Electronic Supplementary Information

Caspase-3 controlled assembly of nanoparticles for fluorescence to turn on

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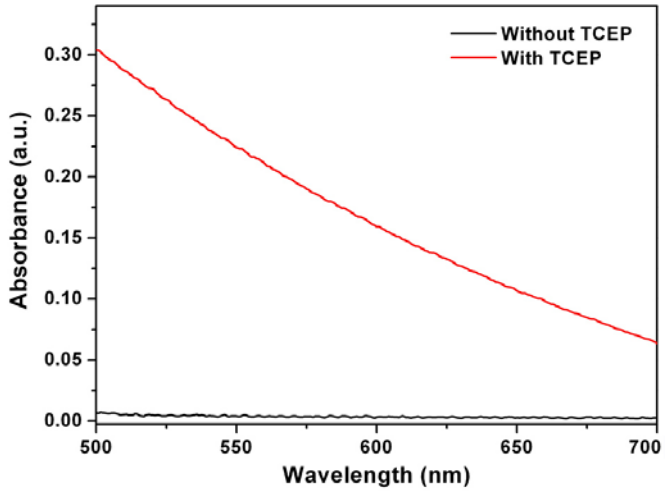


Figure S1. UV-Vis absorption spectra (500-700 nm due to the light scattering) of self-assembled condensation products of **1** at 1mM with (Red) or without (black) the addition of 2 equiv. of TCEP at pH 7.4.

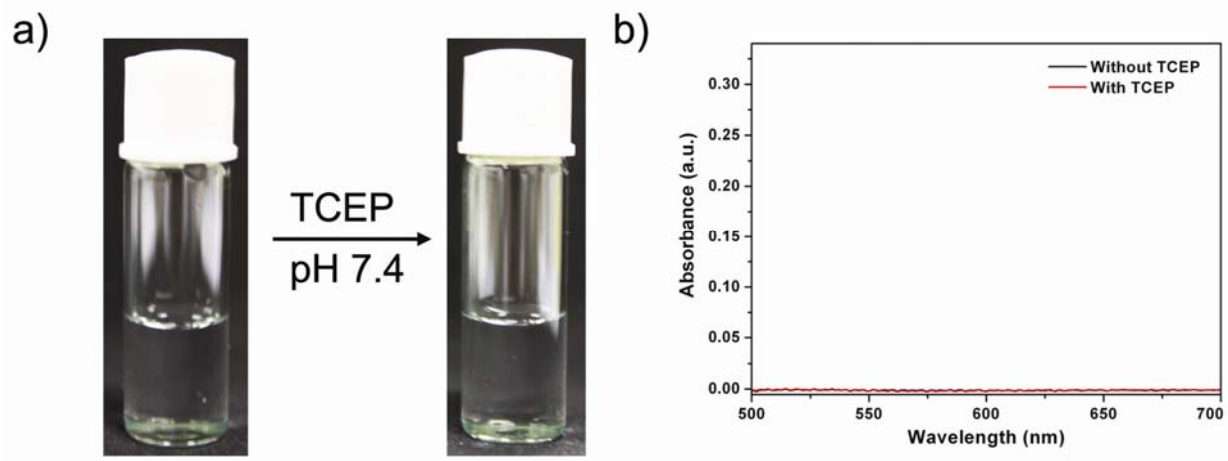


Figure S2. a) Photographs of the solution of **2** at 1 mM dissolved in water with 2% DMSO and after the addition of 2 folds of TCEP and the pH value was adjusted to 7.4 for 5 mins. b) UV-Vis absorption spectra (500-700 nm due to the light scattering) of **1-Ac** at 1mM with (Red) or without (black) the addition of 2 folds of TCEP at pH 7.4.

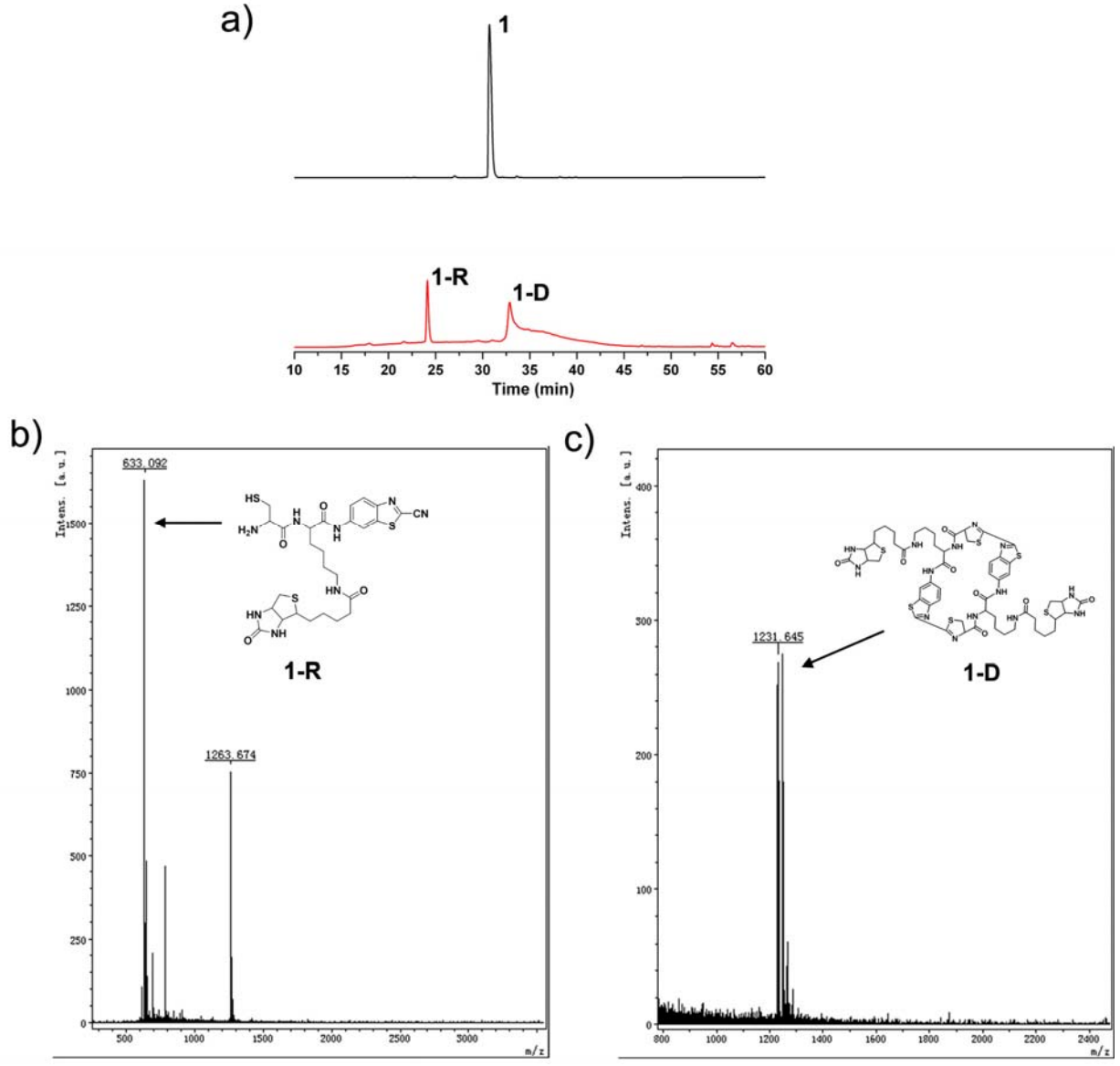


Figure S3. a) HPLC traces of **1** (upper) and **1** treated with 2 equiv. of TCEP for 5 min at pH 7.4 (lower). b, c) MALDI-Mass spectra of HPLC peaks at 24.1 min and 32.9 min respectively in a.

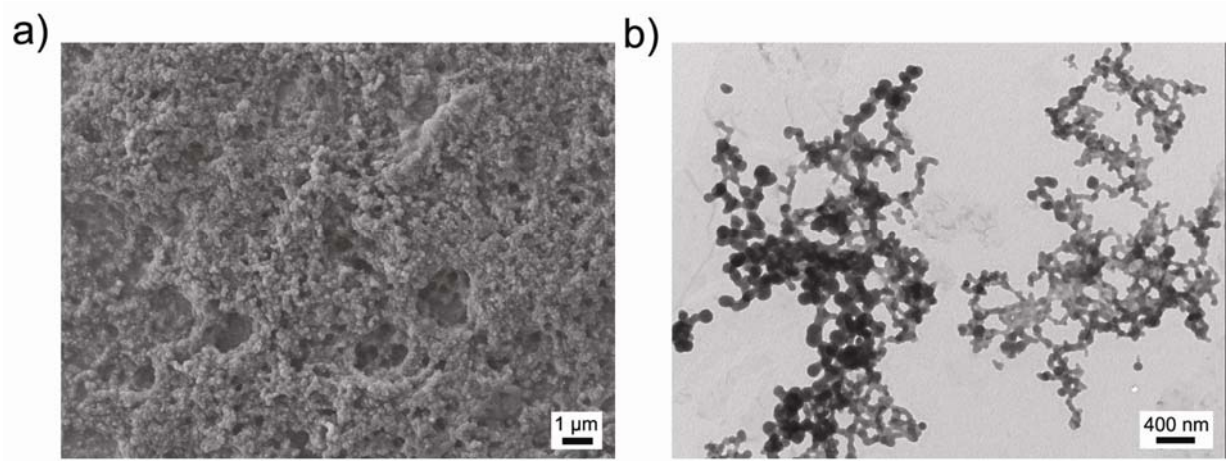


Figure S4. a, b) SEM and TEM images of the nanoparticles in the dispersion of figure 1a.

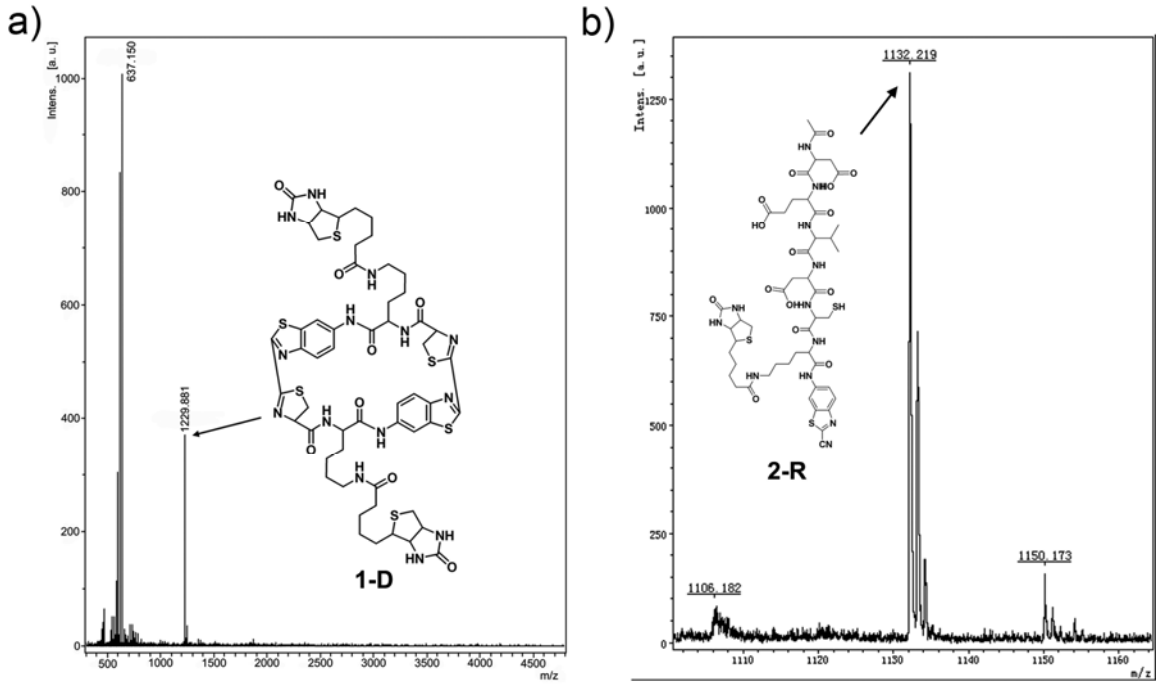


Figure S5. a, b) MALDI-Mass spectra of HPLC peaks at 32.8 min and 37.0 min of figure 3a respectively.

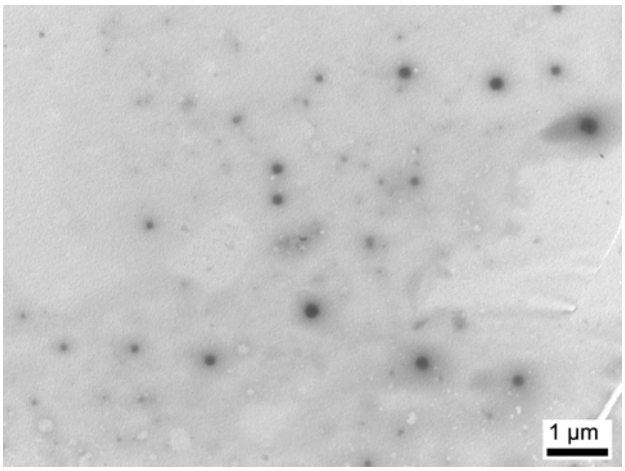


Figure S6. TEM image of the nanoparticles in the dispersion of figure 3a.

Supplementary Table S1. HPLC condition for the purification of compound **1, 1-Ac, 2, and 2-Scr**

Time (minute)	Flow (ml/min.)	H_2O %	CH_3OH %
0	7.0	30	70
3	7.0	30	70
35	7.0	0	100
37	7.0	0	100
38	7.0	30	70
40	7.0	30	70

Supplementary Methods

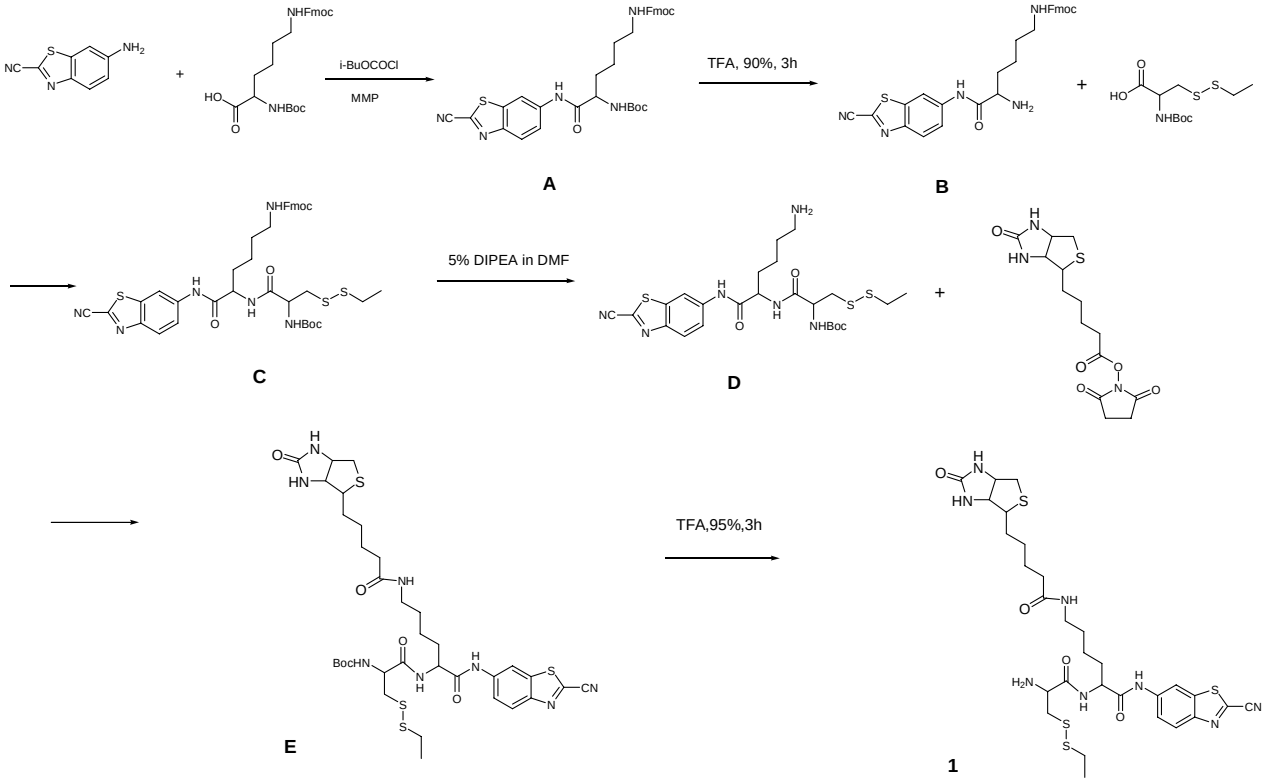
General methods. All the starting materials were obtained from Adamas or Sangon Biotech. Commercially available reagents were used without further purification, unless noted otherwise. All other chemicals were reagent grade or better. Caspase-3 was obtained from BioVision chemicals (~300,000 U/mg, 1 U corresponds to the amount of enzyme which cleaves 1 nmol of the caspase substrate DEVD-*p*NA per hour at 37 ^{o}C). Streptavidin-FITC was obtained from Southern Biotech Chemicals. ^{1}HNMR spectra were obtained on a 300MHz Bruker AV 300. MALDI-TOF/TOF mass spectra were obtained on a time-of-flight Ultrflex Ⅱ mass spectrometer (Bruker Daltonics), HPLC analyses were performed on an Agilent 1200 HPLC system equipped with a G1322A pump and in-line diode array UV detector using a YMC-Pack ODS-AM column with CH_3OH (0.1% of TFA) and water (0.1% of TFA) as the eluent. Dynamic light scattering (DLS) was measured on a Zeta Sizer Nano Series (Malvern Instruments). Scan electron micrograph (SEM) images were obtained on JEOL-JSM-6700F electron microscope at an accelerating voltage of 5.0KV. Transmission electron micrograph (TEM) images were obtained on a JEOL 2010 electron microscope, operating at 100 kV. The cryo-dried samples were prepared as following: a copper grid coated with carbon was dipped into the suspension solvent and placed into a vial, which was plunged into liquid nitrogen until no bubbles were apparent. Then water was removed from the frozen specimen by a freeze-drier. Fluorescence images were taken on an IVIS Lumina (Caliper LifeSciences) equipped with a cooled charge-coupled device (CCD) camera.

Chemical synthesis and characterization of monomers 1, 1-Ac, 2, and 2-Scr

The preparations of compound **1, 1-Ac, 2, 2-Scr** 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 Cys(SEt)-Lys(Biotin)-CBT (**1**)[1]:

Scheme S1. Synthetic route for compound **1**.



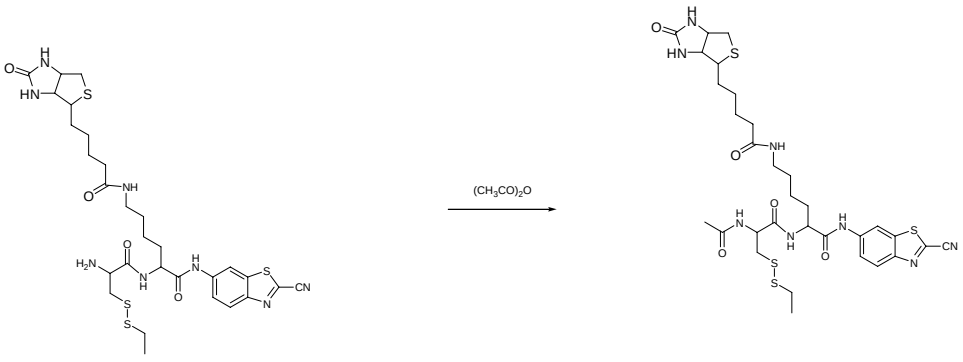
*Synthesis of **A***: The isobutyl chloroformate (109 mg, 0.8 mmol) was added to a mixture of Boc-Lys(Fmoc)-OH (375 mg, 0.8 mmol) and MMP (4-methylmorpholine, 151mg, 1.5 mmol) in THF (5.0 mL) at 0 °C under N_2 and the reaction mixture was stirred for 20 min. The solution of 2-cyano-6-aminobenzothiazole (140 mg, 0.8 mmol) was added to the reaction mixture and further stirred for 1 h at 0 °C then overnight at room temperature. Water (50 mL) was added and the reaction mixture was extracted with ethyl acetate (2 X 100 mL). The combined organic phase was dried by Na_2SO_4 and then evaporated. The pure product **A** (195 mg, yield: 39%) was obtained after normal flash chromatography (eluent: AcOEt : Hexane = 1 : 1).

*Synthesis of compound **B***: The Boc protecting group of **A** was cleaved with 90% TFA in CH_2Cl_2 for 3 h. Precipitated from the cleavage solution using cold diethyl ether, the amino CBT compound **B** was obtained in good yield which was directly used for next step reaction. The mixture of **B** (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 **C** was obtained (355 mg, yield: 75%). Treated with 50% DIPEA in DMF overnight, the desired amino compound **D** (176 mg, yield: 69%) was obtained after HPLC purification. MS: calc. M^+ = 566.7, obsvd. ESI MS: m/z 567.0 [M^+].

*Synthesis of compound **1***: The mixture of amino compound **D** (67.4 mg, 0.129 mmol), Biotin-NHS (52.9 mg, 0.155 mmol), HBTU (98.2 mg, 0.259 mmol), and DIPEA (10 µL) in DMF (2 mL) was stirred for 50 min at room temperature, and then purified by HPLC to yield compound **E** (92 mg, yield: 90%). Deprotection of **E** with 90% TFA in DCM in the presence of 1% triisopropylsilane at

room temperature for 2 h produced compound **1** after HPLC preparation (66.7 mg, yield: 83 %). MS: calculated for $C_{39}H_{41}N_8O_4S_4$ $[(M+H)^+]$: 693.2134; obsvd. HR-MALDI-TOF/MS: m/z 693.2135.

Scheme S2. Synthetic route for compound **1-Ac**



1 **1-Ac**

*Synthesis of Acetyl-Cys(SEt)-Lys(Biotin)-CBT(**1-Ac**)*: **1** (10mg, 0.014 mmol) was acetylated in DMF and then purified with HPLC to yeild **1-Ac** (9.1mg, yield: 86%). ^{1}HNMR of compound **1-Ac** (d_6-DMSO, 300 MHz, Fig. S7): 10.44 (s, 1 H), 8.71 (d, J = 1.2 Hz, 1 H), 8.25 (q, J = 19.1 Hz, 2 H), 8.15 (dd, J_1 = 14.8 Hz, J_2 = 4.4 Hz, 1 H), 7.69-7.72 (m, 2 H), 6.38 (s, 1 H), 6.32 (s, 1 H), 4.48-4.55 (m, 1 H), 4.32-4.39 (m, 1 H), 4.23-4.27 (m, 1 H), 4.05-4.08 (m, 1 H), 3.10 (t, J = 12.1 Hz, 1 H), 2.94-3.05 (m, 4 H), 2.73-2.84 (m, 2 H), 2.63-2.70 (t, J = 21.86 Hz, 2 H), 2.52 (d, J = 12.1 Hz, 1 H), 1.97 (t, J = 15.3 Hz, 2 H), 1.84 (s, 3 H), 1.30-1.45 (m, 6 H), 1.15-1.25 (m, 8 H). MS: calculated for $C_{31}H_{43}N_8O_5S_4$ $[(M+H)^+]$: 735.2239; obsvd. HR-MALDI-TOF/MS: m/z 735.2241.

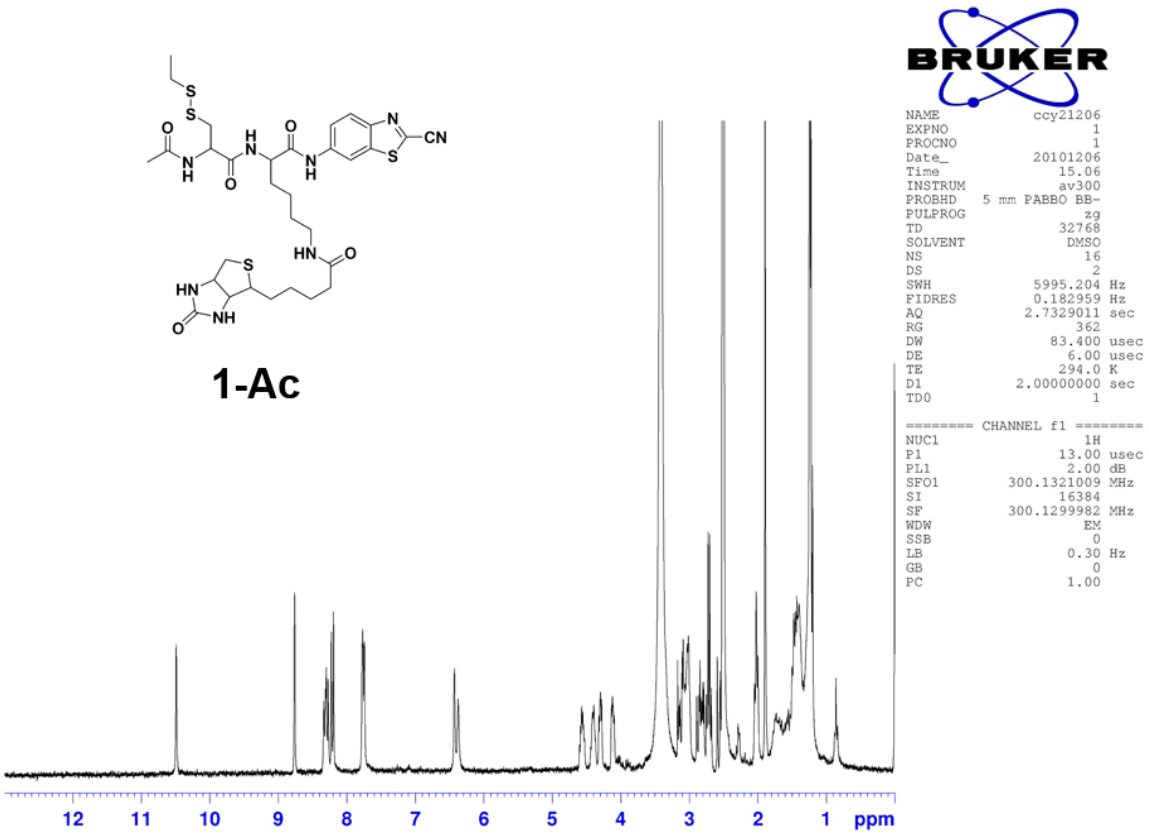
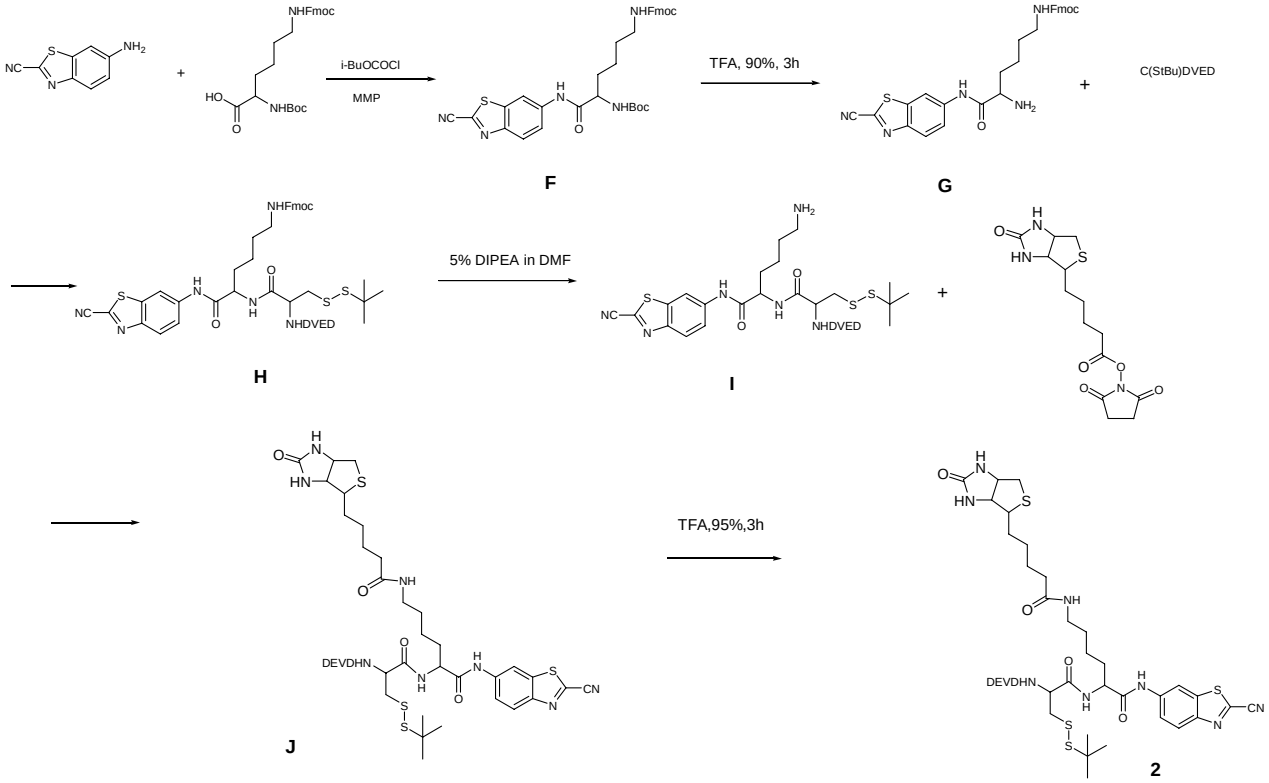


Figure S7. ^{1}HNMR spectrum of compound **1-Ac**.

Scheme S3. Synthetic route for compound **2**.



*Synthesis of Acetyl-Asp-Glu-Val-Asp-Cys(StBu)-Lys(Biotin)-CBT (**2**)*: Peptide C(StBu)DVED (88 mg, 0.10 mmol) was prepared by solid phase peptide synthesis (SPPS) and then coupled to **G** with the same method as above, followed by labeling with biotin NHS ester in DMF for 2hrs, then purified by HPLC to yield **2** (9.6 mg, yield: 7.8%). ^{1}HNMR of compound **2** (d_6-DMSO, 300 MHz, Fig. S8): 12.28 (s, 3 H), 10.40 (s, 1 H), 8.75 (d, J = 1.5 Hz, 1 H), 8.37 (dd, J_1 = 8.4 Hz, J_2 = 4.1 Hz, 1 H), 8.11-8.24 (m, 4 H), 7.99 (d, J = 8.4 Hz, 1 H), 7.69-7.78 (m, 3 H), 6.43 (s, 1 H), 6.37 (s, 1 H), 4.45-4.63 (m, 3 H), 4.25-4.42 (m, 3 H), 4.09-4.18 (m, 2 H), 2.88-3.16 (m, 6 H), 2.67-2.84 (m, 2 H), 2.55-2.63 (m, 2 H), 2.18-2.31 (m, 2 H), 1.94-2.04 (m, 3 H), 1.83 (s, 3 H), 1.66-1.77 (m, 2 H), 1.33-1.50 (m, 6 H), 1.18-1.31 (m, 15 H), 0.75-0.90 (m, 6 H). MS: calculated for $C_{51}H_{73}N_{12}O_{15}S_4$ [$(M+H)^+$]: 1221.4201; obsvd. HR-MALDI-TOF/MS: m/z 1221.4203.

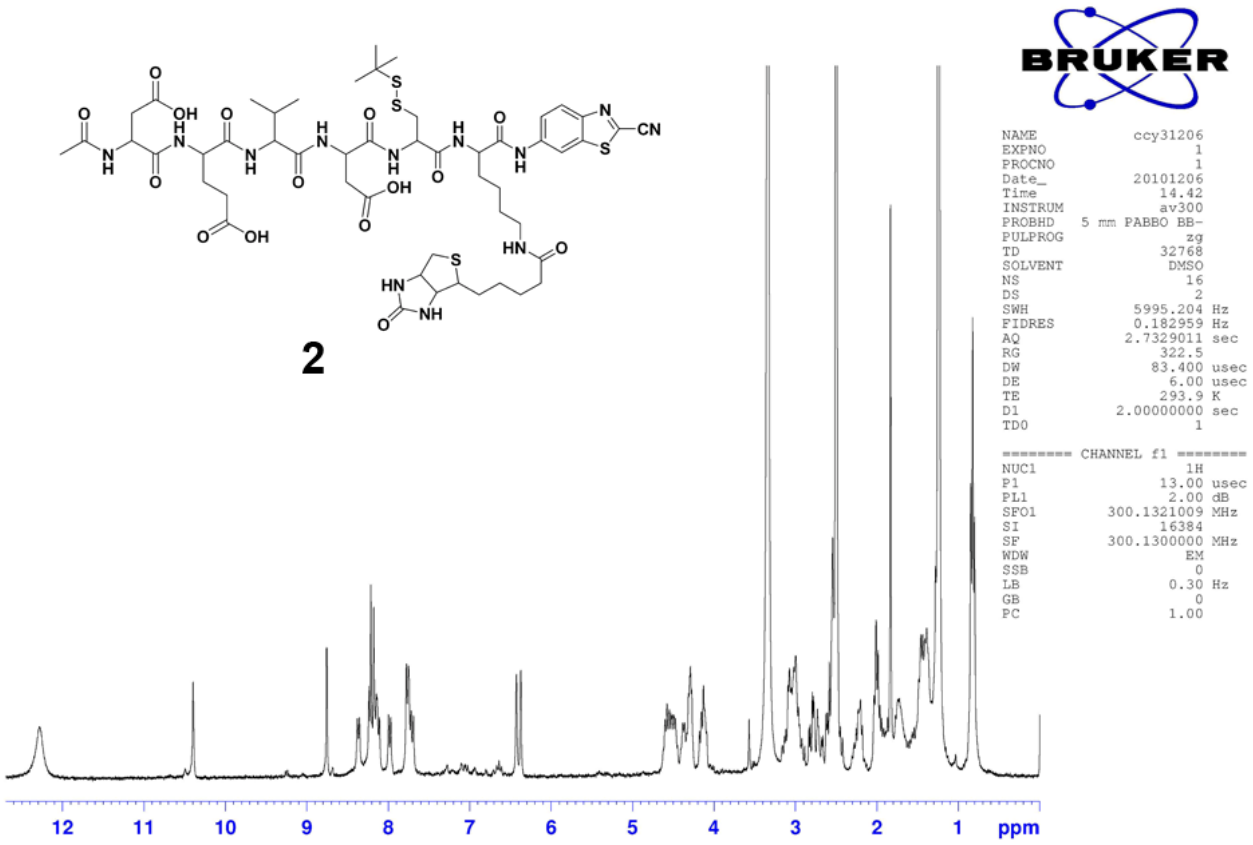
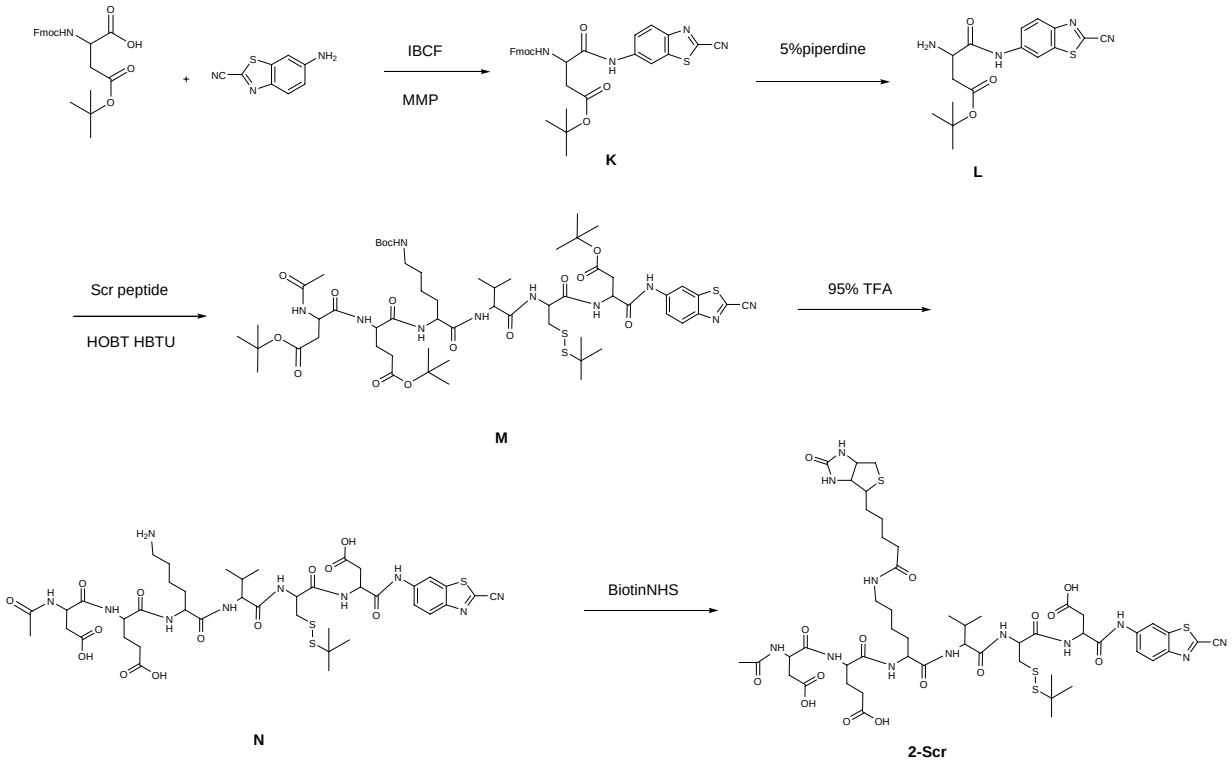


Figure S8. ^{1}HNMR spectrum of compound **2**.

Scheme S4. Synthetic route for compound **2-Scr**



*Preparation of Acetyl-Asp-Glu-Lys(Biotin)-Val-Cys(StBu)-Asp-CBT (**2-Scr**)*:
*Synthesis of **K***: The isobutyl chloroformate (54 mg, 0.4 mmol) was added to a mixture of Fmoc-Asp(OtBu)-OH (165 mg, 0.4 mmol) and MMP (4-methylmorpholine), (151mg, 1.5 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 (70 mg, 0.4 mmol) was added to the reaction mixture and further stirred for 1 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 with Na_2SO_4 and then evaporated. The pure product **K** (65.9 mg, yield: 29 %) was obtained after normal flash chromatography (eluent: AcOEt : Hexane = 1 : 1). MS: calc. M^+=567.6, obsvd. ESI MS: m/z 566.5, $[(M-H)^-]$

*Synthesis of compound **M***: The Fmoc protecting group of **K** was cleaved with 5% Piperdine in DMF for 5 min and then purified by HPLC to obtain compound **L** in good yield. **L** (34.6 mg, 0.1 mmol), CVKED (112 mg, 0.12 mmol) and HBTU (45.5 mg, 0.12 mmol) in DMF (3 mL) were stirred overnight in presence of DIPEA (100 mg, 0.9 mmol). After normal workup, the compound **M** (97 mg, yield: 78%) was obtained. Treated with 90% TFA in CH_2Cl_2 3h, the desired amino compound **N** (62 mg, yield: 80%) was obtained after HPLC purification. MS: calc. M^+ = 996.1, obsvd. ESI MS: 995.3 $[(M-H)^-]$.

*Synthesis of compound **2-Scr***: Biotin-NHS ester (4.1mg, 0.012mmol) was coupled with **N** (9.95 mg, 0.01 mmol) in DMF for 3hrs then purified with HPLC to yield **2-Scr** (10.6 mg, yield: 95%). ^{1}HNMR of compound **2-Scr** (d_6-DMSO, 300 MHz, Fig. S9): 12.30 (s, 3 H), 10.40 (s, 1 H), 8.75 (d, J = 1.5 Hz, 1 H), 8.47 (dd, J_1 = 8.3 Hz, J_2 = 4.4 Hz, 1 H), 8.18-8.26 (m, 3 H), 7.85-7.94 (m, 3 H), 7.70-7.79 (m, 2 H), 6.43 (s, 1 H), 6.38 (s, 1 H), 4.67-4.74 (q, J = 21.73 Hz, 1 H), 4.47-4.55 (m, 2 H), 4.21-4.32 (m, 3 H), 4.09-4.18 (m, 2 H), 3.16 (s, 1 H), 3.05-3.12 (m, 2 H), 2.92-3.04 (m, 3 H), 2.72-2.85 (m, 3 H), 2.57-2.70 (m, 3 H), 2.18-2.27 (m, 2 H), 1.83 (s, 3 H), 1.39-1.67 (m, 7 H), 1.20-1.30 (m, 14 H), 0.77-0.88 (m, 7 H). MS: calculated for $C_{51}H_{73}N_{12}O_{15}S_4$ $[(M+H)^+]$: 1221.4201; obsvd. HR-MALDI-TOF/MS: m/z 1221.4203.

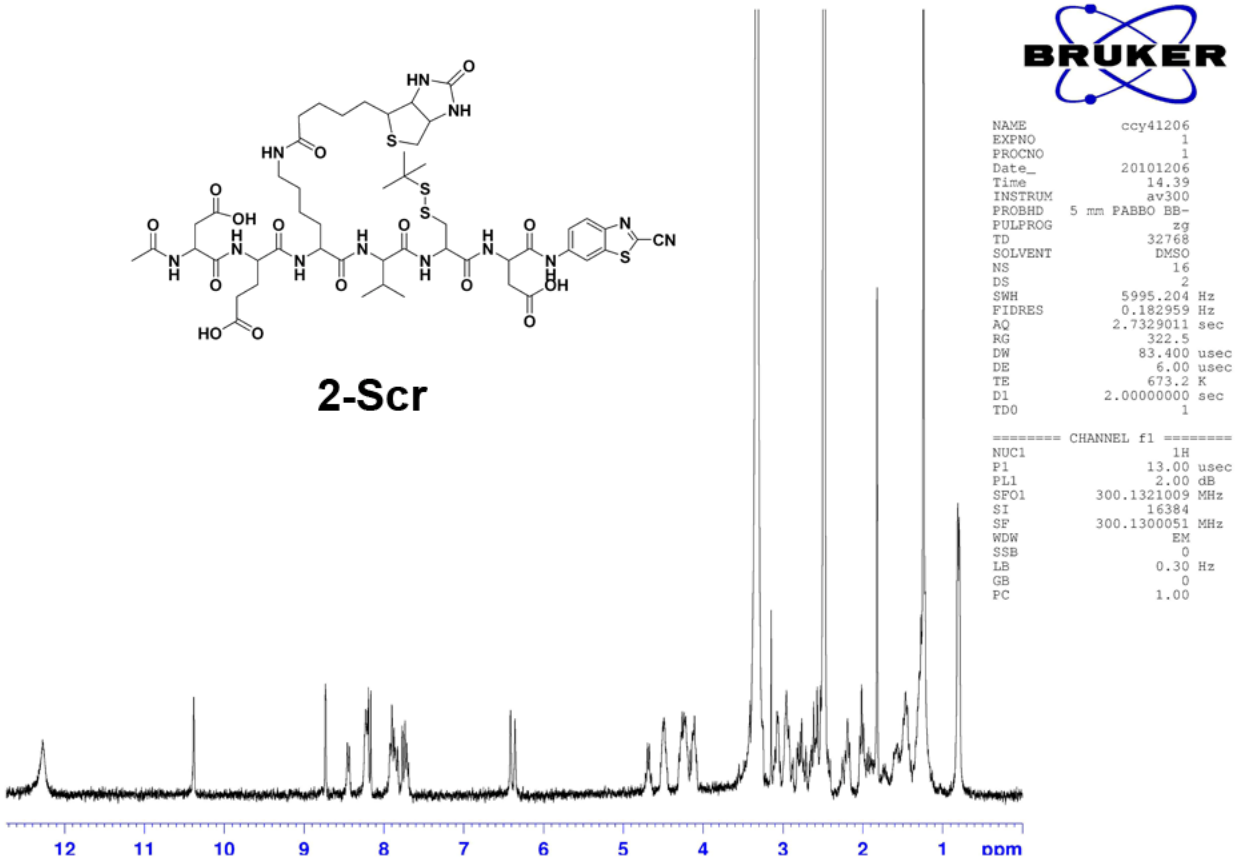


Figure S9. [1]HNMR spectrum of compound **2-Scr**.

Reference:

[1] Liang, G. L.; Ren, H. J.; and Rao, J. H. *Nat. Chem.* **2010**, 2: 54-60.