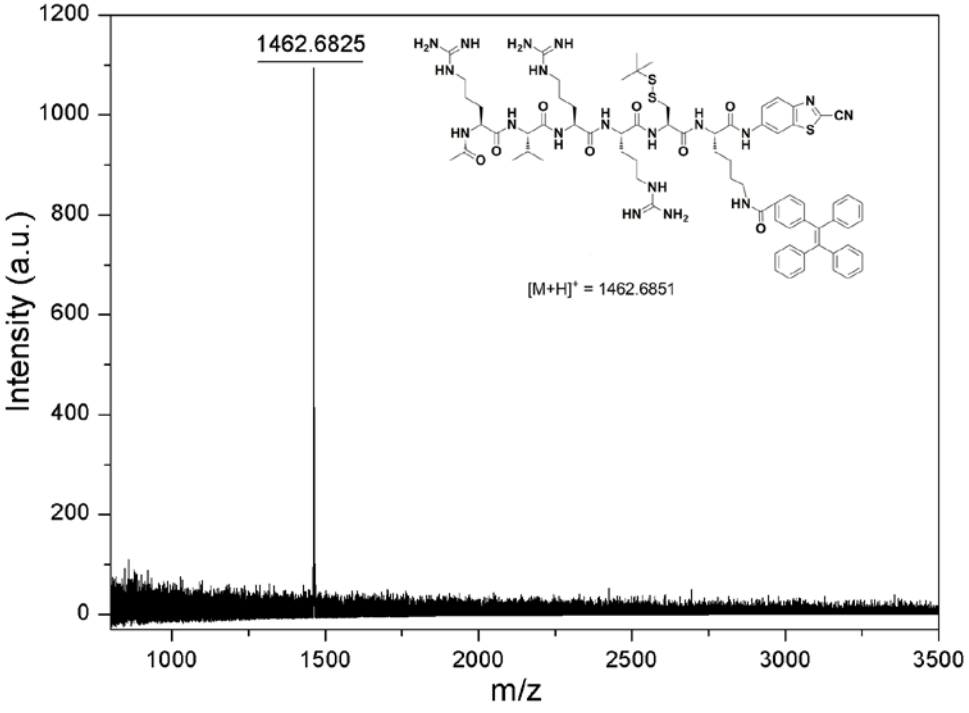


Figure S5. HR-MALDI-TOF/MS spectrum of **1**.

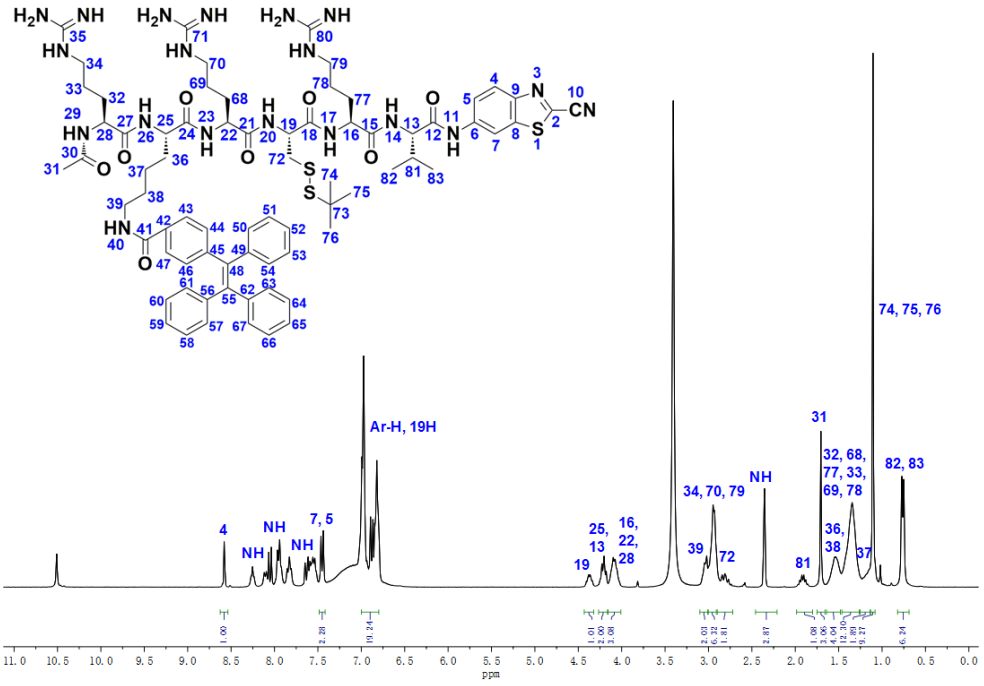


Figure S6. ^{1}H NMR spectrum of **1-Scr** in d_6-DMSO.

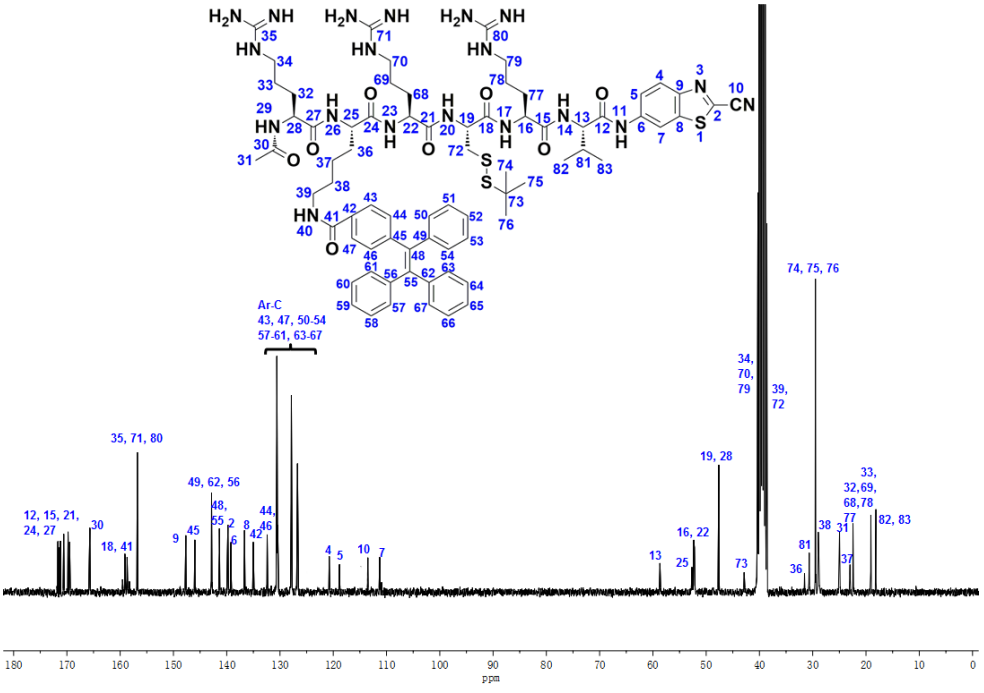


Figure S7. ^{13}C NMR spectrum of **1-Scr** in d_6-DMSO.

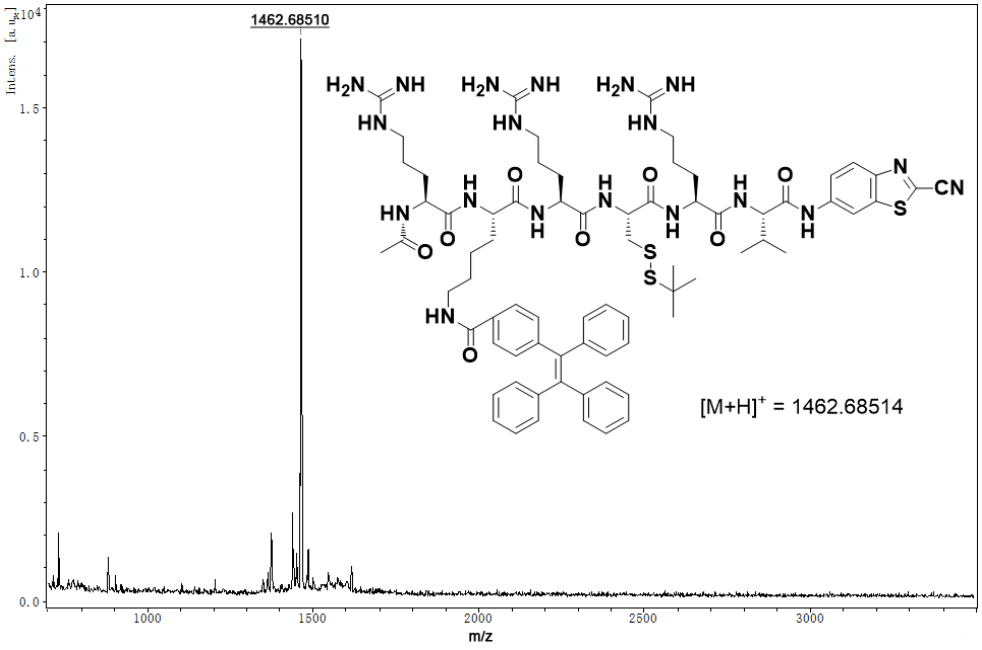


Figure S8. HR-MALDI-TOF/MS spectrum of **1-Scr**.

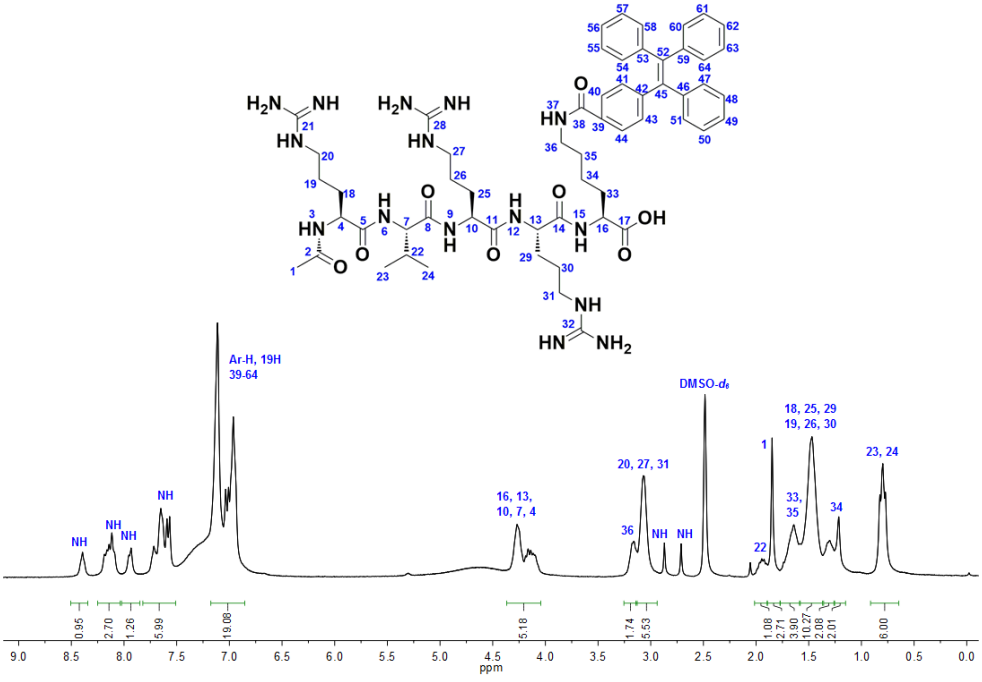


Figure S9. ^{1}H NMR spectrum of **1-Ctrl** in d_6-DMSO.

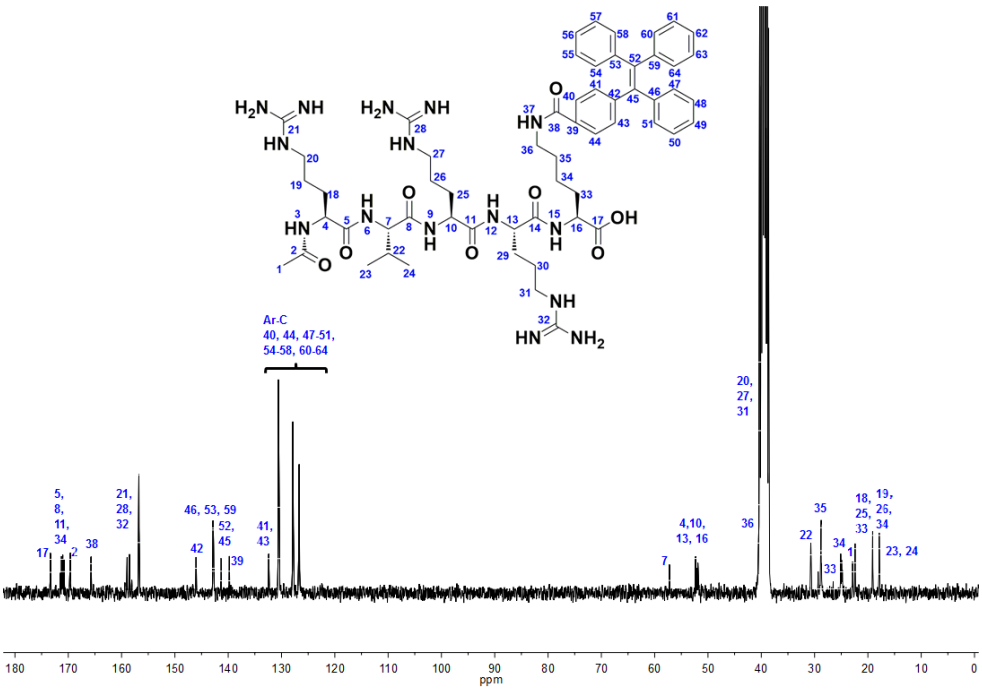


Figure S10. ^{13}C NMR spectrum of **1-Ctrl** in d_6-DMSO.

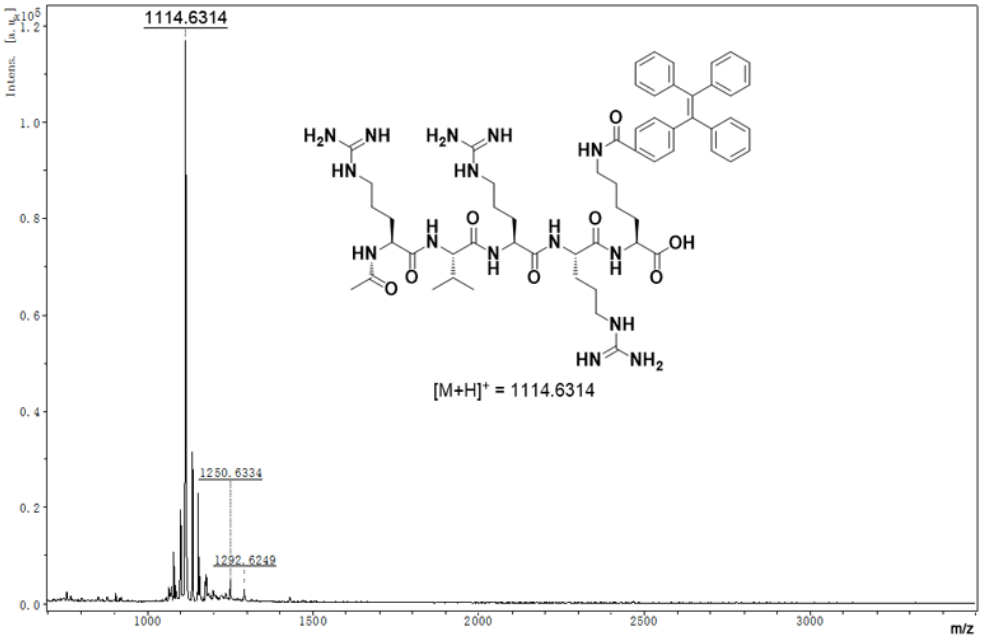


Figure S11. HR-MALDI-TOF/MS spectrum of **1-Ctrl.**

3. Supporting figures and tables

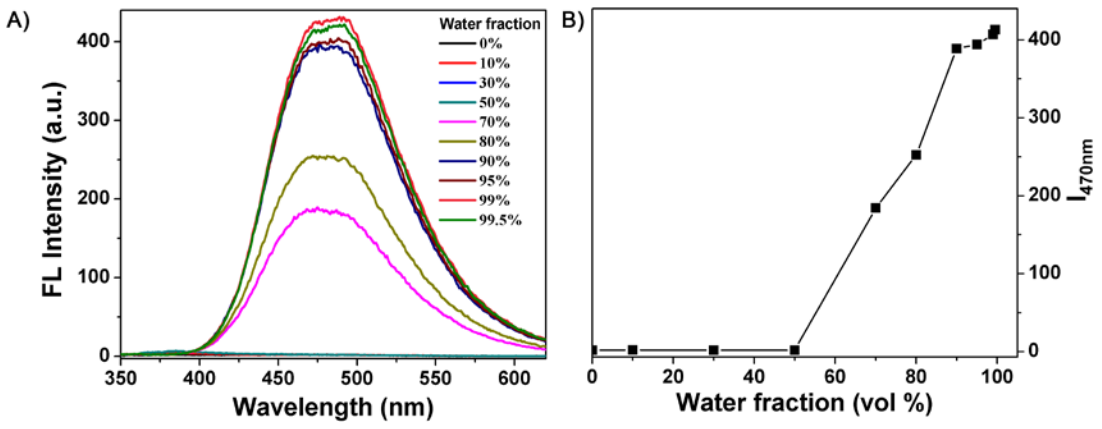


Figure S12. (A) Fluorescence spectra of 100 μM TPE-COOH in DMSO/H_2O mixture at various water fractions; (B) Variation of fluorescence intensity at 470 nm against water fractions.

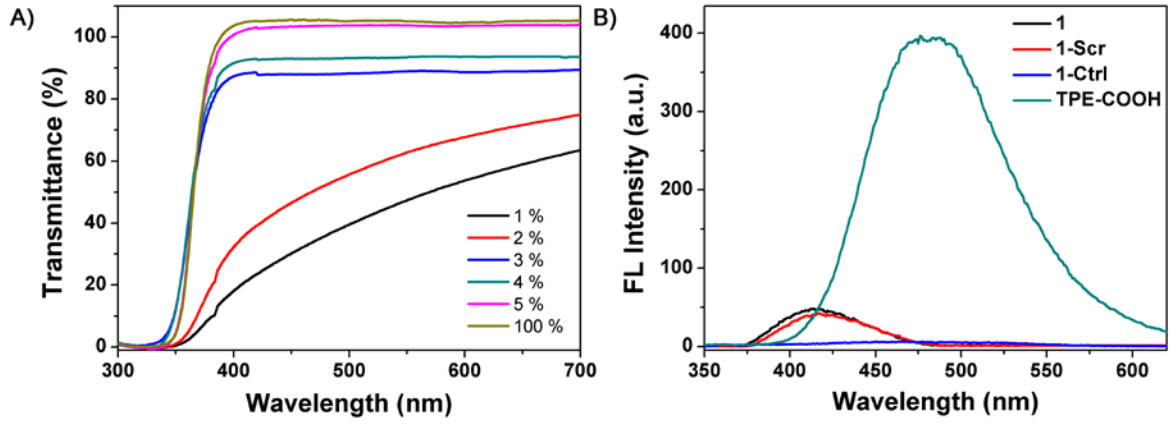


Figure S13. (A) UV−vis transmittance spectra of **1** in DMSO/water mixture at various DMSO fractions. (B) FL spectra of TPE-COOH, **1**, **1-Scr**, and **1-Ctrl** in the aqueous solution containing 5% DMSO. Excitation wavelength: 320 nm.

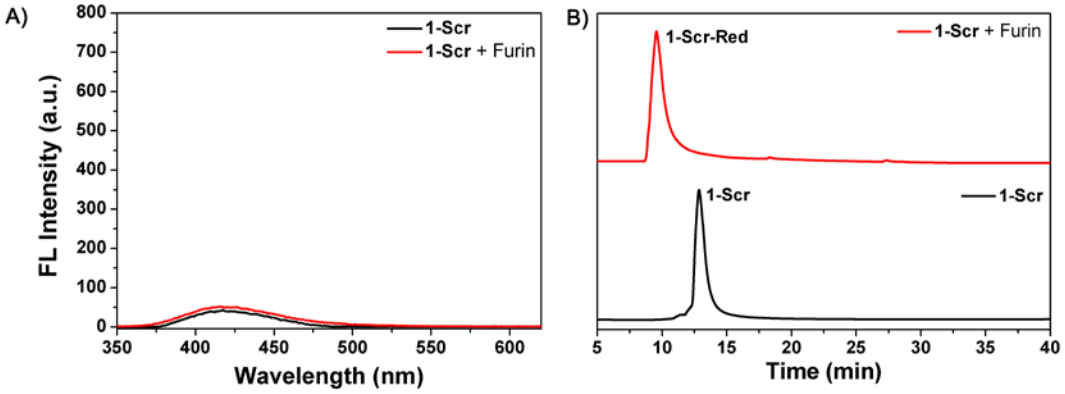


Figure S14. (A) Fluorescence spectra of 100 μM **1-Scr** (black), 100 μM **1-Scr** treated with 1 nmol U^{-1} furin at 37 °C for 4 h in furin buffer (red). Excitation wavelength: 320 nm. (B) HPLC trace of 100 μM **1-Scr** (black), the incubation mixture of 100 μM **1-Scr** treated with 1 nmol U^{-1} furin at 37 °C for 4 h (red).

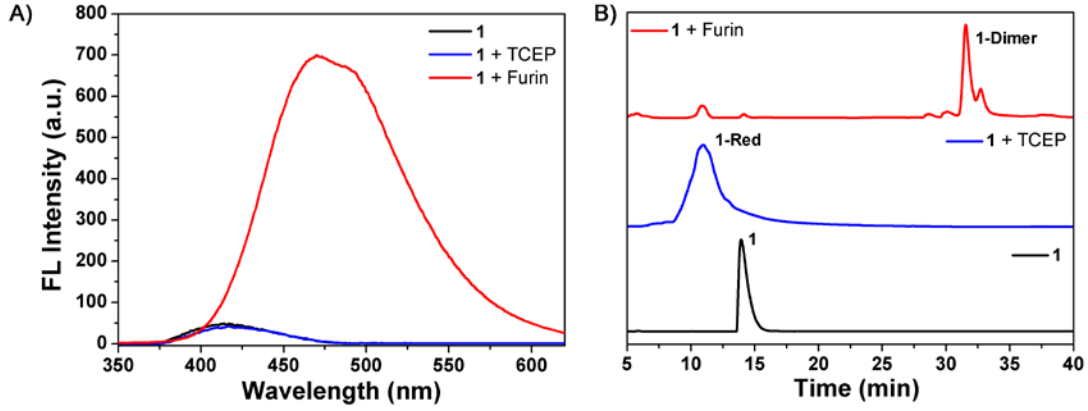


Figure S15. (A) Fluorescence spectra of 100 μM **1** (black), 100 μM **1** treated with 1 mM TCEP (blue), and 100 μM **1** treated with 1 nmol U^{-1} furin at 37 °C for 4 h in furin buffer (red). Excitation wavelength: 320 nm. (B) HPLC trace of 100 μM **1** (black), the mixture of 100 μM **1** incubated with 1 mM TCEP (blue), and the incubation mixture of 100 μM **1** treated with 1 nmol U^{-1} furin at 37 °C for 4 h (red).

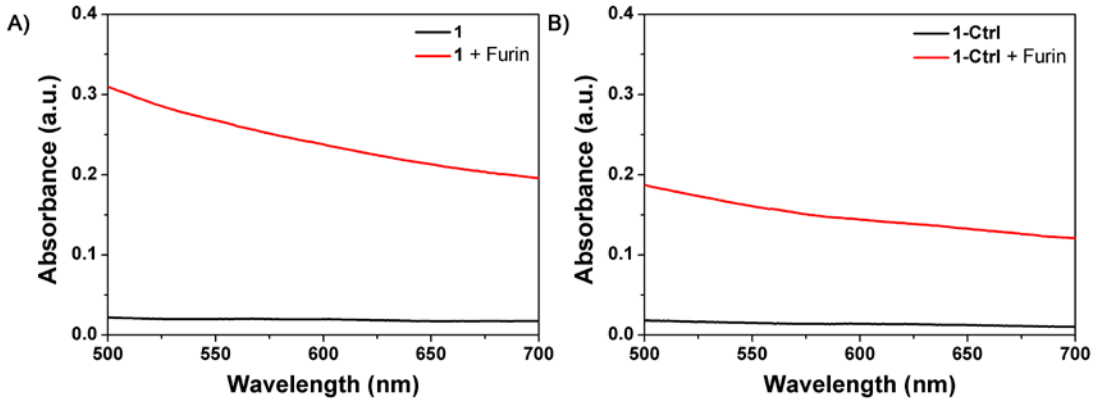


Figure S16. (A) UV-vis absorption spectra (500-700 nm due to the light scattering) of 100 μM **1** (black) and 100 μM **1** treated with 1 nmol U^{-1} furin at 37 °C for 4 h (red). (B) UV-vis absorption spectra (500-700 nm due to the light scattering) of 100 μM **1-Ctrl** (black) and 100 μM **1-Ctrl** treated with 1 nmol U^{-1} furin at 37 °C for 4 h (red).

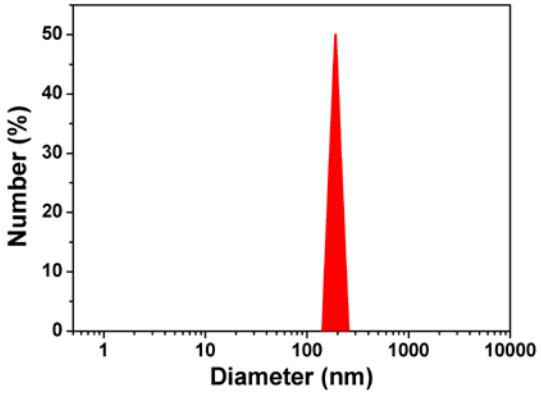


Figure S17. Dynamic light scattering (DLS) measurements of the dual aggregated products of **1** treated with 1 nmol U^{-1} furin at 37 °C for 4 h in furin buffer (i.e., **1-NPs**).

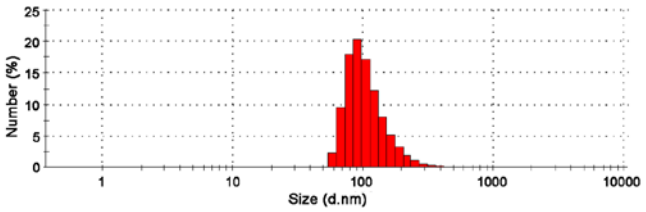


Figure S18. Dynamic light scattering (DLS) measurements of the single aggregated products of **1-Ctrl** treated with 1 nmol U^{-1} furin at 37 °C for 4 h in furin buffer (i.e., **1-Ctrl-NPs**).

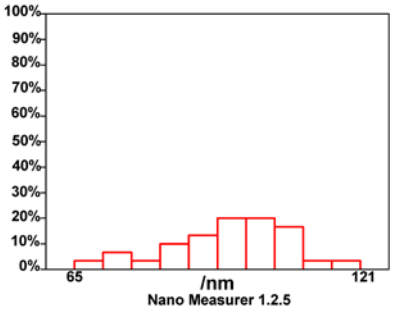


Figure S19. Histogram of diameters of **1-NPs** in the TEM image of Figure 2C.

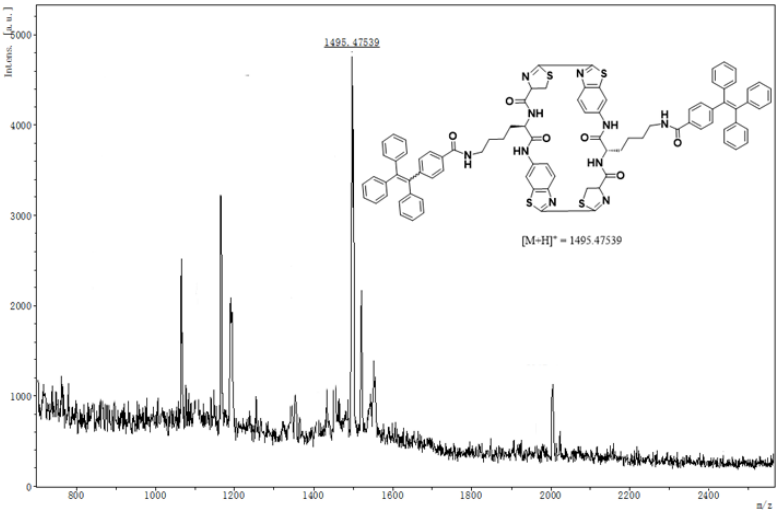


Figure S20. HR-MALDI-TOF/MS spectrum of the **1-Dimer** peak at retention time of 31.5 min in the blue HPLC trace in Figure 2D, confirming the aggregated product of **1** by furin was **1-Dimer**.

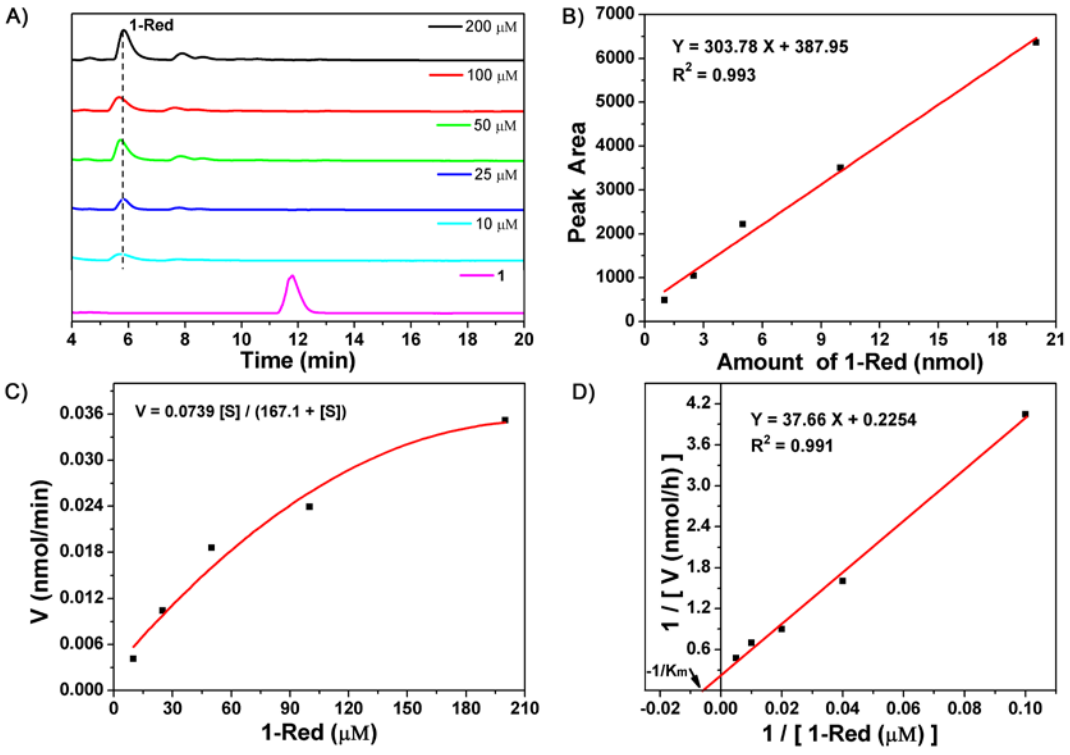


Figure S21. (A) HPLC traces of the incubation mixture of **1** at different concentrations after 1 h incubation with 1 mM TCEP at 37 °C, and HPLC trace of **1** in water (pink). Absorbance: 320 nm. (B) Plotting of the HPLC peak areas vs. the **1-Red** amounts in A. (C) Non-linear regression analysis of furin cleavage rate V (nmol/min) as a function of **1-Red** concentration and fitted to the Michaelis-Menten model to give formula: V = 0.0739*[S] / (167.1 + [S]). [S] = concentration of substrate used. (D) Lineweaver-Burk plot for the enzymatic kinetics of furin towards **1-Red** in C. The Km (167 μM) and Vmax (0.0739 nmol/min) were obtained from the formula above. The enzymatic parameters K_{cat} and K_{cat}/K_m of furin towards **1** were calculated to be 7.84 min^{-1} and 0.047 $\mu M^{-1}min^{-1}$, respectively.

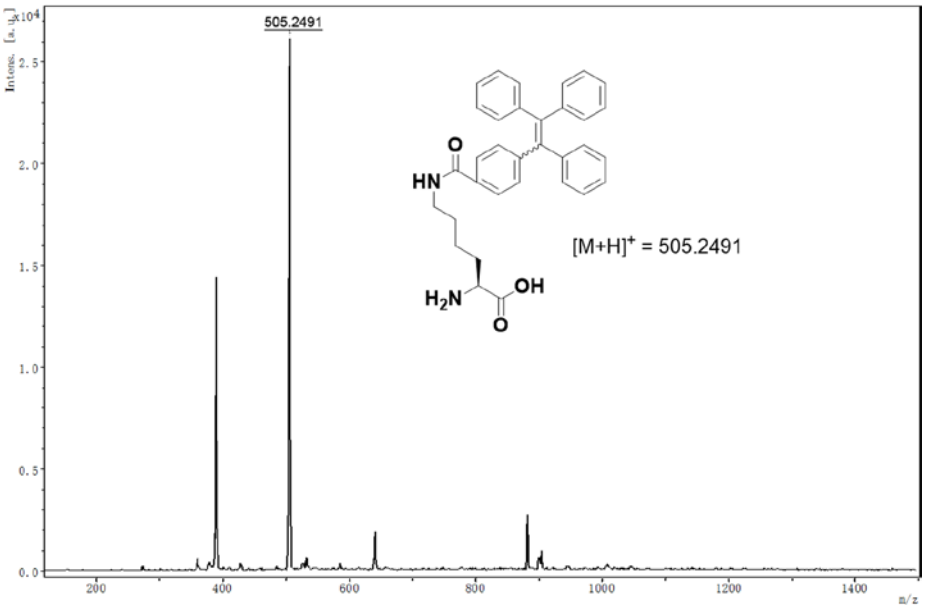


Figure S22. HR-MALDI-TOF/MS spectrum of the **1-Ctrl-Cleaved** peak at retention time of 19.7 min in the green HPLC trace in Figure 2D, suggesting the peak was **1-Ctrl-Cleaved**.

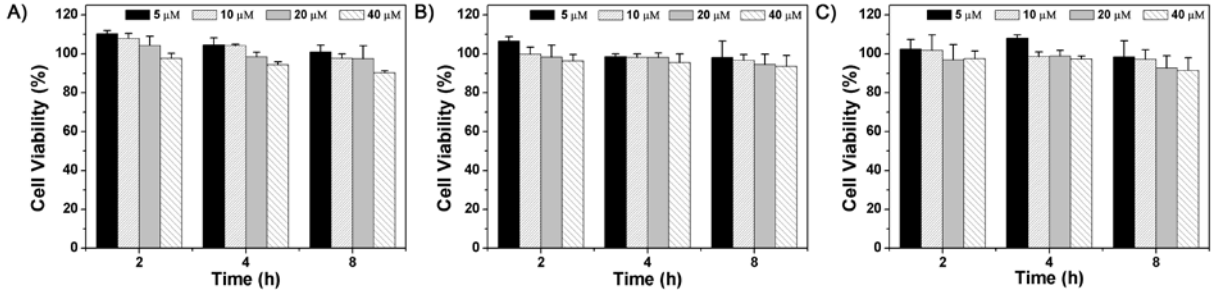


Figure S23. MTT assay of **1** (A), **1-Scr** (B), and **1-Ctrl** (C) on MDA-MB-468 cells. Cell viability values (%) were estimated by MTT proliferation test at compound concentrations of 5, 10, 20, or 40 μM. MDA-MB-468 cells were cultured in the presence of **1**, **1-Scr**, or **1-Ctrl** for 2, 4, or 8 h at 37 °C under 5% CO_2, respectively. These experiments were performed in triplicate. Results are representative of three independent experiments. Error bars represent standard deviations.

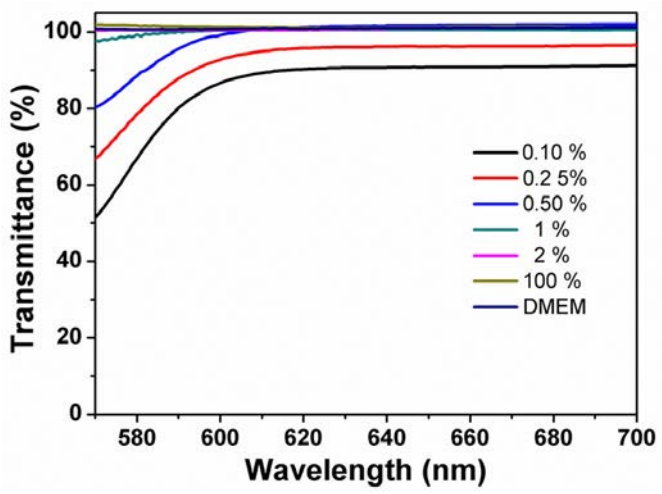


Figure S24. UV−vis transmittance spectra of **1** in DMSO/DMEM mixture at various DMSO fractions.

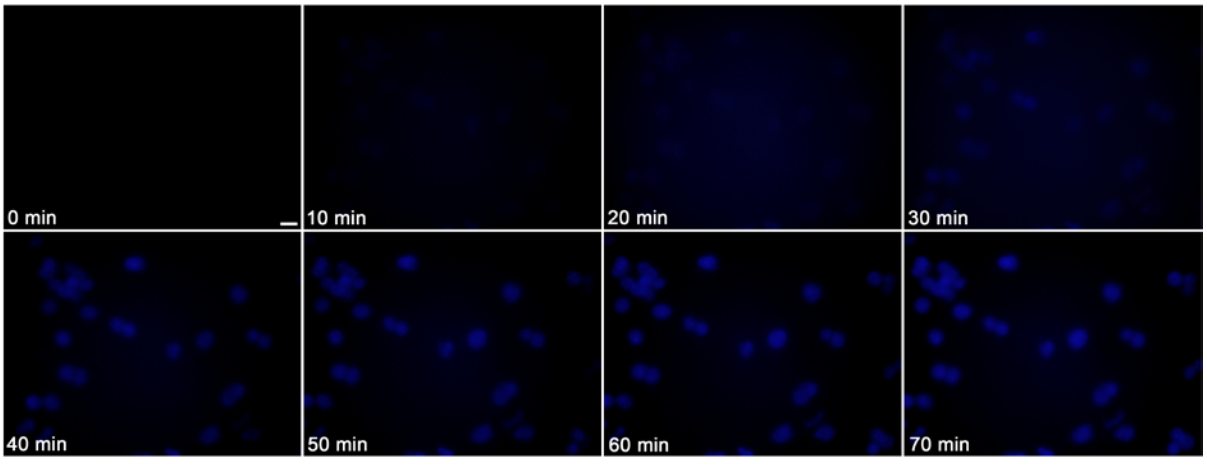


Figure S25. Time course fluorescence-microscopic images of MDA-MB-468 cells incubated with 5 μM **1** (1% DMSO) and 50 μM **C**. All images have the same scale bar: 20 μm

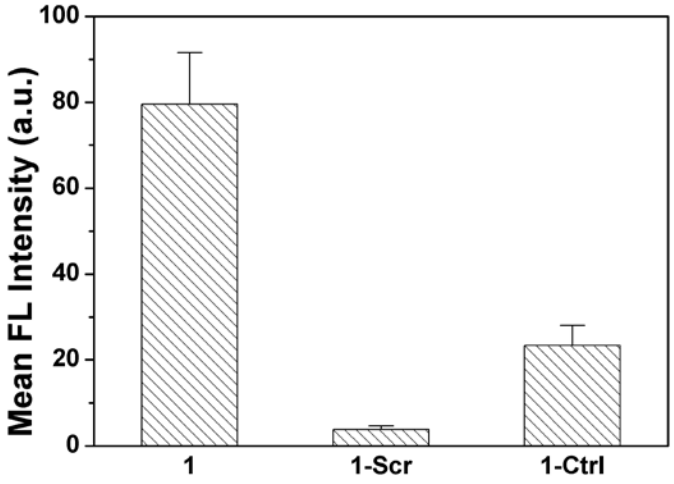


Figure S26. Mean fluorescence intensities of cell images from the DAPI channel in Figure 3 (middle row). Fluorescence intensities of the images were analyzed with ImageJ (Universal Imaging Corp.).

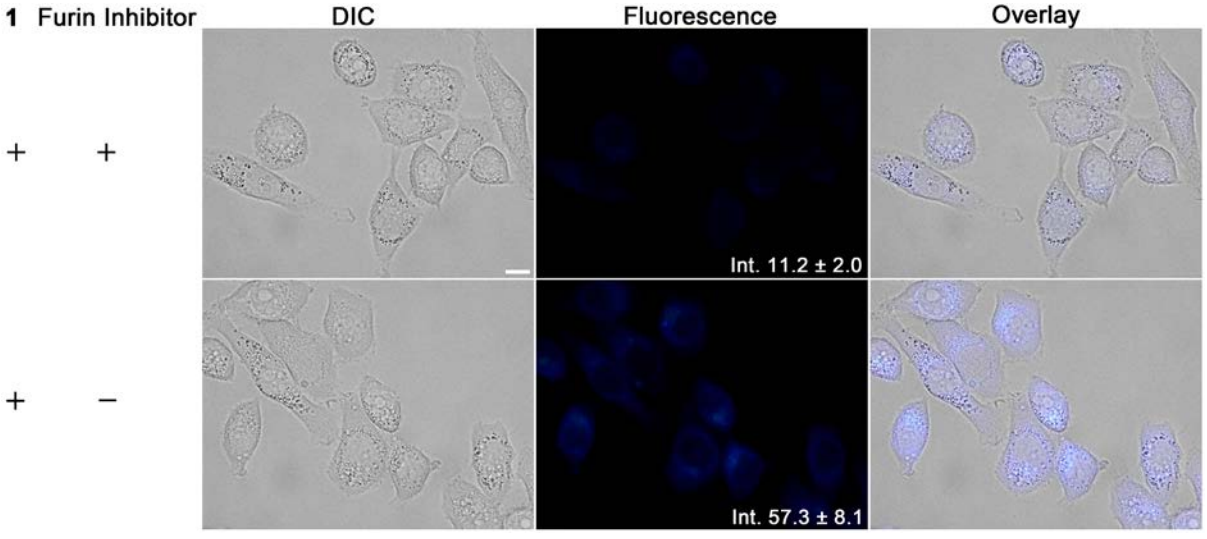


Figure S27. Differential interference contrast (DIC) images (left column), fluorescence images (middle column, DAPI channel), and merged images (right column) of MDA-MB-468 cells pretreated with (top row) or without (bottom row) 500 μM furin inhibitor II (H-(D)Arg-Arg-Arg-Arg-Arg-Arg-NH_2) for 30 min in serum-free medium at 37 °C and washed with PBS for three times, then incubated with 5 μM **1**

(co-incubated with 50 μM **C**) for 1 h, washed with PBS for another three times prior to imaging, respectively. All images have the same scale bar: 10 μm.

Table S1. HPLC condition for the purifications of **1**, **1-Scr**, and **1-Ctrl**.

Time (minute)	Flow (mL/min.)	H_2O% (0.1% TFA)	CH_3CN% (0.1% TFA)
0	3.0	50	50
3	3.0	50	50
35	3.0	5	95
37	3.0	5	95
38	3.0	50	50
40	3.0	50	50

Table S2. HPLC condition for the enzymatic assay of **1** and **1-Scr** in Fig. 2D and S15.

Time (minute)	Flow (mL/min.)	H_2O% (0.1% TFA)	CH_3CN% (0.1% TFA)
0	1.0	50	50
3	1.0	50	50
35	1.0	5	95
37	1.0	5	95
38	1.0	50	50
40	1.0	50	50

Table S3. HPLC condition for enzymatic assay of **1-Ctrl** in Fig. 2D.

Time (minute)	Flow (mL/min.)	H_2O% (0.1% TFA)	CH_3CN% (0.1% TFA)
0	1.0	70	30
3	1.0	70	30
35	1.0	10	90
37	1.0	10	90
38	1.0	70	30
40	1.0	70	30

4. References

1 G. D. Liang, J. W. Y. Lam, W. Qin, J. Li, N. Xie and B. Z. Tang, *Chem. Commun.*, 2014, **50**, 1725.