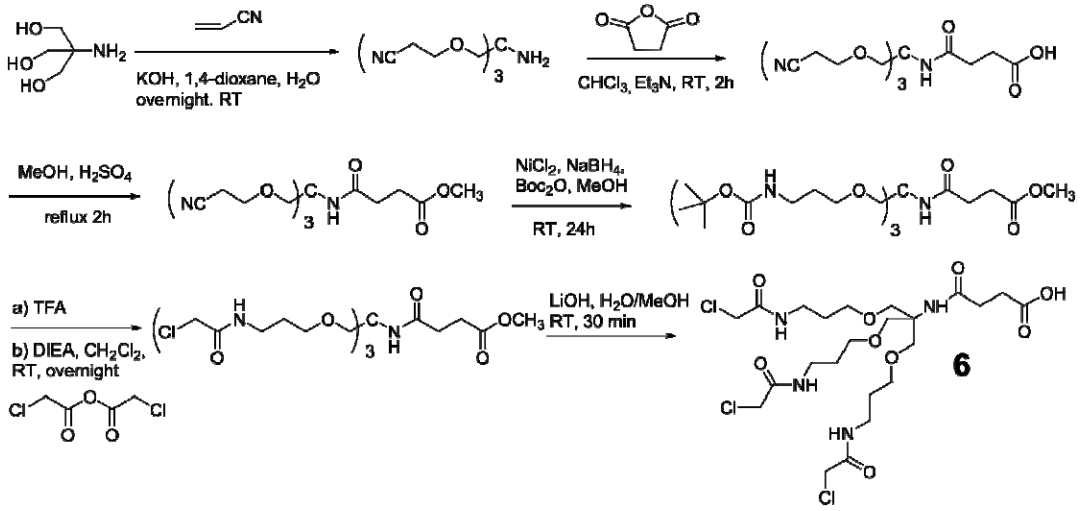
SUPPORTING INFORMATION

A. Synthetic procedures and compound characterization data



Scheme I: Synthesis of compound 6 as a starting material

Compound **6**,[1] compound **7**,[2] M-SH (compound **10**)[3] and CA-SH (compound **11**)[3] were synthesized following published procedures.

Compound 6

^{1}H NMR ($CDCl_3$) δ 1.69 (m, 6H, 3 CH_2); 2.48 (m, 4H, 2 CH_2); 3.30 (m, 6H, 3 CH_2); 3.40 (m, 6H, 3 CH_2); 3.61 (s, 6H, 3 CH_2); 3.95 (s, 6H, 3 CH_2); 6.48 (s, 1H, NH); 7.12 (br s, 3H, 3 NH). ^{13}C NMR ($CDCl_3$) 175.13, 172.72, 166.43, 69.91, 69.23, 59.85, 53.92, 50.41, 42.36, 37.77, 29.0. Molecular formula: $C_{23}H_{39}Cl_3O_9N_4$. Calculated exact mass 620.58, M+H 621.59, ESI-MS found 621.36.

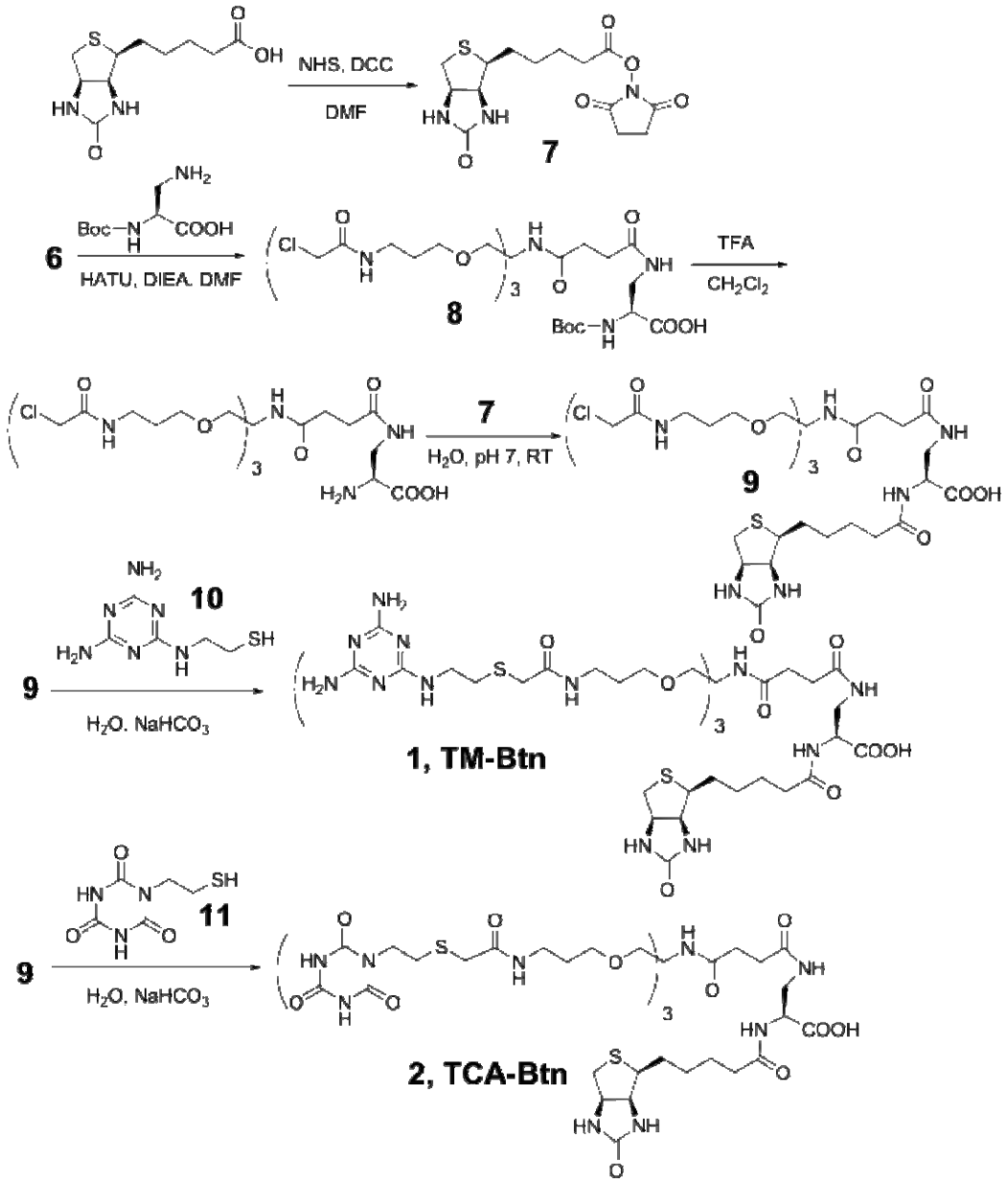
Compound 7

^{1}H NMR ($CDCl_3$): δ1.5−1.6 (m, 2H); 1.6−1.9 (m, 4H); 2.58−2.70 (m, 2H); 2.75 (d, 1H); 2.86 (s, 4H); 2.87−2.97 (m, 1H); 3.16 (m, 1H); 4.33 (m, 1H); 4.52 (m, 1H); 4.96 (s, 1H); 5.23 (s, 1H). ^{13}C NMR ($CDCl_3$) 170.2, 168.4, 165.3, 62.2, 60.1, 56.7, 42.4, 31.8, 28.4, 25.3, 24.1, 23.2. Molecular formula: $C_{14}H_{19}N_3O_5S$. Calculated exact mass 341.10, M+H 342.1, ESI-MS found 342.5.

Compound 8

O-(7-Azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HATU, 380 mg, 1.0 mmol) and diisopropylethylamine (DIEA, 0.13 g, 1.0 mmol) were added into the solution of compound **6** (620 mg, 1.0 mmol) in 5 mL DMF. After stirred at room temperature for 20 min, N^{α}-Boc-L-2,3-diaminopropionic acid (224 mg, 1.1 mmol) and DIEA (0.13 g, 1.0 mmol) in 5 mL DMF was added dropwise. The solution was stirred at room temperature for 2 h. Solvent was removed under reduced pressure, the residue was dissolved in 20 mL CH_2Cl_2, and washed by by 0.2 M HCl (2×20 mL) and water

(2×20 mL). The organic layer was dried by Na_2SO_4 and the solvent evaporated to yield a yellow oil. The crude product was purified by silica gel flash column, using CH_2Cl_2:MeOH = 15:1 to 5:1 as eluent. Compound **8** (0.53 g, yield 65%) was collected as a yellow oil. ^{1}H NMR ($CDCl_3$) δ 1.30 (s, 9H); 1.68 (m, 6H); 2.38 (m, 4H); 3.28 (m, 6H); 3.38 (m, 7H); 3.58 (br s, 7H); 3.94 (s, 6H); 4.10 (m, 1H); 5.98 (s, 1H); 6.60 (s, 1H); 7.38 (br s, 3H), 7.45 (s, 1H). ^{13}C NMR ($CDCl_3$) 174.4, 173.4, 172.5, 165.3, 156.9, 78.2, 69.1, 68.8, 59.2, 56.4, 42.4, 41.3, 37.8, 32.7, 31.4, 29.0, 28.4. Molecular formula: $C_{31}H_{53}Cl_3N_6O_{12}$. Calculated exact mass 806.28, M+H 807.28, ESI-MS found 807.3.



Scheme II: Synthesis of TM-Btn 1 and TCA-Btn 2.

Compound 9

Trifluoroacetic acid (TFA, 1 mL) and water (1 drop) was adding into 2 mL CH_2Cl_2 solution of compound **8** (0.4 g, 0.5 mmol). After stirring for 1 h at RT, solvent was removed under reduced pressure to yield a yellow oil. 10 mL water was added to dissolve the oil and the pH was adjusted to be 7 by saturated $NaHCO_3$ solution. A solution of compound **7** (204 mg, 0.6 mmol) in 10 mL CH_3CN was added. The reaction was monitored by ESI-MS. After stirred at RT for 2 h, the reaction was completed and the pH was adjusted to be 1 by 1M HCl. CH_3CN was removed under reduced pressure. The remaining aqueous solution was extracted by CH_2Cl_2 (3×10 mL) to remove most of the biotin and biotin-NHS (compound 4). Compound **9** was very hydrophilic and could not be purified by silica gel flash column. Therefore, the aqueous solution (compound **9** and some NHS and sodium chloride) was concentrated to 5 mL and used for next reaction without further purification. Molecular formula: $C_{36}H_{59}Cl_3N_8O_{12}S$. Calculated exact mass 932.3, M+Na 955.3, ESI-MS found 955.3.

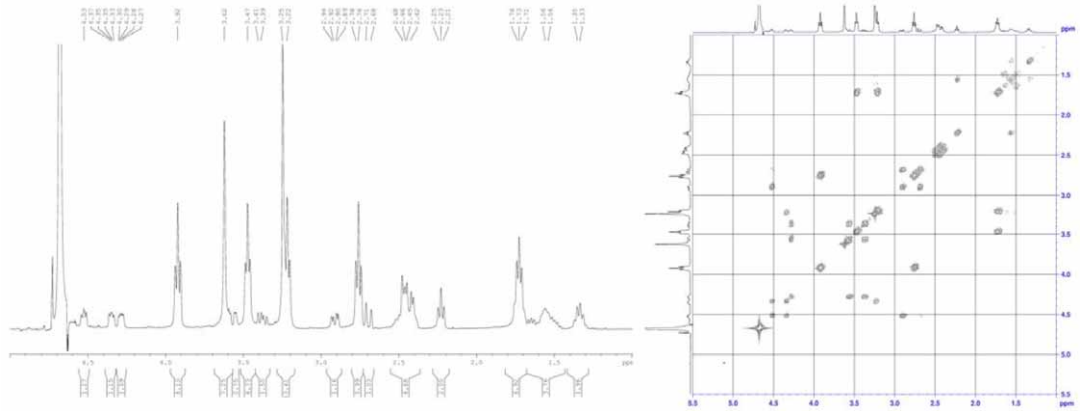


Figure S1. ^{1}H NMR (left) and ^{1}H-^{1}H COSY (right) of compound **TCA-Btn 2** in D_2O.

Compound 2 (TCA-Btn)

Compound **9** (about 0.1 mmol in 1 mL water) and compound **11** (95 mg, 0.5 mmol) was mixed in 2 mL saturated $NaHCO_3$ solution in a 4-mL glass vial by sonication. The vial was capped and the solution was stirred overnight at room temperature. The reaction mixture was purified on a C18 semi-prep HPLC column using a gradient of 0–40% solvent B over 40 min (solvent A = 1% acetonitrile, 99% water with 0.05% NH_3, solvent B = 90% acetonitrile, 10% water with 0.05% NH_3). The purified product was lyophilized to give the compound **2** as white solid (37 mg, yield 27%). ^{1}H NMR (D_2O) δ. 1.30-1.38 (m, 2H); 1.45-1.70 (m, 4H); 1.73 (m, 6H); 2.23 (t, J = 8Hz, 2H); 2.52 (m, 4H); 2.71 (m, 1H); 2.76(t, J = 6Hz, 6H); 2.92 (d×d, J_1= 12 Hz, J_2 = 4Hz, 1H); 3.20-3.27 (m, 13H); 3.40 (d×d, J_1= 12 Hz, J_2 = 8Hz, 1H); 3.47 (m, 6H); 3.62 (s, 7H); 3.92 (t, J = 6Hz, 6H); 4.28 (m, 1H); 4.35 (m, 1H); 4.53 (m, 1H). ^{13}C NMR

(D_2O) δ. 176.3, 176.2, 175.2, 174.4, 172.2, 165.3, 155.8, 154.6, 69.1, 68.8, 62.1, 60.3, 60.1, 55.3, 54.9, 41.1, 39.9, 39.7, 37.0, 35.6, 35.0, 31.6, 31.0, 29.7, 28.2, 28.0, 27.7, 25.1. TCA-Btn Molecular formula: $C_{51}H_{77}N_{17}O_{21}S_4$. Calculated exact mass 1391.44, $M+H^+$ m/e = 1392.4441, HR ESI-MS found 1392.3831; $M+2H^{2+}$ m/e =696.7260, HR ESI-MS found 696.7064.

Compound 1 (TM-Btn)

Compound **9** (about 0.1 mmol in 1 mL water) and compound **10** (94 mg, 0.5 mmol, together with oxidized TCEP and salt) was mixed with 2 mL saturated $NaHCO_3$ solution in a 4-mL glass vial by sonication. The vial was capped and the solution was stirred overnight at room temperature. The reaction mixture was purified on a C18 semi-prep HPLC column using a gradient of 0–30% solvent B over 30 min (solvent A = 1% acetonitrile, 99% water with 0.05% TFA, solvent B = 90% acetonitrile, 10% water with 0.05% TFA). The purified product was lyophilized to give the compound **1** as white solid (33 mg, yield 24%). ^1H NMR (D_2O, as a TFA salt) δ 1.30-1.38 (m, 2H); 1.42-1.70 (m, 4H); 1.73 (m, 6H); 2.24 (t, J = 8Hz, 2H); 2.45 (m, 4H); 2.65-2.71 (m, 7H); 2.92 (d×d, J_1= 12 Hz, J_2 = 4Hz, 1H); 3.21 (m, 7H); 3.26 (s, 6H); 3.45 (m, 7H); 3.53 (m, 6H); 3.61 (s, 7H); 4.34 (m, 1H); 4.44 (m, 1H); 4.53 (m, 1H). ^{13}C NMR (D_2O, as a TFA salt, the signal of TFA was not included) δ 176.7, 175.4, 174.4, 173.5, 172.3, 165.3, 160.4, 156.8, 69.1, 68.8, 62.1, 60.3, 60.1, 55.4, 52.7, 40.0, 39.8, 39.6, 36.9, 35.3, 34.9, 31.5, 31.3, 30.8, 28.2, 28.0, 27.8, 25.1. TM-Btn Molecular formula: $C_{51}H_{86}N_{26}O_{12}S_4$. Calculated exact mass 1382.5801, $M+H^+$ m/e = 1383.5880, HR ESI-MS found 1383.5468; $M+2H^{2+}$ m/e = 692.2979, HR ESI-MS found 692.2917; $M+3H^{3+}$ m/e = 461.8679, HR ESI-MS found 461.8564.

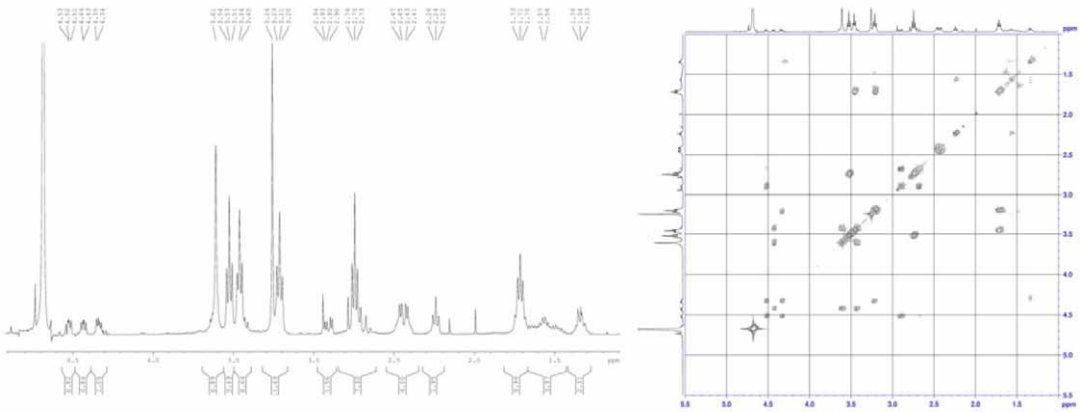


Figure S2. ^1H NMR (left) and ^1H-^1H COSY (right) of **TM-Btn 1** in D_2O.

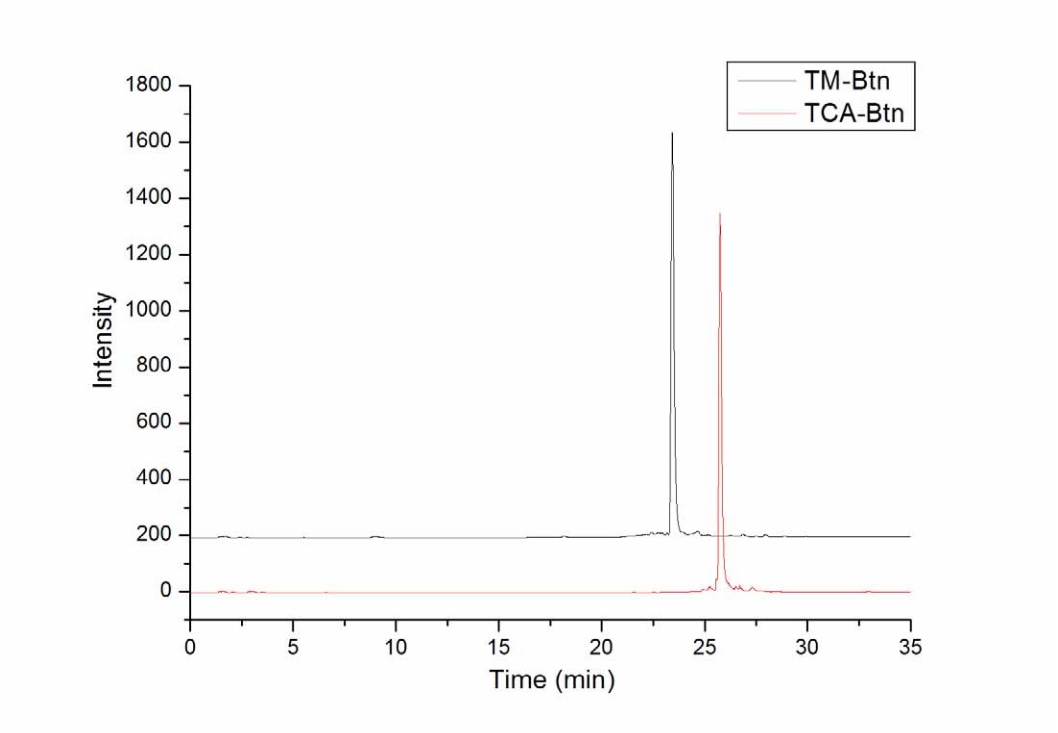


Figure S3. Analytical HPLC trace of **TM-Btn 1** and **TCA-Btn 2** on a C-18 analytical HPLC column. For **TM-Btn 1,** a gradient of 0–30% solvent B over 30 min (solvent A = 1% acetonitrile, 99% water with 0.05% TFA, solvent B = 90% acetonitrile, 10% water with 0.05% TFA) was used. For **TCA-Btn 2**, a gradient of 0–40% solvent B over 40 min (solvent A = 1% acetonitrile, 99% water with 0.05% NH_3, solvent B = 90% acetonitrile, 10% water with 0.05% NH_3) was used. The peak was collected and the compound was identified by ESI-MS.

Compound **12-16** were synthesized following published procedures.[4]

Compound 12

^{1}H NMR (D_2O, as a TFA salt) δ. 1.71 (m, 6H); 2.47 (m, 4H); 2.73 (t, J = 6.6 Hz, 6H); 3.20 (t, J = 7 Hz, 6H); 3.26 (s, 6H); 3.45 (t, J = 6.6 Hz, 6H); 3.55 (t, J = 7 Hz, 6H); 3.59 (s, 6H), 3.62 (dd, J = 6, 14 Hz, 1 H); 3.78 (dd, J = 4, 14 Hz, 1 H); 4.13 (dd, J = 4, 6 Hz, 1 H). ^{13}C NMR (D_2O, as a TFA salt, the signal of TFA was not included) δ 176.3, 174.5, 172.3, 170.1, 160.4, 156.8, 69.1, 68.7, 60.2, 53.4, 39.6, 39.0, 36.9, 34.9, 31.1, 30.5, 28.2, 24.5. Molecular formula: $C_{41}H_{72}N_{24}O_{10}S_3$. Calculated exact mass 1156.51, M+H, 1157.51, MALDI found 1157.3.

Compound 13

^{1}H NMR (D_2O) δ. 1.72 (m, 6H); 2.48 (m, 4H); 2.78 (t, J = 6.8 Hz, 6H); 3.22 (t, J = 7 Hz, 6H); 3.37 (s, 6H); 3.45 (t, J = 7 Hz, 6H); 3.51 (dd, J = 8, 14 Hz, 1 H); 3.61 (s, 6H); 3.72 (dd, J = 4, 14 Hz, 1 H); 3.84

(dd, J = 4, 8 Hz, 1 H); 3.92 (t, J = 6.8 Hz, 6H). ^{13}C NMR (D_2O) δ. 176.1, 175.1, 174.6, 172.6, 158.0, 155.6, 69.1, 68,8, 60.4, 55.2, 40.1, 39.8, 36.9, 35.1, 31.3, 30.7, 29.9, 28.2. Molecular formula: $C_{41}H_{63}N_{15}O_{19}S_3$. Calculated exact mass 1165.35, M+Na, 1188.34, MALDI found 1188.2.

Compound 15

^{1}H NMR (D_2O, as a TFA salt) δ. 1.72 (m, 6H); 2.45 (m, 4H); 2.75 (t, J = 6.6 Hz, 6H); 3.21 (t, J = 7 Hz, 6H); 3.27 (s, 6H); 3.46 (m, 7H); 3.51 (t, J = 7 Hz, 6H); 3.61 (s, 6H), 3.65 (m, 7H); 4.18 (s, 2H); 4.51 (dd, J = 4, 6 Hz, 1H). Molecular formula: $C_{43}H_{73}ClN_{24}O_{11}S_3$. Calculated exact mass 1232.47, M+H, 1233.47, MALDI found 1233.2.

Compound 16

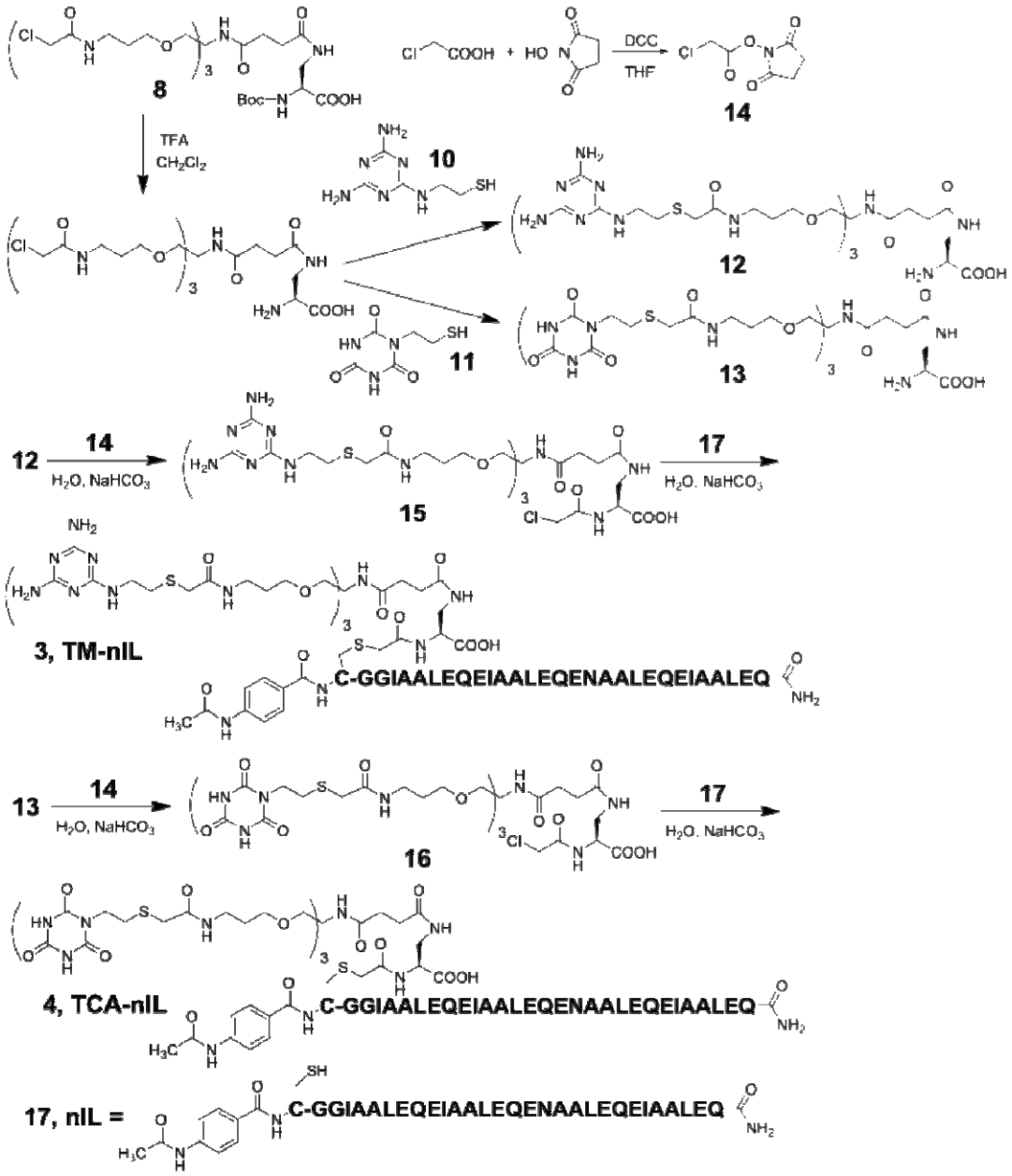
^{1}H NMR (D_2O) δ. 1.72 (m, 6H); 2.45 (m, 4H); 2.76 (t, J = 6 Hz, 6H); 3.22 (t, J = 7 Hz, 6H); 3.37 (s, 6H); 3.40 (dd, J = 8, 12 Hz, 1 H); 3.49 (t, J = 7 Hz, 6H); 3.62 (m, 7H); 3.92 (t, J = 6.8 Hz, 6H); 4.11 (s, 2H); 4.31 (dd, J = 4, 8 Hz, 1 H). Molecular formula: $C_{43}H_{64}ClN_{15}O_{20}S_3$. Calculated exact mass 1241.33, M+Na, 1264.32, MALDI found 1264.2.

Peptide pIL 5 (cc2) and peptide nIL 17 (cc1)

Peptide synthesis was performed on an AAPPTEC apex peptide synthesizer using a Rink amide resin (0.34 mmol/g, typically 0.15 g resin for one peptide sequence) with standard Fmoc chemistry utilizing DIC/HOBt in NMP for coupling steps and 50% piperidine/NMP for Fmoc deprotection. The N-terminal of peptide **pIL 5** and peptide **nIL 17** were capped by 4-acetamidobenzoic acid. Then the resin was washed by DMF, CH_2Cl_2 and MeOH and dried under vacuum. Peptides were cleaved from resin using a TFA:water = 95:5 mixture (2 mL for 0.15 g resin) for 3h at RT. Resin was removed by filtration through cotton and the peptide was precipitated by chilled ether (20 ml), centrifuged, and washed with chilled ether (20 ml). Crude peptides were then dissolved in pure water and purified on a C18 semi-prep column using a gradient of 20–80% solvent B over 30 min (solvent A = 1% acetonitrile, 99% water with 0.05% TFA, solvent B = 90% acetonitrile, 10% water with 0.05% TFA). The purified product was lyophilized to give pure peptide **pIL 5** and peptide **nIL 17** as white powder.

For the synthesis of **nIL** peptide conjugates, the nIL peptide and the corresponding chloride compound **15** or **16** (3-5 molar equivalent to **nIL**) were dissolved in water in a 4-mL glass vial. Saturated $NaHCO_3$ solution was added to adjust the pH to be around 8. The vial was capped and the solution was stirred overnight at room temperature. Then the solution was acidified by 1 M HCl and purified using the same

condition for purifying **nIL 17 (cc1)** peptide.



Scheme III: Synthesis of TM-nIL 3 (cc1) and TCA-nIL 4 (cc1).

Compound 5 (**pIL**) (**cc2**) Molecular formula: $C_{141}H_{249}N_{41}O_{34}$ Calculated exact Mass: 3060.90, M+H, 3061.90, MALDI found 3061.8.

Compound 17 (nIL) (cc1) Molecular formula: $C_{141}H_{225}N_{37}O_{51}S$. Calculated exact Mass: 3284.59, M+H,

3285.59, MALDI found 3285.7.

Compound 3 (**TM-nIL**) **(cc1)** Molecular formula: $C_{184}H_{297}N_{61}O_{62}S_4$. Calculated exact Mass: 4481.08, M+H, 4482.1, MALDI found 4482.5.

Compound 4 (**TCA-nIL**) **(cc1)** Molecular formula: $C_{184}H_{288}N_{52}O_{71}S_4$. Calculated exact Mass: 4489.94. M+Na, 4502.94, MALDI found 4502.7.

Preparation, characterization and concentration measurement of streptavidin and the conjugates with TCA-Btn and TM-Btn

Streptavidin was purchased from Promega. 1.0 mg lyophilized streptavidin was dissolved in 1.0 mL pure water. 50 μL 0.5 mM TCA-Btn or TM-Btn was added into 0.5 mL 1.0 mg/mL streptavidin solution. Mixed for 1 min, the solution was transferred into a microcon (ultracel YM-10) and centrifuged at 4 °C and 132000 rpm for 40 min. Then 0.5 mL PBS (50 mM sodium phosphate and 150 mM NaCl at pH 6.8) was added. The solution was gently pipetted and centrifuged at 4 °C and 161000 rcf for 40 min. This 'PBS washing' was repeated once. Finally, the remaining protein conjugate was dissolved in 0.5 mL PBS. The final concentration was measured by UV-Vis at 280 nm using an extinction coefficient ε= 3.2 $(mg/mL)^{-1}$ cm^{-1}.[5] The purity and integrity of the protein conjugates was analyzed by electrophoresis on NuPAGE® Novex 12% Bis-Tris Gel, stained by coomassie blue. The protein marker was Amersham full-range rainbow molecular weight marker from 12 kD-225 kD. Generally, 7.5 μL sample solution (0.5 μM protein conjugates) was mixed with 2.5 μL NuPAGE LDS 4× sample buffer, and incubated at 70 °C water bath for 10 min. A 100 μL 1:1 mixture of TM-SA and TCA-SA (0.5 μM of each in PBS) was cooled down to 10 °C and incubated for 30 min, then centrifuged at 10 °C and 161000 rcf for 10 min. The supernatant was withdrawn, 7.5 μL of which was mixed with 2.5 μL sample buffer. The remaining precipitate was mixed with 7.5 μL PBS and 2.5 μL sample buffer for electrophoresis. The bands around 102 kD were streptavidin tetramer, which is actually only about 52 kD. The significant deviation has been reported by other researcher, which is due to the extraordinary resistance to denaturation of streptavidin tetramer.[6] The band around 13 kD was a monomer of streptavidin (denatured). The combined filtrate from streptavidin conjugates preparation show no protein, indicating the filtration was working well.

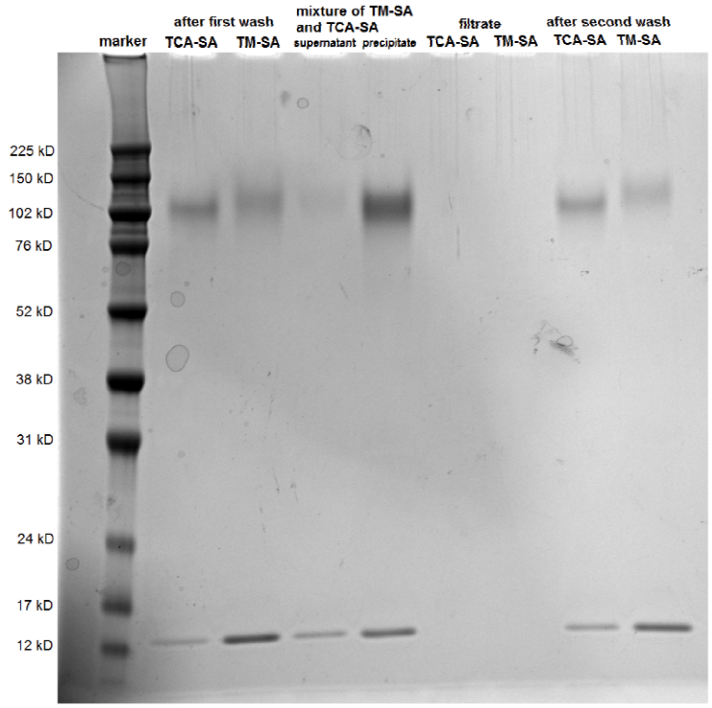


Figure *S4*. SDS-PAGE of the synthetic ligand-streptavidin conjugates.

Preparation, characterization and concentration measurement of IL peptide heterodimers

All the IL peptides have a 4-acetamidobenzamide at their N-terminal, which has an extinction coefficient of 18069 M^{-1} cm^{-1} at 270 nm. Therefore, all the IL peptides concentration was measure by UV. TM-nIL or TCA-nIL was mixed with pIL peptide in PBS pH 7.4 buffer at 1:1 molar ratio. Stable heteromeric peptide dimer TM-IL or TCA-IL were formed and stored at 0.5 mM total peptide concentration at 4 °C. Circular dichroism (CD) spectrum of TCA-IL dimer and TM-IL dimer was measured at 25 °C in PBS 6.8 buffer, with total peptide concentration at 50 μM （Figure S4）. TCA-IL dimer and TM-IL dimer in PBS buffer was injected into a C-18 analytical column, using H_2O/acetonitrile with TFA as solvents, and the UV monitor was at 270 nm for the 4-acetamidobenzamide group (Figure S4). All the peaks were collected and identified using MALDI. And the peak area of pIL was roughly the same as that of TM-nIL or TCA-nIL, consistent with the 1:1 heterodimer structure. The two pIL elution time are different, possibly due to the TM-IL dimer dissociate later or slower than TCA-IL dimer in the HPLC column during the solvent and pH change.

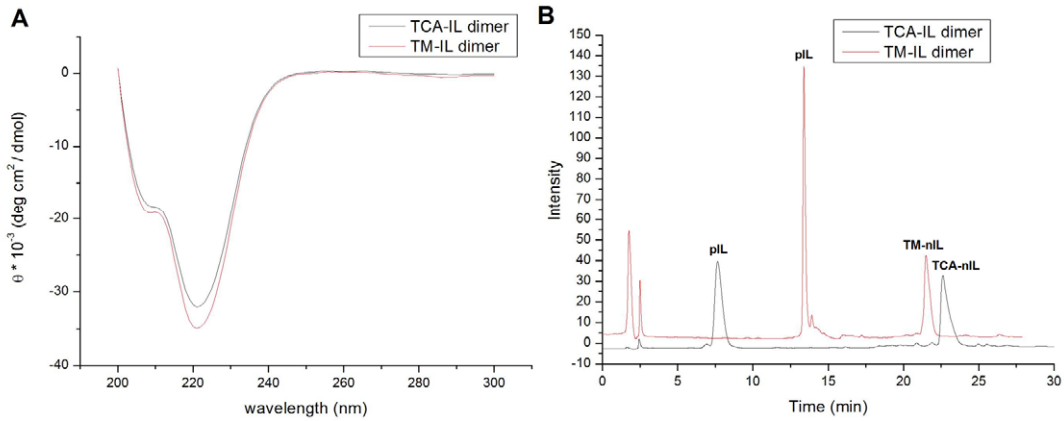


Figure *S5*. (A) CD of the TCA-IL dimer and TM-IL dimer at 25°C in PBS buffer. (B) Analytical HPLC trace of the TCA-IL dimer and TM-IL dimer.

B. Analytical Methods and Procedures

Surface Plasmon resonance (SPR). Surface Plasmon resonance (SPR) was performed on a BIAcore 3000 instrument using a sensor chip SA. The instrument was adjusted to 10 °C and equilibrated for 2 h. All samples and PBS buffer (pH = 6.8) was degassed and filtered through 0.2 μm PVDF filter from Whatman. The flow rate was 10 μL/min for all experiments. The chip surface was washed by 50 mM NaOH in 1 M NaCl for three 1-min injections before functionalization. 20 μL of 0.5 mM TCA-Btn or TM-Btn was injected to functionalize the chip surface. The complimentary streptavidin conjugate was injected at different concentrations to give a binding curve. The surface was regenerated by 1-min injections of 0.5% formic acid in 150 mM NaCl. The baseline and binding capacity was stable up to 10 analysis injections. For the inhibition experiments, 0.1 μM of TCA-SA or TM-SA was incubated with 10 μM complimentary ligands TM or TCA at 10 °C for 10 min, and then the solution was injected. Due to the competition of free ligands TM or TCA, the protein-protein binding was significantly reduced.

Dynamic light scattering (DLS). Dynamic light scattering (DLS) was performed on a Malvern Instruments Zeta Sizer Nano-ZS. Stock solutions of TCA-SA and TM-SA were diluted into PBS buffer and the pH was adjusted to the desired value. Then these samples were filtered through 0.22 μm PVDF syringe filter. For the TCA-SA/TM-SA mixture, the diluted single protein solution was filtered and then two solutions were mixed. The total protein concentration was 1 μM. For temperature trend experiment, the data averaging time at each temperature was 10 min. The particle size was determined by volume data. The polydispersity index (PDI) was usually lower than 0.2, when the particle size was below 100 nm. When the particle size was above 100 nm, meaning the protein has aggregated so much, the PDI value were usually around 0.4-0.7.

Isothermal titration calorimetry (ITC). Isothermal titration calorimetry (ITC) was performed on a

MicroCal VP-ITC microcalorimeter. The titration curve was fit to a 'one set of sites' binding model using Origin program. Stock solutions of TCA-Btn and TM-Btn were diluted in PBS 6.8 buffer, and then filtered through 0.22 μm PVDF syringe filter and degassed before loading. Typically, 0.05 mM TM-Btn was placed in the cell; and TCA-Btn (0.5 mM) was filled into the syringe and injected into the cell. ITC was performed at 10 °C and 25 °C. The control was performing the same measurement using water or buffer replacing one derivative, which gave the reference trace. The final ITC data was after subtraction of the corresponding reference delta H.

Circular dichrism (CD). CD spectra were recorded on a JASCO J-815 Spectrometer under a nitrogen atmosphere over the range of 195-300 nm at 25 °C. Typically, 0.4 mL 50 μM peptide in PBS (50 mM sodium phosphate, 120 mM sodium chloride, pH 6.8) was placed in a quartz cell with a 1 mm path length. The spectra were collected with one data point every 1 nm, and 3 second average time for each data point. Each final spectrum was averaged from three individual parallel scans.

Table *S1* The assembly enthalpy, entropy and dissociation constant of TCA-Btn/TM-Btn interaction in PBS buffer.

Temperature	Assembly enthalpy ΔH (kcal/mol)	Assembly entropy ΔS (cal/mol °C) (based on 1:1 binding model)	Dissociation constant K_d (M) (based on 1:1 binding model)
10 °C	-30.2 ± 0.07	-73.9	$7.0 \pm 1.5\times10^{-8}$
25 °C	-29.2 ± 0.04	-69.5	$5.9 \pm 1.9\times10^{-7}$

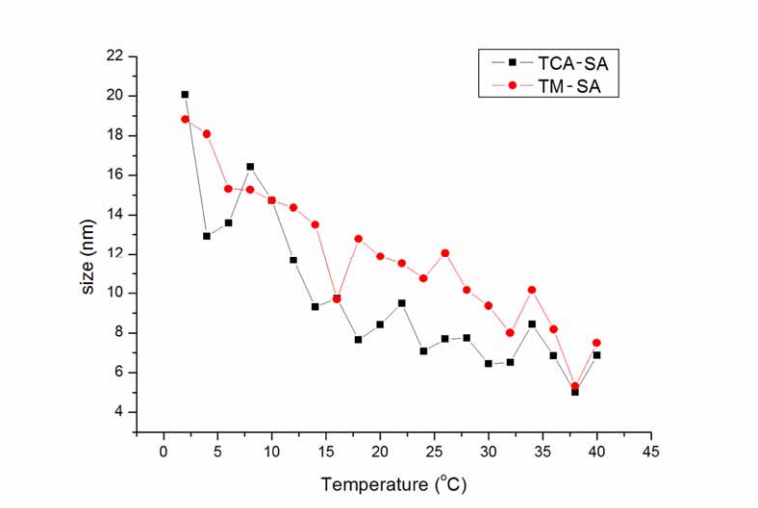


Figure S6. DLS data of SA protein conjugates (both were 1 μM in PBS, pH 6.8) cooling from 40 to 2 °C. The error of size is about 15% when temperature is higher than 20 °C and rises to about 25% when

temperature is lower than 20 °C. For example, for TM-SA at 40 °C, the size is about 7.8 ± 1.2 nm. At 4 °C, the size is about 18.8 ± 4.7 nm.

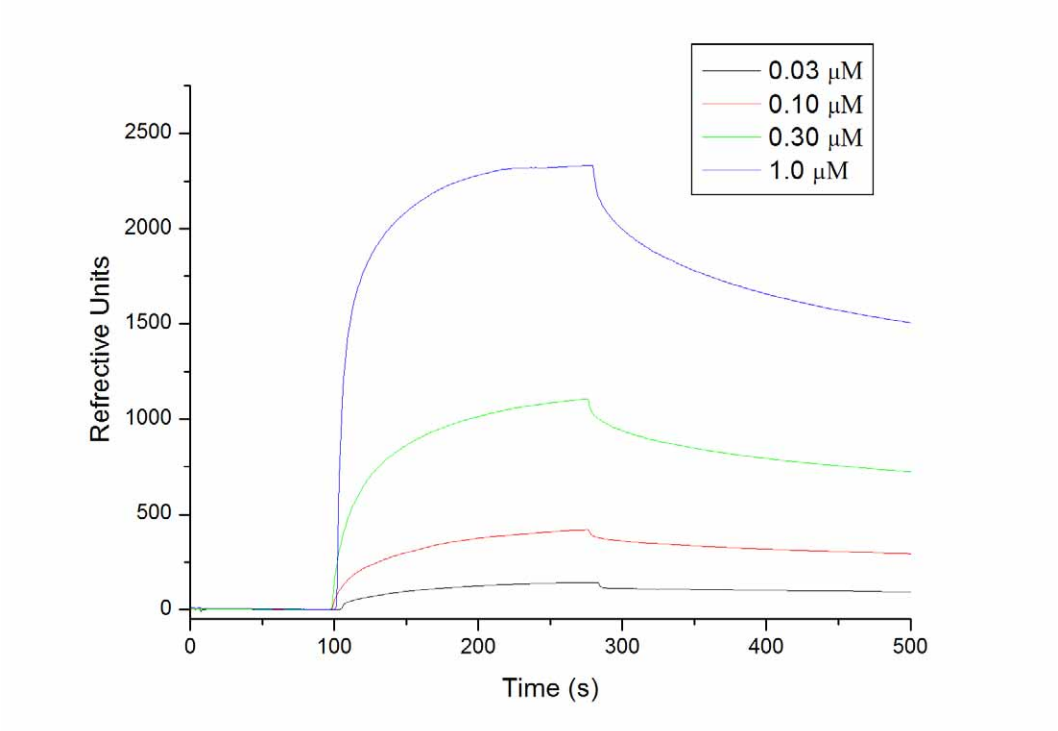


Figure S7. SPR signal as function of soluble TCA-Btn-SA concentration over a TM-Btn-streptavidin SPR chip.

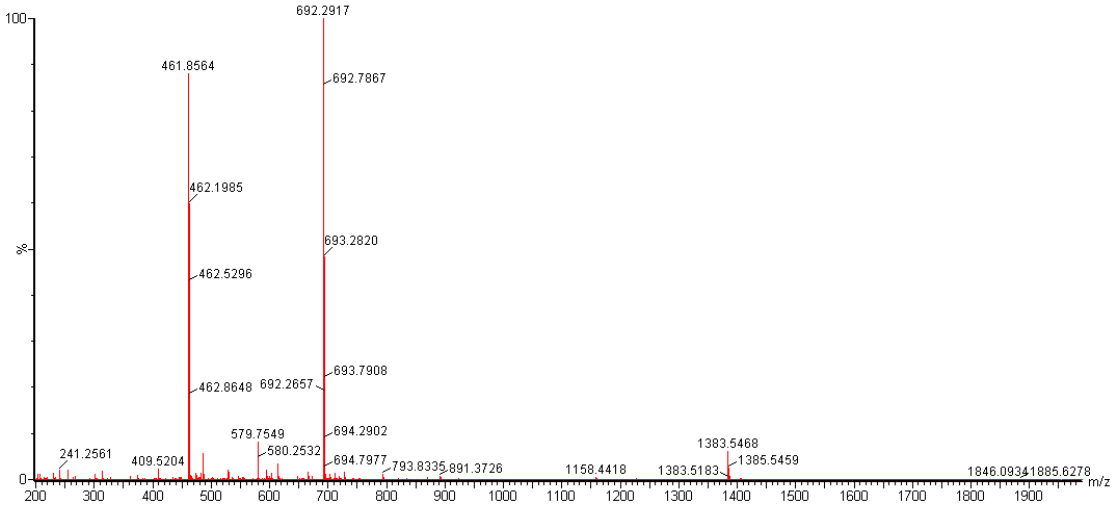


Figure S8. High resolution ESI-MS of **TM-Btn 1**.

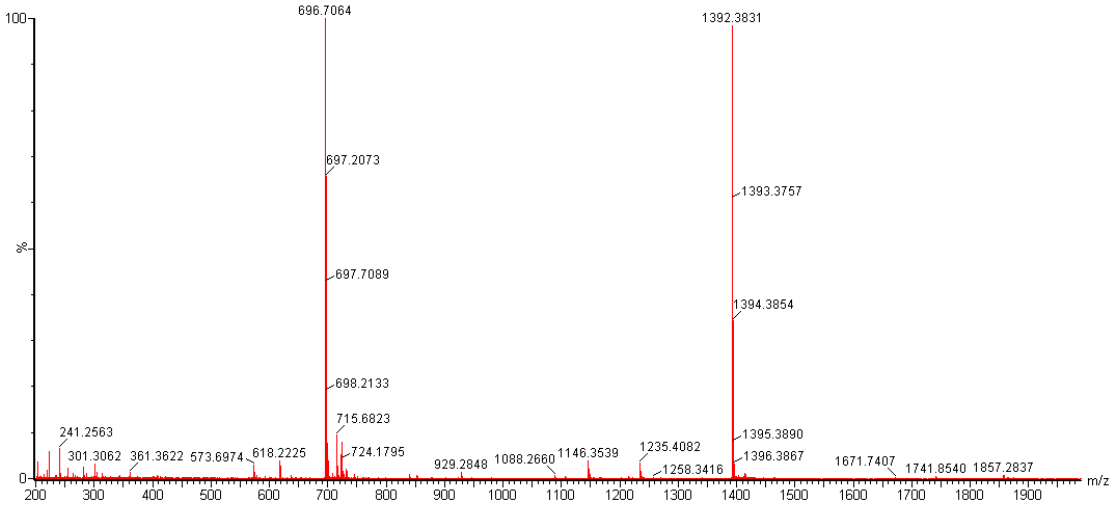


Figure S9. High resolution ESI-MS of **TCA-Btn 2**.

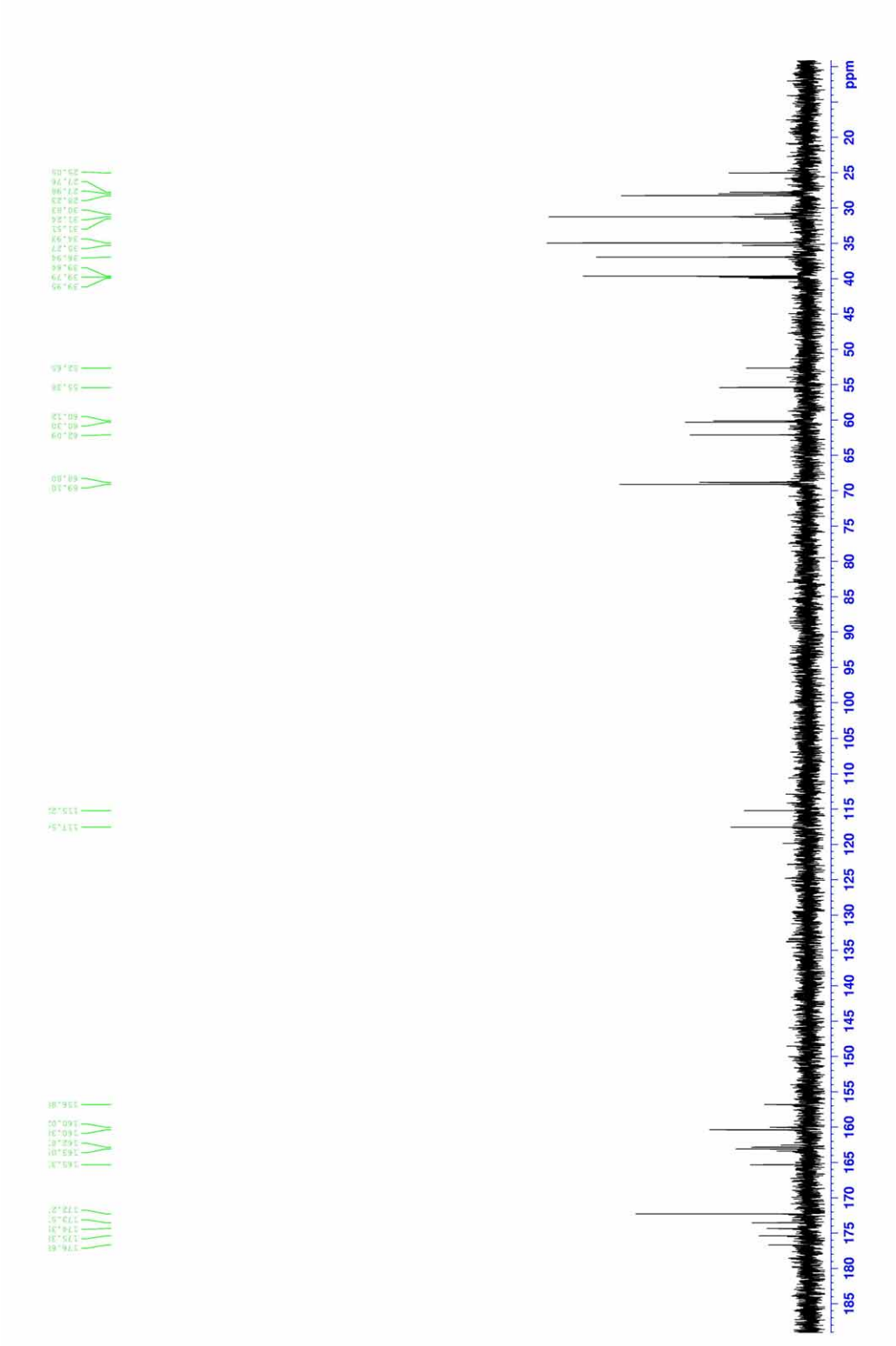


Figure S10. ^{13}C NMR of **TM-Btn 1** in D_2O.

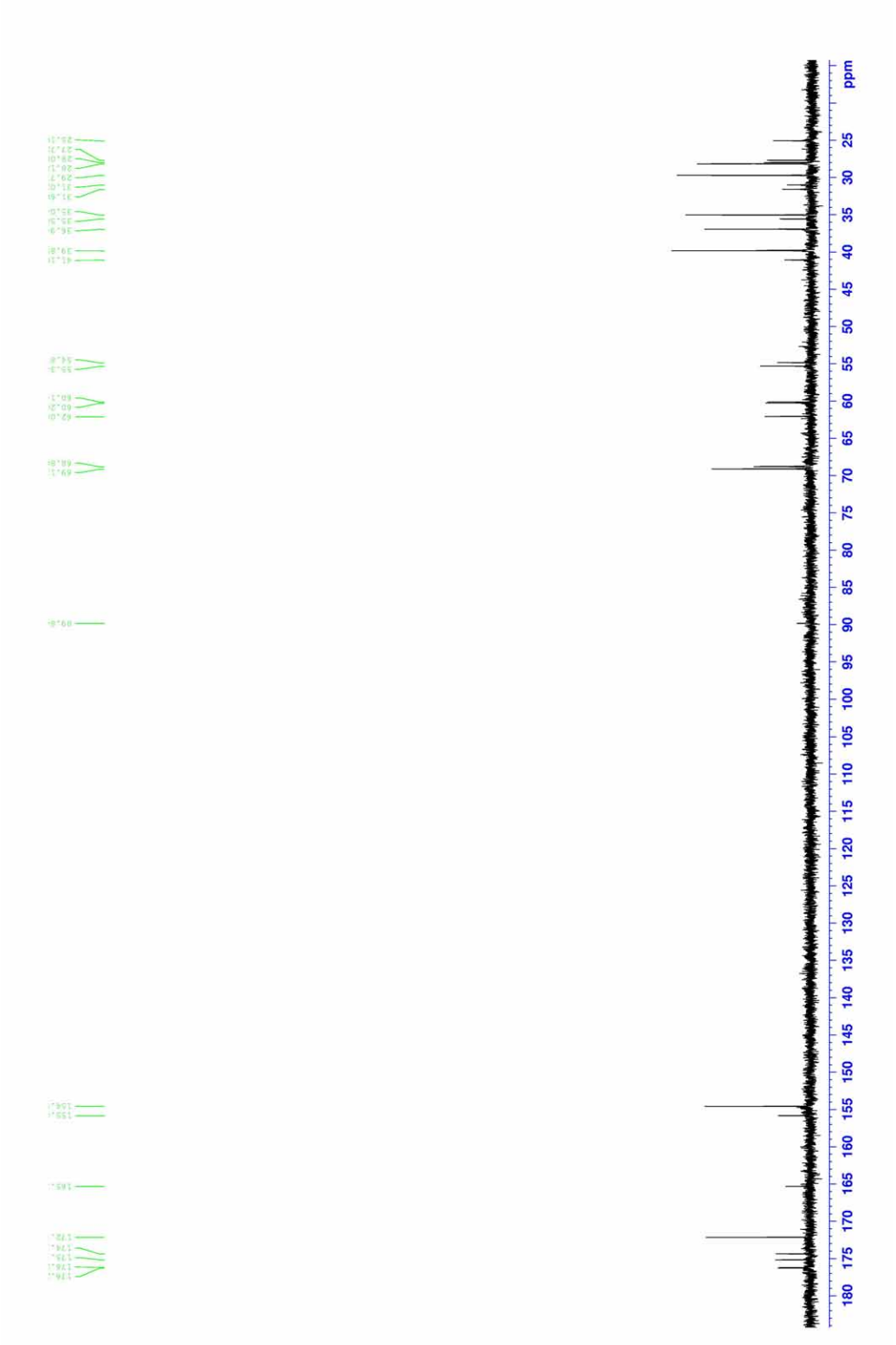


Figure S11. ^{13}C NMR of **TCA-Btn 2** in D_2O.

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