Supplementary Information

A biocompatible condensation reaction for controlled assembly of nanostructures in live cells

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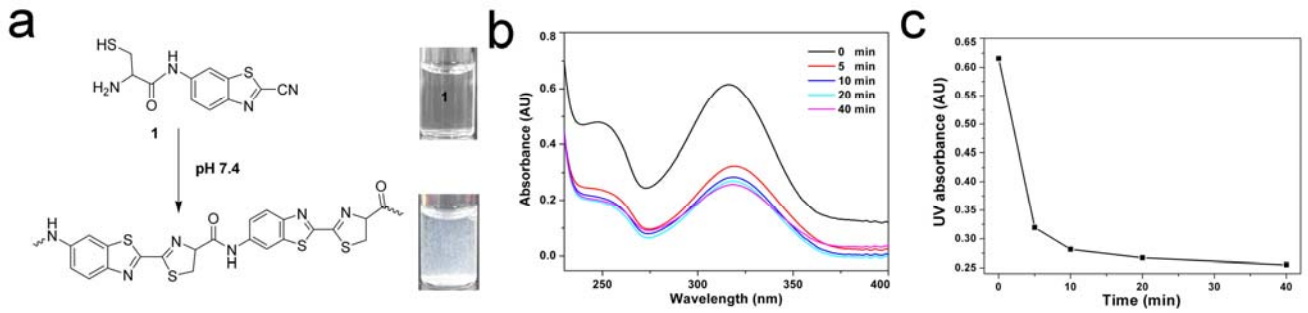
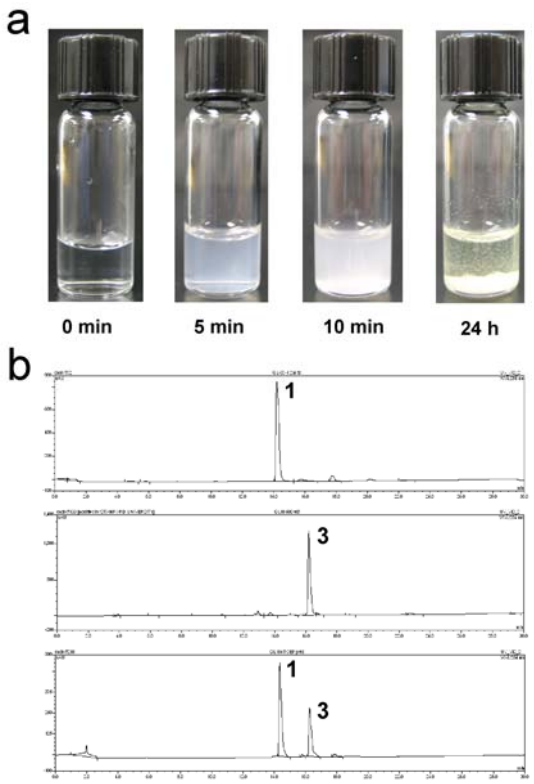
Supplementary Figure 1



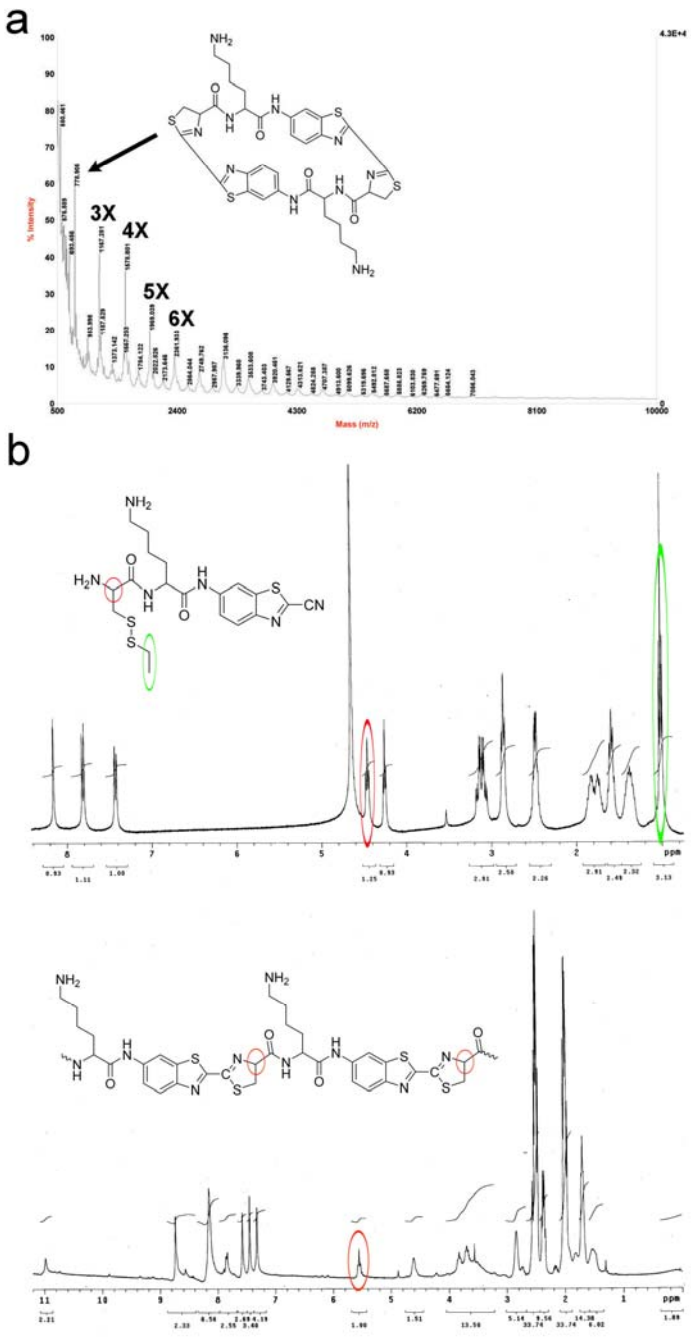
Supplementary Figure 1. Characterizations of the pH controlled condensation reaction of monomer 1 at 200 μM in water. a, Schematic illustration and optical images of the pH controlled condensation of **1** at 200 μM in water: Increasing the pH value of acidic solution of **1** initiates the condensation process and produces the water-insoluble oligomers of monomer **1**. **b**, The UV spectra of the supernatant of the suspension in **a** at indicated time points. **c**, Plot of the UV absorbance at 316 nm vs. time.

Supplementary Figure 2



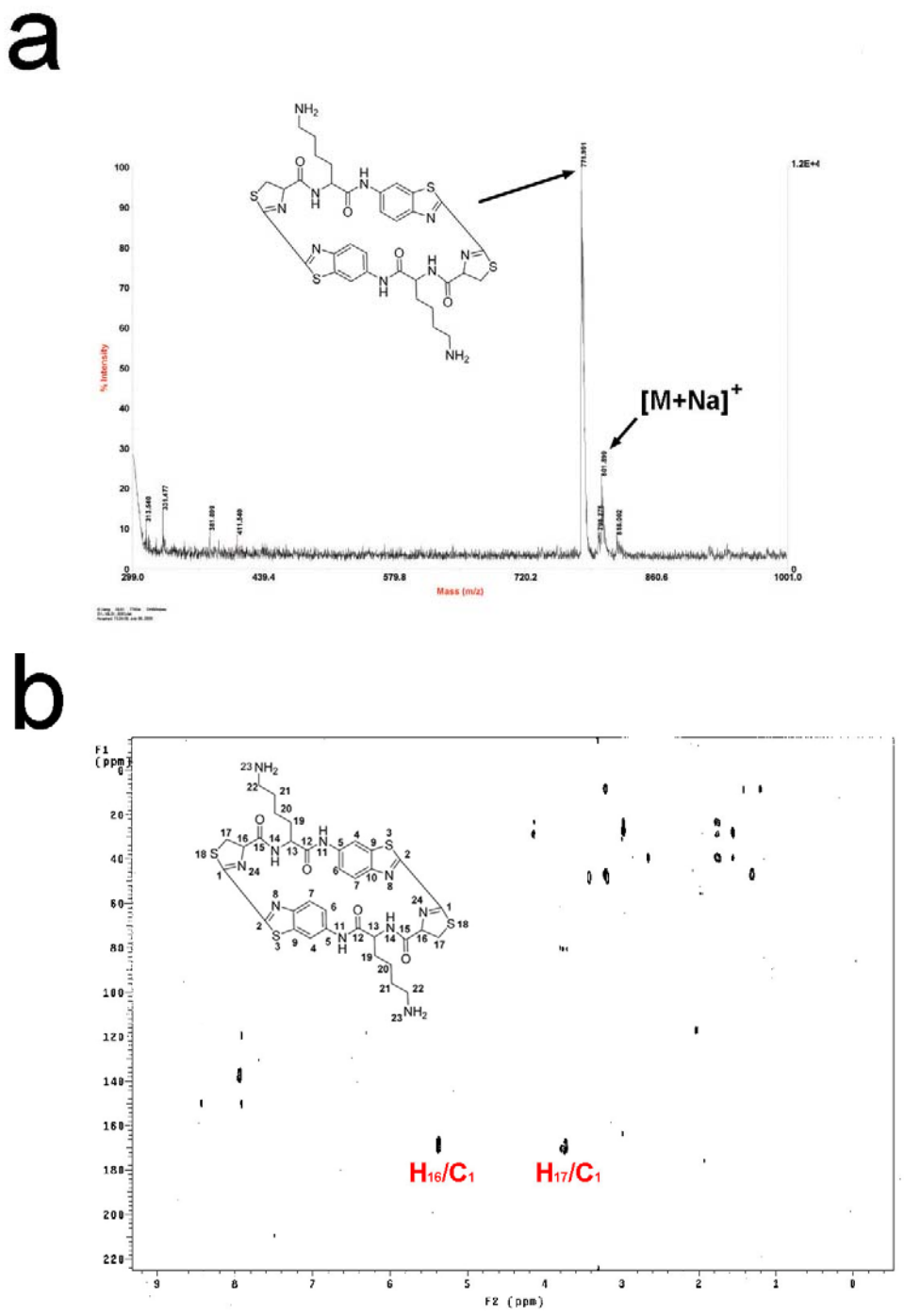
Supplementary Figure 2. **Reduction-controlled condensation of monomer 3. a**, Optical images of condensation process of **3** at 2 mM, pH 7.4, and reduced by tris(2-carboxyethyl)phosphine hydrochloride (TCEP). **b**, HPLC traces of **1** (upper), **3** (middle), and **3** treated with TCEP (lower) for 5 min at pH 5.

Supplementary Figure 3



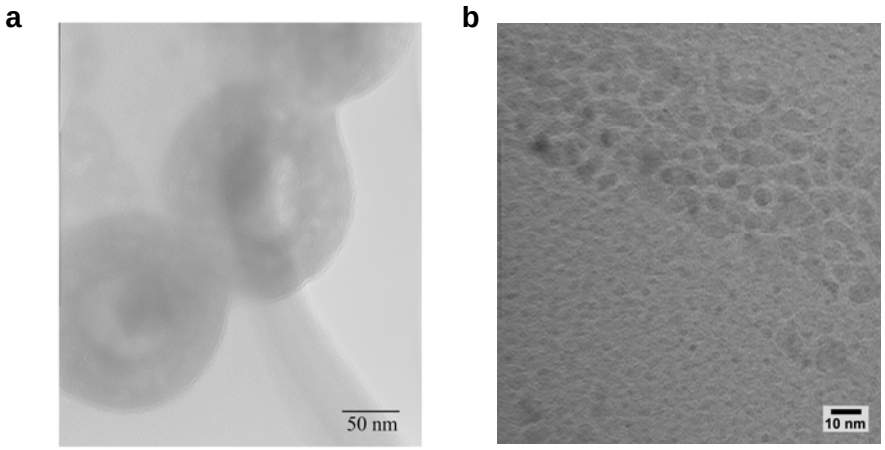
Supplementary Figure 3. **MALDI and NMR characterizations of the reduction-triggered condensation of monomer 4. a**, MALDI mass spectrum of the condensation product of monomer **4**. **b**, ^{1}H-NMR spectra of monomer **4** in D_2O (upper), and of its product in DMSO-d_6 after TCEP reduction (lower). Note the disappearance of the ethyl group on the disulfide in the product (lower), and also the change of the chemical shift of the proton on the alpha carbon of cysteine after condensation.

Supplementary Figure 4



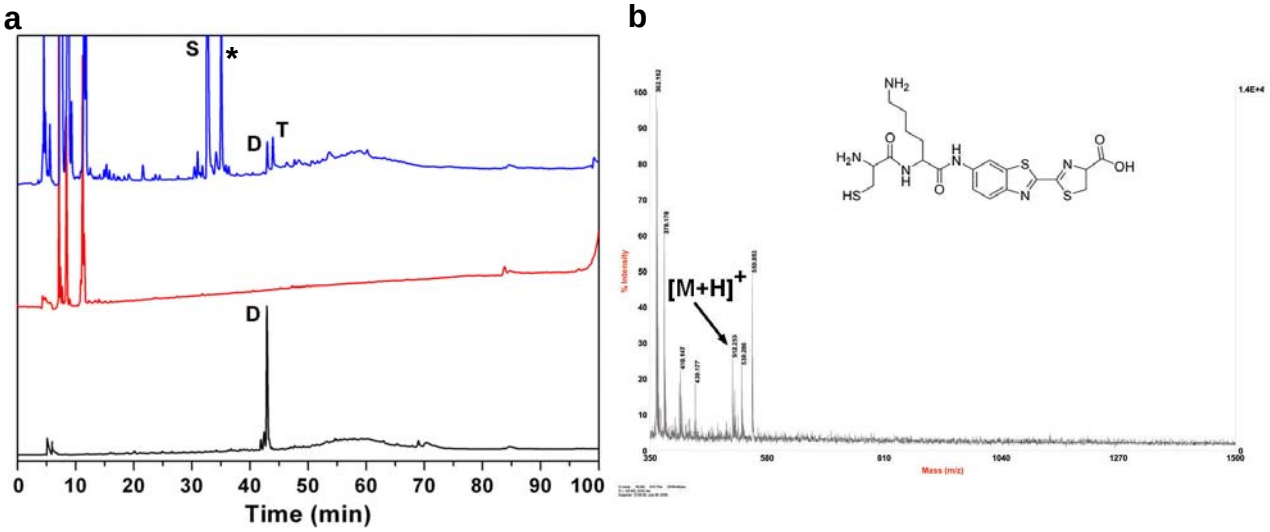
Supplementary Figure 4. **MALDI mass spectroscopic and 2D NMR characterizations of purified cyclic dimer from the oligomers of 4 after condensation**. **a**. MALDI mass spectrum shows the expected molecular weight. **b**. Heteronuclear Multiple Bond Correlation (HMBC) spectrum of the cyclic dimer of **4** proved the formation of ring structure; Both H_{16} and H_{17} have correlations with C_1, indicating the cyclic form of the dimer.

Supplementary Figure 5



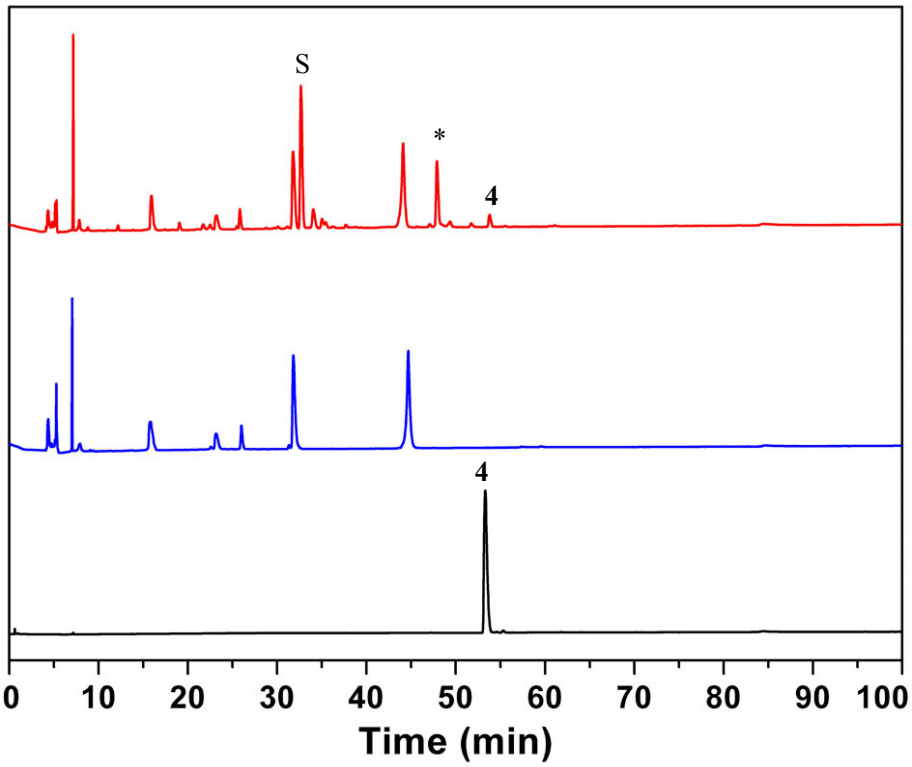
Supplementary Figure 5. **TEM characterization of the nanostructures formed by monomers 3 and 4**. **a**. TEM micrograph of nanoparticles formed during the condensation of monomer **3**; note the donut shape structure. **b**. High-resolution TEM micrograph of hydrogel formed during the condensation of monomer **4**.

Supplementary Figure 6



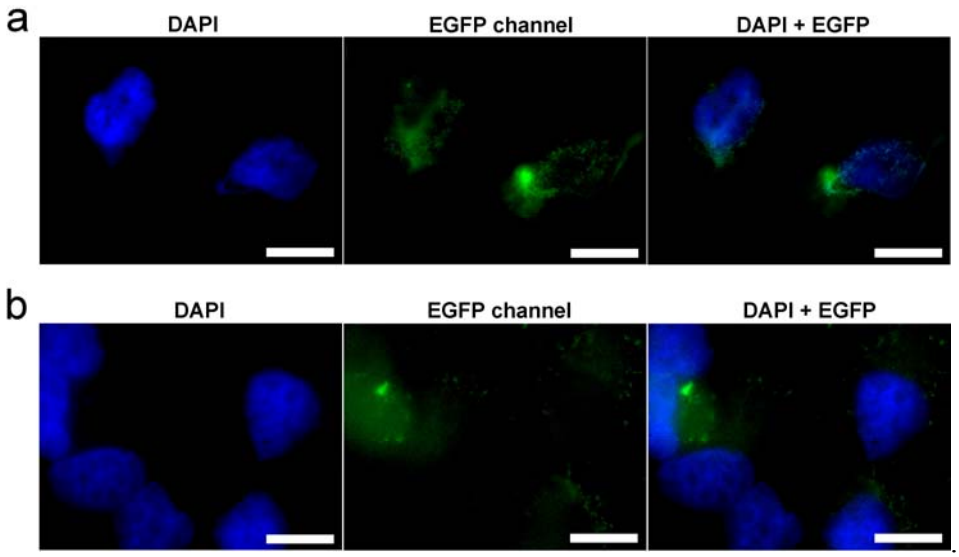
Supplementary Figure 6. **Analysis of the condensation of monomer 4 in the lysates of MDA-MB-468 cells**. **a**, HPLC chromatograms of three samples: 1) upper, cell lysates (from 3 x 10^6 cells) incubated with **4** (200 μM) for 1 h at 37 °C; 2) middle, cell lysates before incubation with monomer **4**; and 3) lower, solution of **4** after TCEP reduction at pH 7.4 (**Figure 4c**). **b**, MALDI TOF mass spectrum of the isolated peak S in **(a)** reveals as the condensation product of monomer **4** with free cysteine in the lysates (observed: m/z 512.2 $[M+H]^+$, and m/z 550.9 $[M+K]^+$; calculated $[M+H]^+$ = 511.7, and $[M+K]^+$ = 549.8). Peak S is assigned as the by-product (structure shown in **b**) formed in the lysate; peak D is the formed cyclic dimer for both in buffers and in lysates; peak * is not identified since its UV-Vis spectrum does not have the typical CBT absorption, suggesting that it is probably not related to monomer **4**; similar broad peaks are observed for both reactions in vitro and in lysates.

Supplementary Figure 7



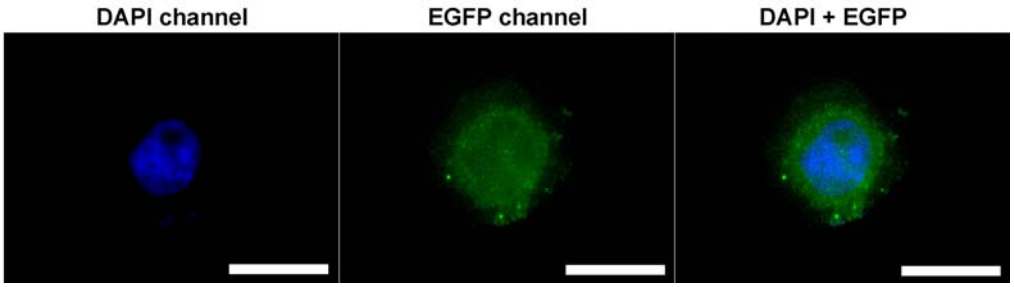
Supplementary Figure 7. **HPLC analysis of the incubation of monomer 4 with MDA-MB-468 cells**. Upper, HPLC trace of culture medium of MDA-MB-468 cells incubated with monomer **4** (200 μM) for 4 h at 37 °C. Middle, HPLC trace of blank culture medium. Lower, HPLC trace of free monomer **4**. Little monomer **4** was remained in the medium after 4 h incubation (upper); Peak S is the condensation product of monomer **4** with free intracellular cysteine, secreted out into medium by cells; and peak * is not identified but is not the dimer.

Supplementary Figure 8



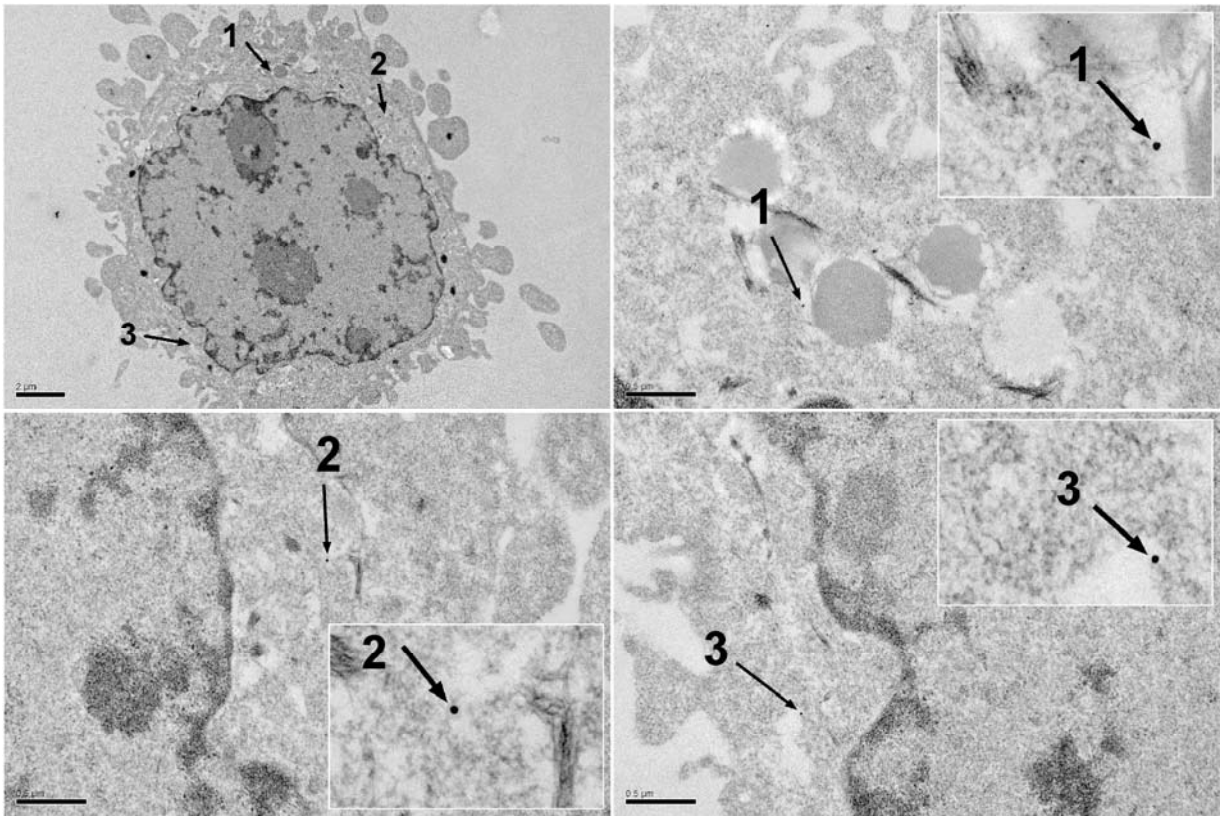
Supplementary Figure 8. Visualization of furin-controlled condensation products in HeLa and COS-7 cells. a, Fluorescence image of HeLa cells incubated with 200 μM of monomer **8** for eight hours and stained with FITC-labeled streptavidin after fixation: DAPI channel (left), EGFP channel (middle), and merged image (right). **b**, Fluorescence image of COS-7 cells incubated with 200 μM of monomer **8** for eight hours and stained with FITC-labeled streptavidin: DAPI channel (left), EGFP channel (middle), and merged image (right). Scale bar: 20 μm.

Supplementary Figure 9



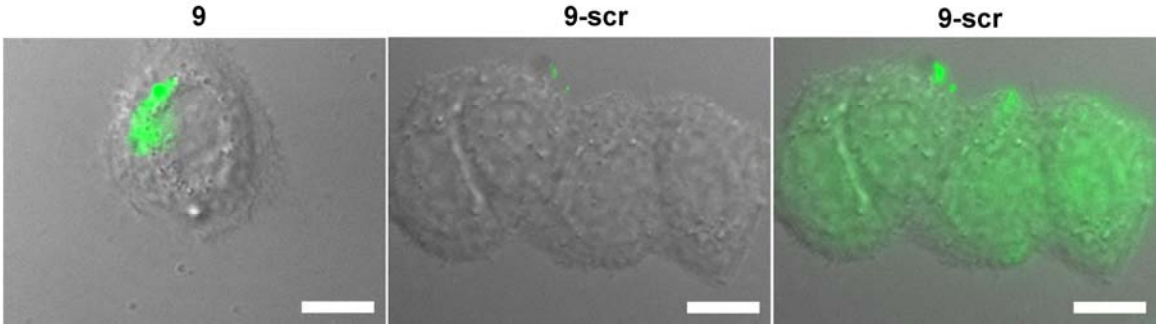
Supplementary Figure 9. Fluorescence image of MDA-MB-468 cells incubated with 20 μM of monomer **8** and 200 μM of furin inhibitor (decanoyl-RVRR-cmk) for eight hours, stained with FITC-labeled streptavidin: DAPI channel (left), EGFP channel (middle), and merged fluorescence image (right). Scale bar: 20 μm.

Supplementary Figure 10



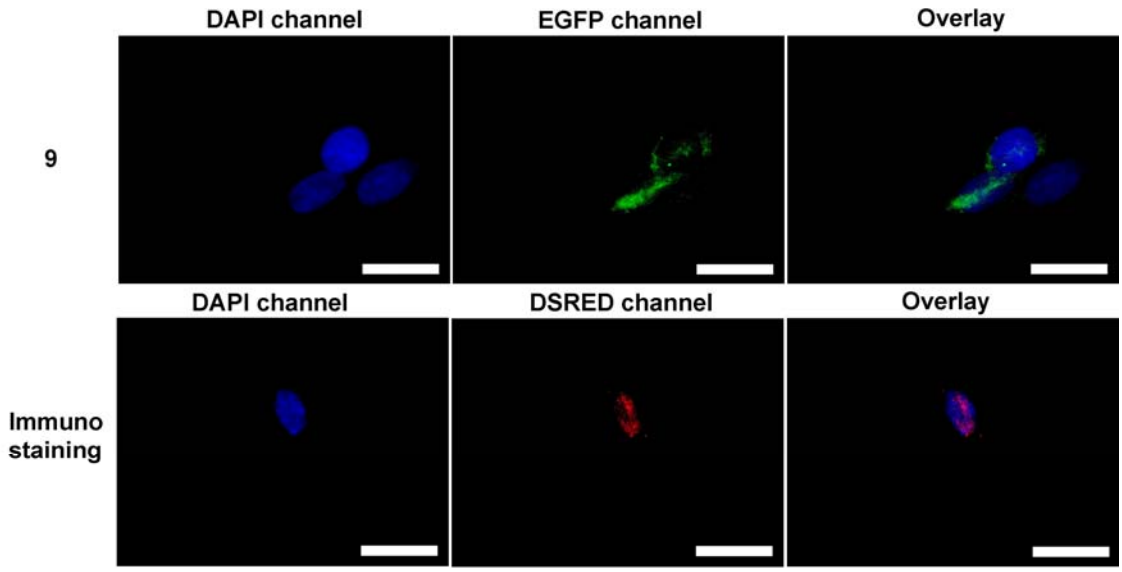
Supplementary Figure 10. Electron microscope images of MDA-MB-468 cell incubated with monomer **8** at 200 μM for 8 hrs and stained with streptavidin-gold nanoparticles (15 nm): upper left, low magnification of EM image showing several gold nanoparticles (arrow indicated) in one cell section (scale bar: 2 μm); upper right and lower panels, high-magnification EM images showing the locations of the gold nanoparticles are at or near Golgi apparatus (scale bar: 0.5 μm).

Supplementary Figure 11



Supplementary Figure 11. Overlaid DIC and fluorescence images of live MDA-MB-468 cells incubated with 2 μM of monomer **9** (left) or **9-scr** (middle) for 2 hours. Fluorescence images at left and middle have the same intensity scale, and fluorescence image at right is scaled down to show a low level but uniform staining of **9-scr** in cells. Scale bar: 10 μm.

Supplementary Figure 12



Supplementary Figure 12. Fluorescence images of C6 cells incubated with 2 μM of monomer **9** (upper) for 2 hrs: upper, DAPI channel (left), EGFP channel (middles) and merged image (right) of C6 cells incubated with monomer **9**; Lower, immunofluorescence staining of C6 cells with rhdomine-labeled antibody against furin: DAPI channel (left), DsRed channel (middle), and merged image (right). Scale bar: 20 μm.

Supplementary Table 1. HPLC analysis of monomer **1** in solution and in precipitates 30 mins after the pH adjustment.

Starting Conc. (μM)	**1** in solution (%)	**1** in precipitate (%)	**1** converted (%)
2	0	26.6	73.4
20	0	11.6	88.4
200	0	8.5	91.5
2000	0.01	2.5	97.5

Supplementary Table 2. HPLC condition for the purification of compound **1-9**.

Time (minute)	Flow (ml/min.)	H_2O %	CH_3CN %
0	7.0	95	5
3	7.0	95	5
35	7.0	30	70
37	7.0	30	70
38	7.0	95	5
40	7.0	95	5

Supplementary Table 3. HPLC condition for the analysis and purification of oligomers formed by condensation of monomer **4**.

Time (minute)	Flow (ml/min.)	H_2O %	CH_3CN %
0	3.0	95	5
3	3.0	95	5
95	3.0	45	55
97	3.0	45	55
98	3.0	95	5
100	3.0	95	5

Supplementary Methods
Chemical synthesis and characterization of monomers 1-9

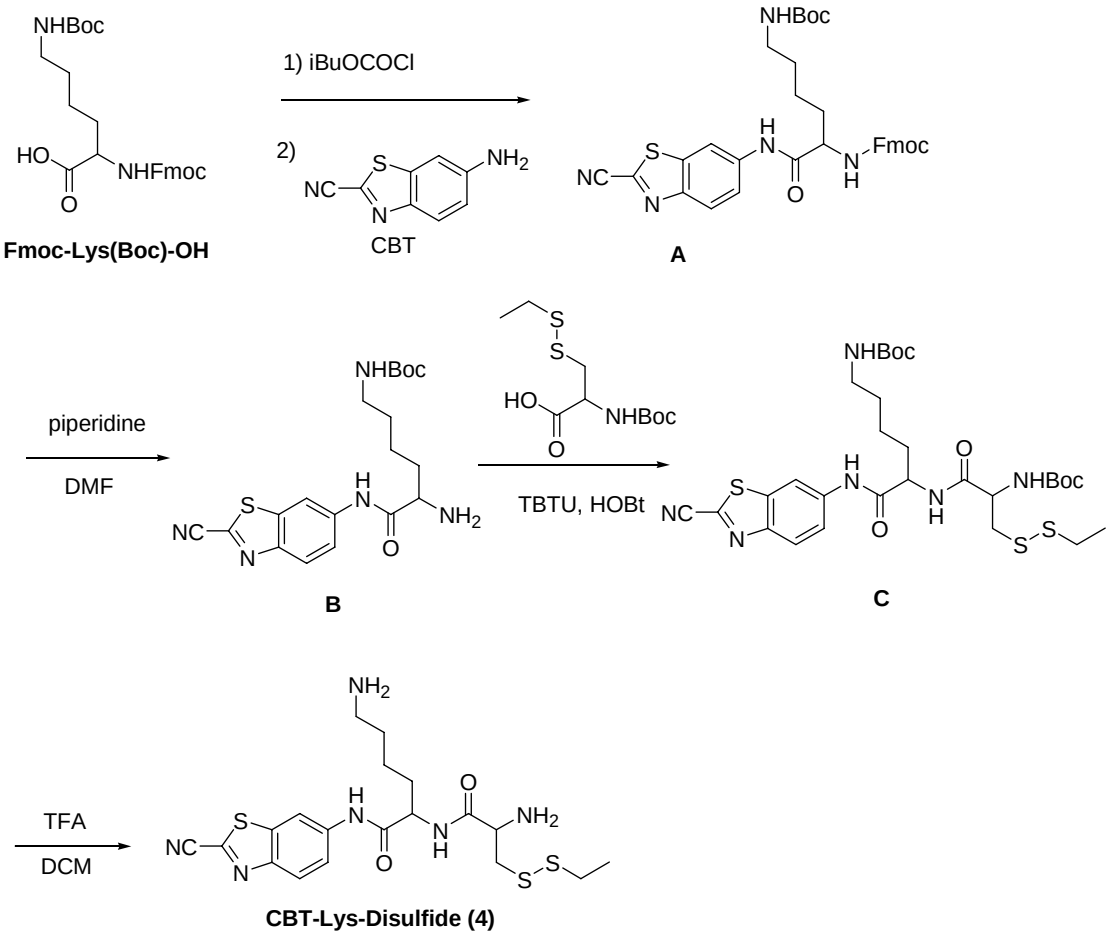
The preparations of compound **1-9** were described as below; 2-cyano-6-aminobenzothiazole (CBT) was synthesized following the literature method (White, E. H., Worther, H., Seliger, H. H., McElroy, W. D. Amino analogs of firefly luciferin and biological activity thereof. *J. Am. Chem. Soc.* 1966, **88**, 2015-2019).

*Preparation of 2-amino-N-(2-cyanobenzo[d]thiazol-6-yl)-3-mercaptopropanamide (**1**)*: Boc-Cys(trt)-OH (463.6 mg, 1 mmol) together with MMP (4-methylmorpholine, 1.2 mmol) were dissolved in 10 ml of dry THF. The solution was cooled with salty ice. Isobutyl chloroformate (1 mmol) was added into the above solution drop-wise under stirring. After the reaction mixture was stirred for additional 0.5 hour, 2-cyano-6-aminobenzothiazole (1.2 mmol) in dry THF was added into the solution in dropwise and the reaction mixture was stirred for another 12 h at r.t. The solvent was removed by rotary evaporation and the protected crude product was purified with a silicon gel column using hexane/ethyl acetate (4:1) as eluent. MS: calc. M+ = 620.8, obsvd. $(M+Na)^+$ = 643.1.
The as-prepared protected compound was dissolved in 8 mL of dry DCM, and 2 mL of TFA was added drop-wise under stirring. The solution was stirred for another 12 h before subject to HPLC purification.(Total yield: 19%). ^{1}H NMR (400 MHz, CD_3OD-d_4): δ 8.78-8.82 (d, 1H), 8.16-8.20 (d, 1H), 7.60-7.70 (dd, 1H), 4.18-4.22 (m, 1H), 3.40-3.55 (dd, 1H), 3.00-3.10 (t, 1H). MS: calc. M^+ = 278.4, obsvd. m/z 279.2 $[(M+H)^+]$, 301.3 $[(M+Na)^+]$.

*CBT-RRVRC (**2**)*: After solid phase peptide synthesis (SPPS), the peptide RRVRC was conjugated to CBT as the same method above (Total yield: 10%). Purity: single peak based on HPLC trace. MS: calc. M^+ = 846.1, obsvd. MALDI-TOF: m/z 847.5 $[(M+H)^+]$.

2-amino-N-(2-cyanobenzo[d]thiazol-6-yl)-3-(2-ethyldisulfanyl)propanamide (**3**): Compound **3** was prepared and purified with the same method as **1** (Total yield: 33%). ^{1}H NMR (400 MHz, CD_3OD-d_4) δ 7.61-7.63 (d, 1H), 7.15-7.22 (d, 1H), 6.85-6.89 (dd, 1H), 4.12-4.19 (m, 1H), 3.35-3.44 (dd, 1H), 3.17-3.25 (t, 1H), 2.85-2.92 (q, 2H), 1.68-1.77 (t, 3H). MS: calc. M^+ = 338.5, obsvd. ESI MS: m/z 338.9 $[M^+]$.

*CBT-Lys-Disulfide (**4**):*



*Synthesis of compound **A***: The isobutyl chloroformate (82 mg, 0.6 mmol) was added to the mixture of Fmoc-Lys(Boc)-OH (234 mg, 0.5 mmol) and NMP (4-methylmorpholine, 101mg, 1.0 mmol) in THF (5.0 mL) at 0^{o}C under N_2 and the reaction mixture was stirred at this temperature for 20 min. The solution of 2-cyano-6-aminobenzothiazole (105 mg, 0.6 mmol) was added to the reaction mixture and stirred for further 2 h at 0 ^{o}C then overnight at room temperature. Water (30 mL) was added and the reaction mixture was extracted with ethyl acetate (2X100 mL). The combined organic phase was dried by Na_2SO_4 and then evaporated. The pure product **A** (yield: 68 %) was obtained after normal flash chromatography (eluent: AcOEt : Hexane = 1 : 1). ^{1}HNMR of compound **A** (d-CDCl3, 400 MHz): 9.24 (bs, 1 H), 8.58 (s, 1 H), 7.99 (d, J = 8.8 Hz, 1 H), 7.75 (d, J = 7.6 Hz, 2 H), 7.74 (d, J = 7.6 Hz ,2 H), 7.20-7.30 (m, 2 H), 7.30-7.45 (m, 2 H), 5.80 (s, 1 H), 4.73 (s, 1 H), 4.42 (d, J = 6.8 Hz ,2 H), 4.36 (bs, 1 H), 4.20 (t, J = 7.2 Hz ,2 H), 3.181 (bs, 1 H), 3.00-3.12 (m, 1 H), 1.40-2.00 (m, 6 H), 1.27 (s, 9 H).

*Synthesis of compound **C***: The protecting group Fmoc of compound **A** was removed using 20% piperidine (in DMF) for 3 h. Water (30 mL) was added and the reaction mixture was extracted with ethyl acetate (2X100 mL). The combined organic phase was dried by Na_2SO_4 and then evaporated.

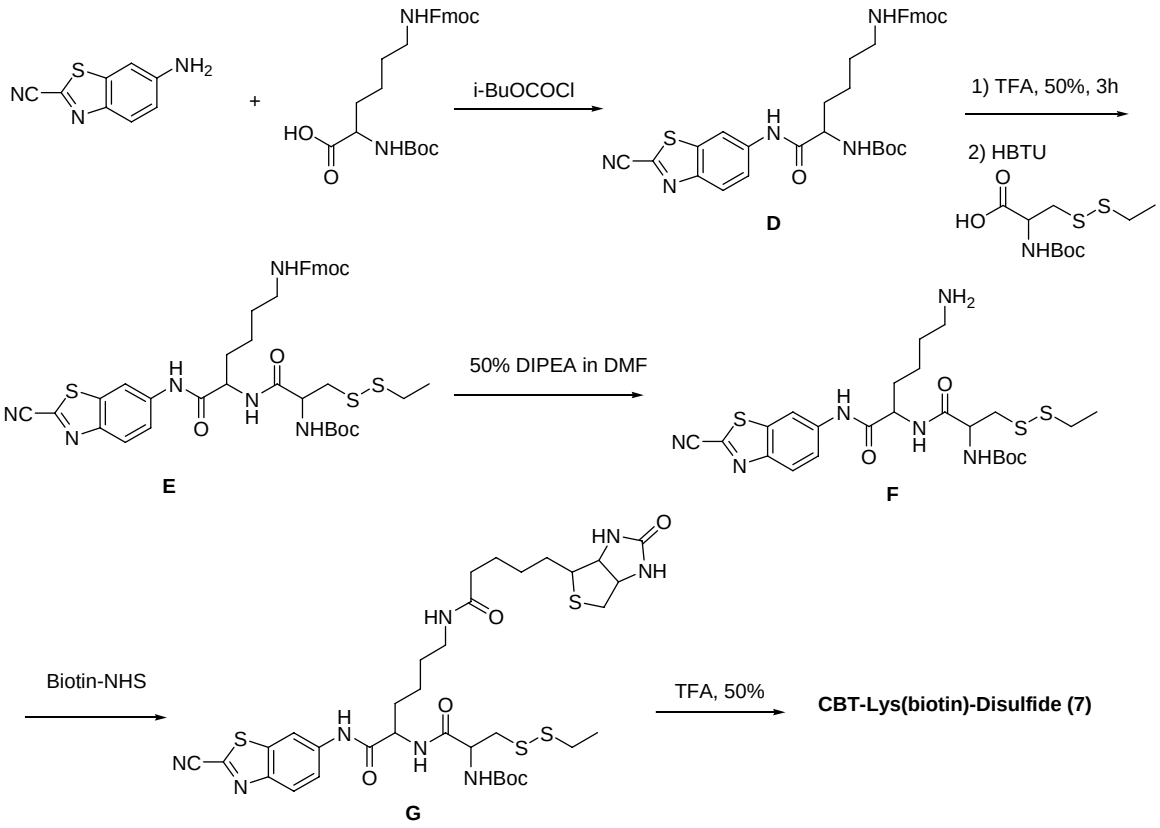
The pure product **B** (yield: 70 %) was obtained after normal flash chromatography (eluent: AcOEt : Hexane = 3 : 2). The mixture of the **B** (70 mg, 0.124 mmol), Boc-Cys(SEt)-OH•DCHA (68.65 mg, 0.148 mmol) and HBTU (56 mg, 0.148 mmol) in DMF (2.0 mL) was stirred overnight in presence of DIPEA (50 mg, 0.45 mmol). Water (30 mL) was added and the reaction mixture was extracted with ethyl acetate (2X50 mL). The combined organic phase was dried by Na_2SO_4 and then evaporated. The pure product C (yield: 68 %) was obtained after normal flash chromatography (eluent: AcOEt : Hexane = 1 : 1). ^{1}HNMR of compound **C** (d-CDCl3, 400 MHz): 9.43 (s, 1 H), 8.63 (s, 1 H), 8.01 (d, J = 9.2 Hz, 1 H), 7.59 (d, J = 8.8 Hz, 1 H), 7.17 (d, J = 6.4 Hz, 1 H), 5.76 (d, J = 6.0 Hz, 1 H), 4.78 (bs, 1 H), 4.57-4.64 (m, 1 H), 4.50 (m, 1 H), 2.95-3.11 (m, 4 H), 2.69 (q, J = 7.2 Hz, 2 H), 1.39-1.83 (m, 6 H), 1.44 (s, 9 H), 1.40 (s, 9 H).

*Preparation of CBT-Lys-Disulfide (**4**)*: The Boc protecting group of **C** was cleaved using 50% TFA in CH_2Cl_2 for 3 h. The CBT-Lys-Disulfide (**4**) was obtained after preparation HPLC (yield: 60 %). ^{1}HNMR of compound **4** (^{4}d-CD_3OD, 400 MHz): 8.16 (s, 1 H), 7.81 (d, J = 8.8 Hz, 1 H), 7.43 (d, J = 8.8 Hz, 1 H), 4.46 (t, J = 7.4 Hz, 1 H), 4.26 (t, J = 6.0 Hz, 1 H), 3.00-3.10 (m, 2 H), 2.86 (t, J = 7.4 Hz, 1 H), 2.40-2.50 (m, 2 H), 1.21-2.00 (m, 6 H), 1.01 (t, J = 7.2 Hz, 1 H). MS: calc. M^+ = 466.1, obsvd. MS (ESI): m/z 467 $[(M+H)^+]$.

*CBT-CRRVR (**5**)*: After solid phase peptide synthesis (SPPS), CRRVR was coupled to CBT to prepare **5** (Total yield: 20%). Its purity was confirmed by HPLC. MS: calc. M^+ = 888.1, obsvd. MALDI-TOF MS: m/z 889.0 $[(M+H)^{+]}$.

*CBT-CDVED (**6**)*: After solid phase peptide synthesis (SPPS), CDVED was coupled to CBT to prepare **6** (Total yield: 23%). Purity: a single peak on HPLC trace. MS: calc. M^+ = 778.8, obsvd. MALDI-TOF MS: m/z 801.5 $[(M+Na)^+]$.

*The synthesis of CBT-Lys(biotin)-Disulfide (**7**)*:
*Synthesis of precursor **D***: The isobutyl chloroformate (204 mg, 1.5 mmol) was added to a mixture of Boc-Lys(Fmoc)-OH (702 mg, 1.5 mmol) and MMP (4-methylmorpholine, 202mg, 2.0 mmol) in THF (5.0 mL) at 0 °C under N_2 and the reaction mixture was stirred for 20 min. The solution of 2-cyano-6-aminobenzothiazole (175 mg, 1.0 mmol) was added to the reaction mixture and further stirred for 2 h at 0 °C then overnight at room temperature. Water (50 mL) was added and the reaction mixture was extracted with ethyl acetate (2X100 mL). The combined organic phase was dried by Na_2SO_4 and then evaporated. The pure product **D** (yield: 79 %) was obtained after normal flash chromatography (eluent: AcOEt : Hexane = 1 : 1). ^{1}HNMR (d-CDCl3, 400 MHz): 9.40 (s, 1 H), 8.50 (s, 1 H), 7.88 (d, J = 8.8 Hz, 1 H), 7.73 (d, J = 7.6 Hz, 2 H), 7.53 (d, J = 7.6 Hz, 2 H), 7.37 (t, J = 7.6 Hz, 2 H), 7.20-7.32 (m, 3 H), 5.47 (d, J = 6.8 Hz, 1 H), 5.01 (t, J = 5.6 Hz, 1 H), 4.21-4.48 (m, 3 H), 4.10-4.19 (m, 2 H), 3.20 (q, J = 6.4 Hz, 2 H), 1.50-2.00 (m, 6 H), 1.43 (s, 9 H).



*Synthesis of compound **F***: The Boc protecting group was cleaved with 50% TFA in CH_2Cl_2 for 3 h. Precipitated from the cleavage solution using cold diethyl ether, the amino CBT compound was obtained in good yield which was directly used for next step reaction. The mixture of the amino CBT compound (300 mg, 0.6 mmol), Boc-Cys(SEt)-OH•DCHA (334 mg, 0.72 mmol) and HBTU (300 mg, 0.72 mmol) in DMF (3 mL) was stirred overnight in presence of DIPEA (100 mg, 0.9 mmol). After normal workup, the compound **E** was obtained. Treated with 50% DIPEA in DMF overnight, the desired amino compound **F** was obtained after HPLC purification. ^{1}HNMR of compound **F** (d-$CDCl_3$, 400 MHz): 8.67 (s, 1 H), 8.12 (d, J = 9.2 Hz, 1 H), 7.73 (d, J = 7.6 Hz, 1 H), 4.49-4.65 (m, 1 H), 4.33-4.43 (m, 1 H), 3.10-3.20 (m, 1 H), 2.81-2.99 (m, 3 H), 2.72 (q, J = 7.2 Hz, 2 H), 1.40-2.10 (m, 6 H), 1.45 (s, 9), 1.29 (t, J = 7.6 Hz, 3 H). MS: calc. M^+ = 566.7, obsvd. MALDI-TOF MS: 567 $[(M+H)^+]$.

*Synthesis of compound **G***: The mixture of amino compound **F** (28 mg, 0.05 mmol), Biotin-NHS (20 mg, 0.06 mmol) and DIEA (10 µL) in DMF (0.2 mL) was stirred for 2 h at room temperature, and then purified by normal flash chromatography (eluent: AcOEt : Hexane = 1 : 1) (90% yield). ^{1}HNMR of compound **G** (d-$CDCl_3$, 400 MHz): 10.17 (s, 1 H), 8.60 (s, 1 H), 8.04 (d, J = 9.2 Hz, 1 H), 7.69 (d, J = 8.8 Hz, 1 H), 7.65 (bs, 1 H), 6.63 (s, 2 H), 6.04 (s, 1 H), 5.75 (s, 1 H), 4.52-4.70 (m, 1 H), 4.40-4.51 (m, 2 H), 4.25-4.34 (m, 1 H), 3.00-3.35 (m, 4 H), 2.80-2.91 (m, 1 H), 2.70-2.80 (m, 1 H), 2.68 (q, J = 7.2 Hz, 2 H), 2.01-2.26 (m, 2 H), 1.30-2.0 (m, 12 H), 1.27 (t, J = 7.2 Hz, 3 H).

*Preparation of CBT-Lys(biotin)-Disulfide (**7**)*: Deprotection of **G** with 50% TFA in DCM in the presence of 10% triisopropylsilane at room temperature for 1 h produced the desired CBT-Lys(biotin)-Disulfide (**7**) in 80% after preparation HPLC. ^{1}HNMR (d_4-CD3OD, 400 MHz): 8.67 (d, J = 1.6 Hz, 1 H), 8.14 (d, J = 8.8 Hz, 1 H), 7.74 (dd, J_1 = 9.2 Hz, J_2 = 2.0 Hz, 1 H), 4.53-4.61 (m, 1 H), 4.44-4.52 (m, 1 H), 4.20-4.32 (m, 2 H), 3.37 (dd, J_1 = 14.8 Hz, J_2 = 4.4 Hz, 1 H), 3.11-3.22 (m, 3 H), 2.99 (dd, J_1 = 14.4 Hz, J_2 = 9.2 Hz, 1 H), 2.91 (dd, J_1 = 12.8 Hz, J_2 = 4.8 Hz, 1 H), 2.69-2.80 (m, 3 H), 2.16 (t, J = 7.2 Hz, 2 H), 1.30-2.00 (m, 12 H), 1.31 (t, J = 7.2 Hz, 3 H). MS: calc. M^+ = 692.2, obsvd. MALDI-TOF: m/z 693 [$(M+H)^+$].

*The synthesis of CBT-Lys(biotin)-Disulfide acetate(**7-Ac**)*: **7** was acetylated and then purified by HPLC. MS: calc. M^+ = 734.2, obsvd. MALDI-TOF MS: m/z 735.8, [$(M+H)^+$].

*CBT-Lys(biotin)-C(StBu)RRVR (**8**)*: Peptide KC(StBu)RRVR was made by solid-phase synthesis and then coupled to CBT with the same method as above, followed by labelling with biotin NHS ester in DMF for 12 hrs. Total yield: 15% after HPLC purification. MS: calc. M^+ = 1330.7, obsvd. MALDI-TOF MS: m/z 1330.7 [M^+].

*CBT-Lys(FITC)-C(StBu)RRVR (**9**)*: After solid phase peptide synthesis (SPPS) of KC(StBu)RRVR, CBT-KC(StBu)RRVR was synthesized with the same method above. After that, CBT-KC(StBu)RRVR was coupled with fluorescein isothiocyanate isomer I in DMF for 12 hrs. Total yield: 16%. Purity: single peak based on HPLC trace (retention time under condition for purification: 33.025 min). MS: calc. M^+ = 1493.8, obsvd. MALDI-TOF MS: m/z 1494.4 [M^+].

*CBT-VR-C(StBu)R Lys(FITC)R (**9-scr**)*: After solid phase peptide synthesis (SPPS) of VRC(StBu)RKR, *CBT-VR-C(StBu)RKR* was synthesized with the same method above. After that, *CBT-VR-C(StBu)RKR* was coupled with fluorescein isothiocyanate isomer I in DMF for 12 hrs. Total yield: 16%. Purity: single peak based on HPLC trace (retention time under condition for purification: 33.467 min). MS: calc. M^+ = 1493.8, obsvd. MALDI-TOF MS: m/z 1493.7 [M^+].

Condensation of monomer 4 in MDA-MB-468 cell lysates

The culture medium for adherent MDA-MB-468cells was carefully removed and the cells were washed with cold PBS (2X). RIPA (RadioImmuno Precipitation Assay) buffer was added to cells using 1 mL of buffer per 5 million cells. Then the cells were kept on ice for 30 min. The cell lysate was gathered, centrifuged at 14,000Xg for 15 min. The supernatant of the cell lysate was incubated with monomer **4** at 200 uM for 1 h and then injected into a HPLC system for analysis.

HMBC experiment

A gradient enhanced HMBC was used to correlate ^{1}H resonances to ^{13}C resonances through 2-bond and 3-bond $^{1}H/^{13}C$ scalar coupling constants. The experiment was acquired with a 21.5 mM of monomer **4** in D_2O on a Varian Inova 600 MHz NMR with 128 scans of 2048 complex points in f2 dimension by 120 real points in f1 with spectral width of 5911 Hz in f2 and 36200 Hz in f1. The data was processed with VNMR 6.1C with linear prediction in f1 to 256 points with zero-filling to a matrix of 2048 by 2048, using sinebell squared weighting functions in both dimensions. The data showed correlations between the proton on carbon 16 to carbon 1 (**Supplementary Figure 4**) confirming that these two atoms are separated by 2 or 3 bonds. Similarly, the data also showed correlations between the protons on carbon 17 to carbon 1 confirming that these 2 atoms are separated by 2 or 3 bonds. Both of these two correlations indicated the cyclic form of the dimer of monomer **4**.

Furin immunofluorescence staining. Cells were fixed with 4% paraformaldehyde for 20 min. Subsequently, the cells were washed (3x) with DPBS and permeabilized with 1% Triton-100 in PBS for 20 min. Cells were then blocked with 1% non-fat milk solution in PBS for 20 min at room temperature. This solution was as used as antibody diluents. After blocking, the cells were incubated with primary antibody (1:100, anti-furin rabbit polyclonal antibody, Biomol International) for 1 h at 37 °C. After washing with PBS (3x5 min) and with 1% milk (1x5 min), cells were incubated with secondary antibody (1:100, goat polyclonal anti-rabbit IgG rhodamine-conjugate) for 30 min at 37 °C followed by washing with PBS (3x) and distilled water (1x) before mounted on a glass slide with DAPI-containing mounting media. Images were acquired using a Zeiss fluorescence microscope equipped with a 63x objective. The exposure time was 150 ms for DAPI, 600 ms for FITC, and 600 ms for rhodamine-conjugated IgG, respectively.