Antioxidant Shape Amphiphiles for Accelerated Wound Healing

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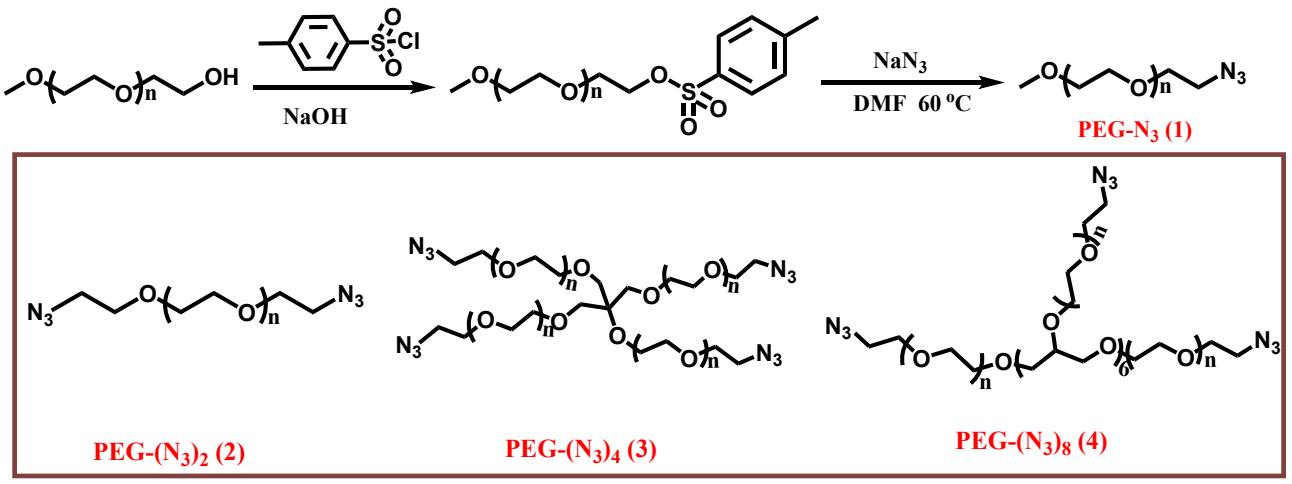
Synthetic Procedures



Scheme S1. Synthetic scheme towards PEG-$(N_3)_n$ (n=1, 2, 4, 8).

PEG-N_3 (1): PEG-OH (M_w: 2 k, 2 g, 1 mmol) and 10 mL THF were placed in 100 mL round bottom flask with a magnetic stirred. The reaction mixture was cooled to 0 °C and the 5 mL of 6 M NaOH was added. A 15 mL THF of tosyl chloride (382 mg, 2 mmol) was then slowly added to the reaction mixture solution under N_2. After stirring at 0 °C for 1 h, the reaction mixture was allowed to reach room temperature and stirred for another 12 h. The resulting mixture was extracted with DCM (300 mL) and the organic layer was washed with 1 M NaOH (150 mL) and saturated NaCl solution. After drying over $MgSO_4$, the solution was evaporated under vacuum to obtain white sample PEG-PTSC.

Dissolving all the obtained PEG-PTSC sample and NaN_3 (260 mg, 4 mmol) with 20 mL DMF. The reaction system was heated 65 °C for 24 h, and then the mixture was evaporated and concentrated to 5 mL solution. After that, which was dispersed into 300 mL of DCM and extracted successively with deionized water and saturated brine. The organic phase was collected and evaporated under vacuum to obtain crude sample. The crude sample was further purified by precipitation in ice ethyl ether to give white solid sample (1860 mg). SEC: M_n = 3.3 kg mol^{-1}, PDI = 1.02.

PEG-$(N_3)_2$ (2): PEG-$(OH)_2$ (M_w:2 k, 2.00 g, 1 mmol), tosyl chloride (764 mg, 4 mmol) and NaN_3 (390 mg, 6 mmol) were used to synthesize white target molecule by a similar method (1870 mg). SEC: M_n = 3.2 kg mol^{-1}, PDI = 1.02.

PEG-$(N_3)_4$ (3): PEG-$(OH)_4$ (M_w:10 k, 1.00 g, 0.1 mmol), tosyl chloride (228 mg, 1.2 mmol) and NaN_3 (260 mg, 4 mmol) were used to synthesize white target molecule by a similar method (890 mg). SEC: M_n = 15.5 kg mol^{-1}, PDI = 1.01.

PEG-$(N_3)_8$ (4): PEG-$(OH)_8$ (M_w:10 k, 1.00 g, 0.1 mmol), tosyl chloride (456 mg, 2.4 mmol), NaN_3 (520 mg, 8 mmol) were used to synthesize white target molecule by a similar method (912 mg). SEC: M_n = 10.2 kg mol^{-1}, PDI = 1.01.

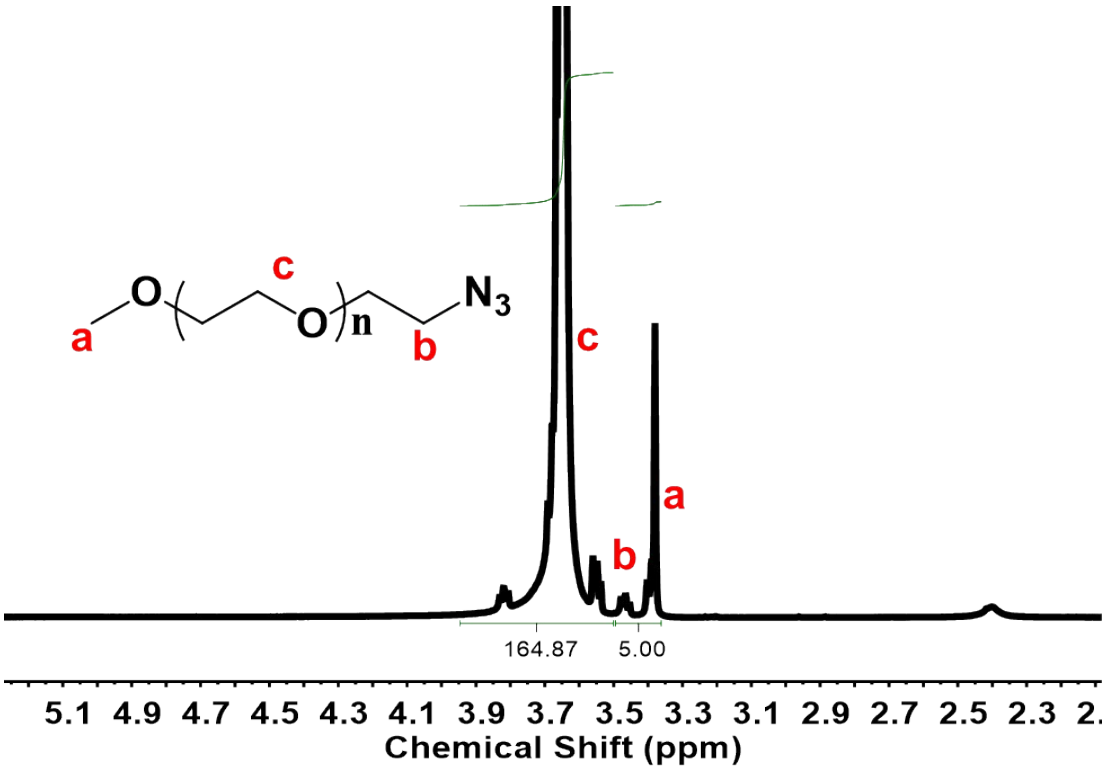


Figure S1. The ^1H NMR spectrum ($CDCl_3$) of PEG-N_3.

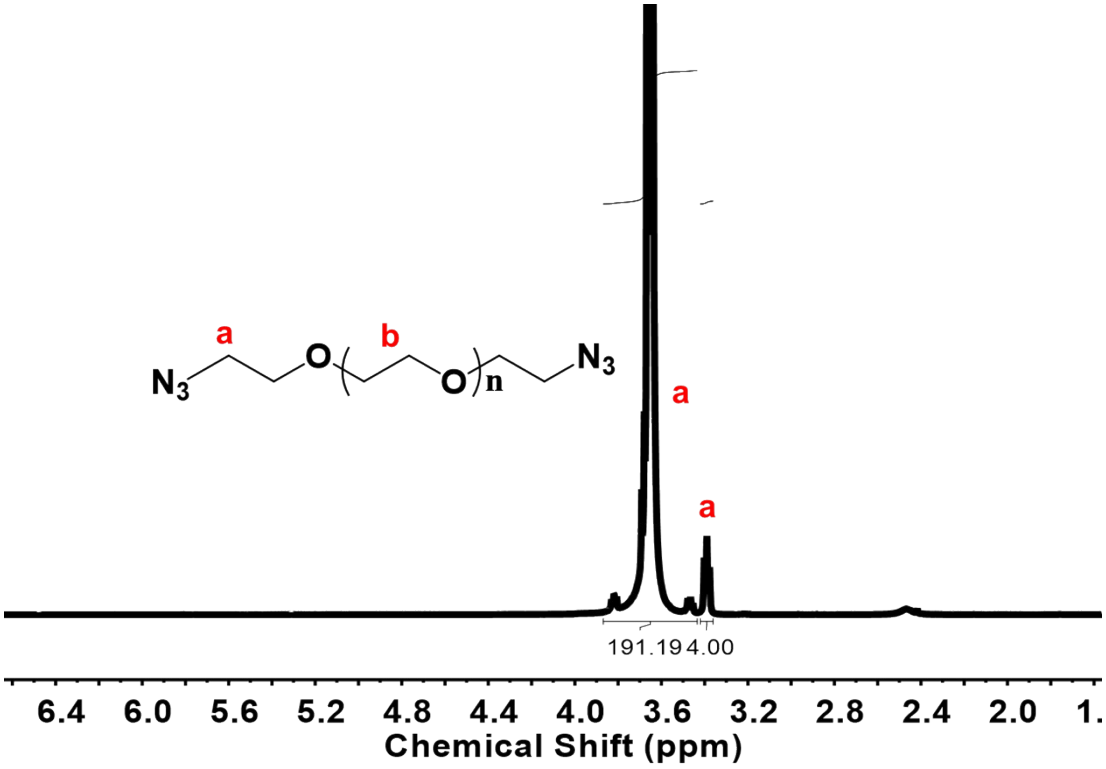


Figure S2. The ^{1}H NMR spectrum ($CDCl_3$) of PEG-$(N_3)_2$.

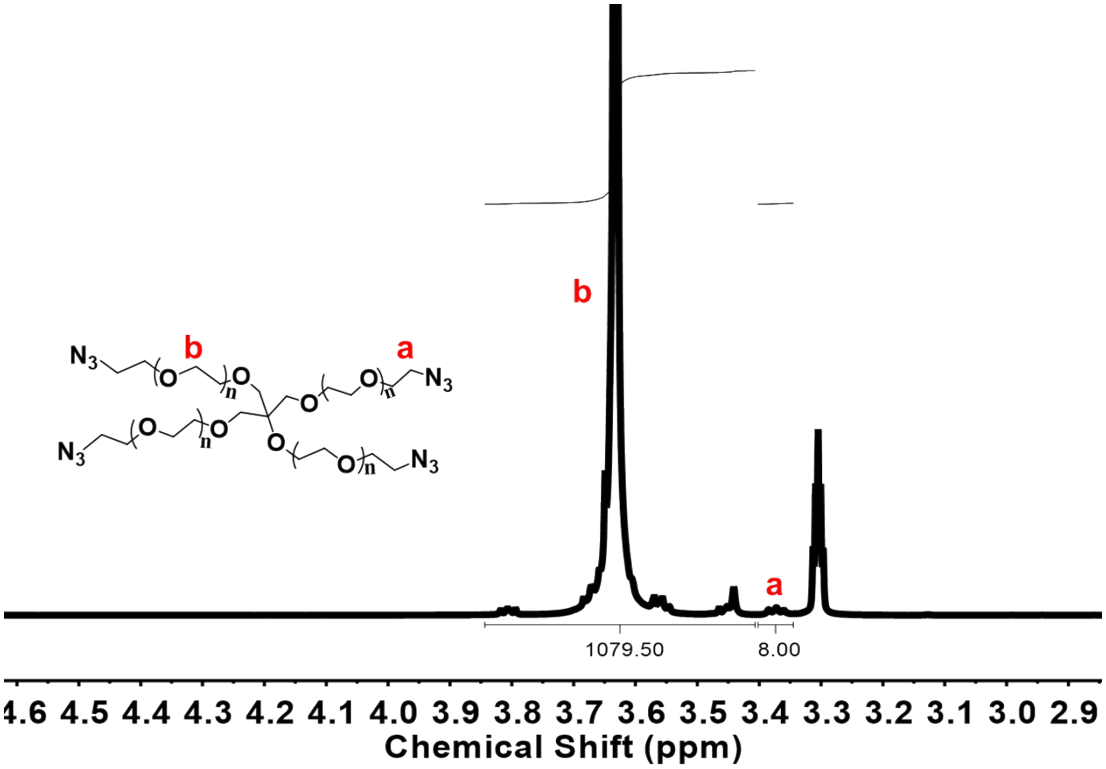


Figure S3. The ^{1}H NMR spectrum (Methanol-D4) of PEG-$(N_3)_4$.

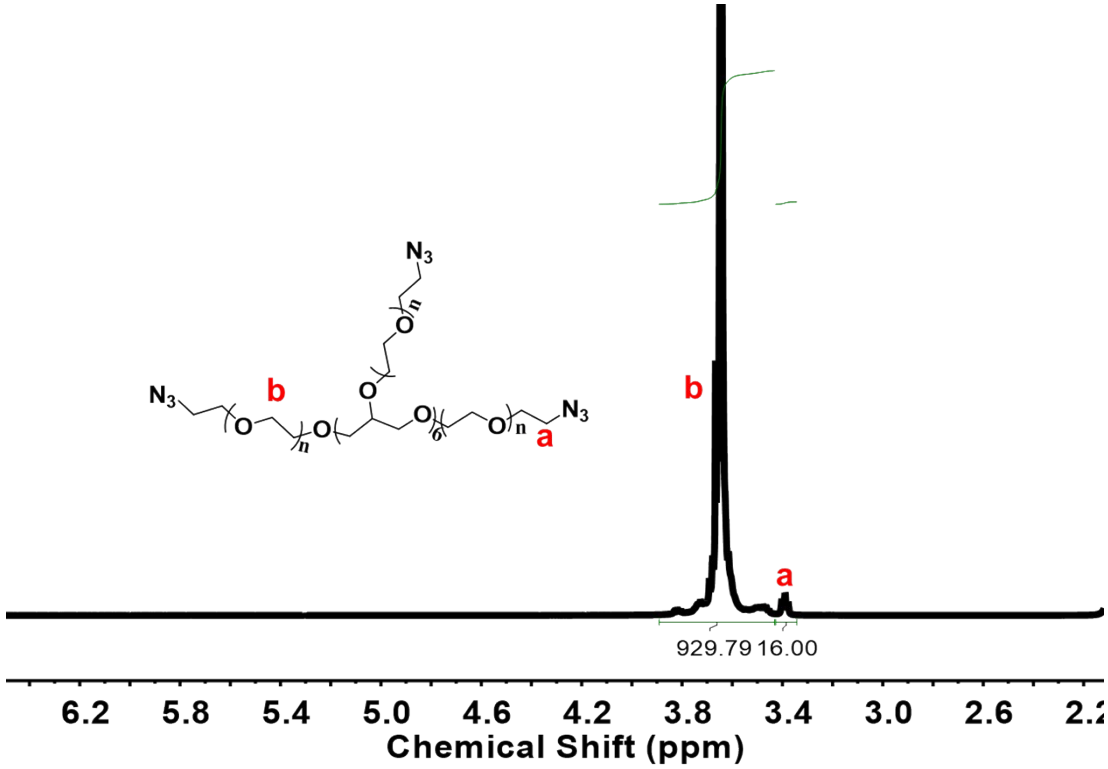
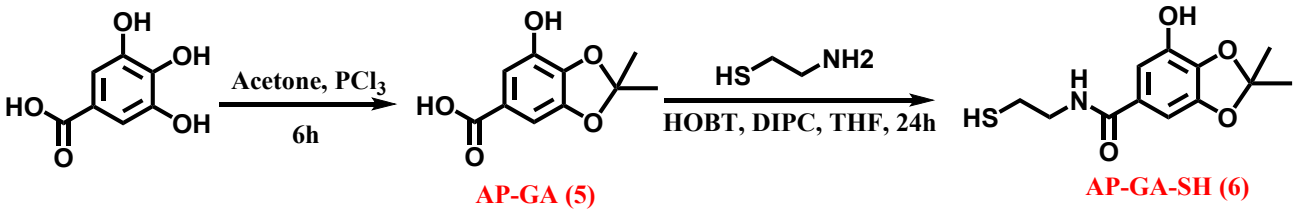


Figure S4. The ^{1}H NMR spectrum ($CDCl_3$) of PEG-$(N_3)_8$.



Scheme S2. Synthesis of AP-GA-SH.

Synthesis of acetonide protected gallic acid **AP-GA (5)**: The gallic acid (5.00 g, 29.41 mmol) was completely dissolved in 60 mL acetone, and then PCl_3 (2.00 mL, 22.98 mmol) was slowly added dropwise *via* a syringe to the solution system under ice bath condition. Following the mixed system was stirred continuously at room temperature for 6 hours. The solution was dried under reduced pressure, after which the crude product was further purified by flash column chromatography on silica

gel with EtOAc/PE (v/v=1:2) as eluent to afford the target product as white powder (2560 mg, 41.45%). ^{1}H NMR (400 MHz, Methanol-D_4, ppm, δ): 7.16 (d, H, CHarom), 6.93 (d, H, CHarom), 1.68 (s, 6H, CH3-).^{13}C NMR (100 MHz, Methanol-D_4, ppm, δ): 168.20, 148.45, 140.20, 138.50, 123.70, 119.10, 113.21, 101.66, 24.50.

AP-GA-SH (6): AP-GA (500 mg, 2.38 mmol), 2-Aminoethanethiol (275 mg, 3.57 mmol) and HOBT (506 mg, 3.75 mmol) were completely dissolved in 5 mL anhydrous dried THF and stirred for 10 min in an ice bath. Following the DIPC (451 mg, 3.75 mmol) was slowly added to mixed solution system. The mixture was allowed to warm up to room temperature and stirred for another 24 hours. After that, the mixture reaction solution was filtered, washed with 1M HCl and saturated NaCl solution, and the organic phase was collected and spin-dried under reduced pressure. The residue was further purified by flash column chromatography on silica gel with EtOAc/PE (v/v=2:1) as eluent to afford the target product (480 mg, 75%). ^{1}H NMR (400 MHz, Methanol-D_4, ppm, δ): 6.95 (d, H, CHarom), 6.78 (d, H, CHarom), 3.47 (t, 2H, -C$\underline{H}_2$CH_2SH), 2.68 (t, 2H, -CH_2C$\underline{H}_2$SH), 1.67 (s, 6H, C$\underline{H}_3$-). ^{13}C NMR (100 MHz, Methanol-D_4, ppm, δ): 168.43, 148.60, 140.33, 137.24, 127.55, 118.93, 110.76, 99.27, 43.04, 24.48, 23.01.

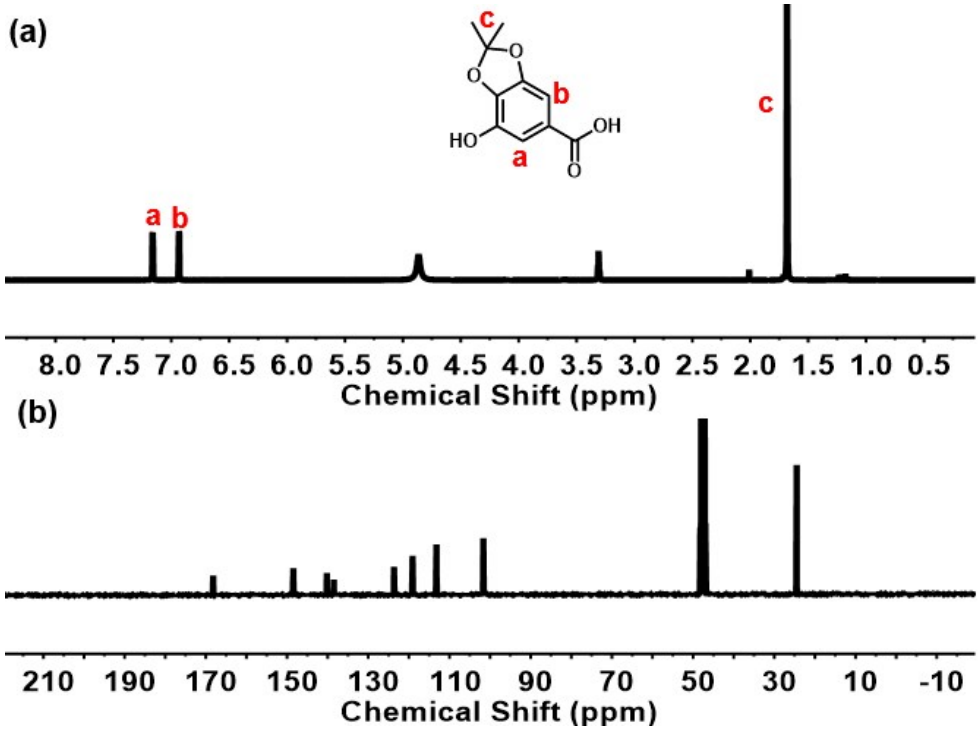


Figure S5. The (a) ^{1}H NMR (b) ^{13}C NMR spectrum (Methanol-D4) of AP-GA.

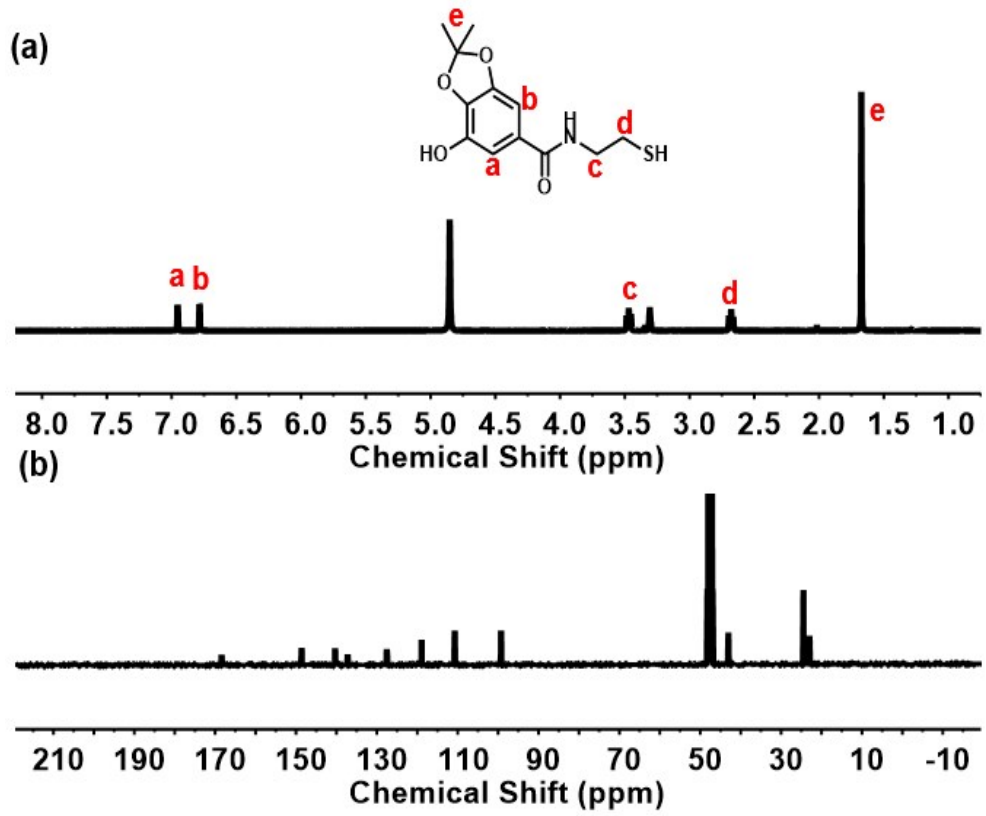


Figure S6. The (a) ^{1}H NMR (b) ^{13}C NMR spectrum (Methanol-D4) of AP-GA-SH.

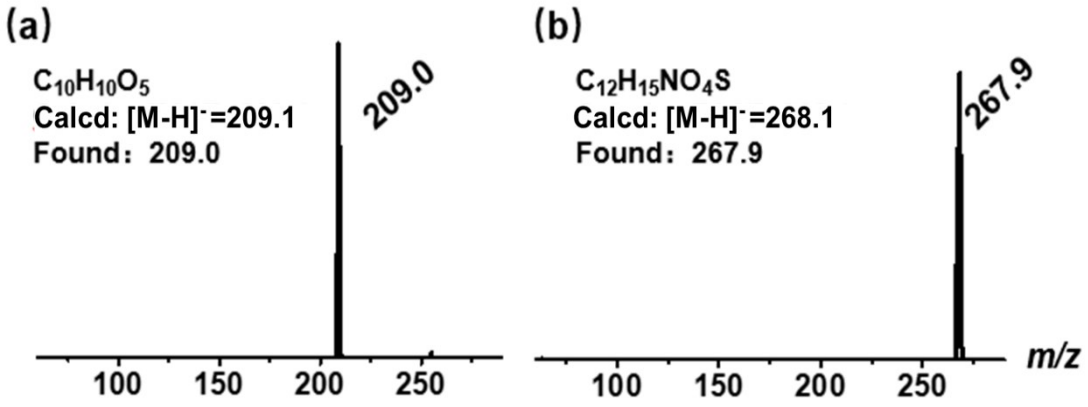
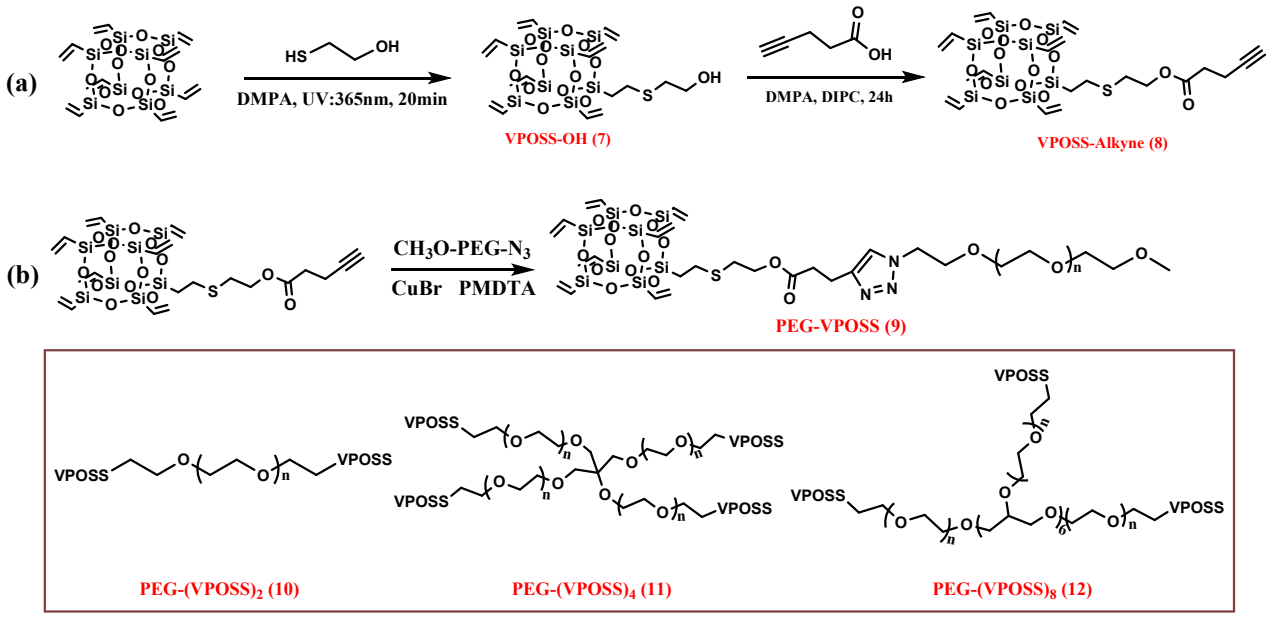


Figure S7. Mass spectra of (a) AP-GA and (b) AP-GA-SH.



Scheme S3. Synthetic scheme towards (a) VPOSS-alkyne and (b) PEG-$(VPOSS)_n$ (n=1,2,4,8).

VPOSS-OH (7): VPOSS (10 g, 15.8 mmol), 2-Mercaptoethanol (1.2 g, 15.8 mmol) and 250 mL THF were charged to a 250 mL beaker with a magnetic stirred. The DMPA (404 mg, 1.58 mmol) was added to the mixed solvent and completely dissolved. Following the reaction system irradiated under a UV 365 nm lamp at room temperature for about 30 min. Removed solvent by reduced pressure and the purified by flash chromatography on silica gel using DCM:PE (v/v=1:3) as the eluent to afford the

white powder sample (2.23 g, 19.9%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm, δ): 6.03 (m, 21H, C$\underline{H_2}$=C$\underline{H}$-arom), 3.71 (t, 2H, -C$\underline{H_2}$OH), 2.74-2.65 (m, 4H, -C$\underline{H_2}$SC$\underline{H_2}$-), 1.08 (m, 2H, -C$\underline{H_2}$CH$_2$S-).

VPOSS-Alkyne (8): VPOSS-OH (1g, 1.41 mmol), 4-Pentynoicacid (207 mg, 2.11 mmol) and DMAP (258 mg, 2.11 mmol) were added to a 50 mL round bottom flask. And the 10 mL anhydrous DCM was added to fully dissolve the all sample in ice bath. After 10 min, the 1 mL DIPC was added and reacted at room temperature for 24 h. The solvent was removed and further purified by flash chromatography on silica gel using DCM:PE (v/v=1:1) as the eluent to afford the white powder sample (950 mg, 85%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm, δ): 6.03 (m, 21H, C$\underline{H_2}$=C$\underline{H}$-arom), 4.24 (t, 2H, -C$\underline{H_2}$OCO), 2.76-2.53 (m, 8H, -C$\underline{H_2}$SC$\underline{H_2}$- , -C$\underline{H_2}$C$\underline{H_2}$CCH), 1.97 (t, H, -CC$\underline{H}$) 1.07 (m, 2H, -C$\underline{H_2}$CH$_2$S-).

PEG-VPOSS (9): The whole reaction process was carried out in oxygen-free glove box. The detailed steps are as follows: PEG-N3 (200 mg, 0.1 mmol), VPOSS-Alkyne (119 mg, 0.15 mmol) and CuBr (2 mg) were added to vial with a magnetic stirring bar and completely dissolved in 10 mL freshly distilled toluene. Then 26 μL PMDTA dripped into the mixed solution system and reacted 36 h under argon atmosphere. After the reaction was completed, the solution was directly transferred onto the top of a silica gel column. And then the DCM was used as eluent to fully remove the unreacted starting materials. Following the mixed solution system DCM/CH_3OH (v/v=20:1) was selected to elute the sample off the column. After the solvent removal, the crude product was precipitated into cold ethyl ether. The product was collected and dried under vacuum to obtain the sample (279 mg, 81%). SEC: M_n = 4.2 kg mol^{-1}, PDI = 1.02.

PEG-(VPOSS)$_2$ (10): PEG-(N_3)$_2$ (150 mg, 0.075 mmol), VPOSS-Alkyne (178 mg, 0.225 mmol), CuBr (2 mg) and PMDTA (26 μL) were selected to synthesize white powder PEG-(VPOSS)$_2$ by a similar method (268 mg, 90%). SEC: M_n = 5.0 kg mol^{-1}, PDI = 1.02.

PEG-(VPOSS)$_4$ (11): PEG-(N$_3$)$_4$ (200 mg, 0.02 mmol), VPOSS-Alkyne (126 mg, 0.16 mmol), CuBr (2 mg) and PMDTA (26 μL) were selected to synthesize white powder PEG-(VPOSS)$_4$ by similar method (215 mg, 82%). SEC: M_n = 17.7 kg mol^{-1}, PDI = 1.01.

PEG-(VPOSS)$_8$ (12): PEG-(N$_3$)$_8$ (200 mg, 0.02 mmol), VPOSS-Alkyne (190 mg, 0.24 mmol), CuBr (2 mg) and PMDTA (26 μL) were selected to synthesize the white powder PEG-(VPOSS)$_8$ by similar method (215 mg, 86%). SEC: M_n = 14.2 kg mol^{-1}, PDI = 1.06.

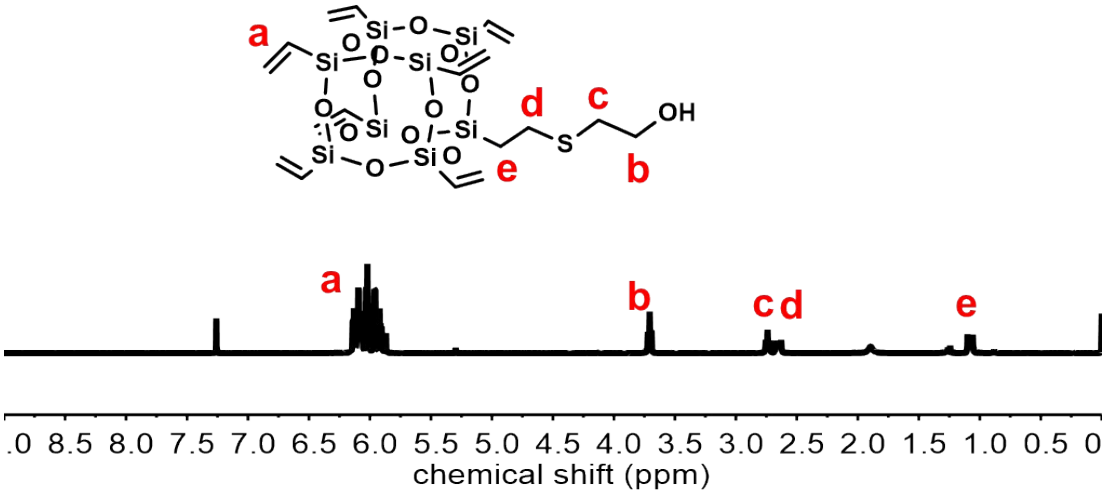


Figure S8. The ^{1}H NMR spectrum of ($CDCl_3$) VPOSS-OH.

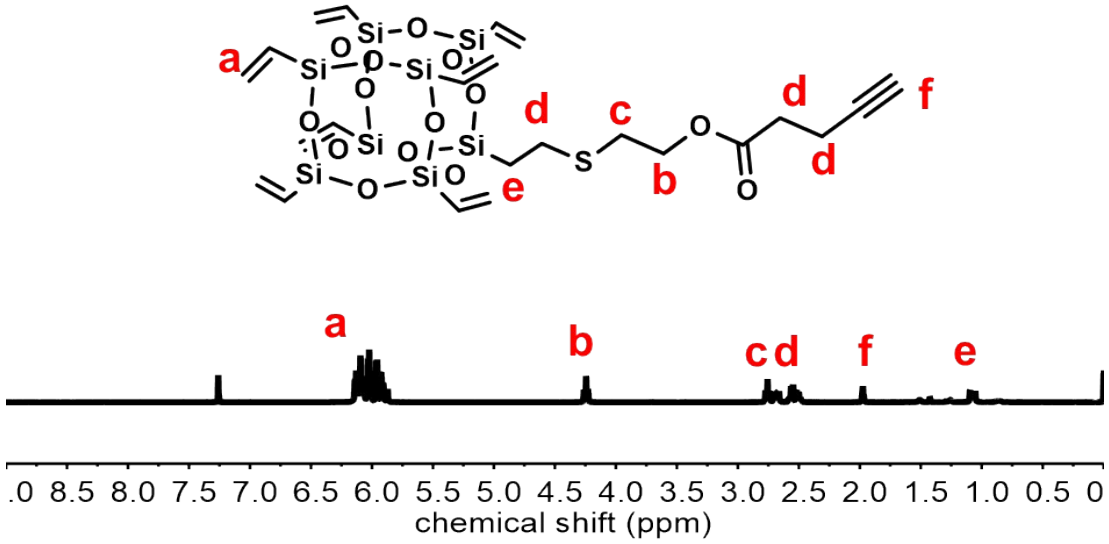


Figure S9. The ^{1}H NMR spectrum of ($CDCl_3$) VPOSS-Alkyne.

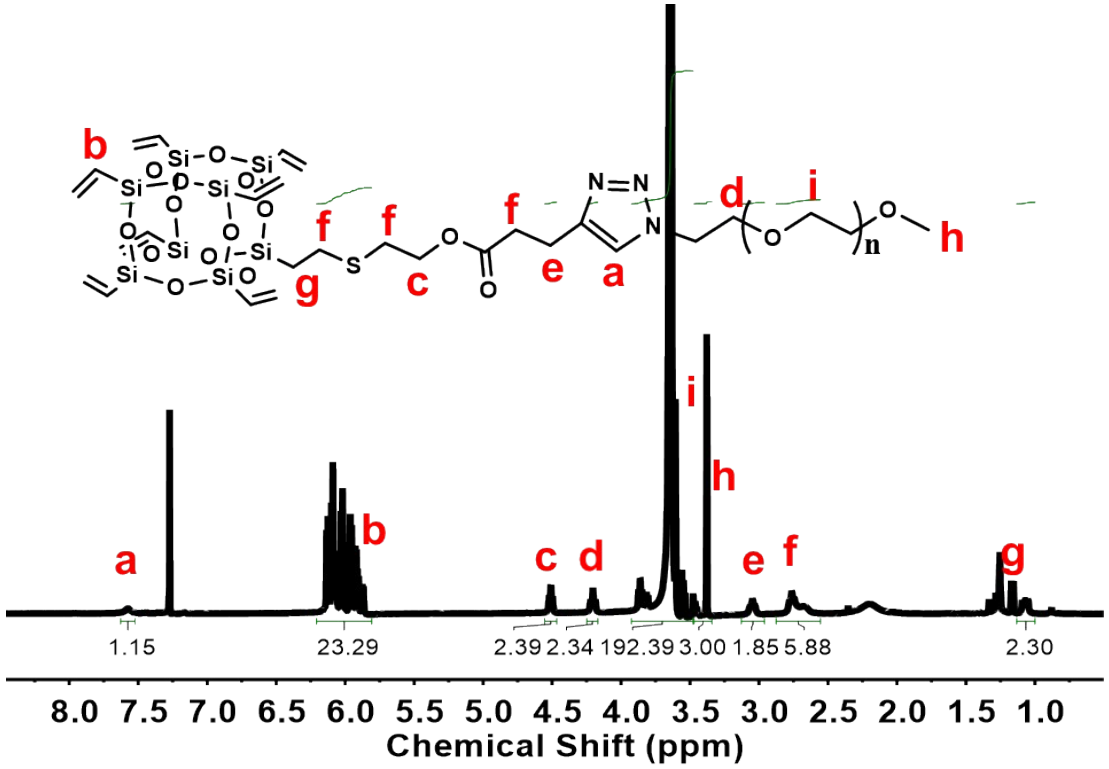


Figure S10. The ^{1}H NMR spectrum of ($CDCl_3$) PEG-VPOSS.

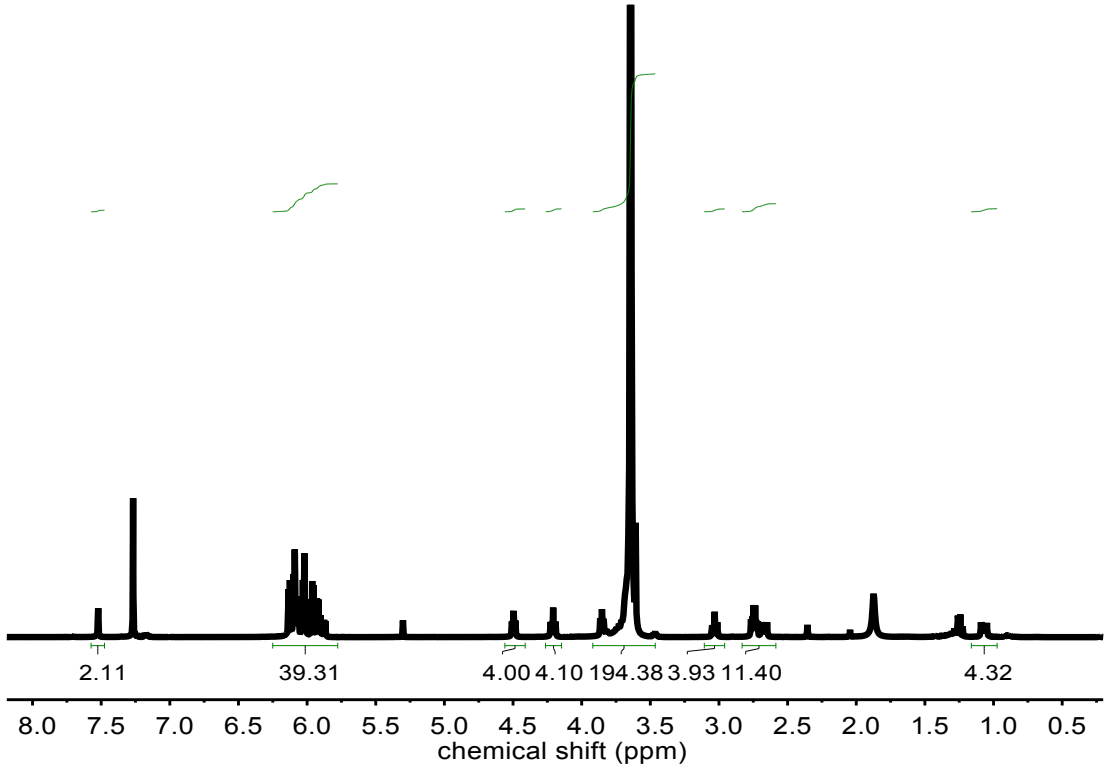


Figure S11. The ^{1}H NMR spectrum of ($CDCl_3$) PEG-$(VPOSS)_2$.

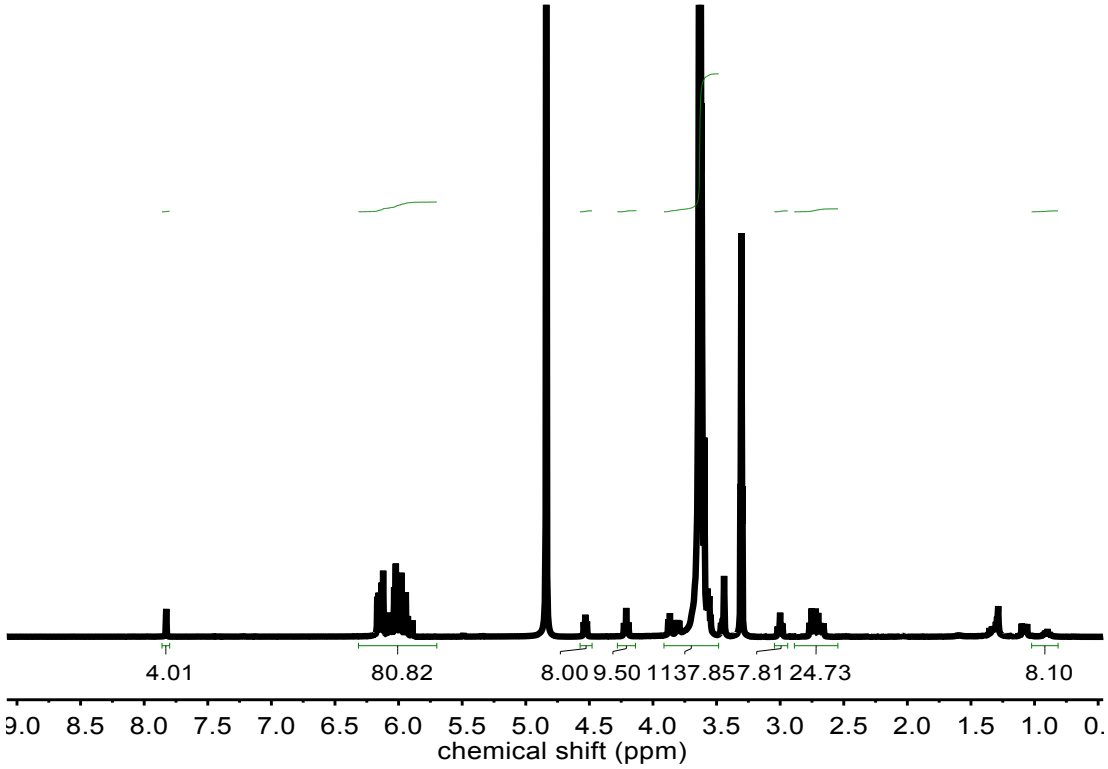


Figure S12. The ^{1}H NMR spectrum of (Methanol-D4) PEG-$(VPOSS)_4$.

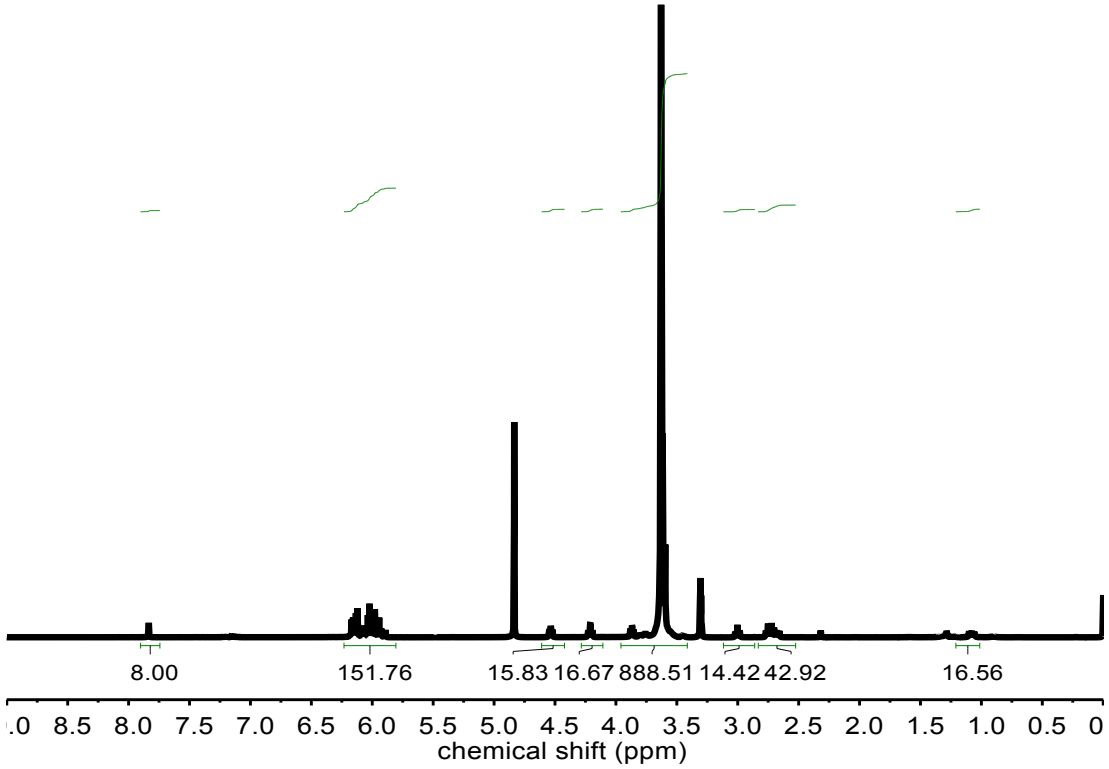
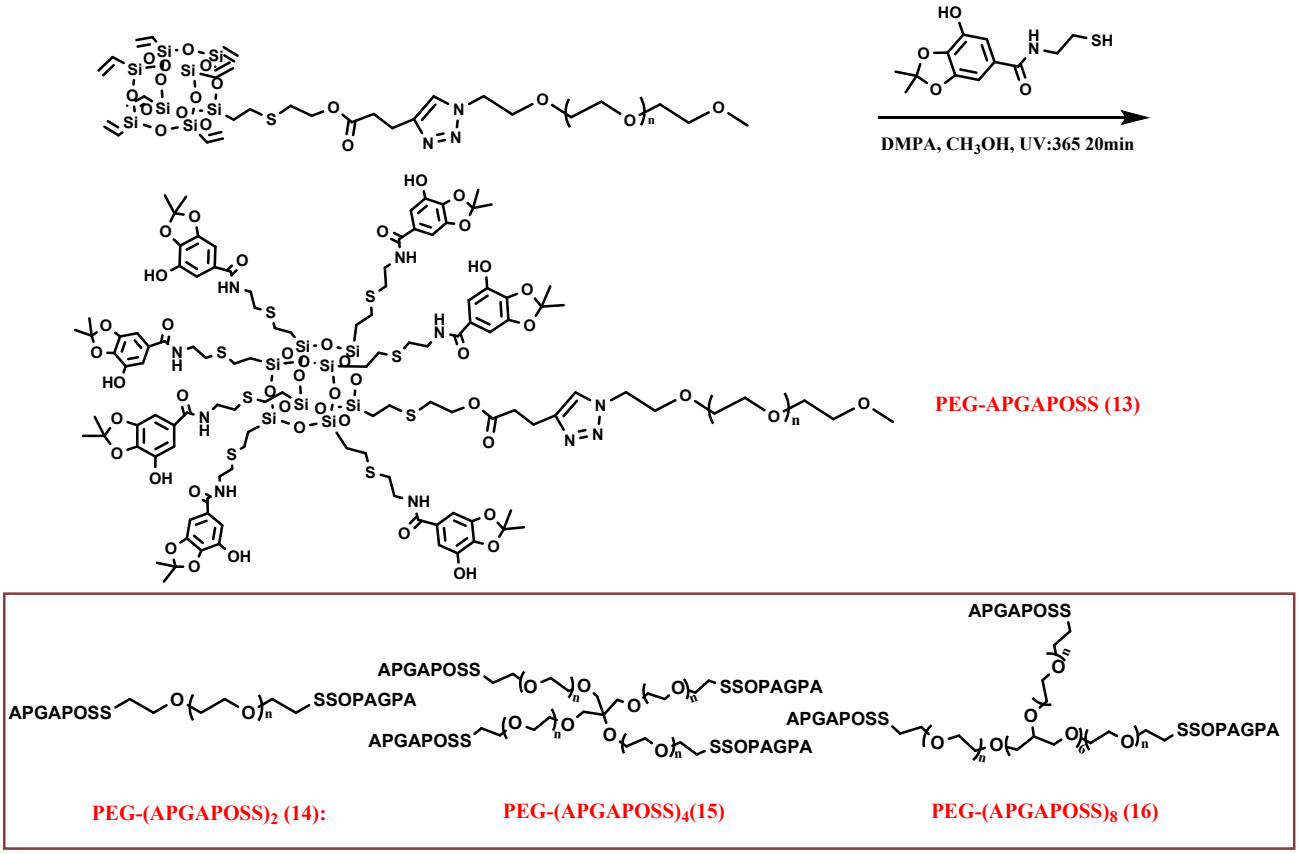


Figure S13. The ^{1}H NMR spectrum of (Methanol-D4) PEG-$(VPOSS)_8$.



Scheme S4. Synthetic scheme towards PEG-$(APGAPOSS)_n$ (n=1, 2, 4, 8).

PEG-APGAPOSS (13): PEG-VPOSS (50 mg, 0.018 mmol), AP-GA-SH (48 mg, 0.18 mmol) and DMPA (5 mg) were dissolved in 3 mL of methanol, followed by irradiation with UV 365 nm for 30 min. The mixed solution was then precipitated into cold ethyl ether three times, the sample PEG-APGAPOSS was collected and dried under vacuum to afford viscous solid (65 mg, 77.4%).

PEG-$(APGAPOSS)_2$ (14): PEG-$(VPOSS)_2$ (50 mg, 0.014 mmol), AP-GA-SH (75 mg, 0.28 mmol) and DMPA (5 mg) were dissolved in 3 mL of methanol, followed by irradiation with UV 365 nm for 30 min, the mixed solution was then precipitated into cold ethyl ether three times, the sample PEG-$(APGAPOSS)_2$ was collected and dried under vacuum to afford viscous solid (71 mg, 69.6%).

PEG-$(APGAPOSS)_4$ (15): PEG-$(VPOSS)_4$ (50 mg, 0.004 mmol), AP-GA-SH (41 mg, 0.15 mmol) and DMPA (5 mg) were dissolved in 3 mL of methanol, followed by irradiation with UV 365 nm for 30 min, the mixed solution was then precipitated into cold ethyl ether three times, the sample PEG-$(APGAPOSS)_4$ was collected and dried under vacuum to afford viscous solid (62 mg, 77.5 %).

PEG-$(APGAPOSS)_8$ (16): PEG-$(VPOSS)_8$ (50 mg, 0.003 mmol), AP-GA-SH (66 mg, 0.245 mmol) and DMPA (5 mg) were dissolved in 3 mL of methanol, followed by irradiation with UV 365 nm for 30 min, the mixed solution was then precipitated into cold ethyl ether three times, the sample PEG-$(APGAPOSS)_8$ was collected and dried under vacuum to afford viscous solid (68 mg, 65.3 %).

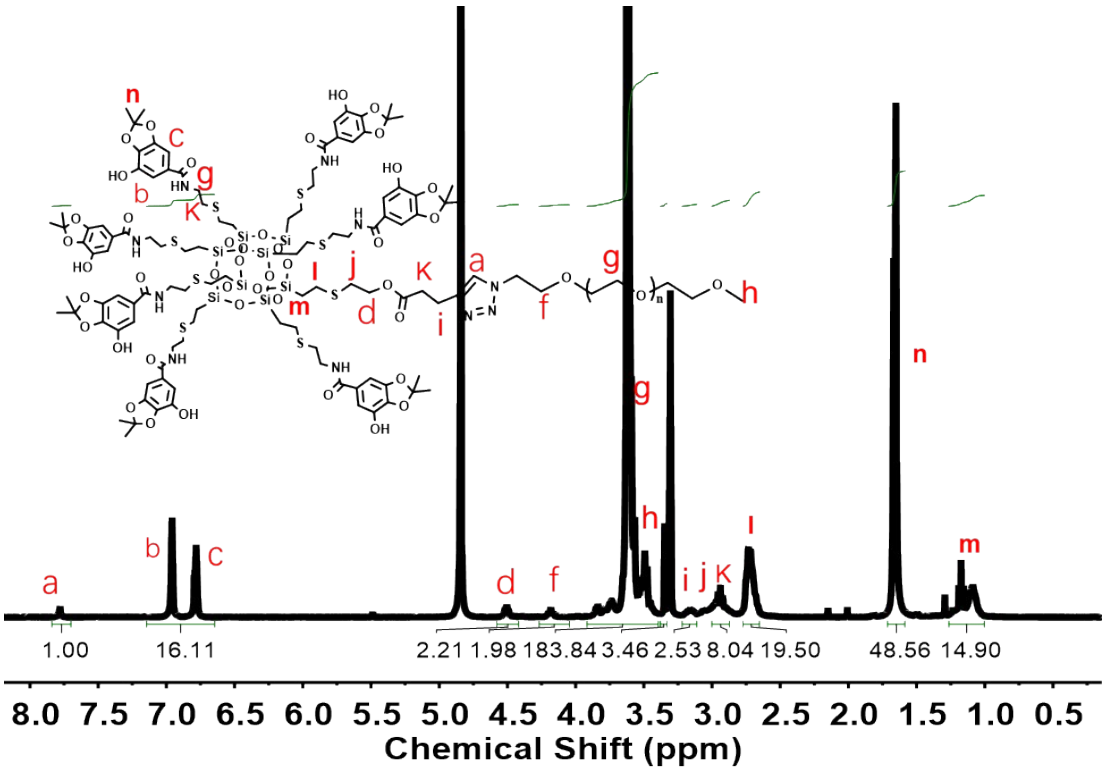


Figure S14. The ^{1}H NMR spectrum of (Methanol-D4) PEG-APGAPOSS.

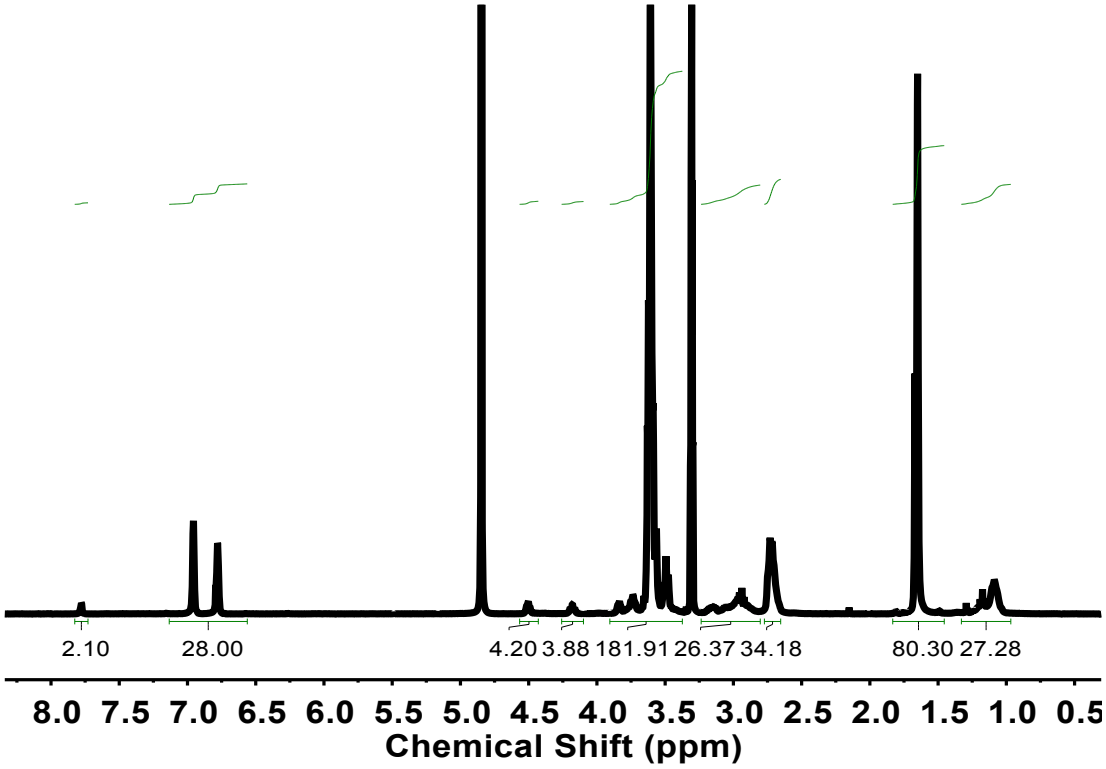


Figure S15. The ^{1}H NMR spectrum of (Methanol-D4) PEG-$(APGAPOSS)_2$.

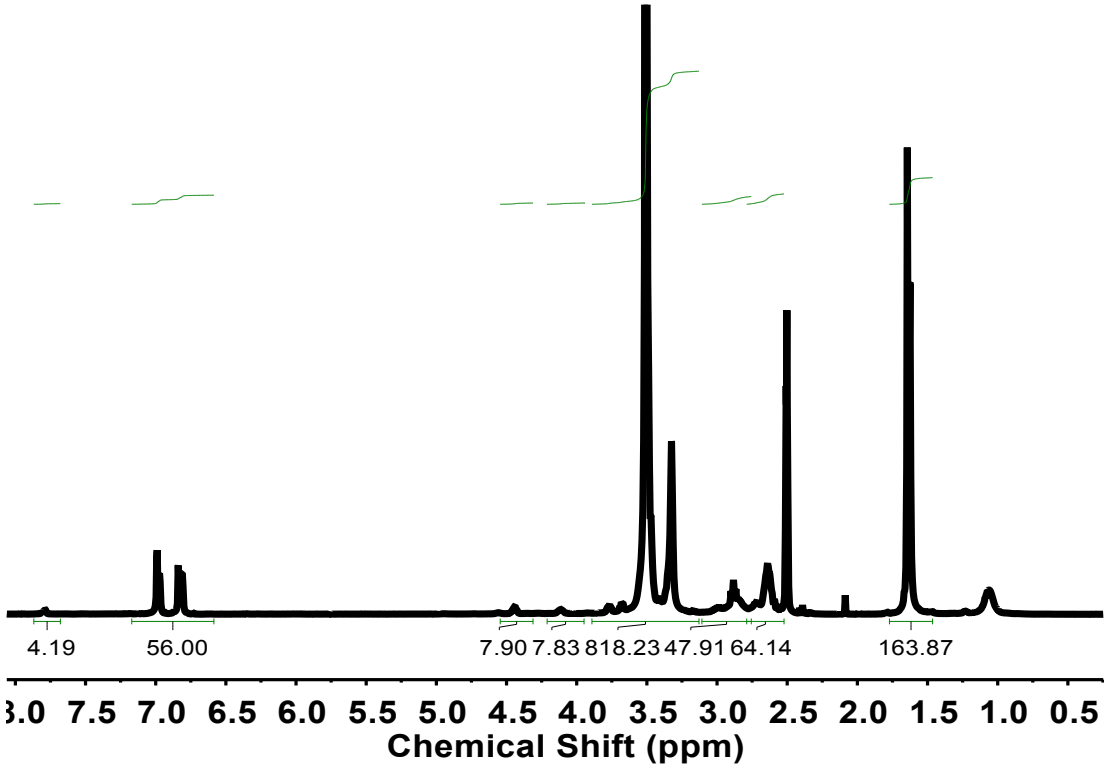


Figure S16. The ^{1}H NMR spectrum of (DMSO-D6) PEG-(APGAPOSS)$_4$.

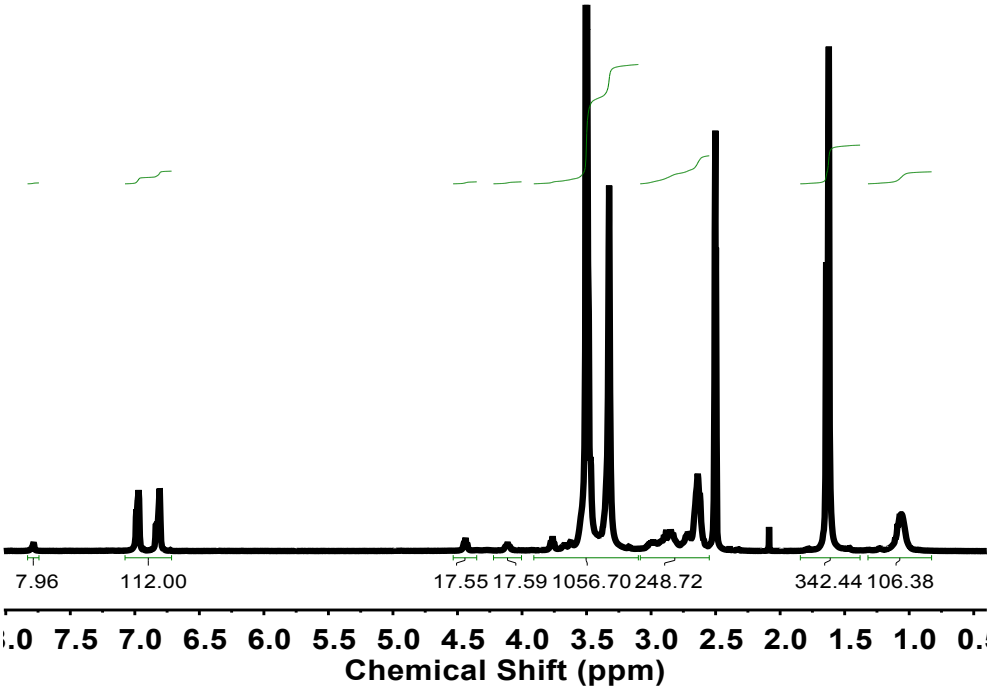
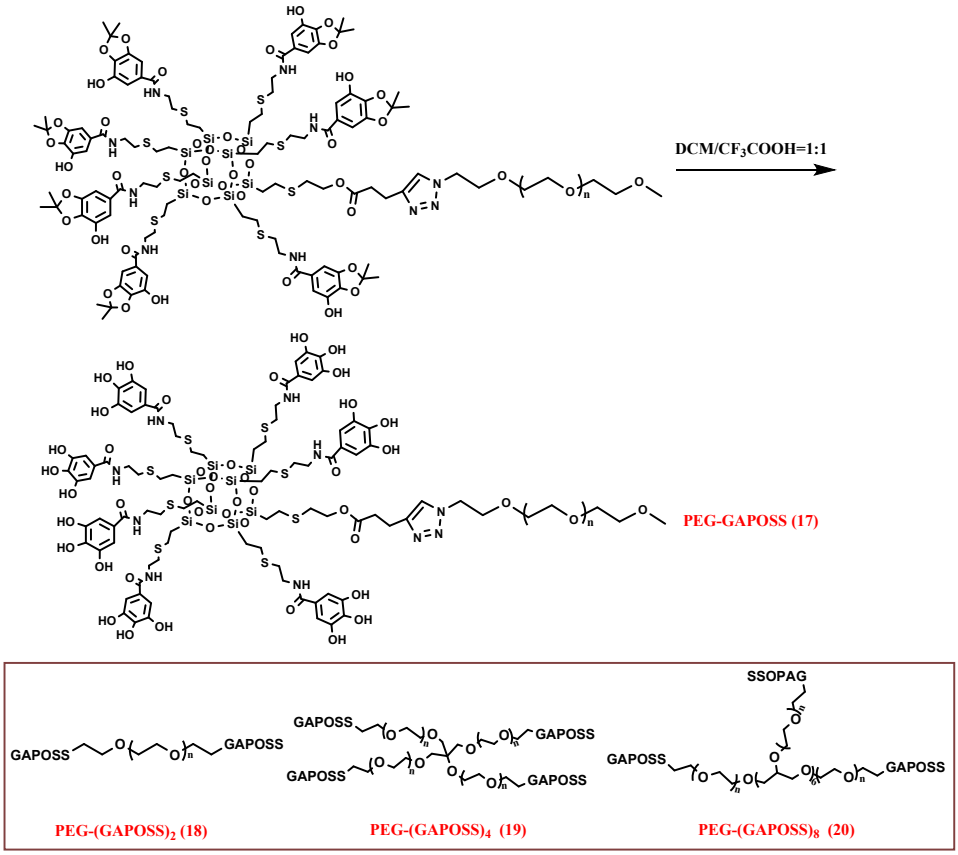


Figure S17. The ^{1}H NMR spectrum of (DMSO-D6) PEG-(APGAPOSS)$_8$.



Scheme S5. Synthetic scheme towards PEG-(GAPOSS)$_n$ (n=1, 2, 4, 8).

PEG-GAPOSS (17): PEG-APGAPOSS (50 mg) was dissolved in mixed solvent of DCM/CF_3COOH (v/v=2:2). After stirring for 5 h, the solvent was evaporated under vacuum to obtain crude product. Which was further purified to give viscous solids PEG-GAPOSS by precipitating into cold ethyl ether. SEC: M_n = 5.6 kg mol^{-1}, PDI = 1.01.

PEG-(GAPOSS)$_2$ (18): PEG-(APGAPOSS)$_2$ (50 mg) was dissolved in mixed solvent of DCM/CF_3COOH (v/v=2:2). After stirring for 5 h, the solvent was evaporated under vacuum to obtain crude product. Which was further purified to give viscous solids PEG-(GAPOSS)$_2$ by precipitating into cold ethyl ether. SEC: M_n = 7.5 kg mol^{-1}, PDI = 1.03.

PEG-(GAPOSS)$_4$ (19): PEG-(APGAPOSS)$_4$ (50 mg) was dissolved in mixed solvent of DCM/CF_3COOH (v/v=2:2). After stirring for 5 h, the solvent was evaporated under vacuum to obtain crude product. Which was further purified to give viscous solids PEG-(GAPOSS)$_4$ by precipitating into cold ethyl ether. SEC: M_n = 19.5 kg mol^{-1}, PDI = 1.02.

PEG-(GAPOSS)$_8$ (20): PEG-(APGAPOSS)$_8$ (50 mg) was dissolved in mixed solvent of DCM/CF_3COOH (v/v=2:2). After stirring for 5 h, the solvent was evaporated under vacuum to obtain crude product. Which was further purified to give viscous solids PEG-(GAPOSS)$_8$ by precipitating into cold ethyl ether. SEC: M_n = 17.7 kg mol^{-1}, PDI = 1.11.

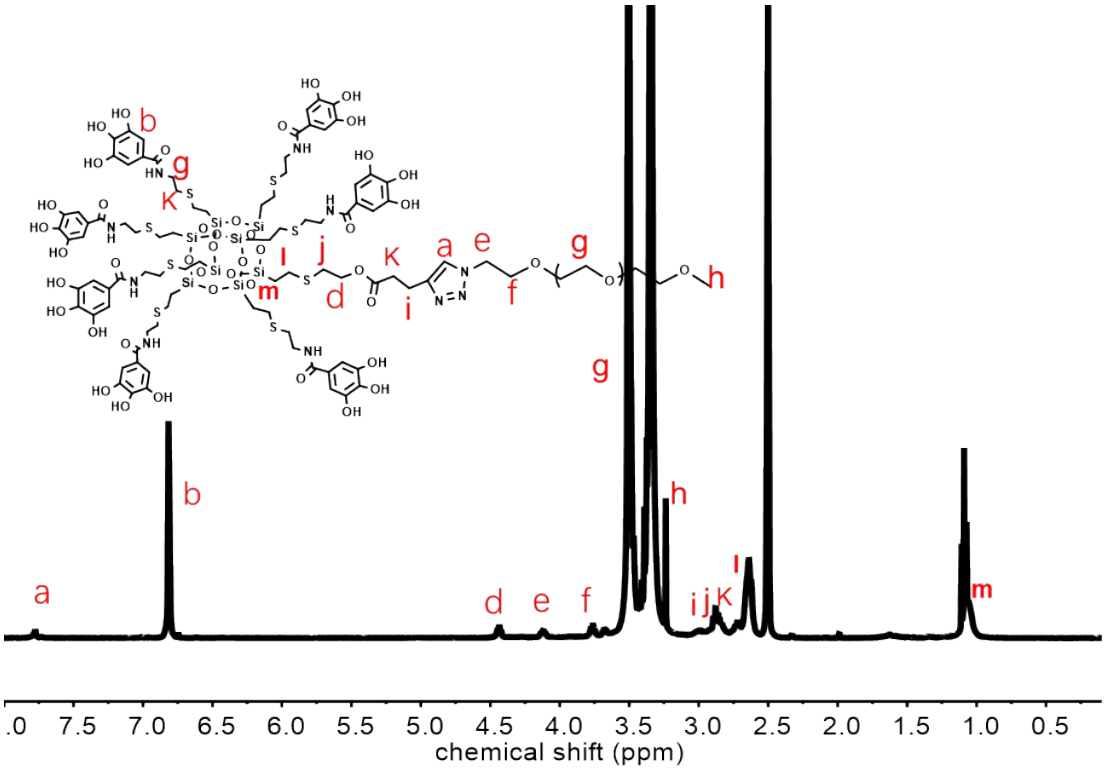


Figure S18. The ^{1}H NMR spectrum (DMSO-D6) of PEG-GAPOSS.

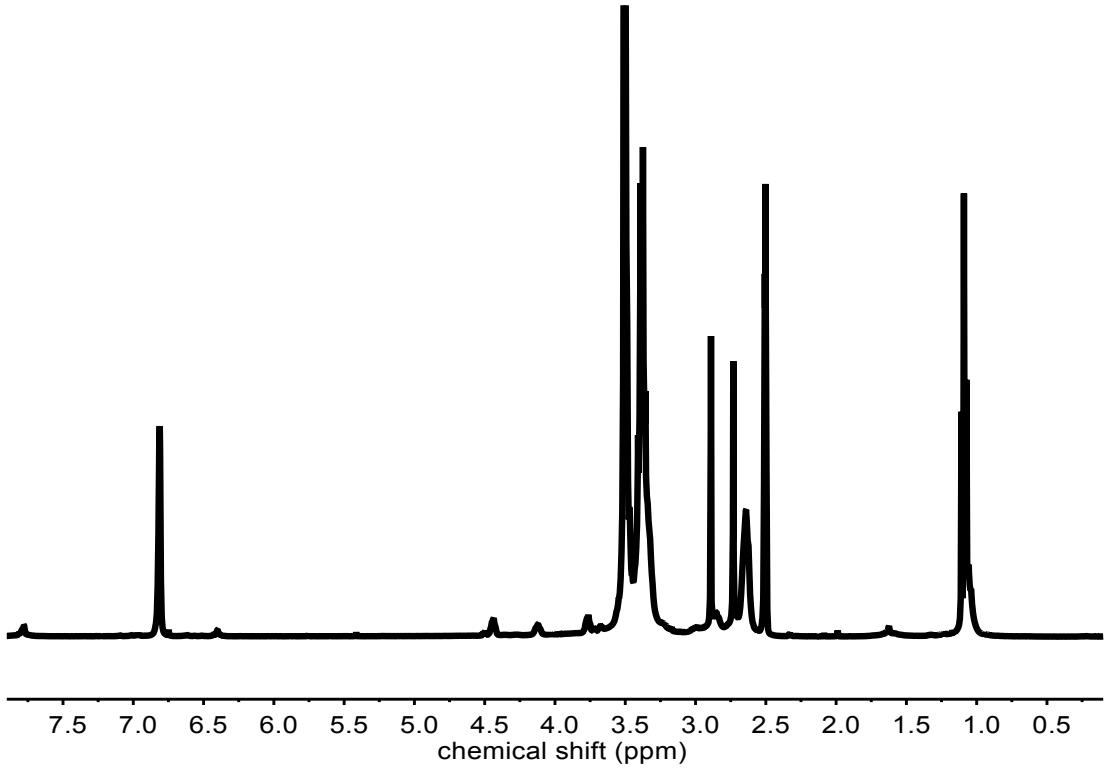


Figure S19. The ^{1}H NMR spectrum (DMSO-D6) of PEG-(GAPOSS)$_2$.

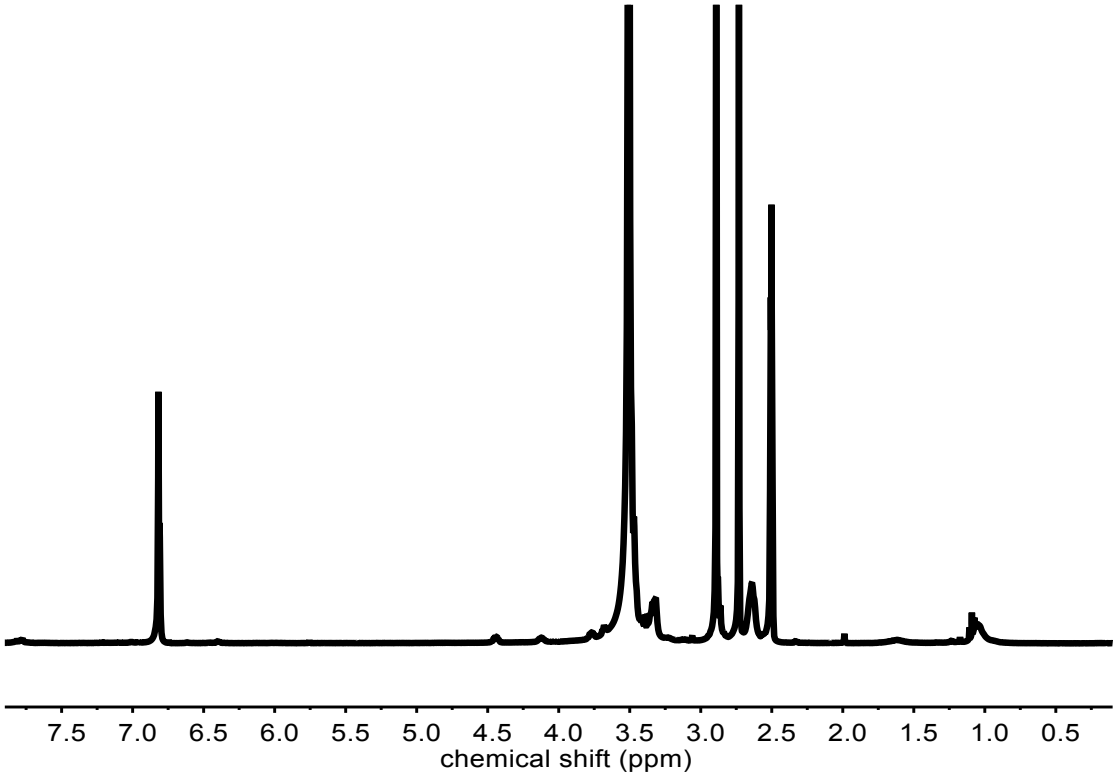


Figure S20. The ^{1}H NMR spectrum (DMSO-D6) of PEG-(GAPOSS)$_4$.

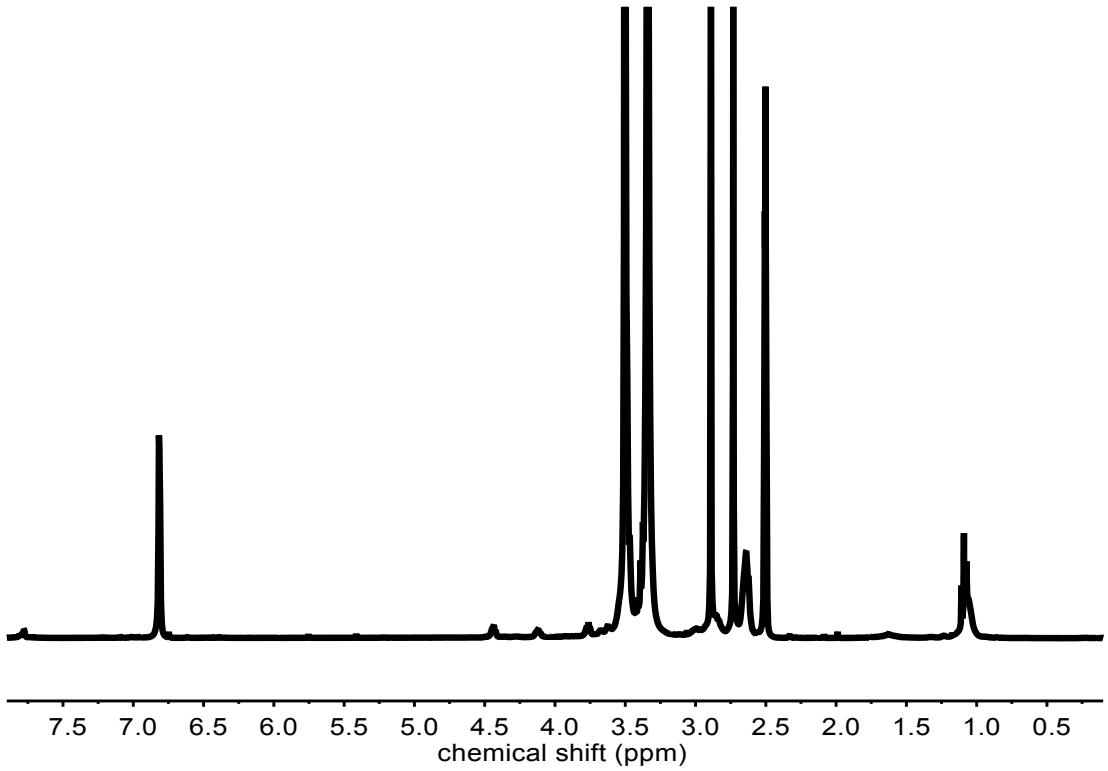


Figure S21. The ^{1}H NMR spectrum (DMSO-D6) of PEG-$(GAPOSS)_8$.

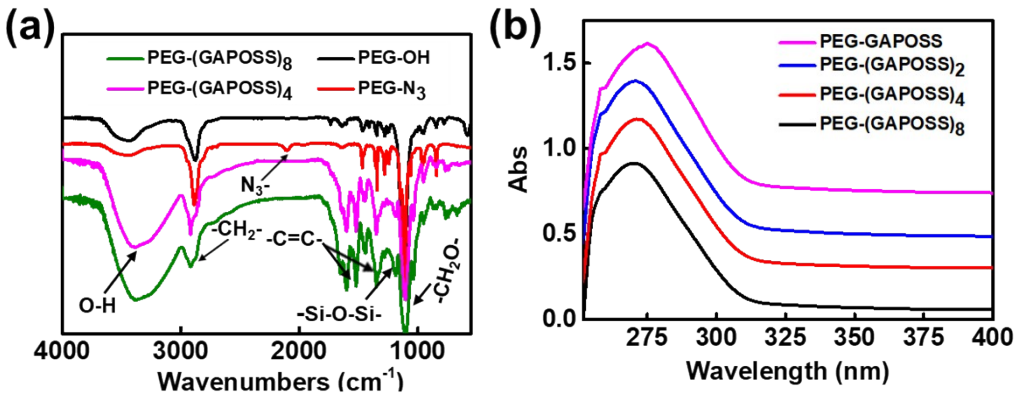


Figure S22. (a) The FT-IR spectra of the PEG, PEG-N_3 and PEG-$(GAPOSS)_n$; (b) the UV spectra of the PEG-$(GAPOSS)_n$ (n=1, 2, 4, 8).

MDA Testing: HUVEC cells were cultured in DMEM medium and incubated in 6 well plate for 12 h. The cells were then treated with PEG-(GAPOSS)4 at different concentrations for another 24 h, and incubated with 100 μM H2O2 for another 24 h. The cells are further digested with trypsin and collected for subsequent treatment. The cells were lysed with cell lysis buffer of Western and IP, and then the lysate was centrifuged with 12000 r/min for 10 minutes to collect the supernatant for follow-up determination. After the preparation of the sample, the BCA protein assay kit can be used to determine the protein concentration, and thiobarbituric acid (TBA) was used to quantitatively detect MDA in the cell lysate. The MDA content in the initial sample can be expressed by the protein content per unit weight.

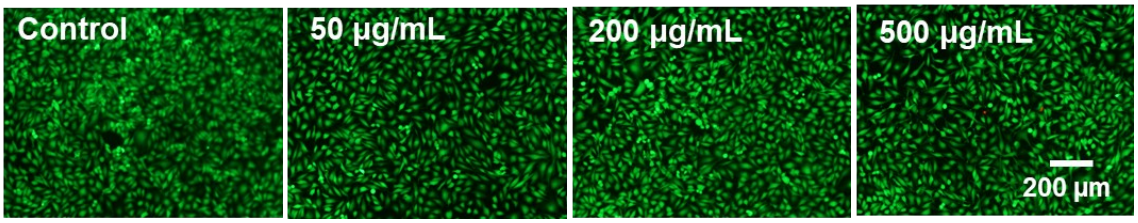


Figure S23. Live/dead staining of HUVECs incubated with PEG-$(GAPOSS)_4$ at various concentrations (50~500 μg/mL) for 24 h.

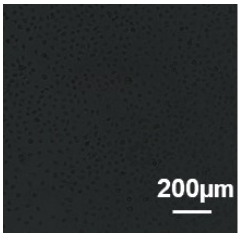


Figure S24. Fluorescence microscope image of ROS level in negative control (NC) group after 24 h cells incubation.