Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

Synthesis and self-assembly of photo-responsive polypeptoid-based copolymers containing azobenzene side-chains

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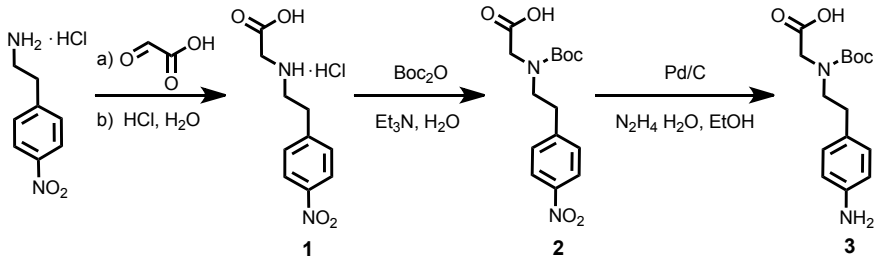
1. Materials

Methoxypoly(ethylene glycol) amine (*m*PEG-NH_2, M_w = 5000 Da) was purchased from Ponsure Biotech, Inc (Shanghai, China). 4-Nitrophenylethylamine hydrochloride was purchased from Accela ChemBio Co., Ltd. Glyoxylic acid monohydrate (99%) and di-*tert*-butyl dicarbonate were purchased from Adamas Reagent Co., Ltd.. 10% Palladium on carbon (wetted with 55% H_2O) was purchased from Bide Pharmatech Ltd. Hydrazine monohydrate (98%) was purchased from Aladdin Reagent. Phosphorus tribromide and triethylamine were purchased from Sinopharm Chemical Reagent Co., Ltd.. Anhydrous *N*,*N*-dimethylformamide (DMF) was purchased from J&K Scientific Ltd. prior to polymerization. All other chemicals were purchased from commercial sources and used as received.

2. Methods

Both ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance III HD 500 MHz NMR spectrometer. DMSO-d_6, $CDCl_3$ and CF_3COOD were used as solvents. Gel permeation chromatography (GPC) was performed at 30 °C on a HLC-8320 GPC system with 0.01 M LiBr in DMF as the eluent at a flow rate of 0.6 mL/min. Conventional calibrations were performed using polystyrene standards (PS). The concentrations of all polymer samples were about 5 mg/mL. FT-IR spectra were performed on a ThermoFisher Nicolet 6700 spectrometer at frequencies ranging from 4000 to 400 nm^{-1} at 25 °C. The samples were thoroughly mixed with KBr crystal and pressed into pellet form. Thermogravimetric analysis (TGA) was conducted using a PerkinElmer Pyris 1 thermogravimetric analyzer. All samples were heated from ambient temperature to 600 °C at 10 °C/min under nitrogen flow (50 mL/min). Differential scanning calorimetry (DSC) was measured using a Netzsch DSC 204 F1. All samples were heated from -50 °C to 250 °C at 5 °C/min for 3 cycles. TEM experiments were performed on a Talos L120C G2 with a Ceta CMOS camera and Thermo Scientific Maps analysis software. 4 μL of polymer solution was pipetted onto holey carbon-coated 300 mesh copper grids. The excess amount of solution was removed. The residual solvent was evaporated for at least 24 hours unless anything noted. AFM studies were preformed on a Bio-Fastscan AFM in ambient air with Nanoscope software. The intensity-averaged hydrodynamic diameter (D_h) and zeta potential of the polymer assemblies was measured at 25 °C by dynamic light scattering (DLS) using a ZS90 Malvern Zetasizer instrument. Fluorescence spectra were recorded on a PerkinElmer LS55 fluorescence spectrometer. The UV-Vis spectra of the samples were measured at room temperature using a Shimadzu UV-1800 spectrometer in the range of 200-600 nm. Circular dichroism (CD) spectra were recorded on a Jasco J815 CD spectrometer with a 1 mm path length quartz cell.

3. Synthesis and characterization of the monomer Azo-NNCA[1-3]



Scheme S1. Synthetic route of monomer 3.

Synthesis of 2-(4-nitrophenethyl amino) acetic acid hydrochloride (1)

Nitrophenethylamine hydrochloride (20.66 g, 0.10 mol) was neutralized with triethylamine (15.30 mL, 0.11 mol) in 150 mL CH_2Cl_2 and stirred for 30 min, in which glyoxylic acid monohydrate (18.41 g, 0.20 mol) was added at room temperature. The mixture was stirred vigorously for 24 h, filtered and the solvent was evaporated. The obtained brown oil was redissolved in 200 mL 1 M HCl and refluxed at 100 °C for over 24 h. The water was removed by rotary evaporation to afford a white solid. Further purification of the crude product by recrystallization (methanol/diethyl ether) afforded a white needle crystal, 2-(4-nitrophenethyl amino) acetic acid hydrochloride. Yield: 21.61 g, 83%. ^{1}H NMR (500 MHz, D_2O) δ (ppm): 3.37 (m, 2H), 3.71 (s, 2H), 3.91 (m, 2H), 7.43 (d, 2H), 8.11 (d, 2H). ^{13}C NMR (125 MHz, D_2O) δ (ppm): 168.9, 167.6, 146.8, 144.2, 144.1, 129.8, 124.1, 53.4, 47.8, 31.4.

Synthesis of 2-((*tert*-butoxycarbonyl)-4-nitrophenethyl amino) acetic acid (2)

(4-Nitrophenethyl amino) acetic acid hydrochloride (21.61 g, 0.083 mol) and triethylamine (57.68 mL, 0.415 mol) were dissolved in 200 mL deionized water, followed by the addition of di-*tert*-butyl dicarbonate (45.44 g, 0.208 mol) and stirring for 30 h at room temperature. The reaction solution was washed with *n*-hexane and acidified by 1 M HCl to pH 3, then the solution was extracted with ethyl acetate (3×50 mL). The organic phase was collected and washed with brine twice, dried over anhydrous Na_2SO_4. Further purification of the crude product by column chromatography (ethyl acetate/petroleum ether = 1:5) afforded a pale yellow oil, 2-((*tert*-butoxycarbonyl)-4-nitrophenethyl amino) acetic acid. Yield: 15.07 g, 56%. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 1.41 (s, 9H), 2.95 (m, 2H), 3.52 (m, 2H), 3.90 (d, 2H), 7.35 (dd, 2H), 8.15 (t, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 174.8, 171.4, 155.6, 154.9, 146.7, 146.6, 129.7, 123.7, 81.1, 50.0, 49.7, 35.0, 28.2.

Synthesis of 2-((*tert*-butoxycarbonyl)-4-anilinoethyl amino) acetic acid (3)

2-((*Tert*-Butoxycarbonyl)-4-nitrophenethyl amino) acetic acid (15.07 g, 0.047 mmol) was added into a suspension of 50 mg Pd/C in ethanol (150 mL), and 40 mL hydrazine hydrate (98%) was added. The reaction mixture was refluxed at 80 °C for 24 h, and the reaction progress was monitored by thin-layer chromatography (TLC). The insoluble precipitate was filtered and washed with ethanol (3×15 mL), the filtrate was concentrated by rotary evaporator and dried in a vacuum oven. Further purification of the crude product by column chromatograghy (ethyl acetate/petroleum ether = 1:1) afforded a white solid, 2-((*tert*-butoxycarbonyl)-4-anilinoethyl amino) acetic acid. Yield: 12.59 g, 92%. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 1.35 (d, 9H), 2.54 (m, 2H), 3.24 (m, 2H), 3.51 (d, 2H), 4.49 (b, 2H), 6.47 (d, 2H), 6.80 (t, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 173.4, 169.0, 155.4, 155.2, 147.0, 129.4, 126.1, 114.4, 78.3, 50.0, 33.8, 33.3, 28.5.

Synthesis of 2-((*tert*-butoxycarbonyl)-2-azobenzeneethyl amino) acetic acid (4)

((*Tert*-butoxycarbonyl)-4-anilinoethyl amino) acetic acid (12.59 g, 0.043 mmol) was dissolved in 100 mL glacial acetic acid, and nitrosobenzene (4.61 g, 0.043 mol) was then added. The solution was overnight stirred at room temperature. The solvent was removed by evaporation and the residual was redissolved in CH_2Cl_2. Further purification of the crude product by column chromatography (ethyl acetate/petroleum ether = 1:10) afforded a dark red oil, 2-((*tert*-butoxycarbonyl)-2-azobenzeneethyl amino) acetic acid. Yield: 10.49 g, 64%. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 1.35 (d, 9H), 2.54 (m, 2H), 3.24 (m, 2H), 3.51 (d, 2H), 7.44 (dd, 2H), 7.53-7.61 (m, 3H), 7.83 (m, 2H), 7.87 (m, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 171.7, 154.8, 152.2, 150.7, 143.7, 130.2, 129.7, 129.6,

122.9, 122.7, 79.2, 49.5, 34.5, 34.1, 28.2.

Synthesis of *N*-azobenzeneethyl *N*-carboxyanhydride (Azo-NNCA)

2-((*Tert*-butoxycarbonyl)-2-azobenzeneethyl amino) acetic acid (10.49 g, 0.027 mmol) was dissolved in 80 mL anhydrous CH_2Cl_2 under Ar atmosphere and cooled in ice bath. Phosphorus tribromide (1.50 mL, 0.016 mmol) was added dropwise over 30 min. After stirring for another 3 h, the solution was filtered and concentrated under vacuum and the yellow solid was obtained. Further purification of the crude product by recrystallization (THF/*n*-hexane) afforded an orange solid, *N*-azobenzeneethyl *N*-carboxyanhydride. Yield: 4.32 g, 51%. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.96 (t, 2H), 3.58 (t, 2H), 4.31 (s, 2H), 3.84 (d, 2H), 7.52 (d, 2H), 7.55–7.63(m, 3H), 7.85 (d, 2H), 7.88 (d, 2H). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 167.6, 152.5, 152.2, 150.9, 142.6, 130.1, 129.7, 129.6, 123.0, 122.7, 49.7, 44.4, 32.9.

Synthesis of homopolymer poly(*N*-azobenzeneethyl glycine) (PAzo) and block copolymer (PEG-*b*-PAzo$_n$)

All polymerizations were carried out in argon-filled gloverbox, and all polymerization flasks were flame-dried. As an example of Azo-NNCA polymerization initiated by *m*PEG-NH_2, Azo-NNCA (0.5 g, 1.62 mmol) was dissolved in anhydrous DMF and placed in a polymerization flask, followed by the addition of *m*PEG-NH_2 (404.4 mg, 0.081 mmol). The flask was sealed and heated at 55 °C for 48 h. The PEG-*b*-PAzo$_n$ was precipitated in cold diethyl ether and collected by centrifugation. After dried in vacuum at 40 °C, an orange solid was obtained, Yield: ~ 65%. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 2.89 (t, 82H), 3.04 (t, 82H), 3.23 (s, 3H), 3.30-3.64 (s, 452H), 3.93 (t, 82H), 7.36–7.67 (m, 105H), 7.71–7.93 (m, 84H).

Preparation of polymer solution

The self-assemblies of PEG-*b*-PAzo$_n$ were prepared via a nanoprecipitation approach. PEG-*b*-PAzo$_n$ copolymers (4 mg) were first dissolved in 2 mL DMSO, and the mixtures were added to 4 mL deionized water under slightly stirring. After the addition was completed, the transparent solution continued to stir for 0.5 h, followed by the removal of DMSO using dialysis method for 2 days, resulting in a series of polymer solutions for further use. The solution was irradiated with a high pressure mercury UV lamp (500 W) and a xenon lamp (800 W), respectively. The temperature of the samples was maintained to 25 °C using a water bath.

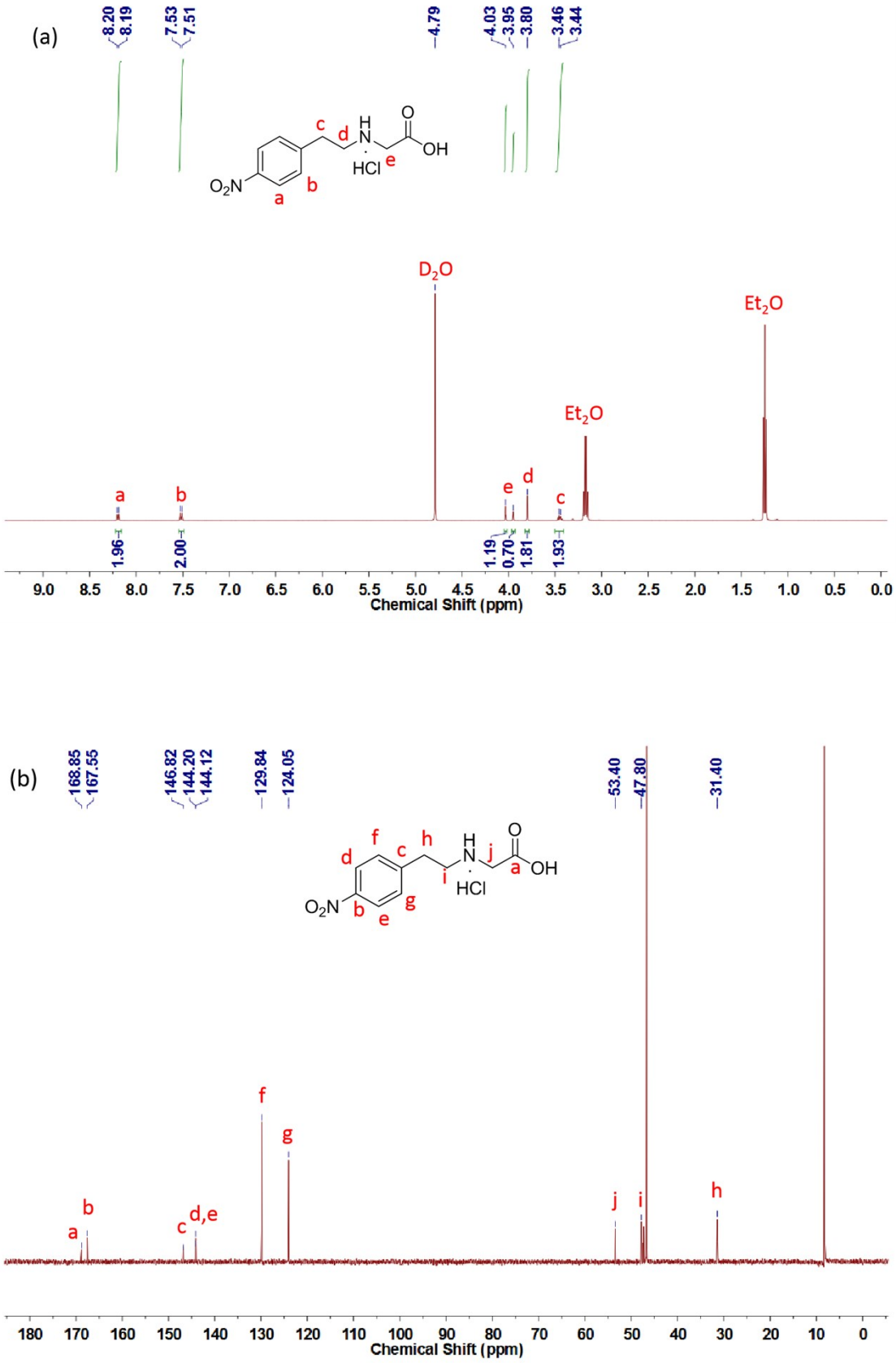


Fig. S1 (a) ^{1}H NMR and (b) ^{13}C NMR spectra of compound **1** in D_2O.

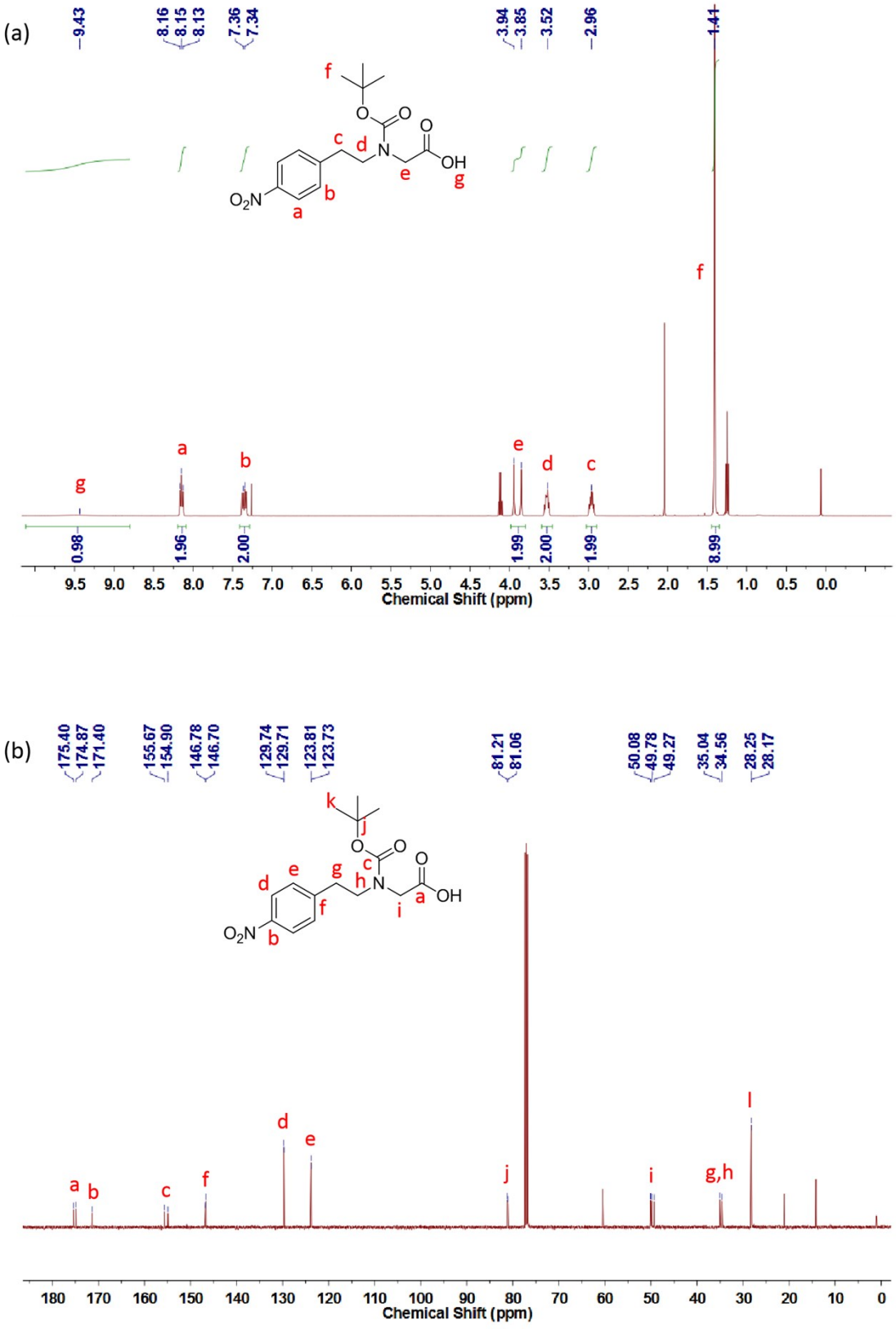


Fig. S2 (a) ^{1}H NMR and (b) ^{13}C NMR spectra of compound **2** in $CDCl_3$.

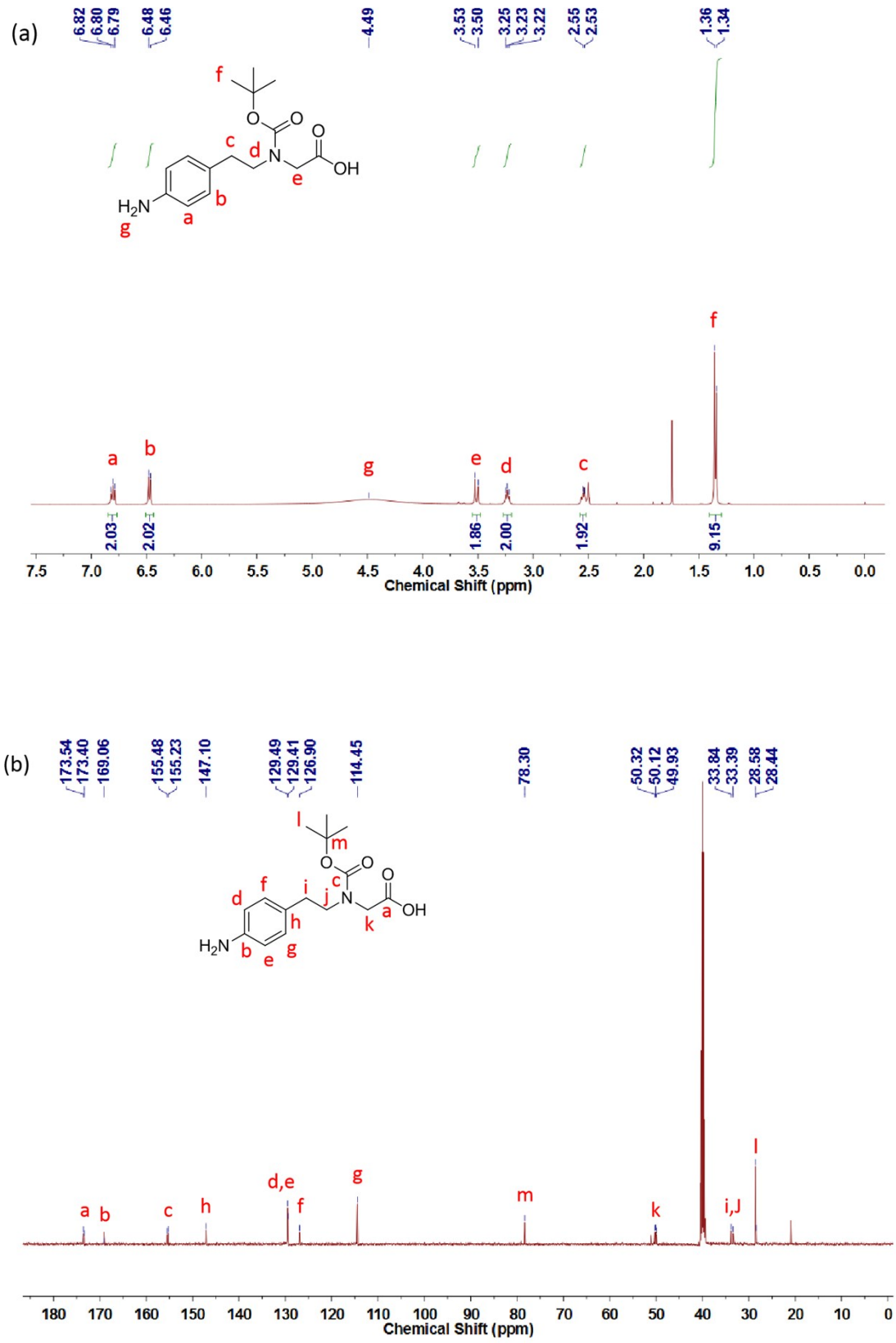


Fig. S3 (a) ^{1}H NMR and (b) ^{13}C NMR spectra of compound **3** in DMSO-d_6.

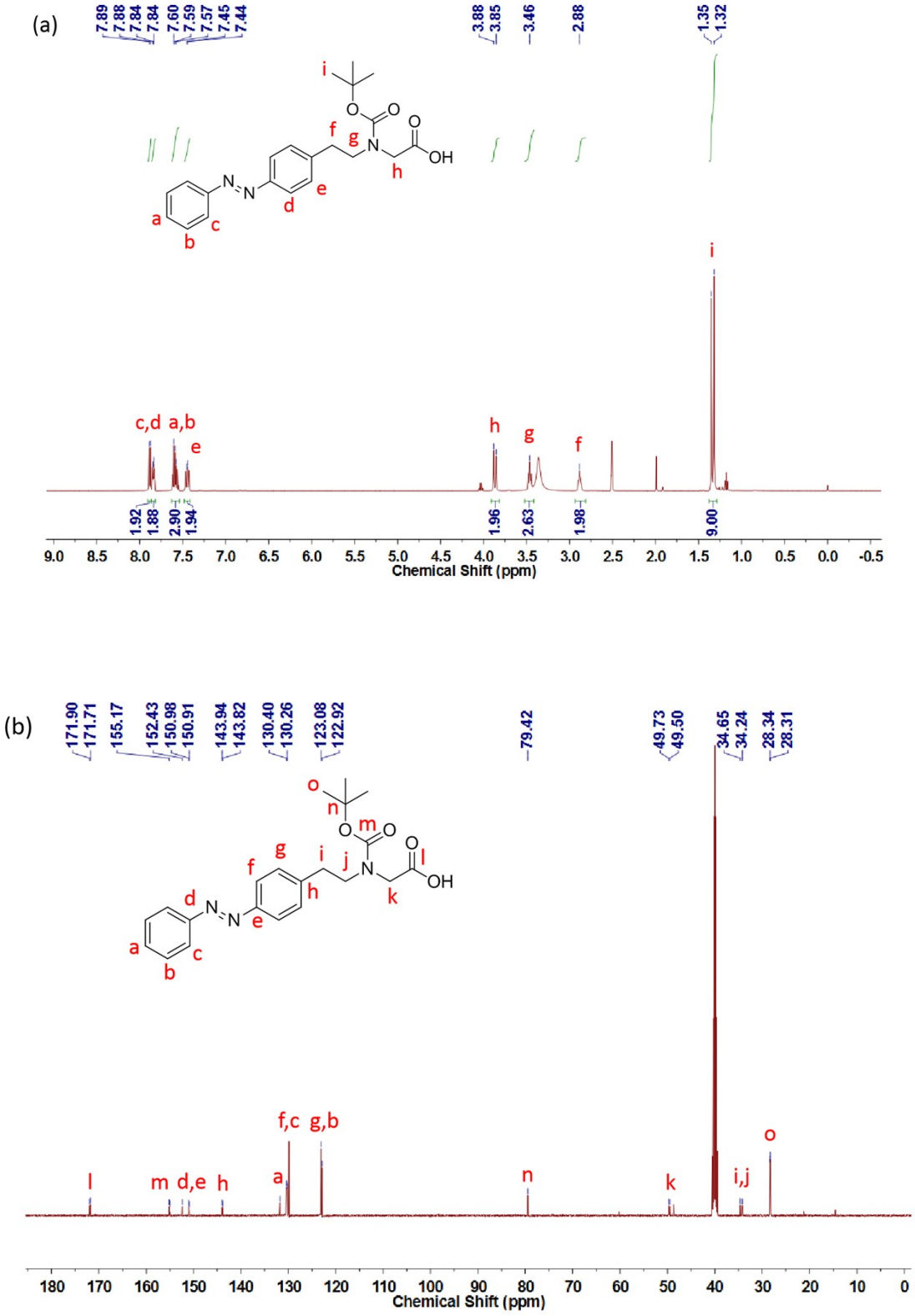


Fig. S4 (a) ^{1}H NMR and (b) ^{13}C NMR spectra of compound **4** in DMSO-d_6.

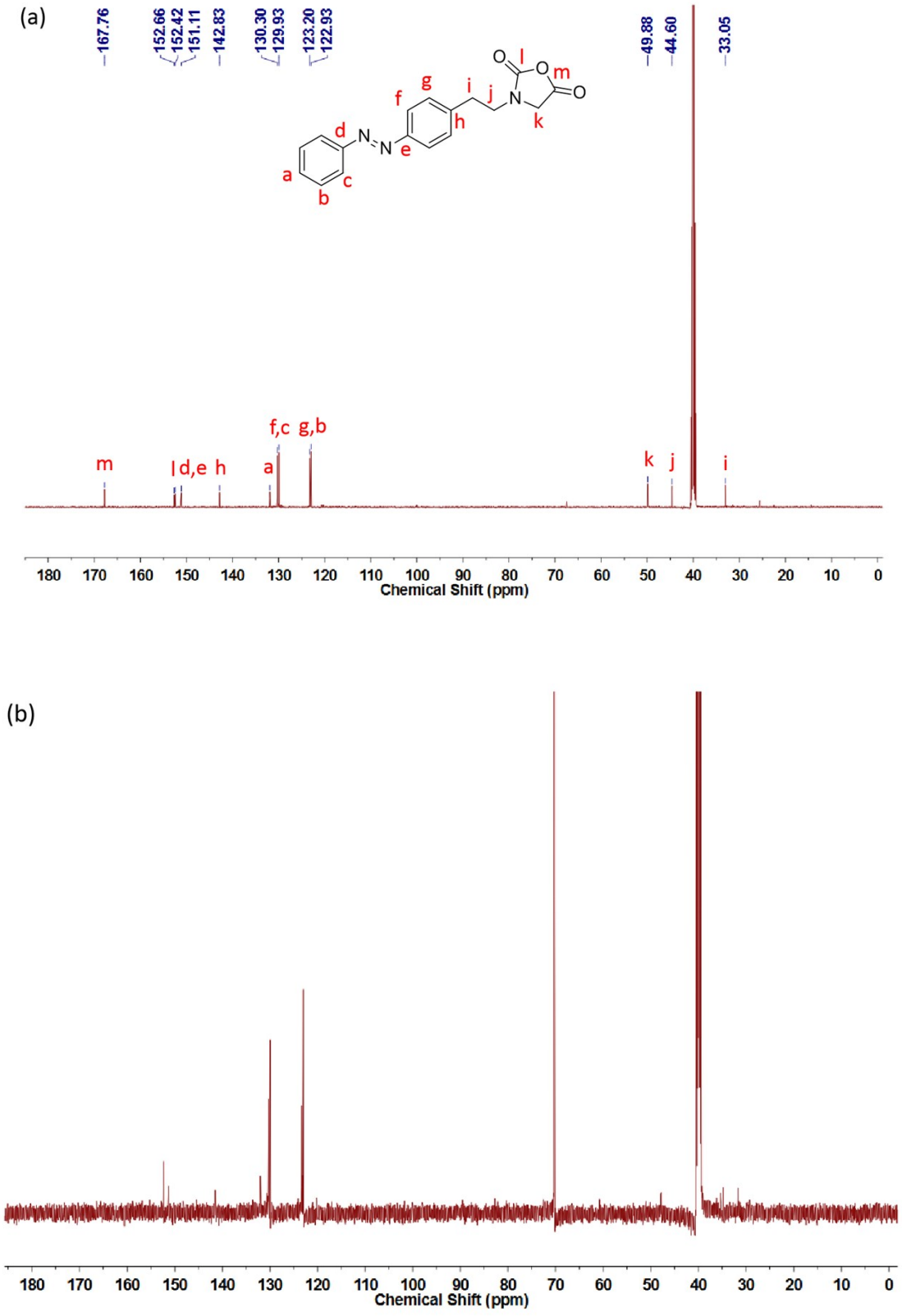


Fig. S5 (a) ^{13}C NMR spectra of Azo-NNCA and (b) PEG-*b*-PAzo$_{21}$ in DMSO-d_6.

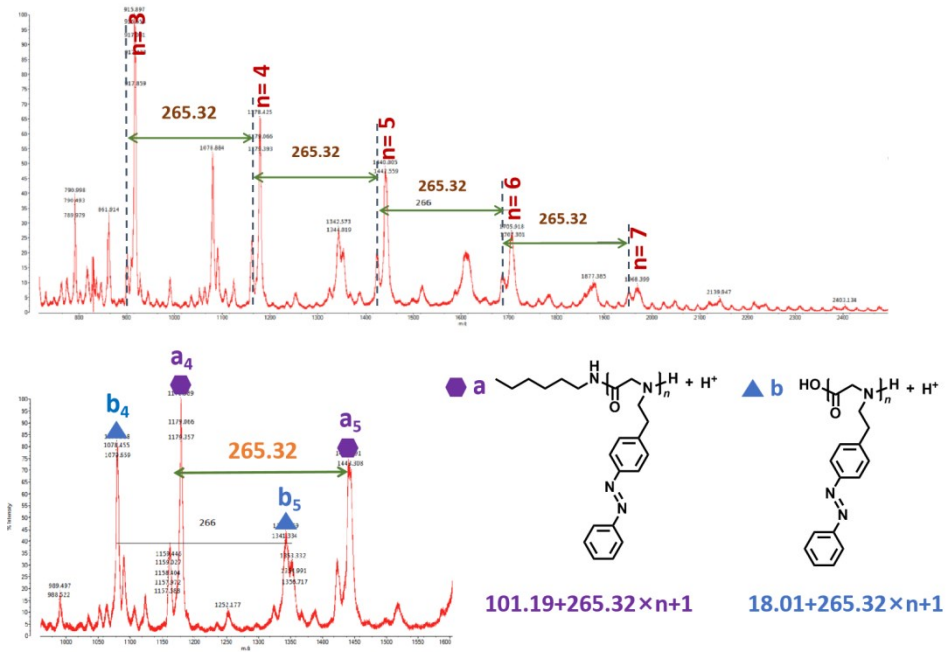


Fig. S6 Representative full and expanded MALDI-TOF spectrum of $PAzo_{21}$. matrix: DCTB.

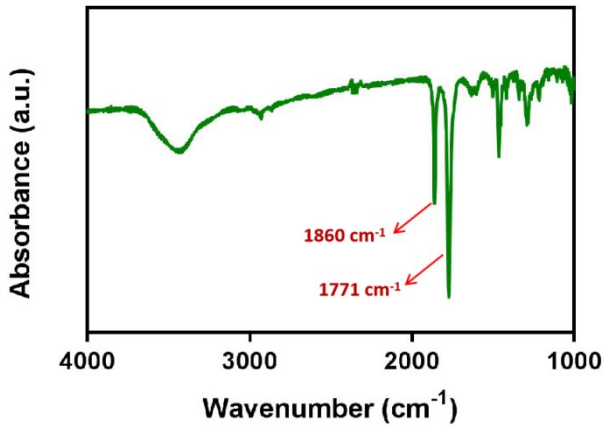


Fig. S7 FT-IR spectrum of monomer Azo-NNCA.

Table S1. Molecular characteristics of homopolymers $PAzo_n$

Entry	Samples	M/I [a]	$M_{n\ theor.}$ [b]	$M_{n\ NMR}$ [c]	$M_{n\ GPC}$ [d]	PDI [d]
1	$PAzo_5$	10	2750	1690	-	-
2	$PAzo_{10}$	20	5400	3020	5690	1.75
3	$PAzo_{21}$	40	10700	5670	7400	1.65

[a] feed molar ratio of the monomer to hexylamine. [b] Determined based on feeding *M/I* ratio. [c] Calculated from ^{1}H NMR spectra. [d] Determined by GPC in 0.1 M LiBr/DMF and conventional calibrations were preformed using polystyrene standard. All samples could be not dissolved in DMF.

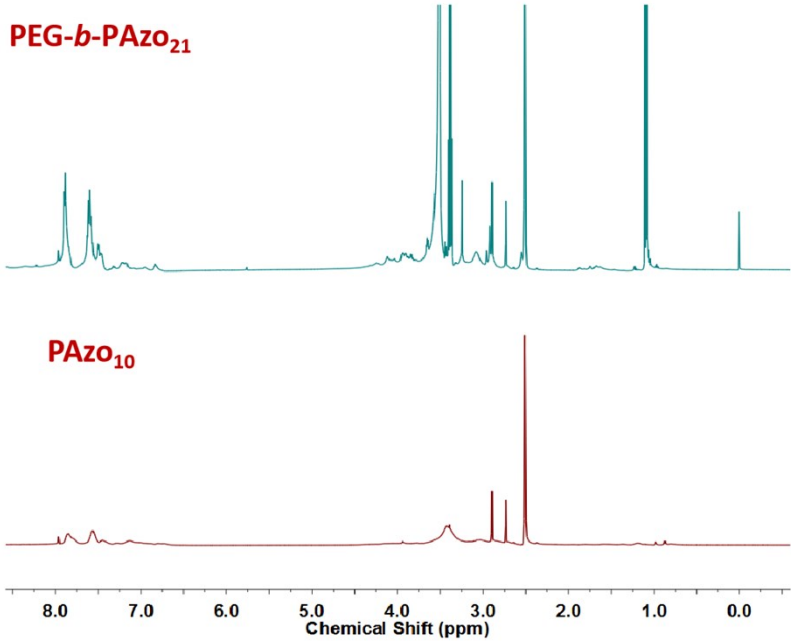


Fig. S8 ^{1}H NMR spectra of PEG-*b*-PAzo$_{21}$ and PAzo$_{10}$.

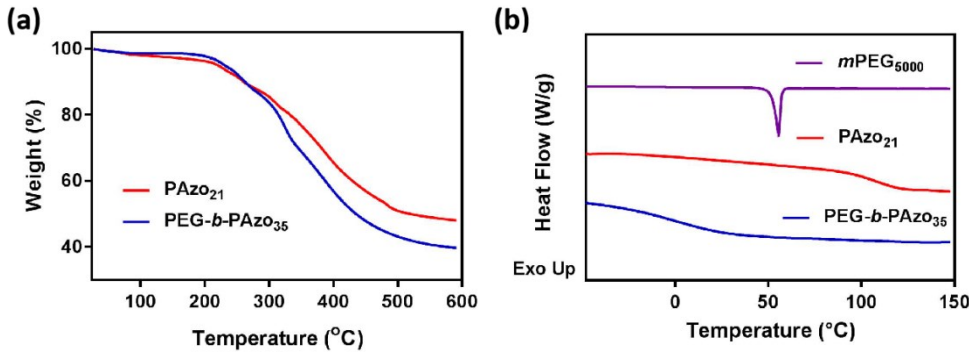


Fig. S9 (a) TGA and (b) DSC curves of PAzo$_{21}$ and PEG-*b*-PAzo$_{35}$.

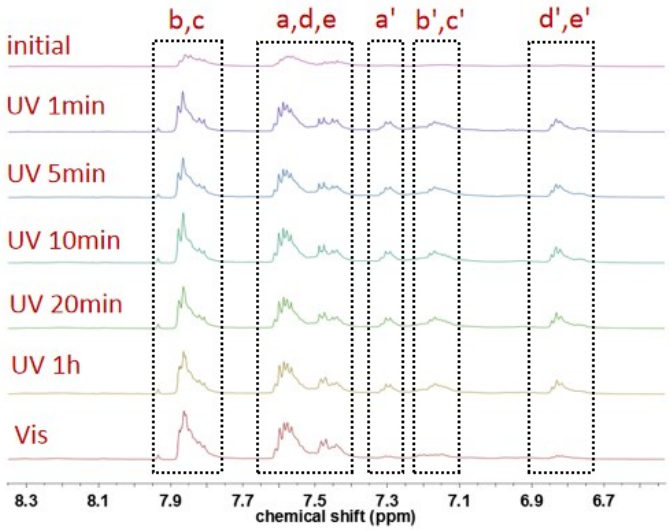


Fig. S10 ^{1}H NMR spectra of PEG-*b*-PAzo$_{21}$ in DMSO-d_6 irradiated by UV and visible light.

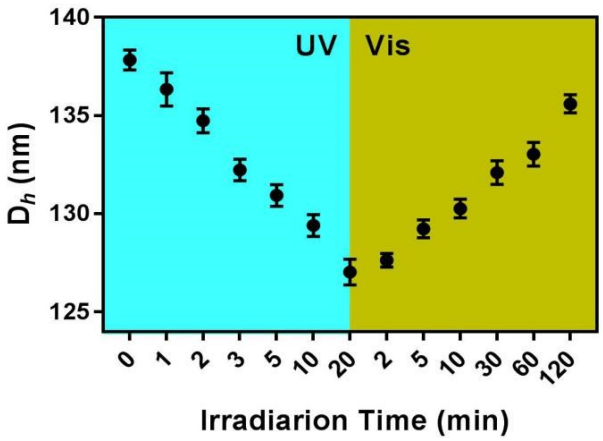


Fig. S11 The hydrodynamic diameter (D_h) of PEG-*b*-PAzo$_{21}$ assemblies after UV and visible light irradiation continuously.

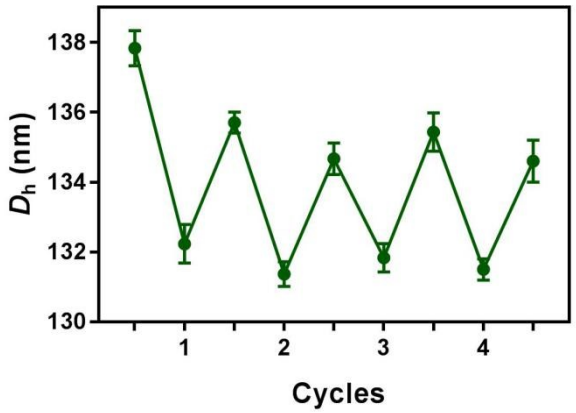


Fig. S12 The hydrodynamic diameter (D_h) of PEG-*b*-PAzo$_{21}$ assemblies after UV and visible light irradiation cycles (A cycle consists of 3 min UV irradiation and 25 min visible light irradiation).

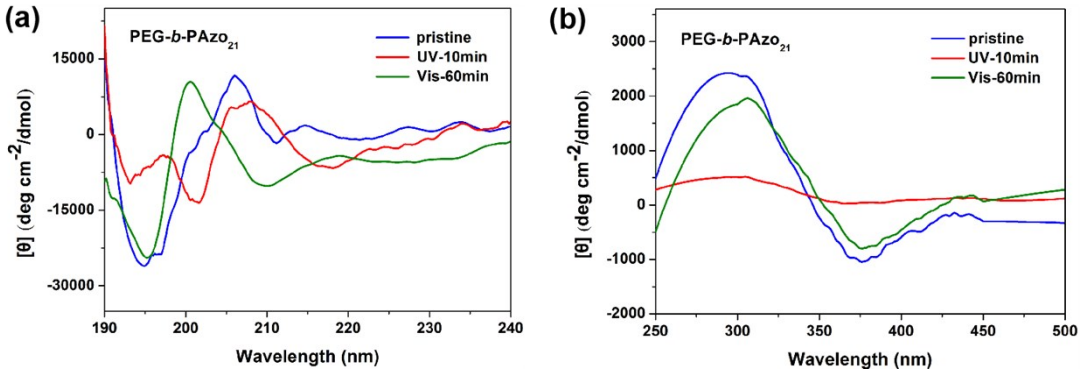


Fig. S13 The circular dichroism (CD) spectra of PEG-*b*-PAzo$_{21}$ in HIFE after UV and visible light irradiation continuously.

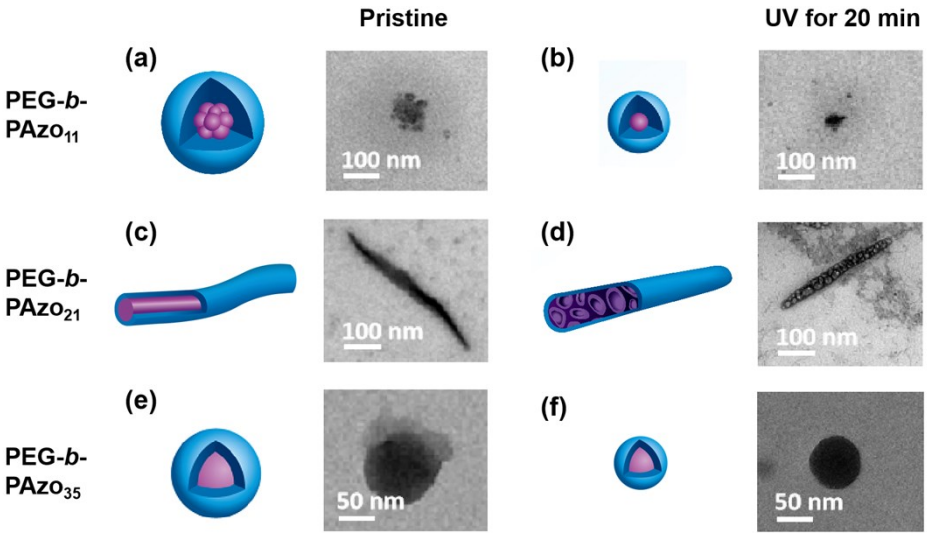


Fig. S14 Schematic and TEM image of PEG-*b*-PAzo$_n$ before and after UV irradiation.

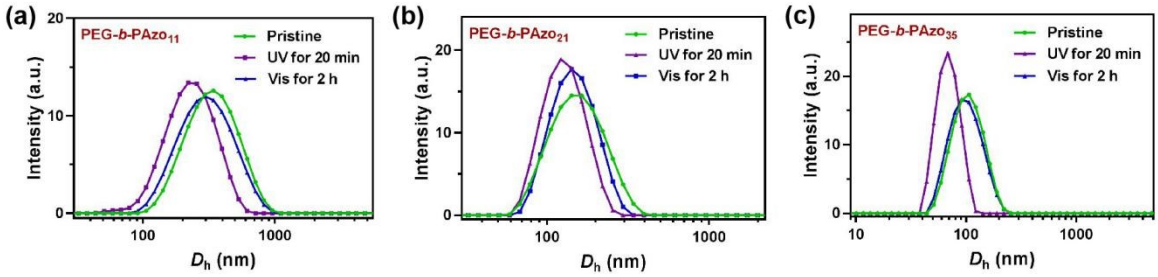


Fig. S15 The D_h distribution of PEG-*b*-PAzo$_n$ without UV irradiation, with 20 min UV irradiation and after 2 h visible light irradiation.

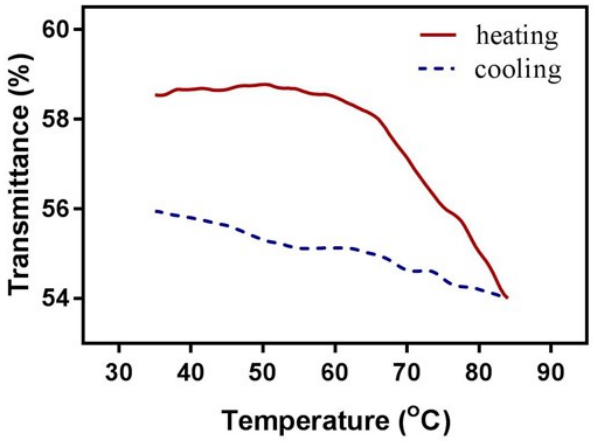


Fig. S16 Plots of transmittance as a function of temperature for PEG-*b*-PAzo$_{11}$ aqueous solution (c = 0.5 mg/mL).

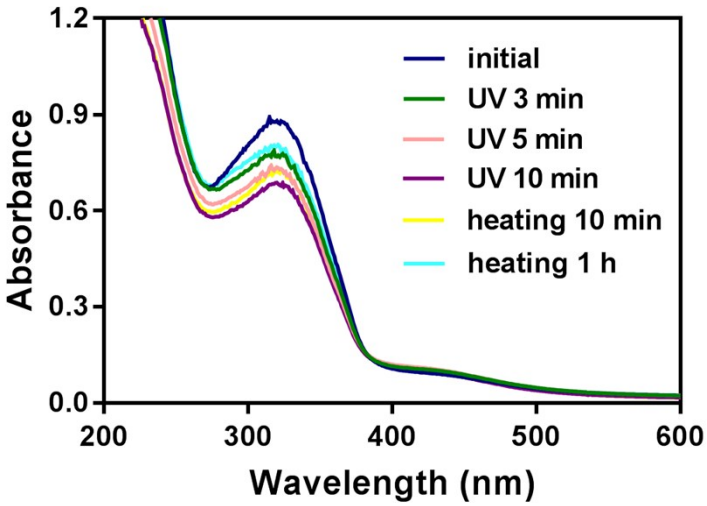


Fig. S17 UV-Vis spectra of PEG-*b*-PAzo$_{21}$ solution (c = 0.15 mg/mL) irradiated by UV light and heating at 70 °C in the dark.

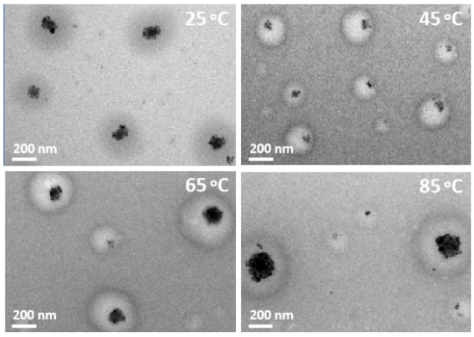


Fig. S18 TEM images of PEG-b-PAzo$_{11}$ micelles after heating at different temperatures

Supplementary References

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3. J. R. Wei, J. Sun, X. Yang, S. F. Ji, Y. H. Wei and Z. B. Li, *Polym. Chem.*, 2020, **11**, 337-343.