

## -Supplemental Information-

### Flexible organic frameworks sequester neuromuscular blocking agents *in vitro* and reverse neuromuscular block *in vivo*

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## General experimental details

### General methods and materials

Commercial reagents were used without further purification.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded with a Bruker AVANCE III HD 400 MHz spectrometer (100 MHz) in the indicated solvents at 298 K. Chemical shifts were referenced to the residual solvent peaks. Dynamic light scattering (DLS) and zeta potential experiments were conducted on a Malvern Zetasizer Nano ZS. Isothermal calorimetric experiments were conducted on a Nano ITC-TA instrument (ITC200). Quantitative analysis of NMBAs was measured by reversed-phase Agilent 1260 High Performance Liquid Chromatography. The melting point of unknown compound was determined by Mettler Toledo melting point meter.

### Isothermal titration calorimetry (ITC) experiments

The FOF-SS1-3 and NMBAs solutions were prepared in PBS (pH 7.4). The concentration of FOF-SS1-3 was calculated based on that of precursors T1-T3. An aqueous solution of FOF-SS1-3 (0.1 mM, while 0.2 mM for FOF-SS1) was placed in the sample cell (300  $\mu\text{L}$ ). Solution of NMBAs (0.8 mM while 1.2 mM for Vec) was dropped in the order of the first injections (0.5  $\mu\text{L}$ ) followed by 19 injections (2  $\mu\text{L}$ ), the heat evolved was recorded at  $T = 298$  K. The binding constants (curve fitting) were fitted using the MicroCal ITC analyze software.

### Dialysis experiment

FOF-SS-1-3 and Cis were mixed in PBS (pH = 7.4), while the former's concentration was 10 mg/mL and the later's concentration was 1 mg/mL. The control group contained only 1 mg/mL Cis. The total volume is 1 mL, and they were put into a dialysis bag (cut-off molecular weight: 1000). The dialysis bag was placed in 20 mL PBS and incubated in a shaker at 310 K. Samples were taken every ten minutes for separation by high performance liquid chromatography. The amount of Cis remaining in the dialysis bag was quantitatively analyzed according to the standard curve of Cis ( $A=30.87c-2.11$ ,  $R^2 = 0.9996$ ).

The dialysis rate was calculated following equation:

$$c = \frac{A + 2.11}{30.87} \times 100\% \quad \text{Equation 1}$$

$$\text{Residual Cis (\%)} = 1 - \frac{20 * c}{1 * 1000} \times 100\% \quad \text{Equation 2}$$

in which A was the integral area in the HPLC spectra,  $c$  was the concentration of Cis in external solution ( $\mu\text{g/mL}$ ).

### Cell cytotoxicity

Cytotoxicity was evaluated by cell counting kit-8 (CCK-8) assay. H9C2 cells and L02 cells were seeded in 96-well plate ( $8 \times 10^3$  cells and 100  $\mu\text{L}$  culture medium in per well) and were incubated for 24 hours at 310 K containing 5%  $\text{CO}_2$ . Then the culture medium was removed and 100  $\mu\text{L}$  culture medium containing FOF-SS1-3 at different concentration was added (4-512  $\mu\text{g/mL}$ ). The culture was removed after 24 h and 100  $\mu\text{L}$  fresh culture medium containing 10% CCK-8 was added. The cells were incubated for another 1 hour and the cell viability was evaluated by absorbance at 450 nm using Allsheng AMR-100 microplate reader.

### Hemolysis experiment

5% human and mouse red blood cells preserved in Alsever's solution were centrifuged in a 1000 r/min centrifuge for ten minutes to obtain red blood cells. Cells were diluted with equal volume of isotonic saline. The mixed solution (140  $\mu\text{L}$ ) were mixed with saline (560  $\mu\text{L}$ , negative control), deionized water (560  $\mu\text{L}$ , positive control), or FOF-SS1-3 solution at different concentrations (4-512  $\mu\text{g/mL}$ ). After incubation for 1 h at 310 K, the samples were centrifuged at 3000 r/min for 10 minutes at 277 K and

the supernatants were obtained, and their hemolysis ratio was evaluated by absorbance at 545 nm using Biotek Synergy H1 microplate reader. The hemolysis rate was calculated following equation:

$$\text{Hemolysis rate (\%)} = \frac{A_s - A_n}{A_p - A_n} \times 100\% \quad \text{Equation 3}$$

in which  $A_s$ ,  $A_n$  and  $A_p$  are the absorbances at 545 nm of the samples, negative control and positive control.

## ***In vivo* experiment**

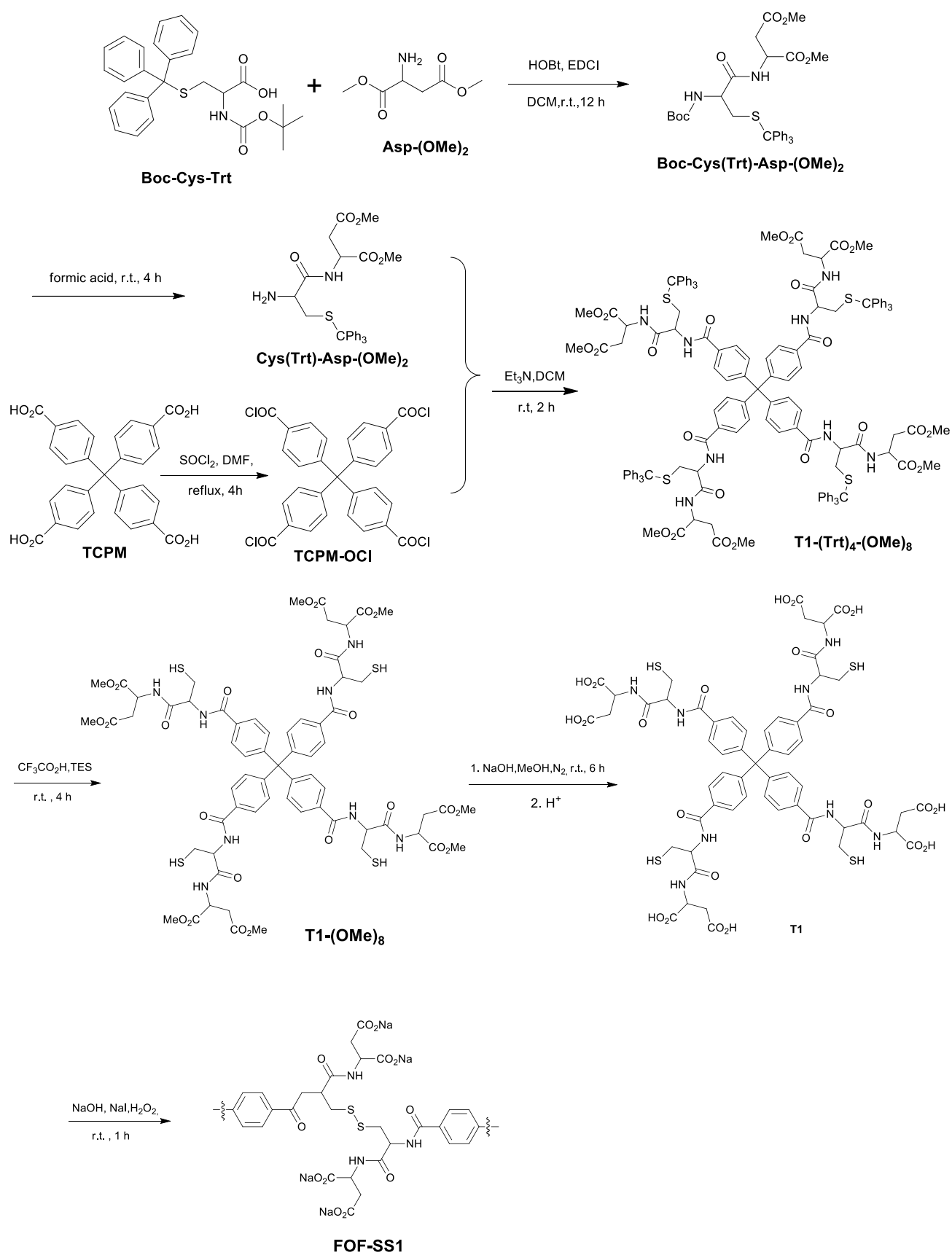
**Ethical statement:** the animal experiments were carried out based on protocols approved by ethical board of Fudan University.

**General surgical procedures:** A total of 144 Sprague-Dawley rats whose weights ranged from 180 to 280 g were used ( $n = 6$ , contained 3 male and 3 female rats). Surgical rats were anesthetized with 5% isoflurane and then maintained anesthesia with 1% isoflurane while the flow rate of isoflurane was controlled at 0.6 L/min. Endotracheal intubation was conducted for mechanical ventilation before injection of NMBAs. Constant airflow containing 1% isoflurane was pumped to maintain anesthesia to eliminate stress response. Rats were positioned supine on a thermostatic heating plate to maintain the rectal temperature at  $37.0 \pm 1$  °C.<sup>17,18</sup>

**Drug administration:** Cis (0.6 mg/kg, two times ED90), Roc (3.5 mg/kg, two times ED90), Vec (0.7 mg/kg, two times ED90), neostigmine/glycopyrrolate (0.06/0.012, 0.12/0.024 and 0.24/0.048 mg/kg) were diluted in 0.5 ml water. **FOF-SS1-3** (40 mg/kg, 80mg/kg and 120 mg/kg) were diluted in 0.5 ml 5% glucose.

**Experimental Protocol:** The right leg was shaved and pasted on electrode patches to stimulate the rat quadriceps femories muscle. The twitch response of the quadriceps muscle was measured by acceleration sensor of the Sigo TOF-Watch Monitor. The transducer was fixed on the tibia of the right leg where the swing amplitude is the largest, which is convenient for accurate measurement of acceleration. The TOF Watch was switched to cal mode, we stimulated the femoral nerve continuously at 1 Hz with 10 mA current until twitch height reached a stable state. Then the TOF Watch was switched to the train-of-four (TOF) mode, and continued to stimulate the femoral nerve at 2 Hz with a 15 s-internal until the T1 stabilized while TOF was > 90%. Rats were intubated and mechanical ventilation with the tidal volume of 8.0 mL at 80 Hz. Corresponding doses of NMBAs was injected into rats through the tail vein. After complete NMB was induced, either placebo (0.5 mL), mentioned dose of neostigmine/glycopyrrolate and **FOF-SS1-3** were administered at maximum twitch depression (T1 = 0). The time of TOF-recovery (>0.9) was measured to evaluate the effect of reversing NMB *in vivo*.

## Synthetic Procedures



**Scheme S1.** Synthesis of **T1** and **FOF-SS1**.

**Boc-Cys(Trt)-Asp-(OMe)<sub>2</sub>**: To a solution of Boc-Cys-Trt (9.28g, 20 mmol) in 100 mL CH<sub>2</sub>Cl<sub>2</sub>, HOBT (2.97 g, 22 mmol), EDCI (4.22 g, 22 mmol) and Asp-(OMe)<sub>2</sub> (3.95 g, 20 mmol) were dissolved. The pH was adjusted to 9 with N-methylmorpholine. The mixture was stirred at room temperature for 12 hours. Then the mixture was washed with saturated NaCl solution (50 mL × 2) and 5% KHSO<sub>4</sub> (50 mL × 2). The organic phase was separated, and anhydrous sodium sulfate was added to remove water. The crude product was separated by column chromatography (EA: Hexane = 1:2) and the Boc-Cys(Trt)-Asp-(OMe)<sub>2</sub> was obtained as white solid (10.70 g, 88%). M.P. 95 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.17 (d, *J* = 8.0 Hz, 1H), 7.40-7.20 (m, 15H), 7.00 (d, *J* = 8.5 Hz, 1H), 4.6 (q, *J* = 6.5 Hz, 1H), 3.92 (q, *J* = 7.5 Hz, 1H), 3.56 (s, 3H), 3.54 (s, 3H), 2.72 (qd, *J* = 16.6, 6.4 Hz, 1H), 2.37-2.28 (m, 2H), 1.38 (s, 9H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): δ 171.18, 170.85, 170.55, 155.36, 144.77, 129.55, 128.51, 127.23, 78.96, 66.38, 53.76, 52.63, 52.08, 48.87, 35.87, 34.17, 28.58. HR-MS (ESI): calcd for C<sub>33</sub>H<sub>38</sub>N<sub>2</sub>O<sub>7</sub>S [M+H]<sup>+</sup>: 607.2472, found 607.2462.

**Cys(Trt)-Asp-(OMe)<sub>2</sub>**: Boc-Cys(Trt)-Asp-(OMe)<sub>2</sub> (2.428 g, 4 mmol) was dissolved in 20 mL formic acid, and stirred at room temperature for 4 h. Then the solution was concentrated by rotary evaporation. The residual oil mixture was dissolved in 20 mL CH<sub>2</sub>Cl<sub>2</sub>, and washed with saturated NaHCO<sub>3</sub> solution to neutralize the residual formic acid until the pH approached to 7. The organic phase was separated, and anhydrous sodium sulfate was added to remove water. CH<sub>2</sub>Cl<sub>2</sub> was removed by rotary evaporation to obtain the Cys(Trt)-Asp-(OMe)<sub>2</sub> as an oil. (1.93 g, 96%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 7.87 (d, *J* = 8.5 Hz, 1H), 7.47-7.41 (m, 6H), 7.32-7.26 (m, 6H), 7.21 (t, *J* = 7.3 Hz, 3H), 4.76 (dt, *J* = 8.9, 4.7 Hz, 1H), 3.03-2.93 (m, 2H), 2.78 (dd, *J* = 17.0, 4.8 Hz, 1H), 2.69-2.57 (m, 2H). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 173.04, 171.32, 171.14, 144.67, 129.71, 128.11, 126.94, 67.08, 53.96, 52.84, 52.10, 48.36, 37.45, 36.24. HR-MS (ESI): calcd for C<sub>28</sub>H<sub>30</sub>N<sub>2</sub>O<sub>5</sub>S [M+H]<sup>+</sup>: 507.1948, found 507.1943.

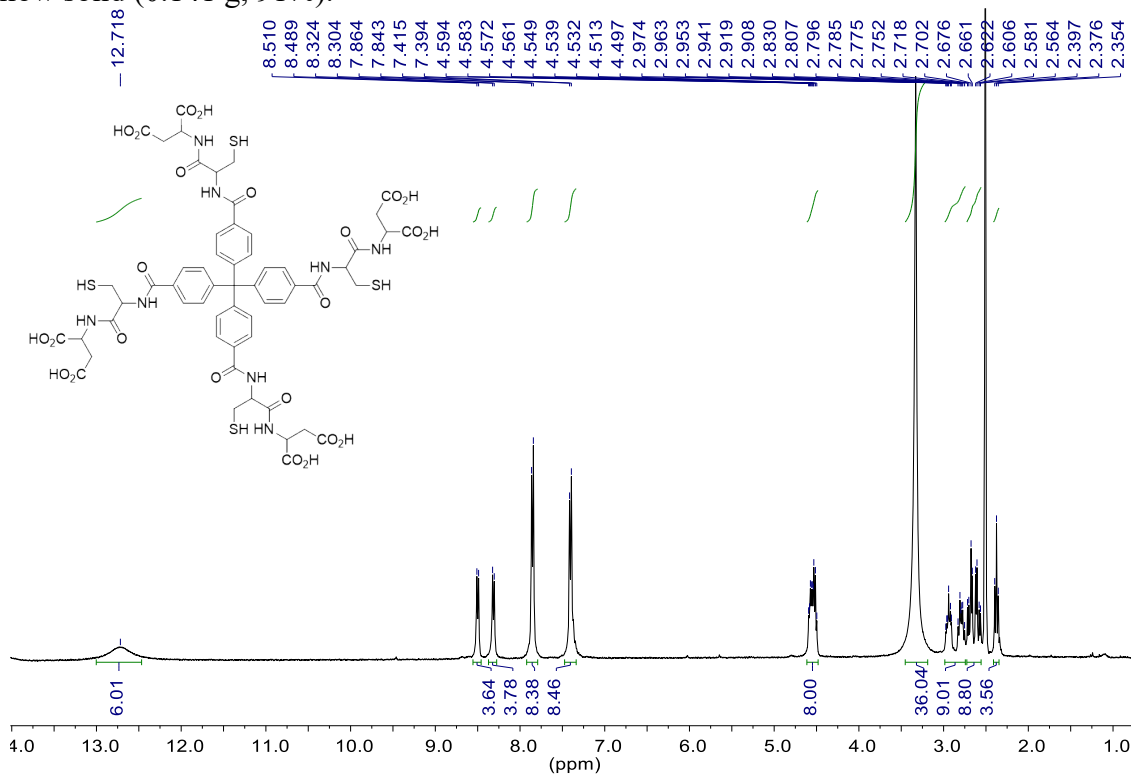
**T1-(Trt)<sub>4</sub>-(OMe)<sub>8</sub>**: To a solution of TCPM (0.248 g, 0.5 mmol) in 12 mL SOCl<sub>2</sub> was added two drops of DMF. The mixture was heated to reflux for 4 hours and cooled to room temperature, SOCl<sub>2</sub> was removed by rotary evaporation. Cys(Trt)-Asp-(OMe)<sub>2</sub> (1.115 g, 2.2 mmol) dissolved in 40 mL dry CH<sub>2</sub>Cl<sub>2</sub> and dry triethylamine (0.606 g, 6 mmol) was added and the resulting mixture was stirred at room temperature under N<sub>2</sub> atmosphere for 2 hours. Then the mixture was washed with saturated NaCl solution (30 mL × 3). The organic phase was separated, and anhydrous sodium sulfate was added to remove water. The crude product was separated by column chromatography (CH<sub>2</sub>Cl<sub>2</sub>: methanol = 50:1) and recrystallized in acetonitrile to obtain T1-(Trt)<sub>4</sub>-(OMe)<sub>8</sub> as light yellow solid (0.686 g, 56%). M.P. 205 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.65 (d, *J* = 8.2 Hz, 4H), 8.29 (d, *J* = 8.0 Hz, 4H), 7.83 (d, *J* = 8.5 Hz, 8H), 7.41 (d, *J* = 8.5 Hz, 8H), 7.35-7.17 (m, 60H), 4.59 (q, *J* = 6.6 Hz, 4H), 4.51-4.43 (m, 4H), 3.52 (s, 12H), 3.48 (s, 12H), 2.69 (ddd, *J* = 53.1, 16.5, 6.6 Hz, 8H), 2.56-2.46 (m, 8H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): δ 169.56, 169.22, 168.58, 164.53, 147.86, 143.14, 130.44, 128.86, 127.94, 126.89, 126.41, 125.60, 64.81, 63.86, 51.14, 51.01, 50.42, 47.32, 34.18, 32.30. HR-MS (ESI): calcd for C<sub>141</sub>H<sub>132</sub>N<sub>8</sub>O<sub>42</sub>S<sub>4</sub> [M+H]<sup>+</sup>: 2449.8310, found 2449.8388.

**T1-(OMe)<sub>8</sub>**: To a solution of T1-(Trt)<sub>4</sub>-(OMe)<sub>8</sub> (0.32 g, 0.13 mmol) in 8 mL trifluoroacetic acid was added triethylsilane (166 μL, 1.044 mmol). The mixture was stirred at room temperature for 4 hours. Then the insoluble by-product triphenylmethane was filtered out. Hexane (5 mL × 2) was added to the filtrate for removing the slightly dissolved triphenylmethane in trifluoroacetic acid. The trifluoroacetic acid was removed by rotary evaporation to obtain T1-(OMe)<sub>8</sub> (0.149 g, yield 79%). M.P. 137 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.51 (d, *J* = 7.9 Hz, 8H), 7.85 (d, *J* = 8.4 Hz, 8H), 7.41 (d, *J* = 8.5 Hz, 8H), 4.66 (q, *J* = 6.9 Hz, 4H), 4.55 (td, *J* = 8.7, 4.6 Hz, 4H), 3.61 (s, 12H), 3.57 (s, 12H), 2.97-2.69 (m, 16H), 2.37 (t, *J* = 8.5 Hz, 4H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): δ 170.91, 170.38, 170.04, 166.13, 148.91, 131.85, 130.00, 127.51, 64.98, 55.86, 52.24, 51.67, 48.56, 35.43, 25.85. HR-MS (ESI): calcd for C<sub>65</sub>H<sub>76</sub>N<sub>8</sub>O<sub>24</sub>S<sub>4</sub>, [M-H]<sup>-</sup>: 1479.3772, found 1479.3776.

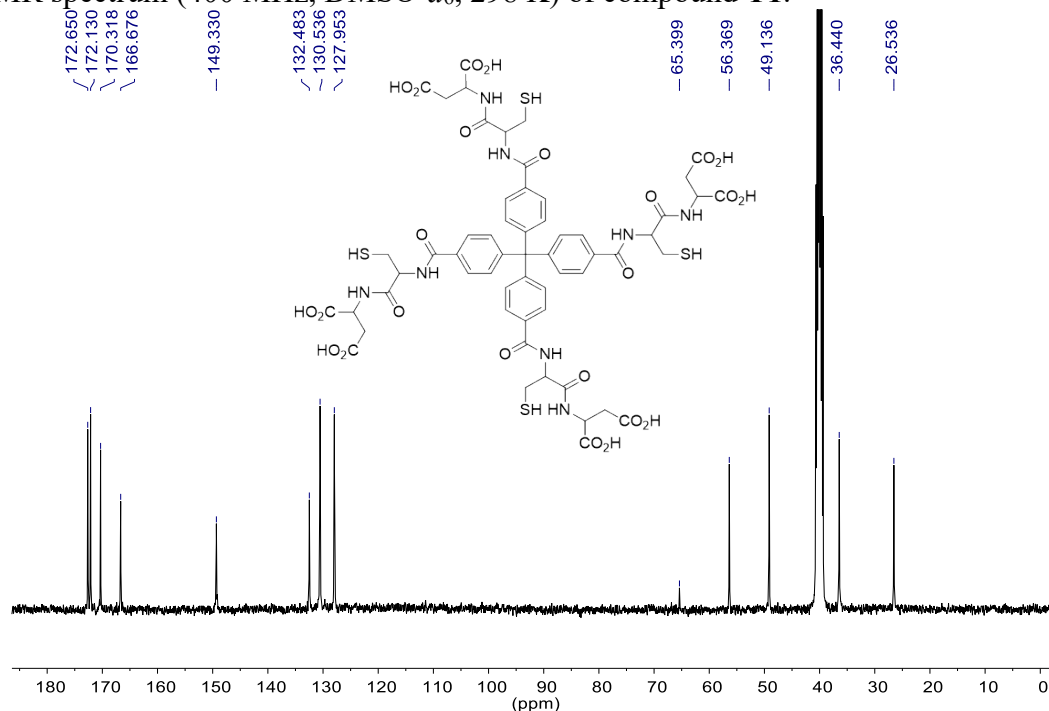
**T1**: To a solution of T1-(OMe)<sub>8</sub> (0.149 g, 0.1 mmol) in 3 mL methanol and 3 mL deionized water was added sodium hydroxide (0.083 g, 2.08 mmol). The resulting mixture was stirred at room temperature for 12 hours under N<sub>2</sub>. Then methanol was removed by rotary evaporation, and 5 mL deionized water was added. The mixed solution was filtered to obtain light yellow solution. 2 M HCl solution was gradually added to the resulting filtrate until the pH approached to 3~4. Precipitate was filtered and dried, and the target molecule **T1** was obtained as light yellow solid (0.123 g, 91%). M.P. > 300 °C

(decomposed).  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  12.72 (s, 8H), 8.50 (d,  $J = 8.2$  Hz, 4H), 8.31 (d,  $J = 7.9$  Hz, 4H), 7.85 (d,  $J = 8.5$  Hz, 8H), 7.40 (d,  $J = 8.5$  Hz, 8H), 4.59-4.50 (m, 8H), 2.97-2.75 (m, 8H), 2.64 (qd,  $J = 16.8, 6.4$  Hz, 8H), 2.37 (t,  $J = 8.5$  Hz, 4H).  $^{13}\text{C}$  NMR (100 MHz,  $\text{DMSO-}d_6$ ):  $\delta$  172.65, 172.13, 170.32, 166.68, 149.33, 132.48, 130.54, 127.95, 65.40, 56.37, 49.14, 36.44, 26.54. HR-MS (ESI): calcd for  $\text{C}_{57}\text{H}_{60}\text{N}_8\text{O}_{24}\text{S}_4$ ,  $[\text{M-H}]^-$ : 1367.2520, found 1367.2512.

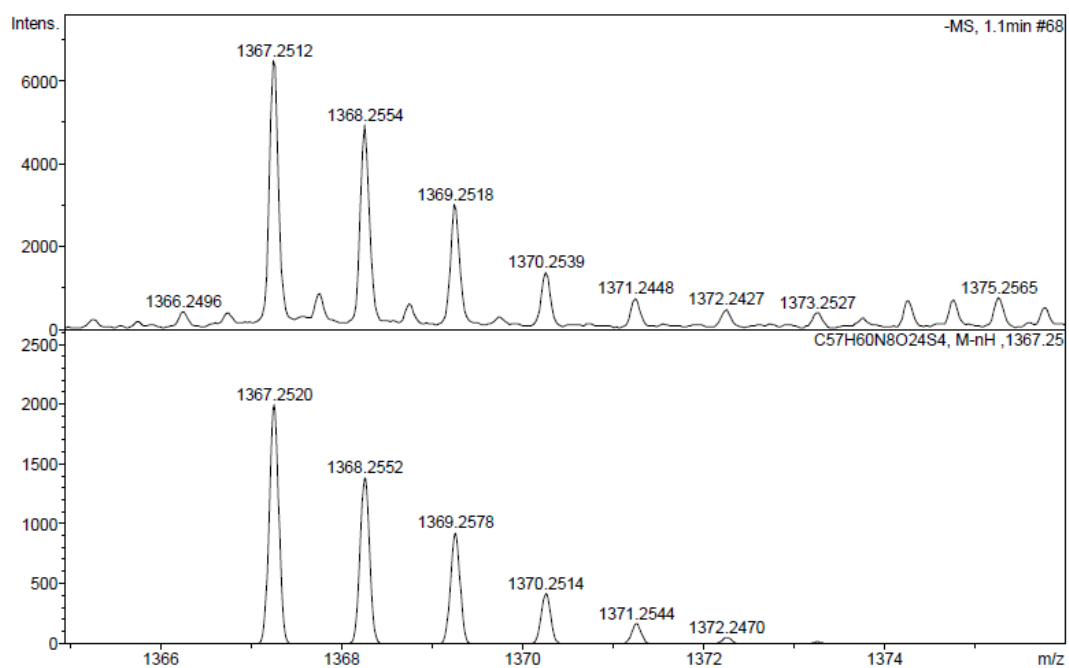
**FOF-SS1:** To a solution of compound **T1** (0.137 g, 0.1 mmol) in 8.6 mL deionized water was added NaOH solution (0.8 mL, 1 M), NaI solution (0.2 mL, 0.1 M),  $\text{H}_2\text{O}_2$  solution (0.4 mL, 1 M). The mixture was stirred at room temperature for 1 hour. The mixture was transferred to dialysis bags to remove excess  $\text{H}_2\text{O}_2$  and inorganic salt. **FOF-SS1** was obtained by rotary evaporation under low temperature as light yellow solid (0.141 g, 91%).



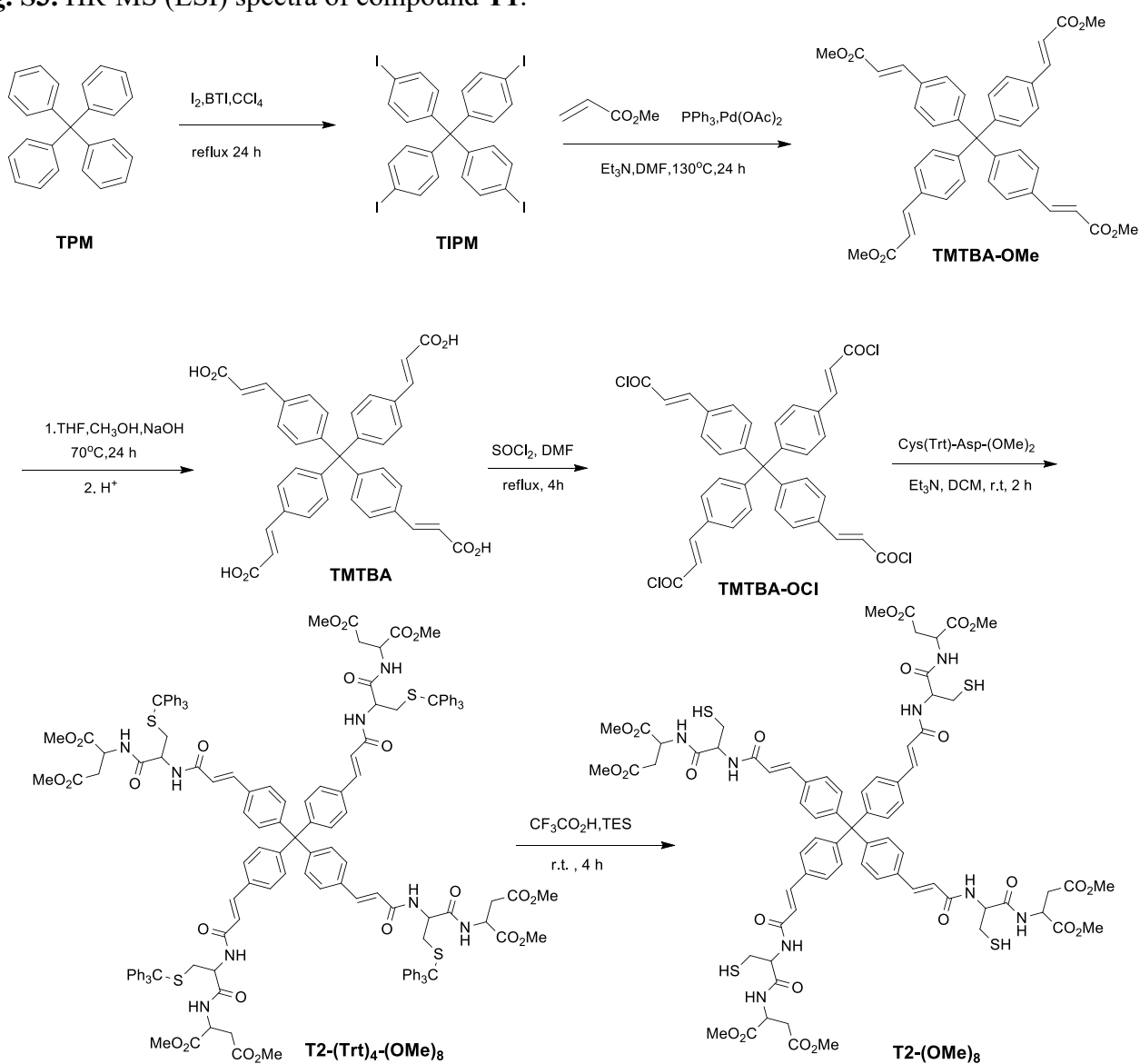
**Fig. S1.**  $^1\text{H}$  NMR spectrum (400 MHz,  $\text{DMSO-}d_6$ , 298 K) of compound **T1**.



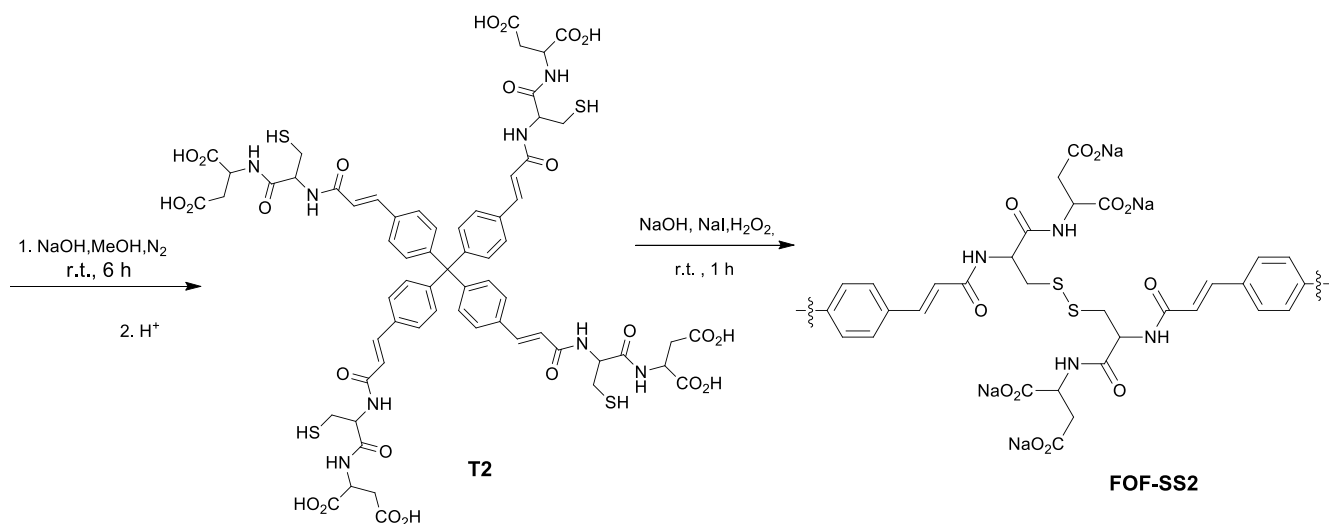
**Fig. S2.**  $^{13}\text{C}$  NMR spectrum (100 MHz,  $\text{DMSO-}d_6$ , 298 K) of compound **T1**.



**Fig. S3.** HR-MS (ESI) spectra of compound **T1**.







**Scheme S2.** Synthesis of **T2** and **FOF-SS2**.

**TIPM**, **TMTBA-OMe** and **TMTBA** were prepared according to the literature procedures.<sup>[1]</sup>

**T2-(Trt)<sub>4</sub>-(OMe)<sub>8</sub>** was prepared as a light yellow solid in 35% yield using the procedures described for **T1-(Trt)<sub>4</sub>-(OMe)<sub>8</sub>**. M.P. 233 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.55 (d, *J* = 7.9 Hz, 4H), 8.44 (d, *J* = 8.1 Hz, 4H), 7.54 (d, *J* = 8.3 Hz, 8H), 7.41 (d, *J* = 15.6 Hz, 4H), 7.38-7.01 (m, 76H), 6.76 (d, *J* = 15.8 Hz, 4H), 4.64-4.52 (m, 8H), 3.55 (s, 12H), 3.54 (s, 12H), 2.85-2.64 (m, 8H), 2.37 (m, 8H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): δ 171.14, 170.80, 170.15, 165.10, 147.55, 144.72, 139.17, 133.30, 131.33, 129.55, 128.53, 127.81, 127.24, 122.43, 66.31, 64.91, 52.63, 52.12, 51.75, 49.00, 35.79, 34.63. HR-MS (ESI): calcd for C<sub>149</sub>H<sub>140</sub>N<sub>8</sub>O<sub>24</sub>S<sub>4</sub>, [M+2Na]<sup>2+</sup> 1299.4324, found 1299.4348.

**T2-(OMe)<sub>8</sub>** was prepared as a yellow solid yield using the procedures described for **T1-(OMe)<sub>8</sub>**. M.P. 144 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.63 (d, *J* = 7.8 Hz, 4H), 8.38 (d, *J* = 8.0 Hz, 4H), 7.55 (d, *J* = 8.3 Hz, 8H), 7.43 (d, *J* = 15.7 Hz, 4H), 7.26 (d, *J* = 8.3 Hz, 8H), 6.78 (d, *J* = 15.8 Hz, 4H), 4.66 (q, *J* = 7.0 Hz, 4H), 4.57 (q, *J* = 7.4 Hz, 4H), 3.63 (s, 12H), 3.61 (s, 12H), 2.90-2.67 (m, 16H), 2.26 (t, *J* = 8.4 Hz, 4H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): δ 171.34, 170.79, 170.25, 165.31, 147.50, 139.03, 133.22, 131.26, 127.77, 122.42, 64.87, 55.15, 52.70, 52.16, 49.03, 35.79, 26.84. HR-MS (ESI): calcd for C<sub>73</sub>H<sub>84</sub>N<sub>8</sub>O<sub>24</sub>S<sub>4</sub>, [M+Na]<sup>+</sup> 1607.4373, found 1607.4373.

**Compound T2** was prepared as a yellow solid yield using the procedures described for **T1** (yield 74% for 2 steps). M.P. >300°C (decomposed). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.68 (s, 8H), 8.46 (d, *J* = 7.8 Hz, 4H), 8.37 (d, *J* = 7.9 Hz, 4H), 7.54 (d, *J* = 8.3 Hz, 8H), 7.42 (d, *J* = 15.6 Hz, 4H), 7.25 (d, *J* = 8.2 Hz, 8H), 6.78 (d, *J* = 15.8 Hz, 4H), 4.61-4.52 (m, 8H), 2.88-2.57 (m, 16H), 2.27 (t, *J* = 8.4 Hz, 4H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) δ 172.64, 172.04, 170.15, 165.39, 147.53, 139.01, 133.32, 131.31, 127.81, 122.60, 64.91, 55.30, 49.19, 36.28, 27.07. HR-MS (ESI): calcd for C<sub>65</sub>H<sub>68</sub>N<sub>8</sub>O<sub>24</sub>S<sub>4</sub>, [M+2Na]<sup>2+</sup> 737.1687, found 737.1680.

**FOF-SS2** was prepared as a yellow solid yield using the procedures described for **FOF-SS1** (yield 89%).

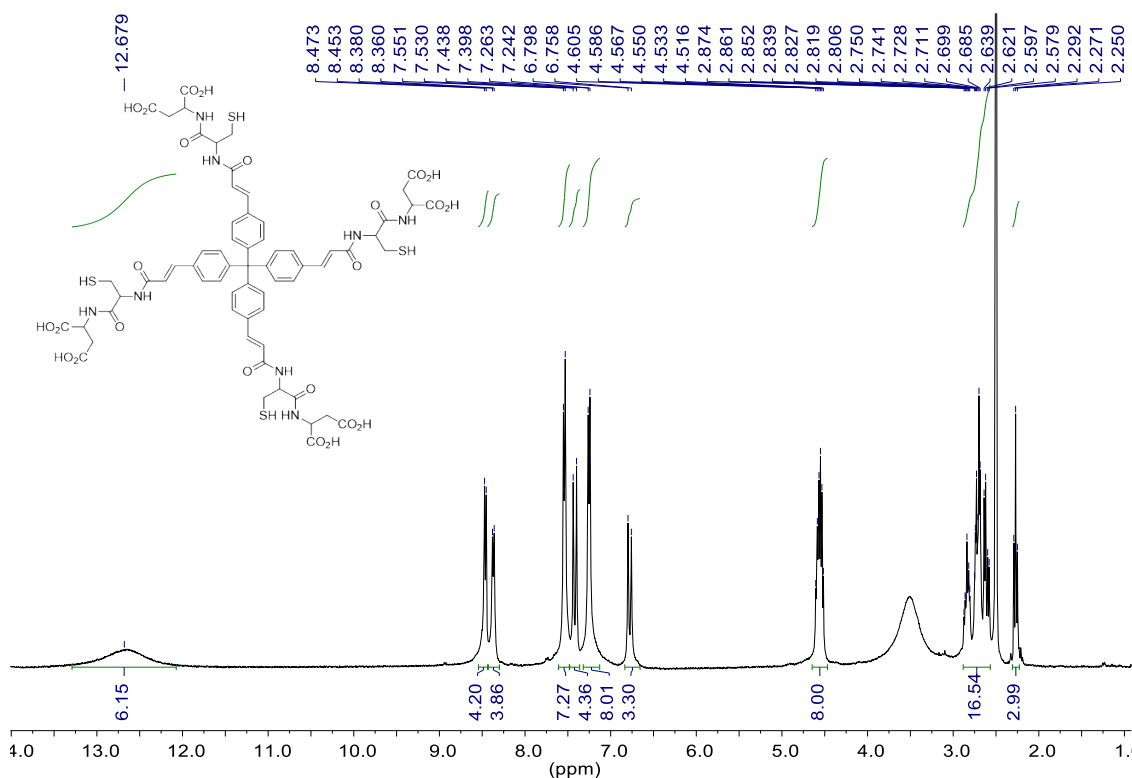


Fig. S4. <sup>1</sup>H NMR spectrum (400 MHz, DMSO-*d*<sub>6</sub>, 298 K) of compound T2.

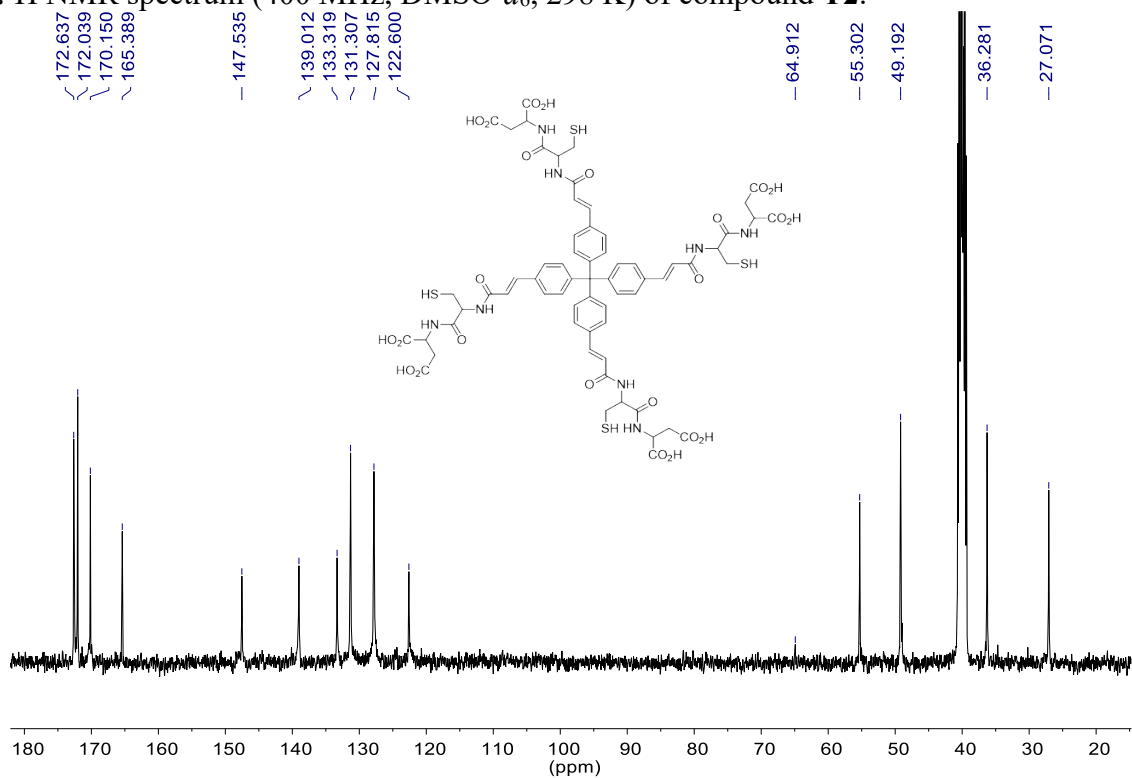
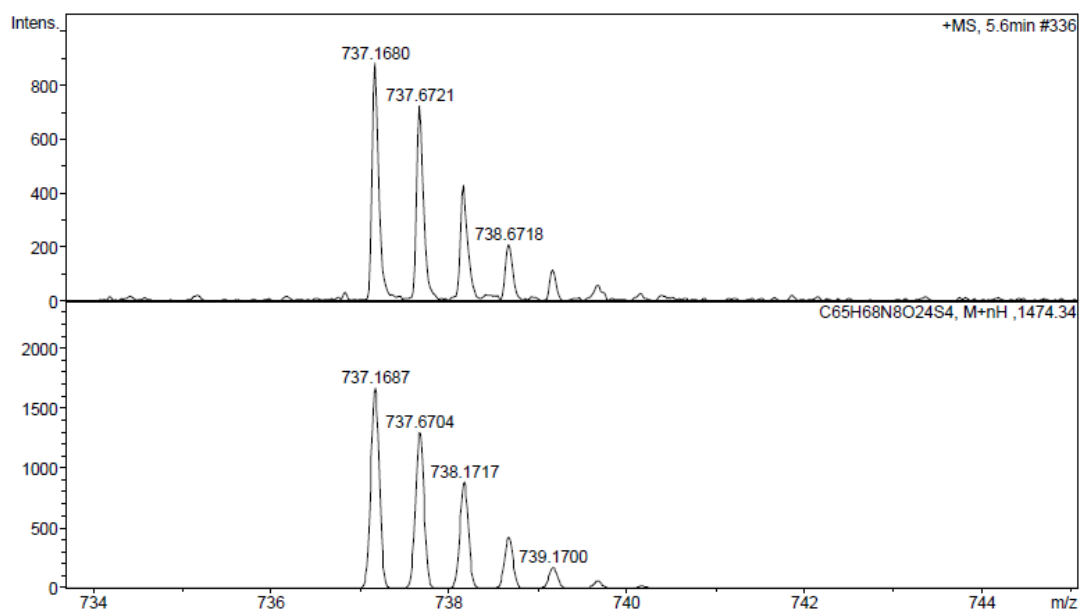
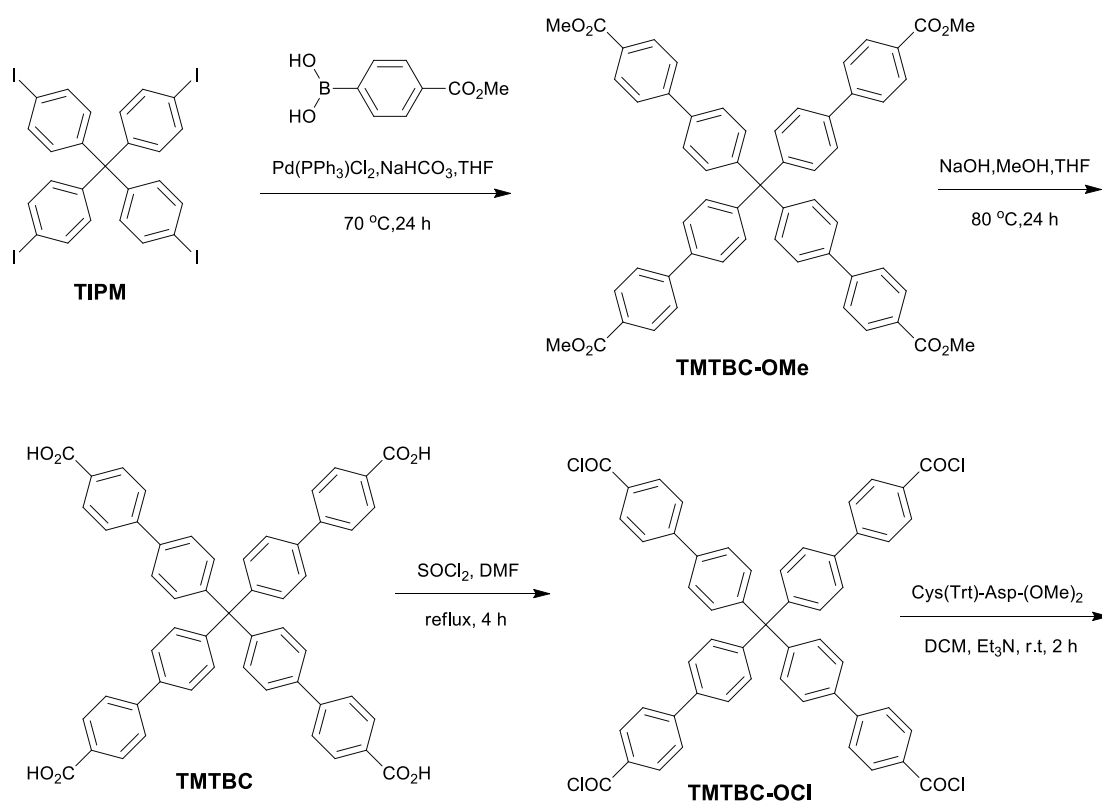
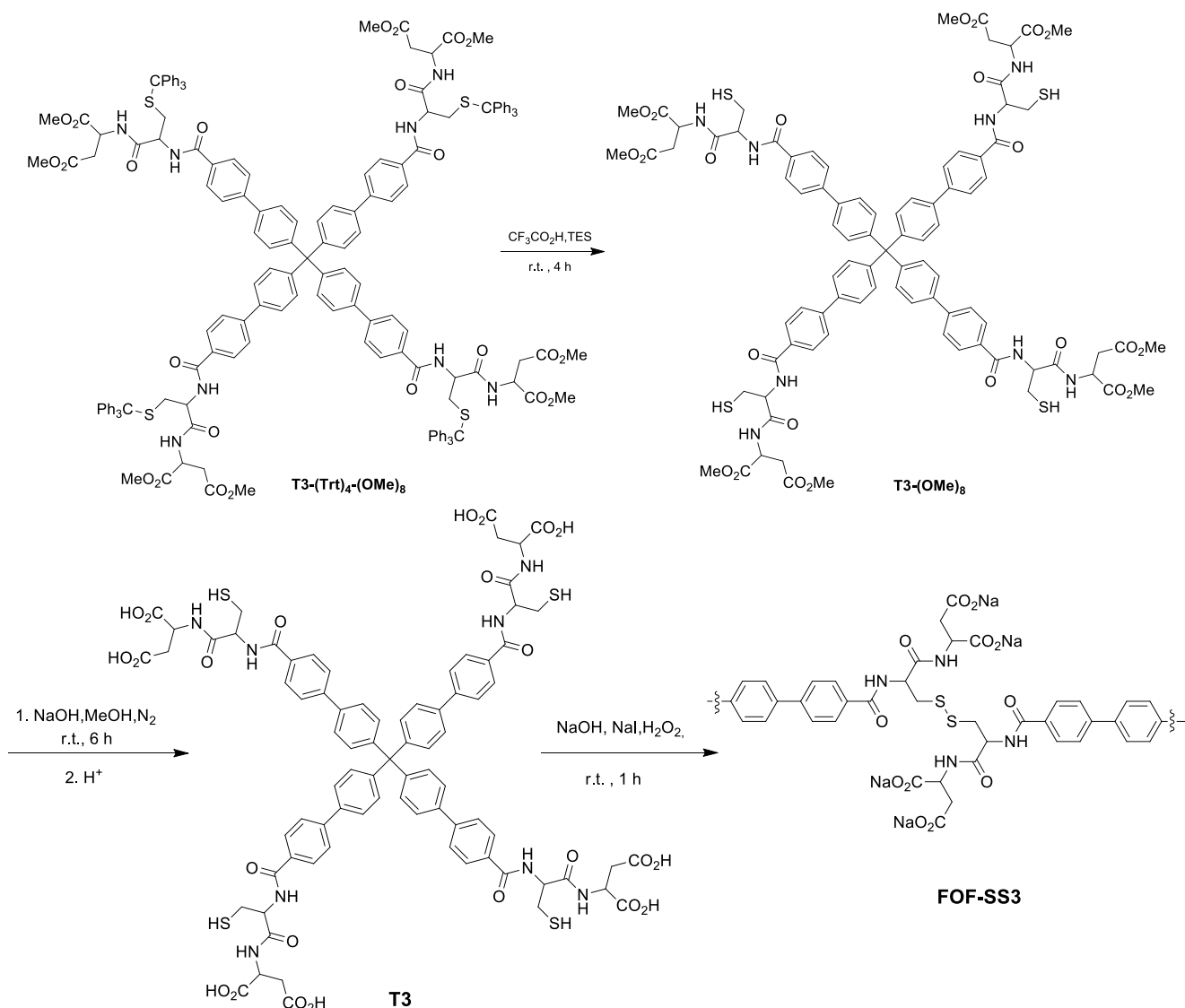


Fig. S5. <sup>13</sup>C NMR spectrum (100 MHz, DMSO-*d*<sub>6</sub>, 298 K) of compound T2.



**Fig. S6.** HR-MS (ESI) spectra of compound **T2**.





**Scheme S3.** Synthesis of **T3** and **FOF-SS3**.

**TMTBC-OMe** and **TMTBC** were prepared according to the literature procedures.<sup>[2]</sup>

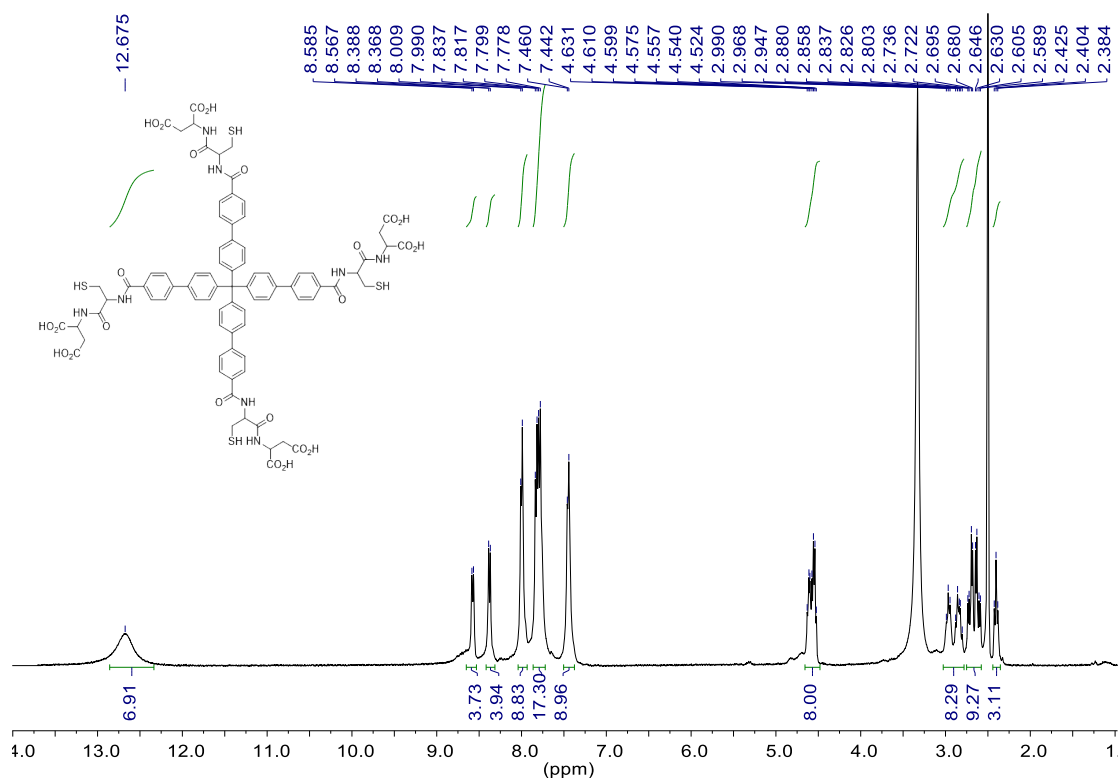
**T3-(Trt)<sub>4</sub>-(OMe)<sub>8</sub>** was prepared as a light yellow solid in 52% yield using the procedures described for **T1-(Trt)<sub>4</sub>-(OMe)<sub>8</sub>**. M.P. 198 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.74 (d, *J* = 8.1 Hz, 4H), 8.35 (d, *J* = 8.0 Hz, 4H), 7.99 (d, *J* = 8.3 Hz, 8H), 7.84 (d, *J* = 8.3 Hz, 8H), 7.79 (d, *J* = 8.4 Hz, 8H), 7.47 (d, *J* = 8.2 Hz, 8H), 7.38-7.20 (m, 60H), 4.64 (q, *J* = 6.6 Hz, 4H), 4.53 (q, *J* = 8.5 Hz, 4H), 3.55 (s, 12H), 3.54 (s, 12H), 2.85-2.65 (m, 8H), 2.64-2.53 (m, 8H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): δ 171.18, 170.83, 170.25, 166.26, 146.51, 144.75, 142.73, 137.20, 132.99, 131.48, 129.53, 128.74, 128.49, 127.20, 126.97, 126.80, 66.41, 64.37, 52.80, 52.61, 52.05, 48.93, 35.80, 33.91. HR-MS (ESI): calcd for C<sub>165</sub>H<sub>148</sub>N<sub>8</sub>O<sub>24</sub>S<sub>4</sub>,, [M+2Na]<sup>2+</sup> 1399.4637, found 1399.4681.

**T3-(OMe)<sub>8</sub>** was prepared as a yellow solid yield using the procedures described for **T1-(OMe)<sub>8</sub>**. M.P. 159 °C. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 8.62-8.52 (m, 8H), 8.01 (d, *J* = 8.4 Hz, 8H), 7.84 (d, *J* = 8.4 Hz, 8H), 7.80 (d, *J* = 8.5 Hz, 8H), 7.46 (d, *J* = 8.4 Hz, 8H), 4.69 (q, *J* = 6.7 Hz, 4H), 4.59 (td, *J* = 8.6, 4.9 Hz, 4H), 3.63 (s, 12H), 3.60 (s, 12H), 3.02-2.70 (m, 16H), 2.41 (t, *J* = 8.5 Hz, 4H). <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>): δ 171.43, 170.89, 170.63, 166.70, 146.54, 142.73, 137.27, 133.23, 131.53, 128.75, 127.00,

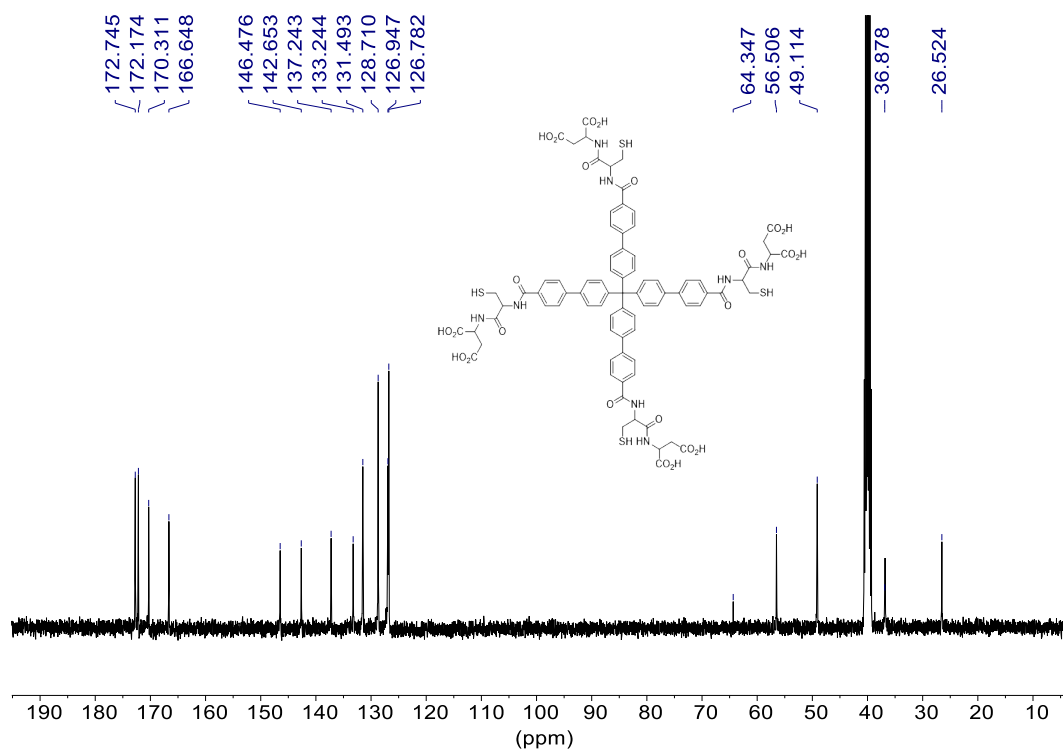
126.83, 64.40, 56.42, 52.74, 52.19, 49.08, 35.95, 26.36. HR-MS (ESI): calcd for  $C_{89}H_{92}N_8O_{24}S_4$ ,  $[M+H]^+$  1785.5180, found 1785.5178.

**Compound T3** was prepared as a light yellow solid yield using the procedures described for **T1** (yield 84% for 2 steps). M.P. >300 °C (decomposed).  $^1H$  NMR (400 MHz,  $DMSO-d_6$ ):  $\delta$  12.67 (s, 8H), 8.58 (d,  $J = 7.4$  Hz, 4H), 8.38 (d,  $J = 7.8$  Hz, 4H), 8.00 (d,  $J = 7.5$  Hz, 8H), 7.84-7.78 (m, 16H), 7.45 (d,  $J = 7.2$  Hz, 8H), 4.63-4.52 (m, 8H), 3.03-2.78 (m, 8H), 2.66 (qd,  $J = 16.6, 6.1$  Hz, 8H), 2.40 (t,  $J = 8.3$  Hz, 4H).  $^{13}C$  NMR (100 MHz,  $DMSO-d_6$ ):  $\delta$  172.74, 172.17, 170.31, 166.65, 146.48, 142.65, 137.24, 133.24, 131.49, 128.71, 126.95, 126.78, 64.35, 56.51, 49.11, 36.88, 26.52. HR-MS (ESI): calcd for  $C_{81}H_{76}N_8O_{24}S_4$ ,  $[M-2H]^{2-}$  835.1844, found 835.1836.

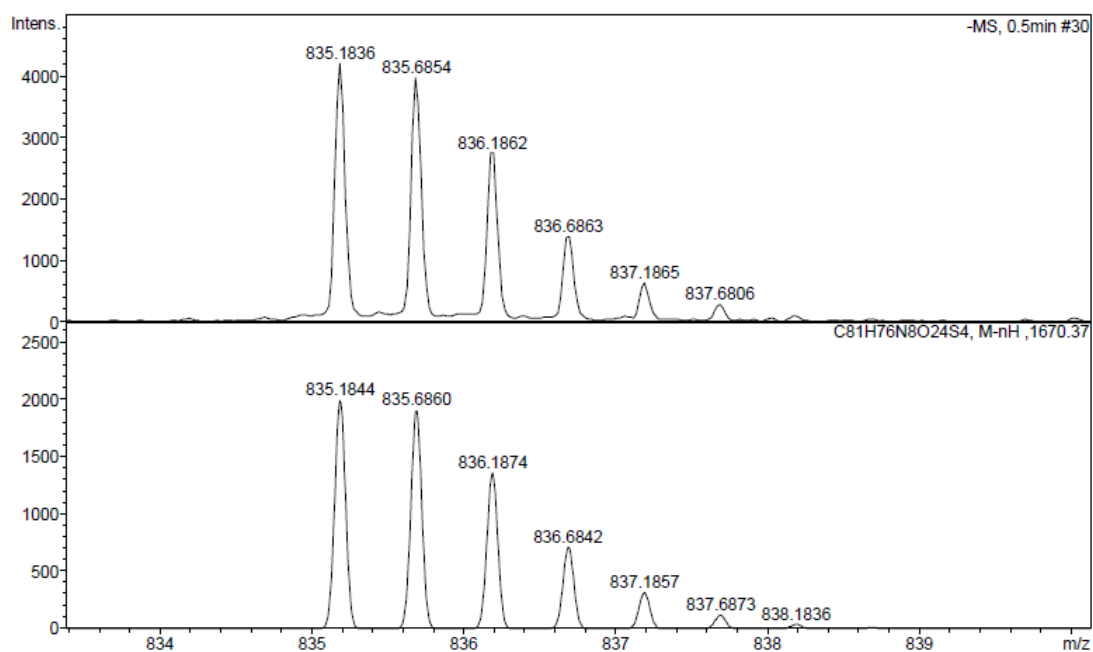
**FOF-SS3** was prepared as a yellow solid yield using the procedures described for **FOF-SS1** (yield 92%).



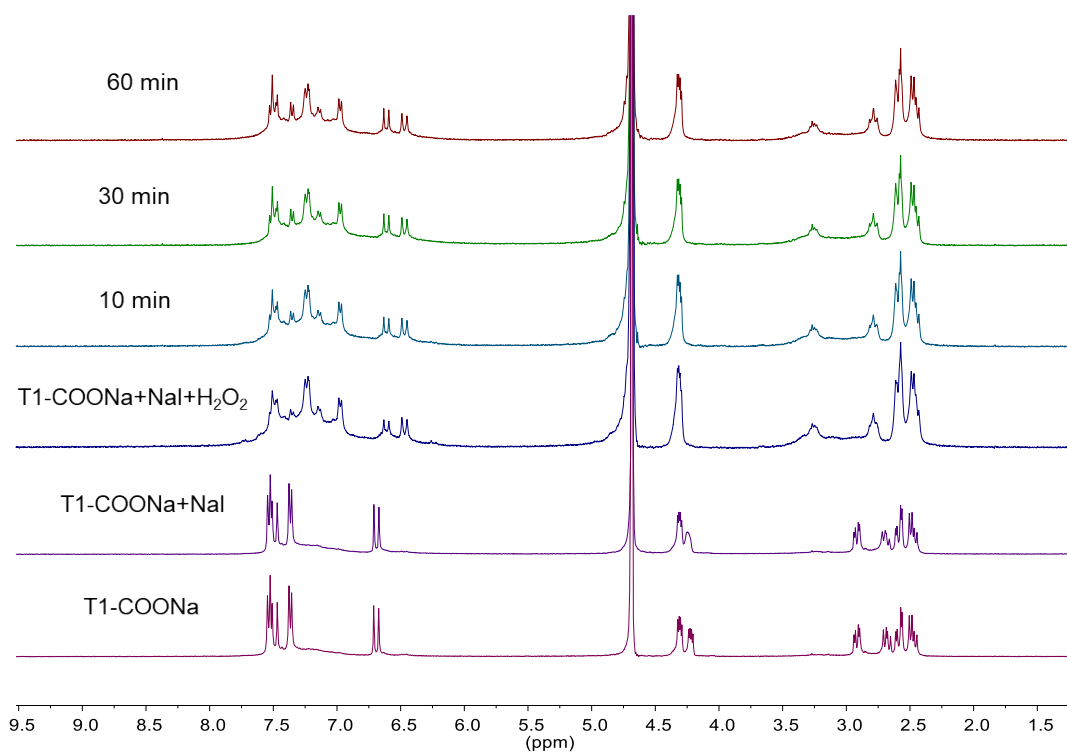
**Fig. S7.**  $^1H$  NMR spectrum (400 MHz,  $DMSO-d_6$ , 298 K) of compound **T3**.



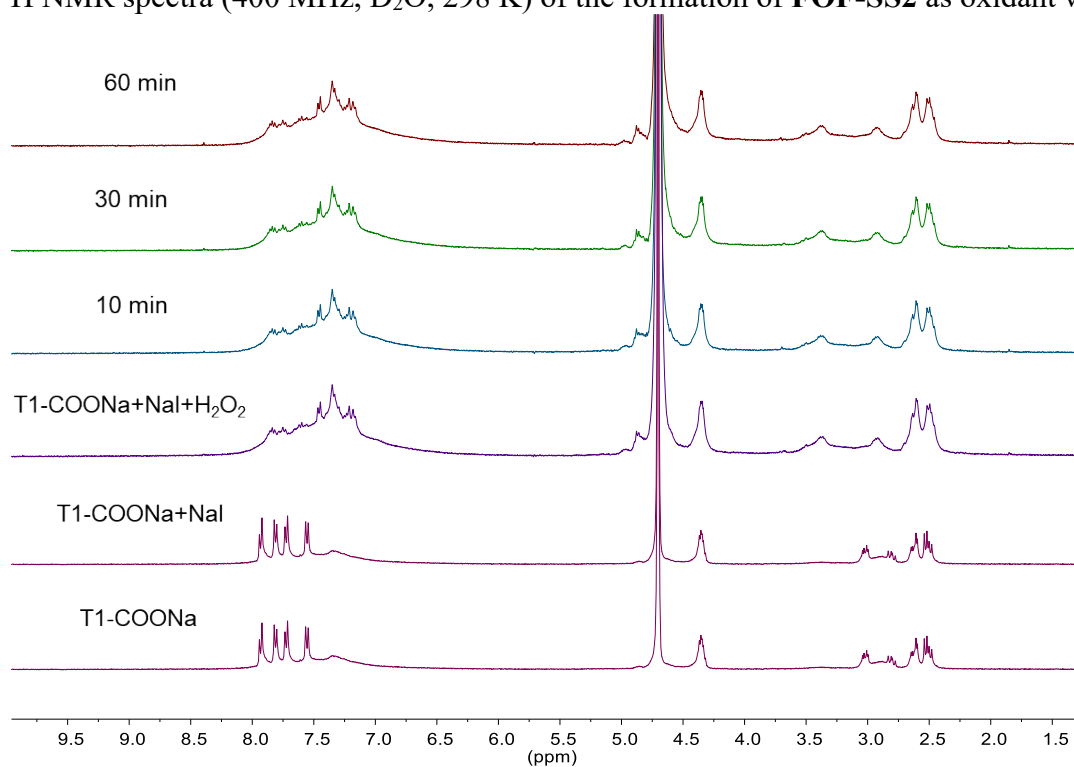
**Fig. S8.** <sup>13</sup>C NMR spectrum (100 MHz, DMSO-*d*<sub>6</sub>, 298 K) of compound T3.



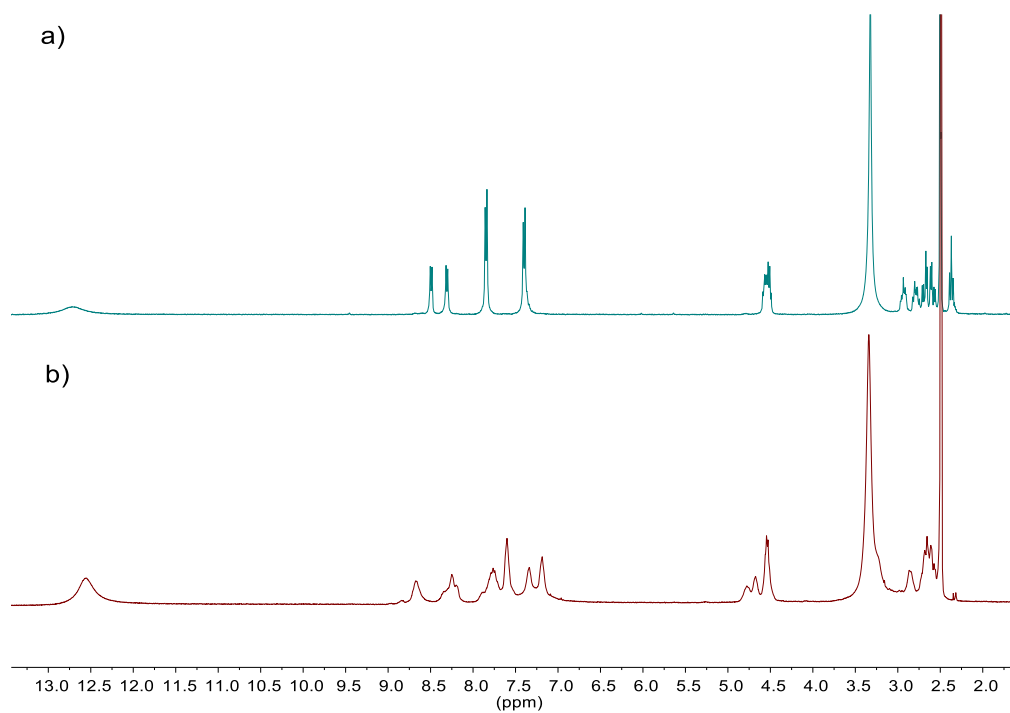
**Fig. S9.** HR-MS (ESI) spectra of compound T3.



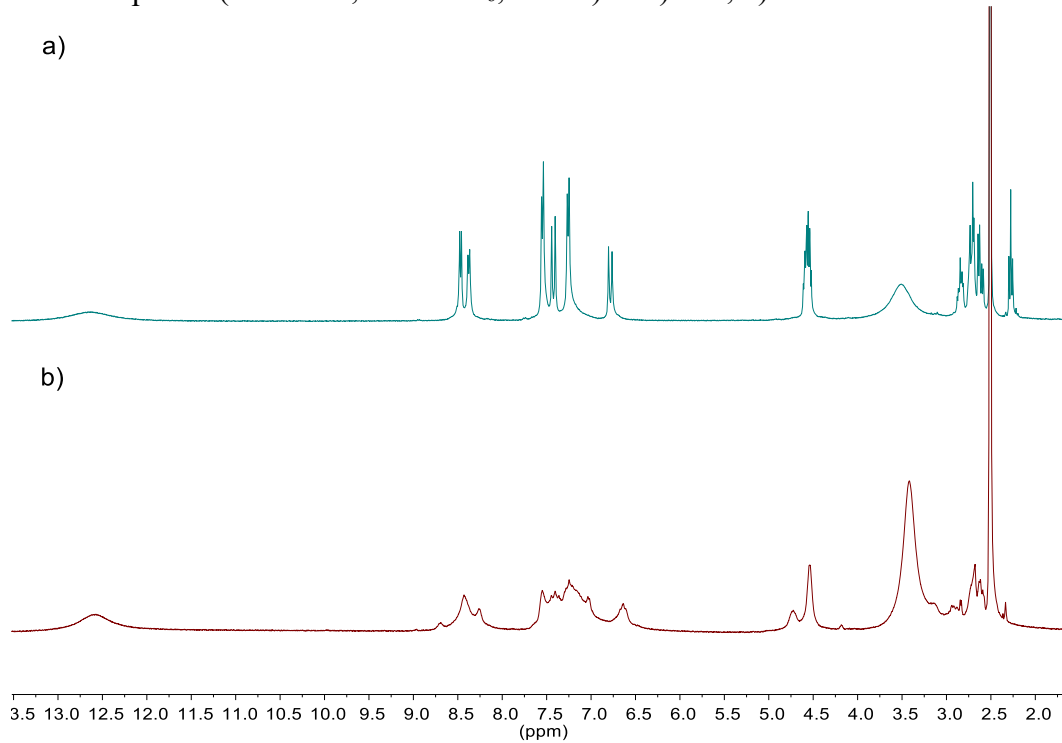
**Fig. S10.**  $^1\text{H}$  NMR spectra (400 MHz,  $\text{D}_2\text{O}$ , 298 K) of the formation of **FOF-SS2** as oxidant was added.



**Fig. S11.**  $^1\text{H}$  NMR spectra (400 MHz,  $\text{D}_2\text{O}$ , 298 K) of the formation of **FOF-SS3** as oxidant was added.

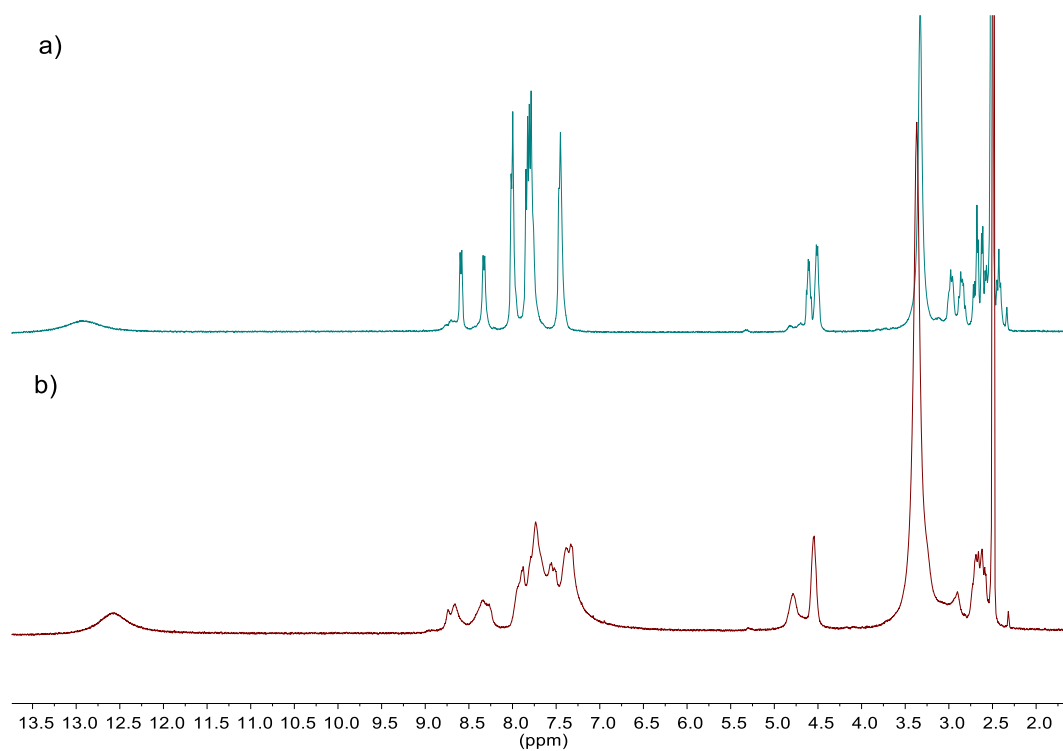


**Fig. S12.** <sup>1</sup>H NMR spectra (400 MHz, DMSO-*d*<sub>6</sub>, 298 K) of a) T-1, b) acidulated FOF-SS1.

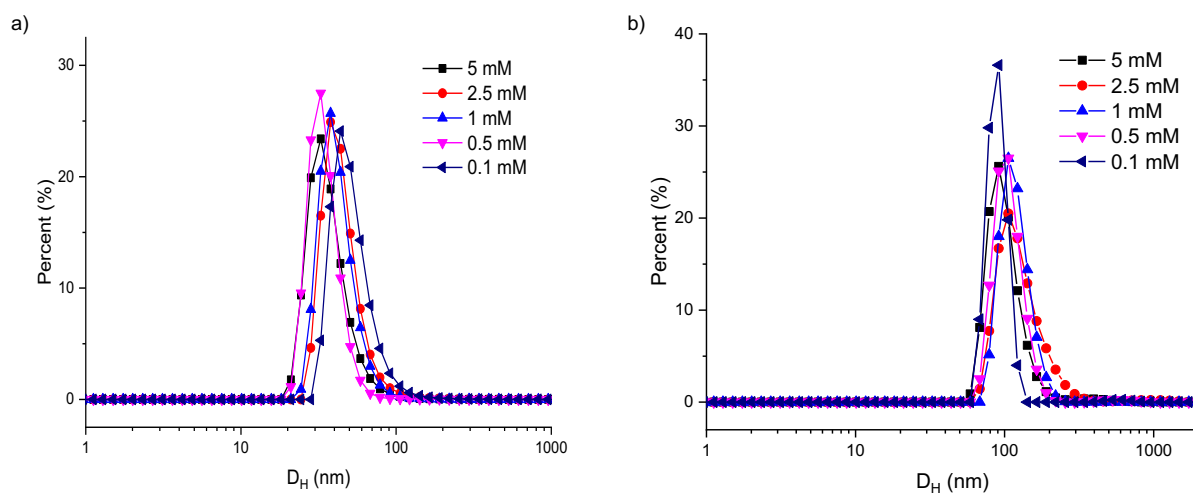


**Fig. S13.** <sup>1</sup>H NMR spectra (400 MHz, DMSO-*d*<sub>6</sub>, 298 K) of a) T-2, b) acidulated FOF-SS2.

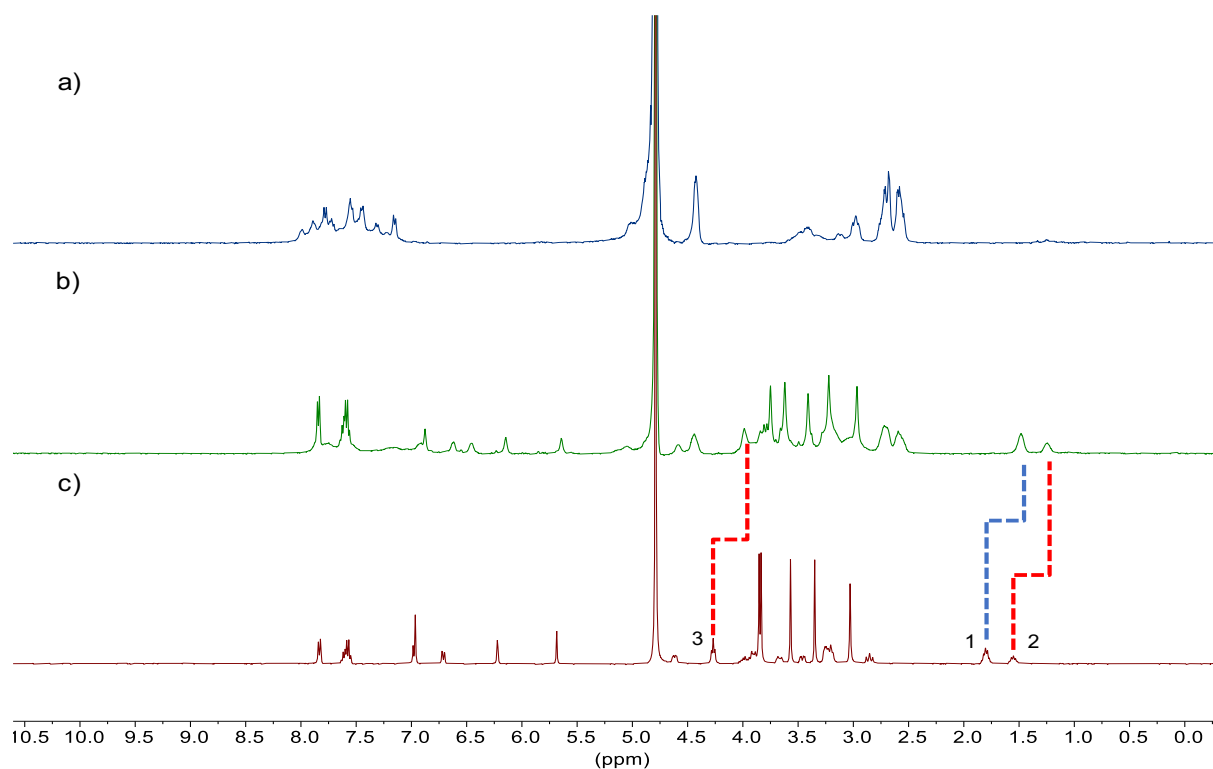




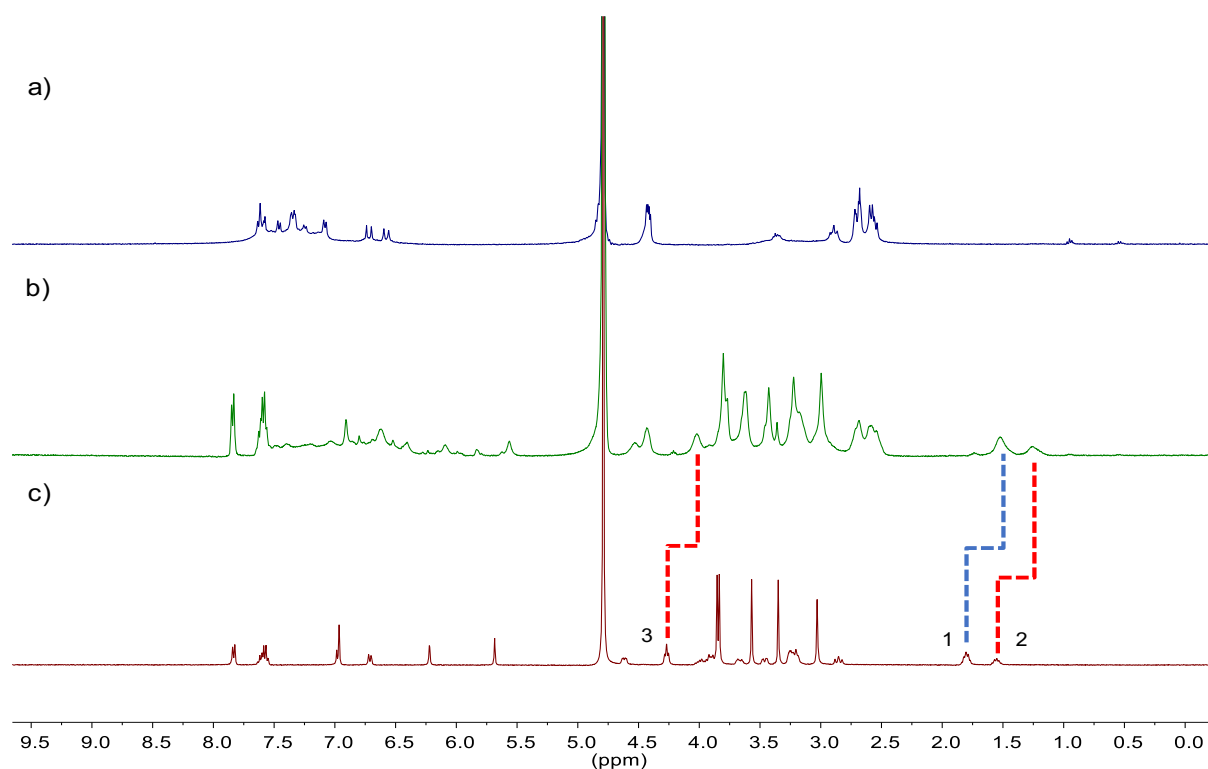
**Fig. S14.** <sup>1</sup>H NMR spectra (400 MHz, DMSO-*d*<sub>6</sub>, 298 K) of a) T-3, b) acidulated FOF-SS3.



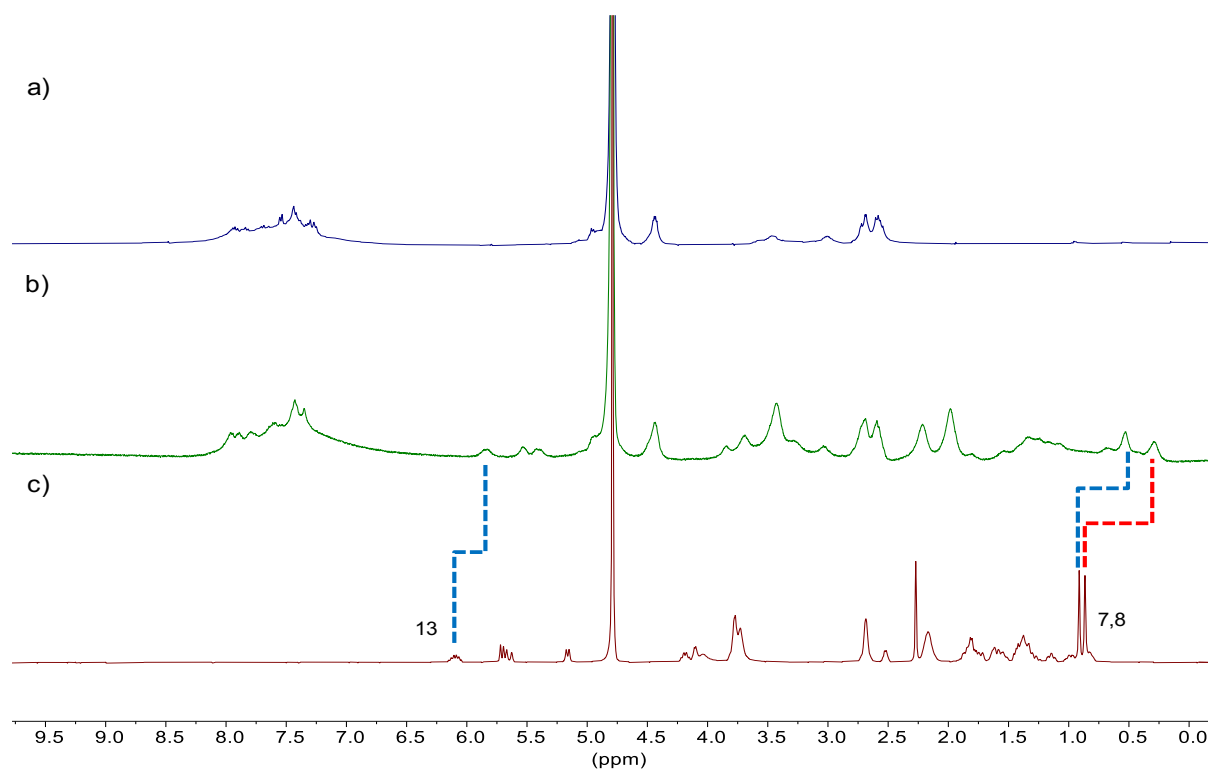
**Fig. S15.** DLS profile of a) FOF-SS2 and b) FOF-SS3 at different concentrations in water (calculated based on [T2]-[T3]).



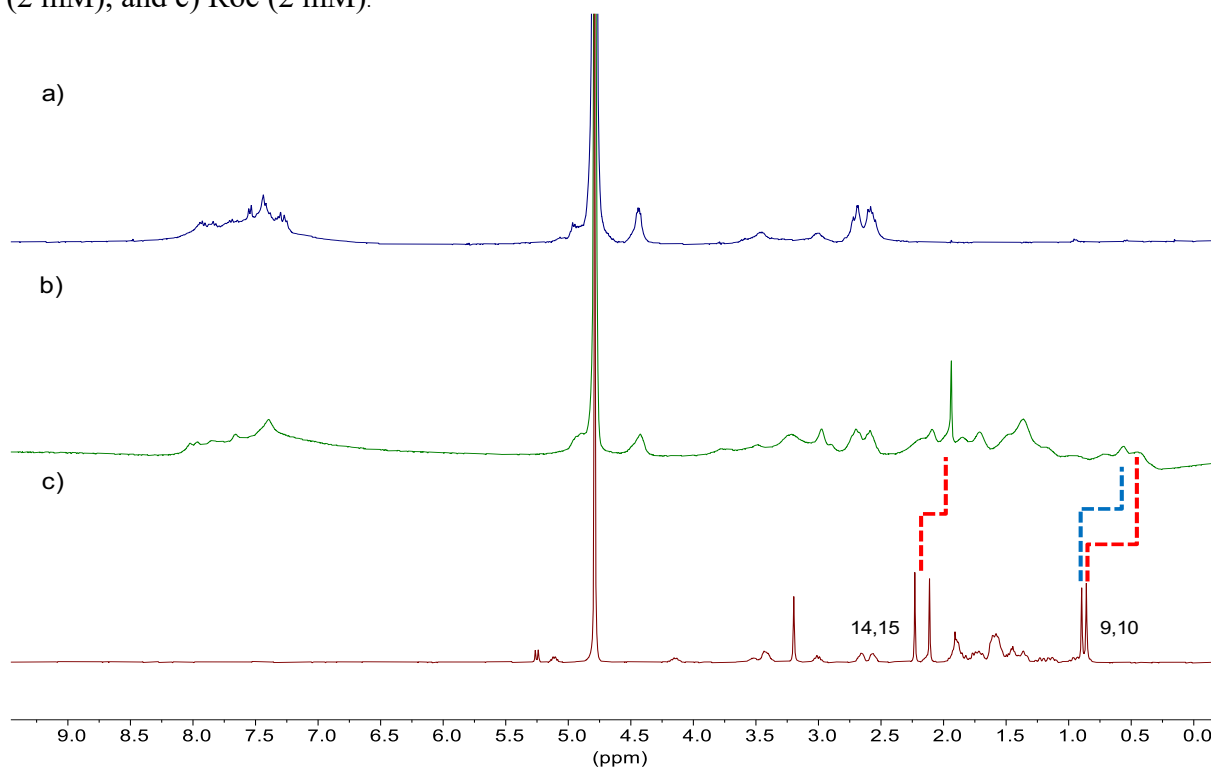
**Fig. S16.**  $^1\text{H}$  NMR spectra (400 MHz,  $\text{D}_2\text{O}$ , 298 K) of a) **FOF-SS1** (2 mM), b) **Cis** (2 mM) and **FOF-SS1** (2 mM), and c) **Cis** (2 mM).



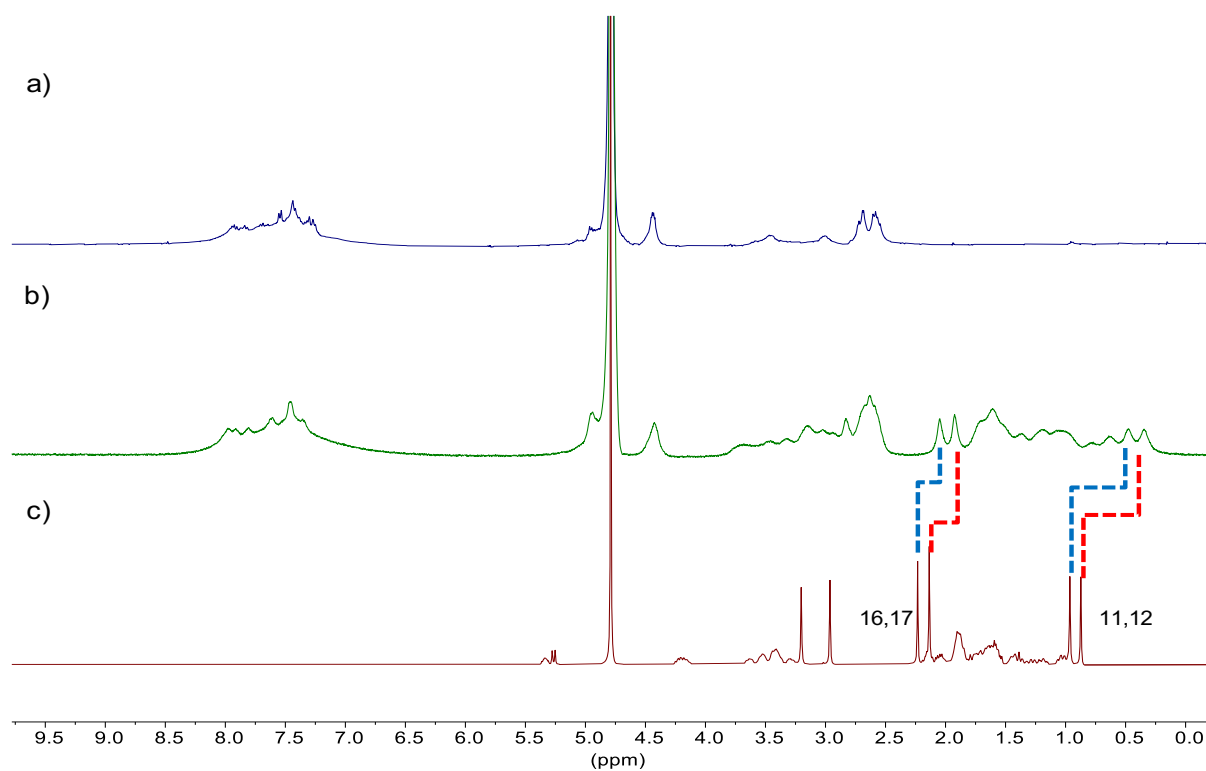
**Fig. S17.**  $^1\text{H}$  NMR spectra (400 MHz,  $\text{D}_2\text{O}$ , 298 K) of a) **FOF-SS2** (2 mM), b) **Cis** (2 mM) and **FOF-SS2** (2 mM), and c) **Cis** (2 mM).



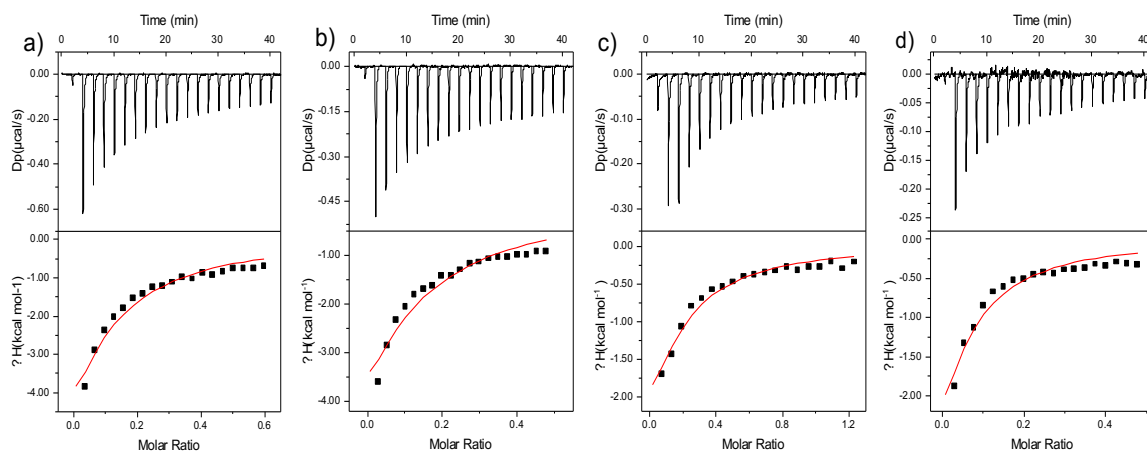
**Fig. S18.**  $^1\text{H}$  NMR spectra (400 MHz,  $\text{D}_2\text{O}$ , 298 K) of a) **FOF-SS3** (2 mM), b) **Roc** (2 mM) and **FOF-SS3** (2 mM), and c) **Roc** (2 mM).



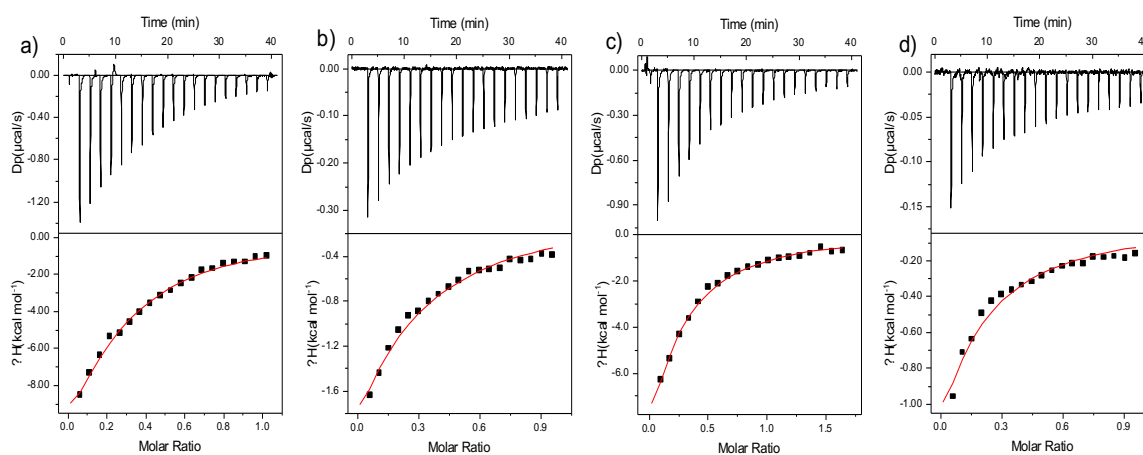
**Fig. S19.**  $^1\text{H}$  NMR spectra (400 MHz,  $\text{D}_2\text{O}$ , 298 K) of a) **FOF-SS3** (2 mM), b) **Vec** (2 mM) and **FOF-SS3** (2 mM), and c) **Vec** (2 mM).



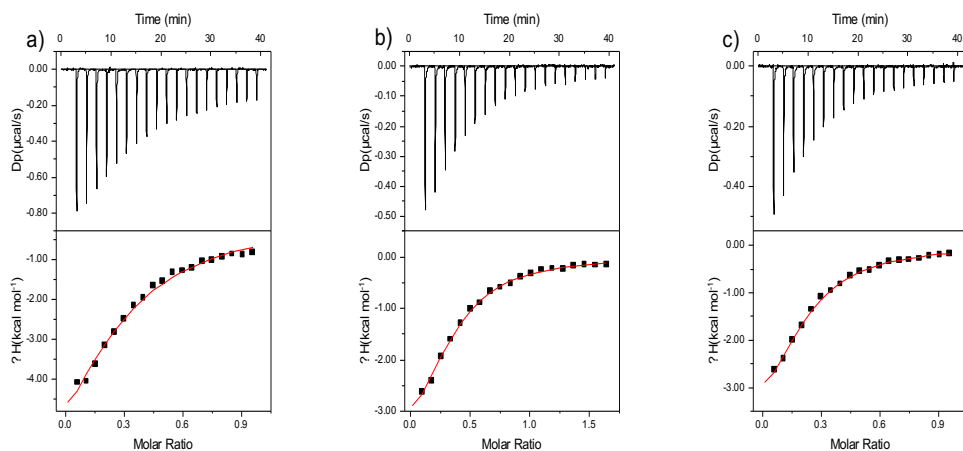
**Fig. S20.**  $^1\text{H}$  NMR spectra (400 MHz,  $\text{D}_2\text{O}$ , 298 K) of a) **FOF-SS3** (2 mM), b) **Panc** (2 mM) and **FOF-SS3** (2 mM), and c) **Panc** (2 mM).



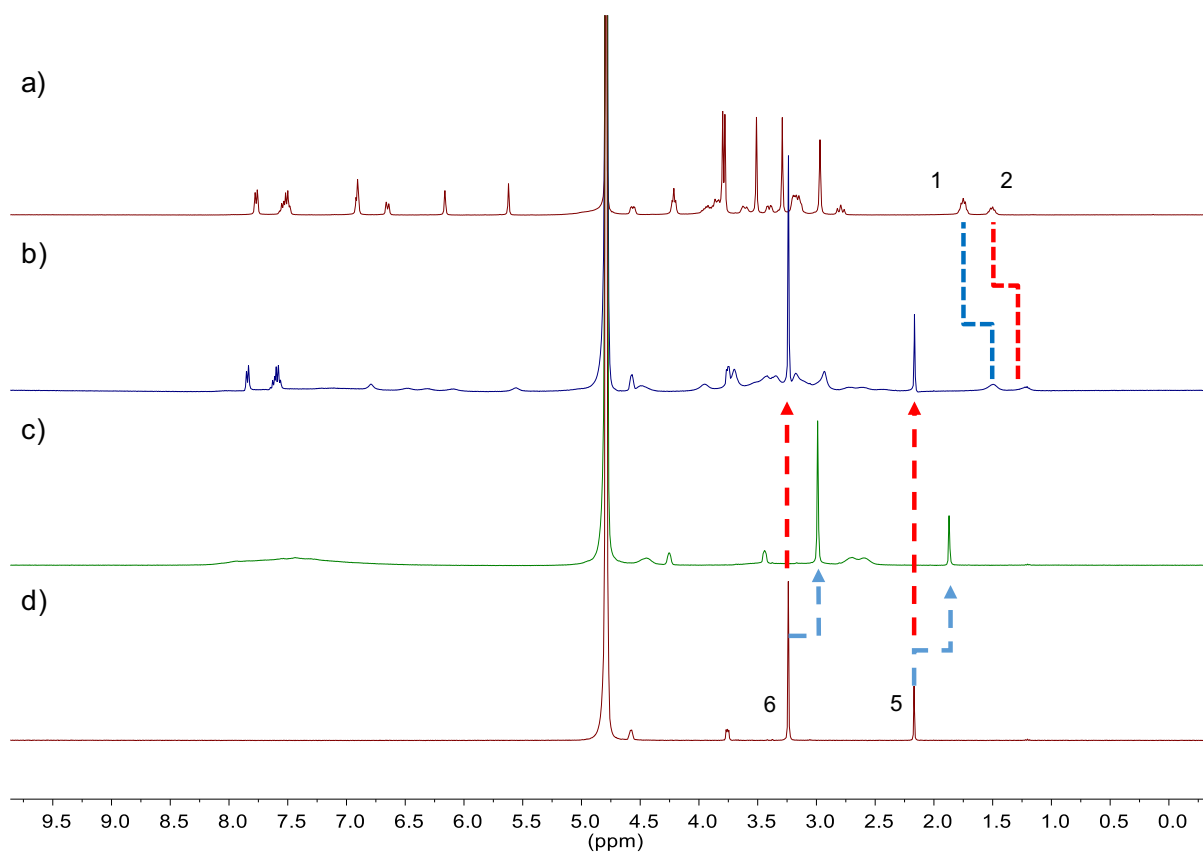
**Fig. S21.** Isothermal titration thermograms of **FOF-SS1** (300  $\mu\text{L}$ , 0.2 mM) titrated with the aqueous solution of a) Cis (40  $\mu\text{L}$ , 0.8 mM), b) Roc (40  $\mu\text{L}$ , 0.8 mM), c) Vec (40  $\mu\text{L}$ , 1.2 mM), d) Panc (40  $\mu\text{L}$ , 0.8 mM) at 298 K.



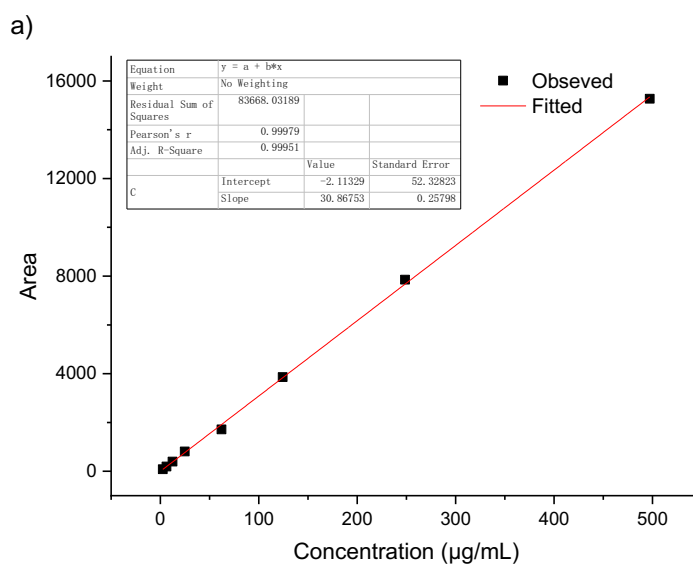
**Fig. S22.** Isothermal titration thermograms of **FOF-SS2** (300  $\mu\text{L}$ , 0.1 mM) titrated with the aqueous solution of a) Cis (40  $\mu\text{L}$ , 0.8 mM), b) Roc (40  $\mu\text{L}$ , 0.8 mM), c) Vec (40  $\mu\text{L}$ , 1.2 mM), d) Panc (40  $\mu\text{L}$ , 0.8 mM) at 298 K.



**Fig. S23.** Isothermal titration thermograms of **FOF-SS3** (300  $\mu\text{L}$ , 0.1 mM) titrated with the aqueous solution of a) Roc (40  $\mu\text{L}$ , 0.8 mM), b) Vec (40  $\mu\text{L}$ , 1.2 mM), c) Panc (40  $\mu\text{L}$ , 0.8 mM) at 298 K.



**Fig. S24.**  $^1\text{H}$  NMR spectra (400 MHz,  $\text{D}_2\text{O}$ , 298 K) of a) Cis (2 mM), b) FOF-SS3 (2 mM), Cis (2 mM), Ach (6 mM), c) Ach (2 mM), FOF-SS3 (2 mM) and d) Ach (2 mM).



**Fig. S25.** Concentration-absorption standard curve of Cis, the red line represents the linear fit of the data ( $R^2 = 0.9996$ ,  $A = 30.867c - 2.113$ ).

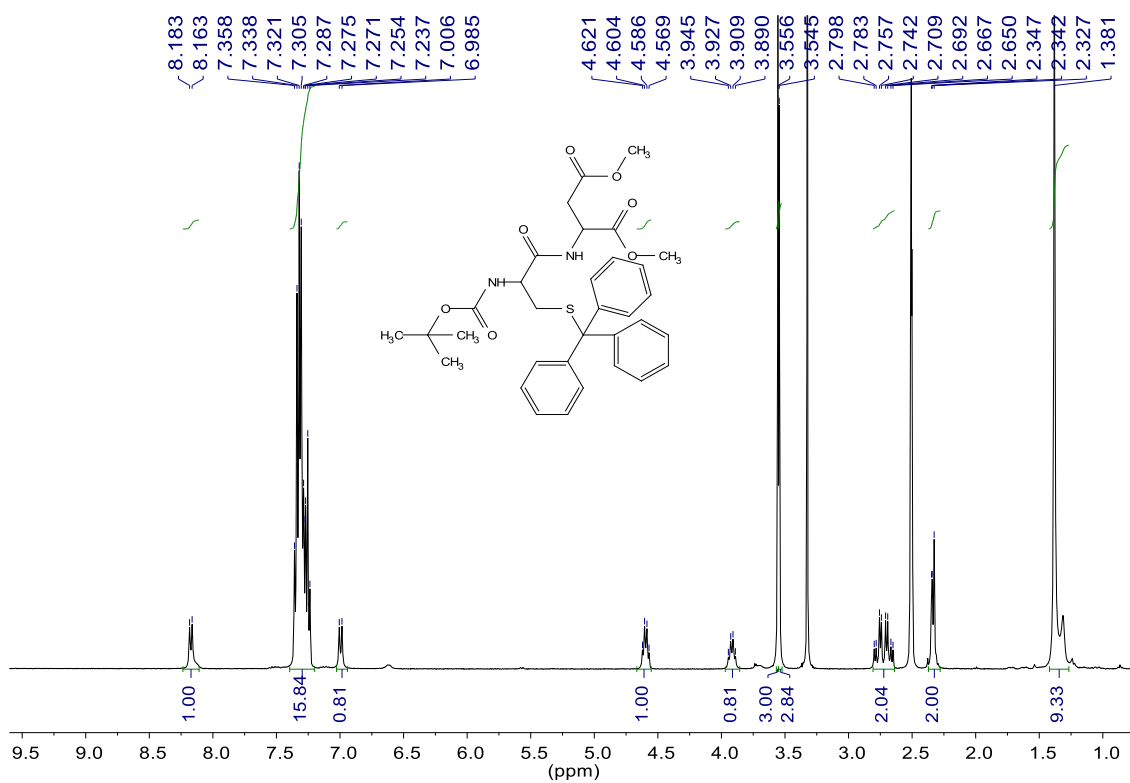


Fig. S26.  $^1\text{H}$  NMR spectrum (400 MHz,  $\text{DMSO-}d_6$ , 298 K) of Boc-Cys(Trt)-Asp(OMe) $_2$ .

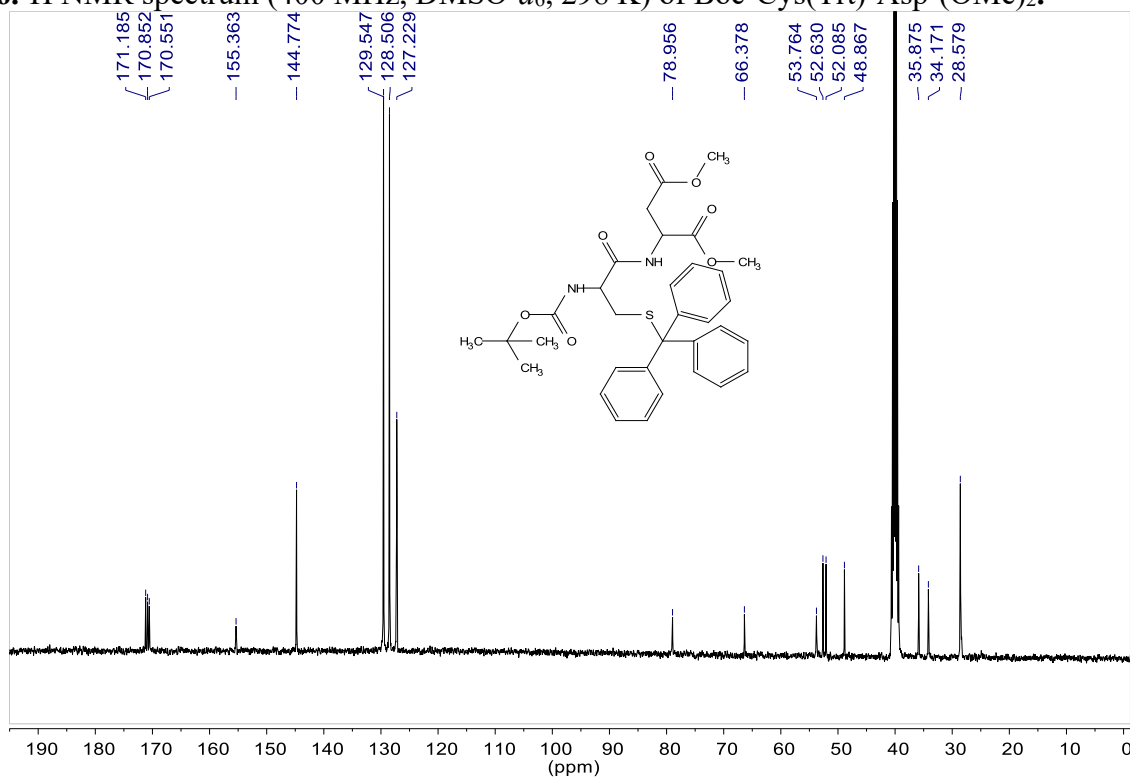


Fig. S27.  $^{13}\text{C}$  NMR spectrum (100 MHz,  $\text{DMSO-}d_6$ , 298 K) of Boc-Cys(Trt)-Asp(OMe) $_2$ .

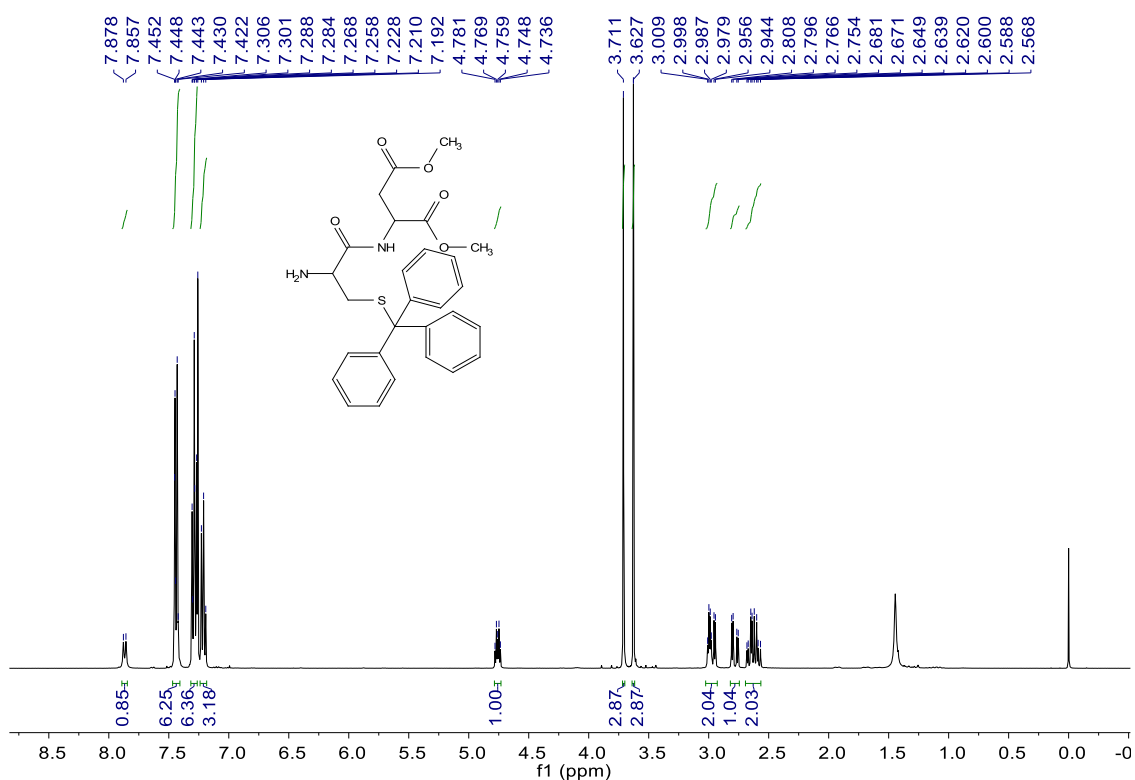


Fig. S28.  $^1\text{H}$  NMR spectrum (400 MHz,  $\text{CDCl}_3$ , 298 K) of Cys(Trt)-Asp-(OMe) $_2$ .

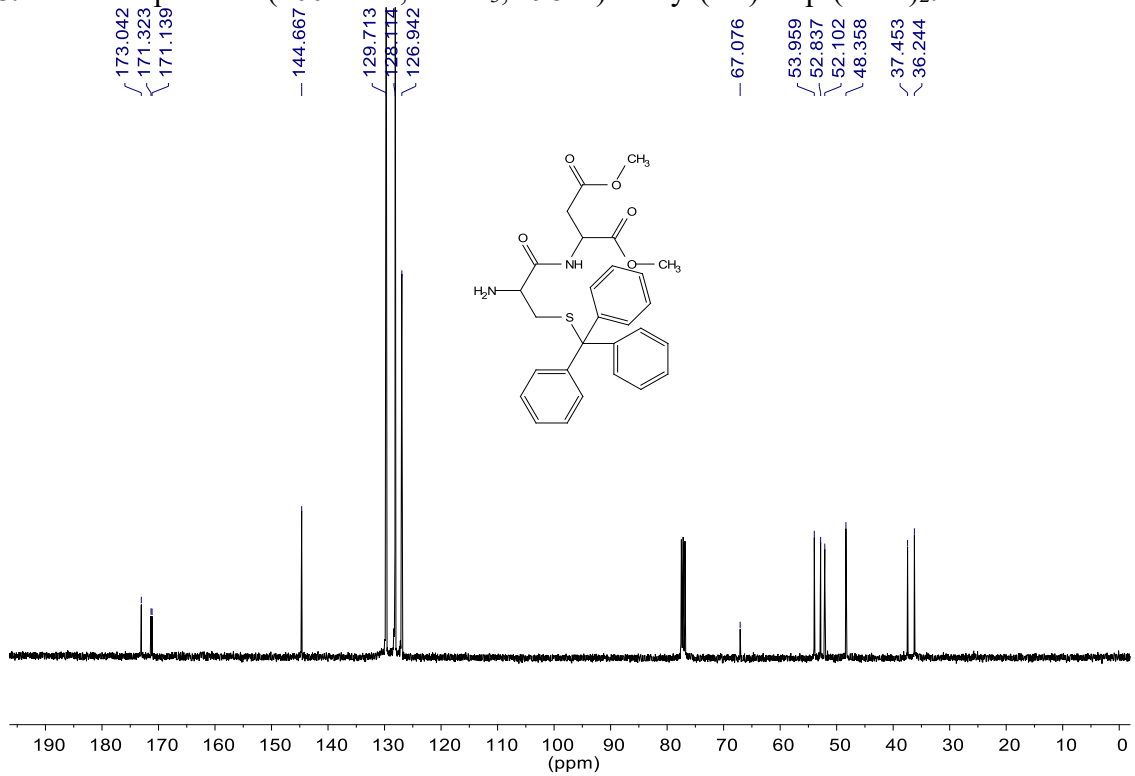


Fig. S29.  $^{13}\text{C}$  NMR spectrum (100 MHz,  $\text{CDCl}_3$ , 298 K) of Cys(Trt)-Asp-(OMe) $_2$ .



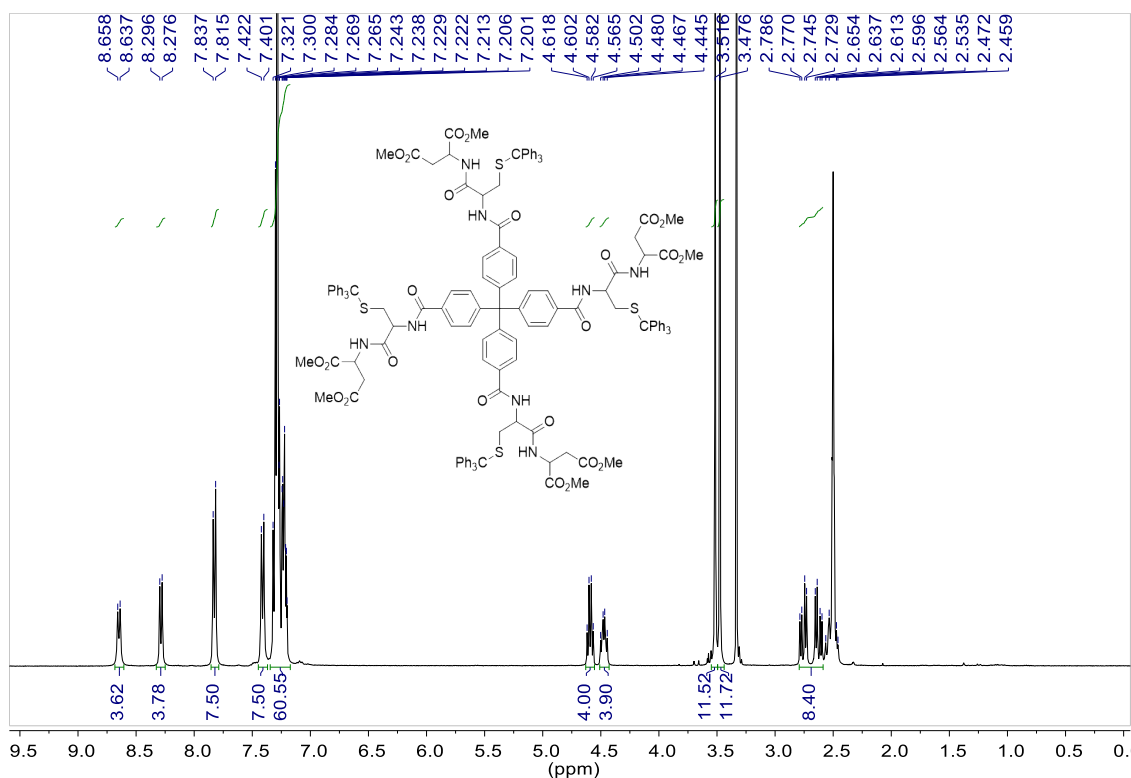


Fig. S30. <sup>1</sup>H NMR spectrum (400 MHz, DMSO-*d*<sub>6</sub>, 298 K) of T1-(Trt)<sub>4</sub>-(OMe)<sub>8</sub>.

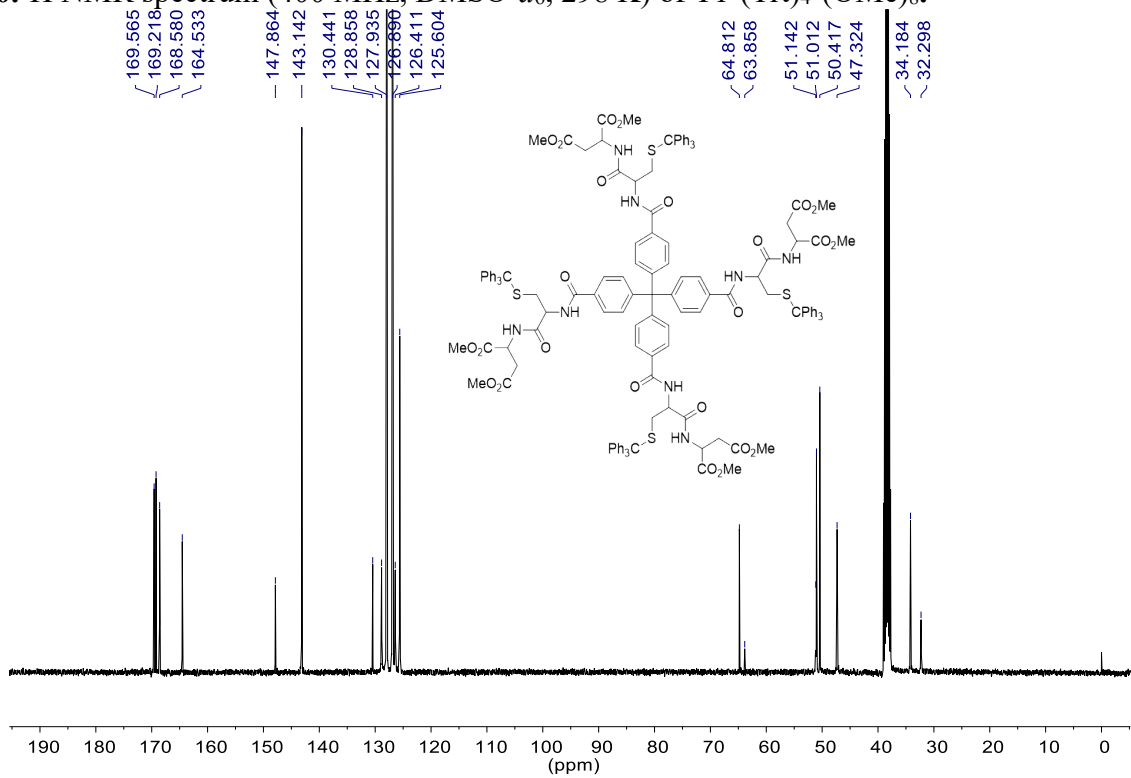


Fig. S31. <sup>13</sup>C NMR spectrum (100 MHz, DMSO-*d*<sub>6</sub>, 298 K) of T1-(Trt)<sub>4</sub>-(OMe)<sub>8</sub>.

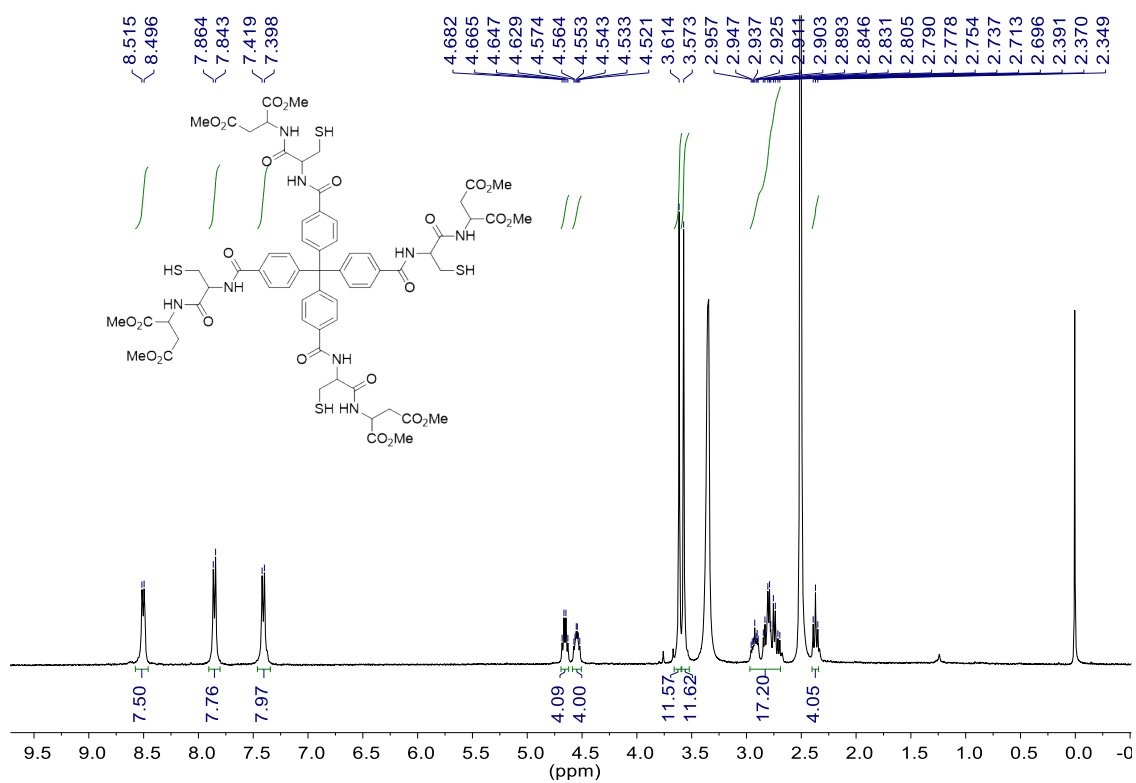


Fig. S32. <sup>1</sup>H NMR spectrum (400 MHz, DMSO-*d*<sub>6</sub>, 298 K) of T1-(OMe)<sub>8</sub>.

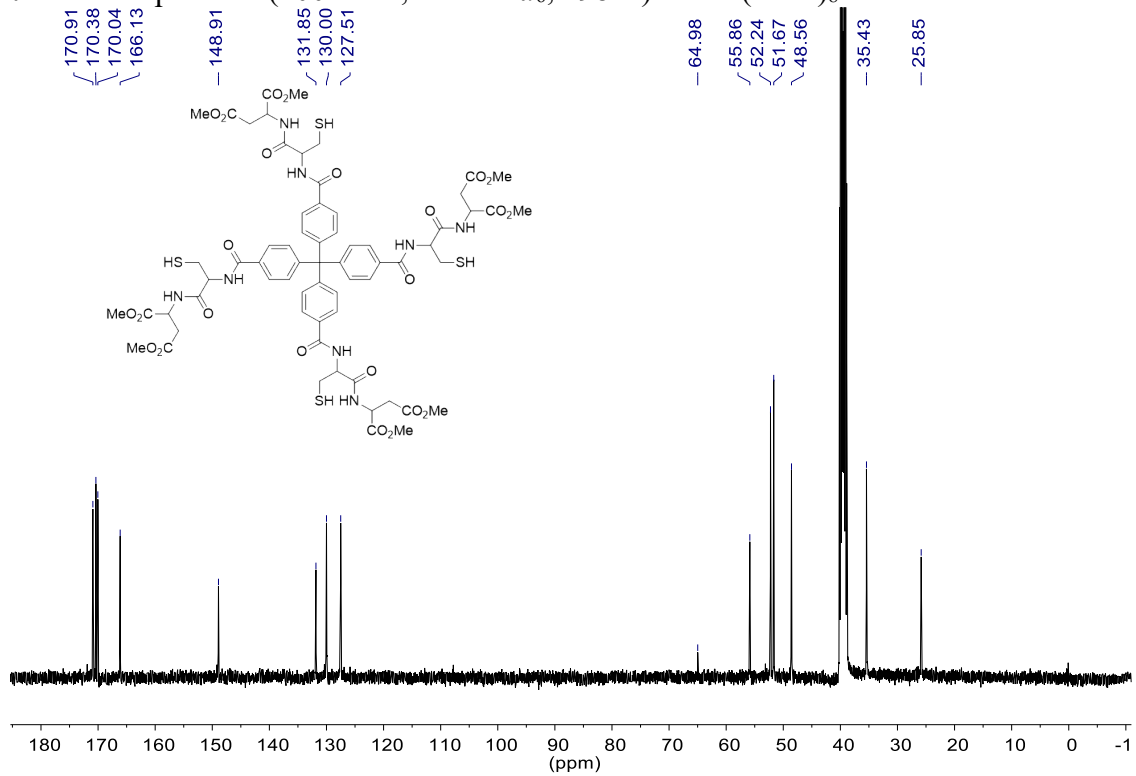
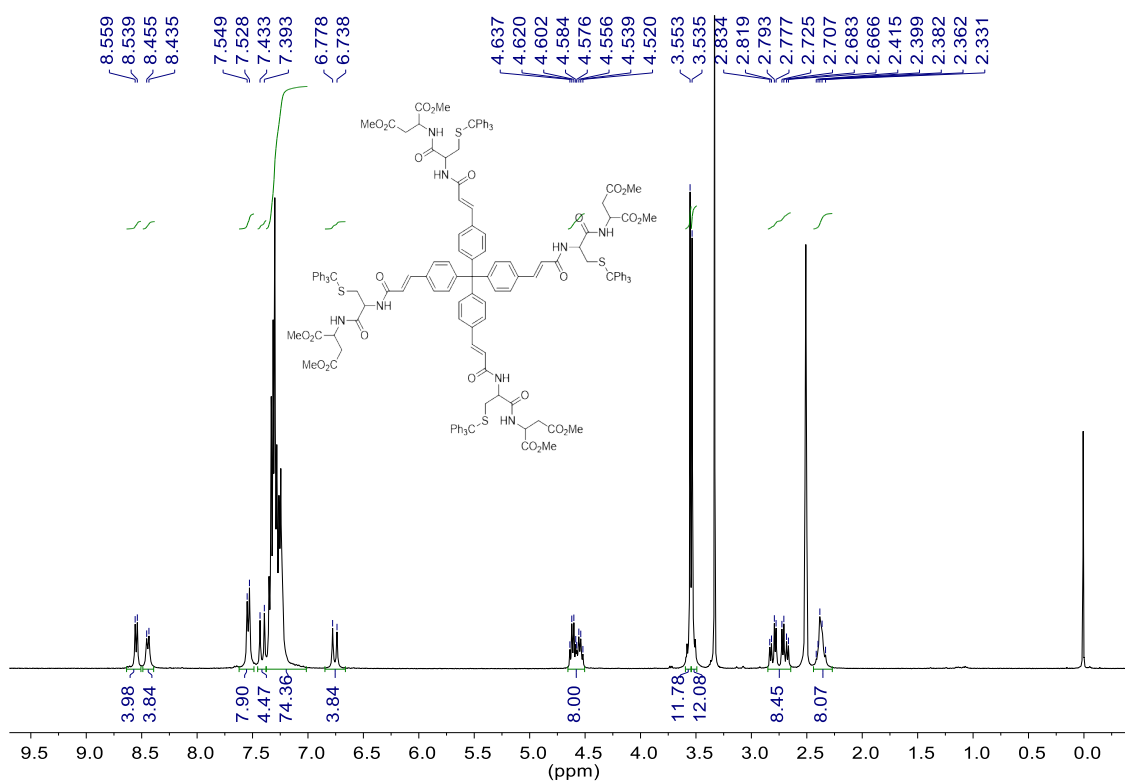
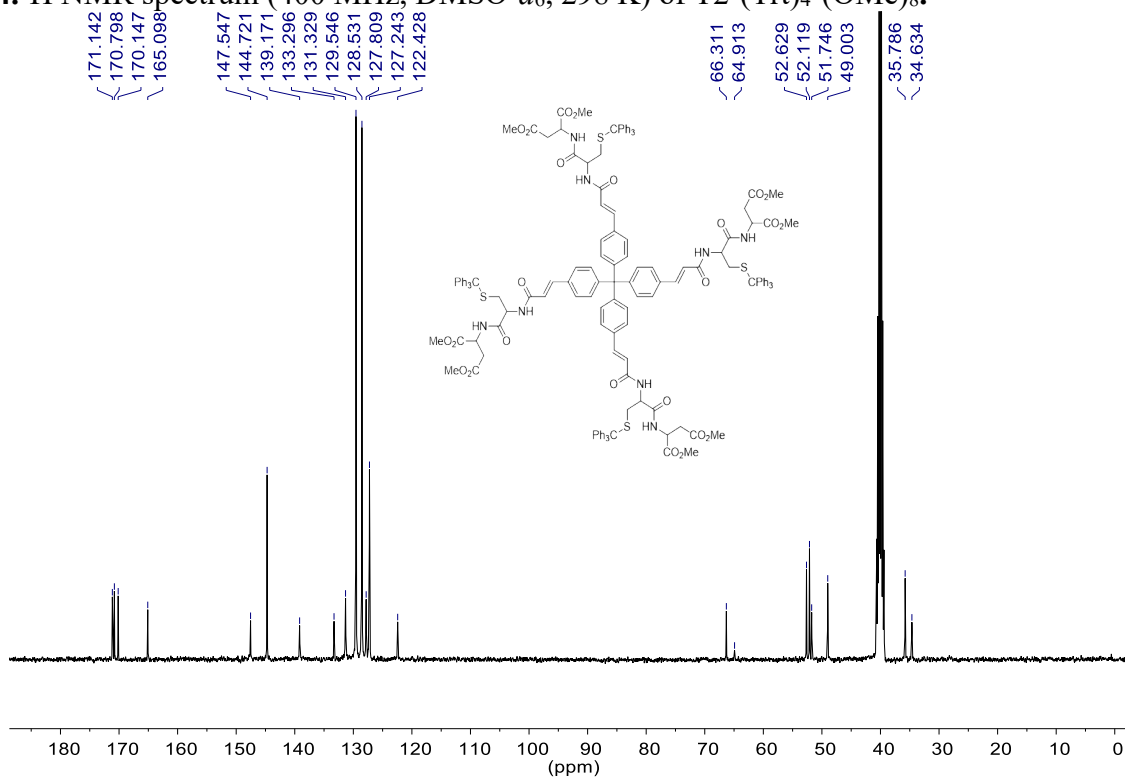


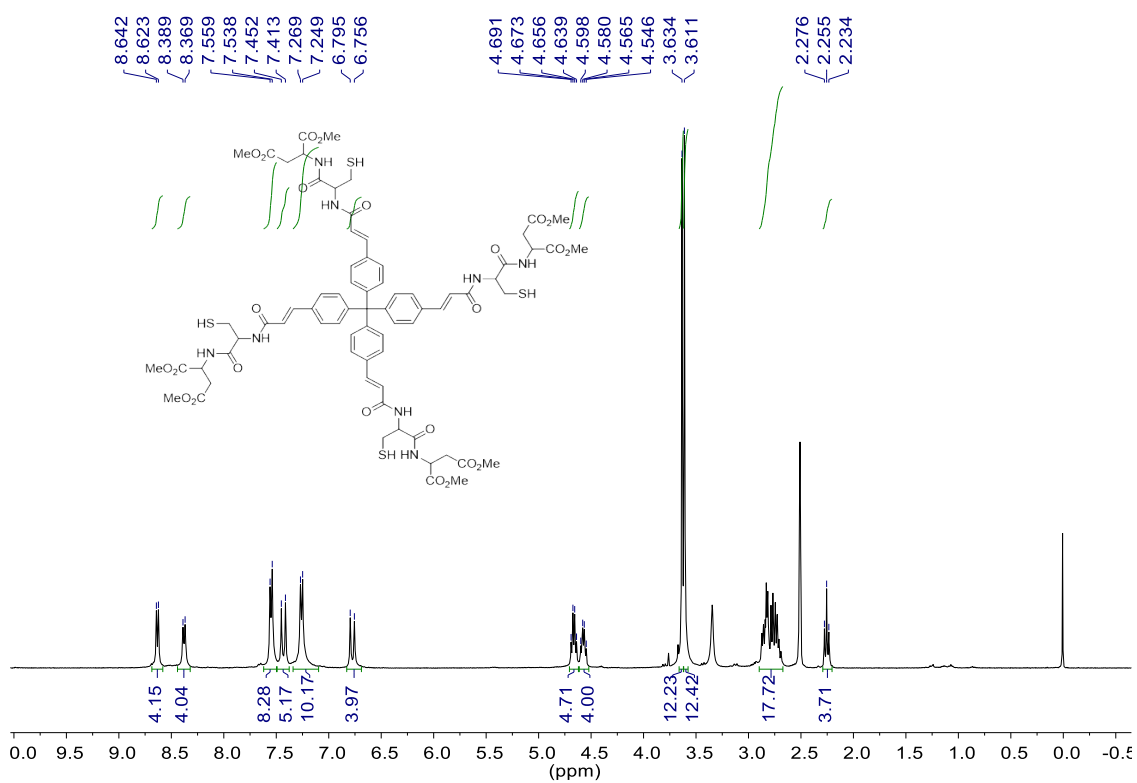
Fig. S33. <sup>13</sup>C NMR spectrum (100 MHz, DMSO-*d*<sub>6</sub>, 298 K) of T1-(OMe)<sub>8</sub>.



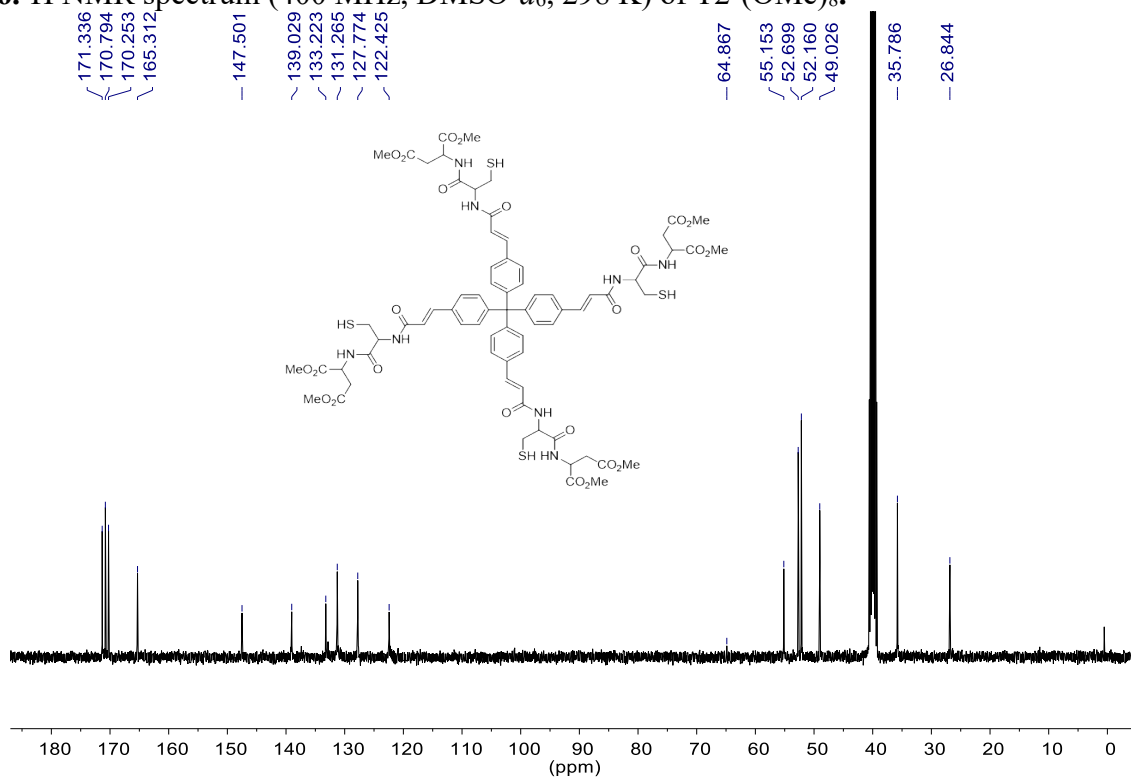
**Fig. S34.** <sup>1</sup>H NMR spectrum (400 MHz, DMSO-*d*<sub>6</sub>, 298 K) of T2-(Trt)<sub>4</sub>-(OMe)<sub>8</sub>.



**Fig. S35.** <sup>13</sup>C NMR spectrum (100 MHz, DMSO-*d*<sub>6</sub>, 298 K) of T2-(Trt)<sub>4</sub>-(OMe)<sub>8</sub>.



**Fig. S36.** <sup>1</sup>H NMR spectrum (400 MHz, DMSO-*d*<sub>6</sub>, 298 K) of T2-(OMe)<sub>8</sub>.



**Fig. S37.** <sup>13</sup>C NMR spectrum (100 MHz, DMSO-*d*<sub>6</sub>, 298 K) of T2-(OMe)<sub>8</sub>.



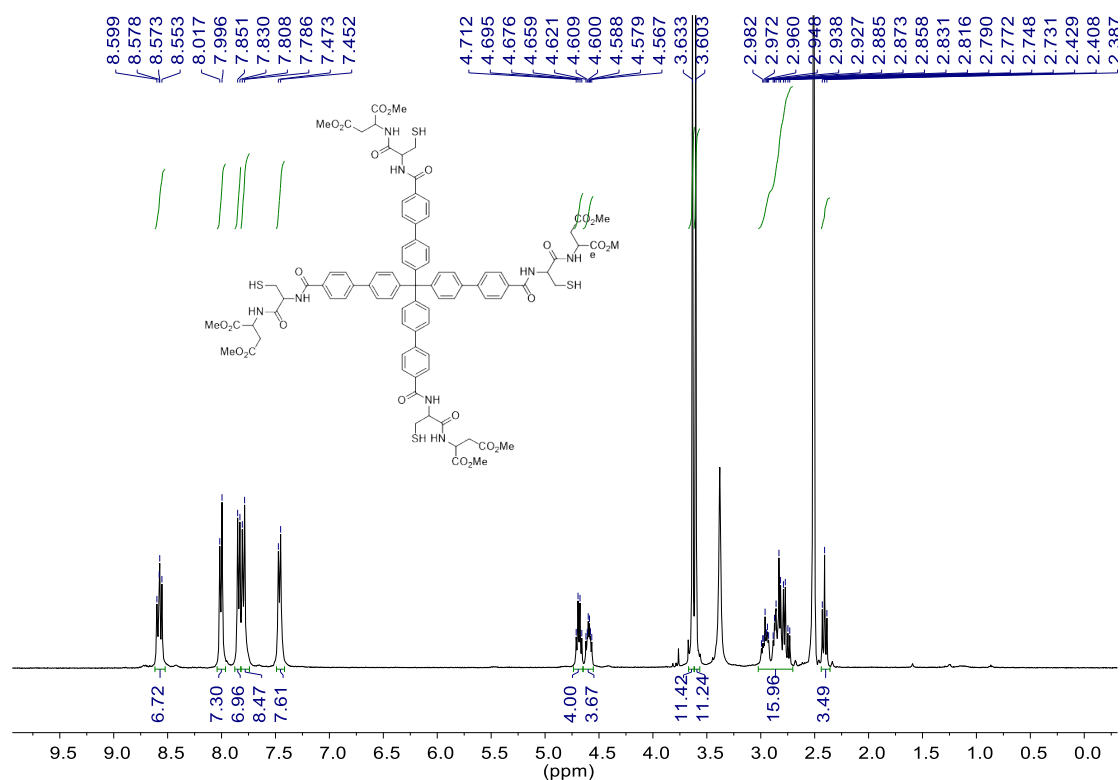


Fig. S40. <sup>1</sup>H NMR spectrum (400 MHz, DMSO-*d*<sub>6</sub>, 298 K) of T3-(OMe)<sub>8</sub>.

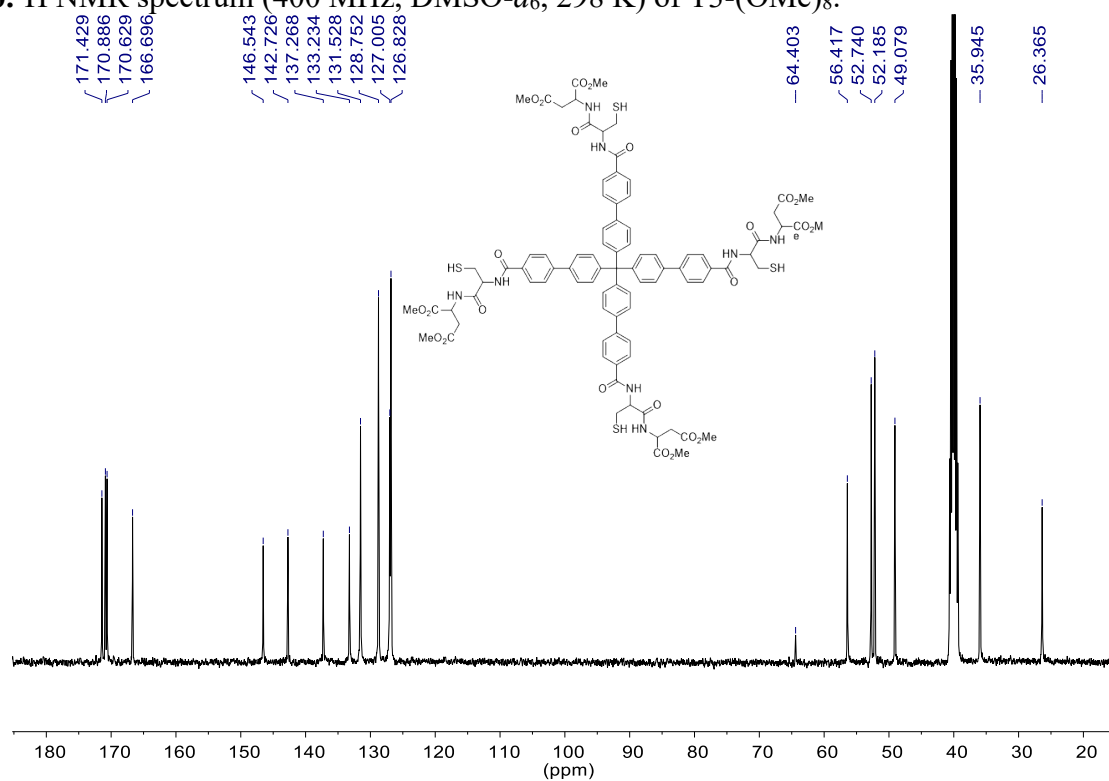


Fig. S41. <sup>13</sup>C NMR spectrum (100 MHz, DMSO-*d*<sub>6</sub>, 298 K) of T3-(OMe)<sub>8</sub>.

## References

- [1] a) L. Felix, U. Sezer, M. Arndt, M. Mayor, *Eur. J. Org. Chem.* **2014**, *31*, 6884-6895; b) D. Liu, Z. Xie, L. Ma, W. Lin, *Inorg. Chem.* **2010**, *49*, 9107-9109.
- [2] a) L. Ma, A. Jin, Z. Xie, W. Lin, *Angew. Chem. Int. Ed.* **2009**, *48*, 9905-9908.