Electronic Supplementary Information (ESI)

Water-soluble mitochondria-targeting polymeric prodrug micelles for fluorescence monitoring and high intracelluar anticancer efficiency

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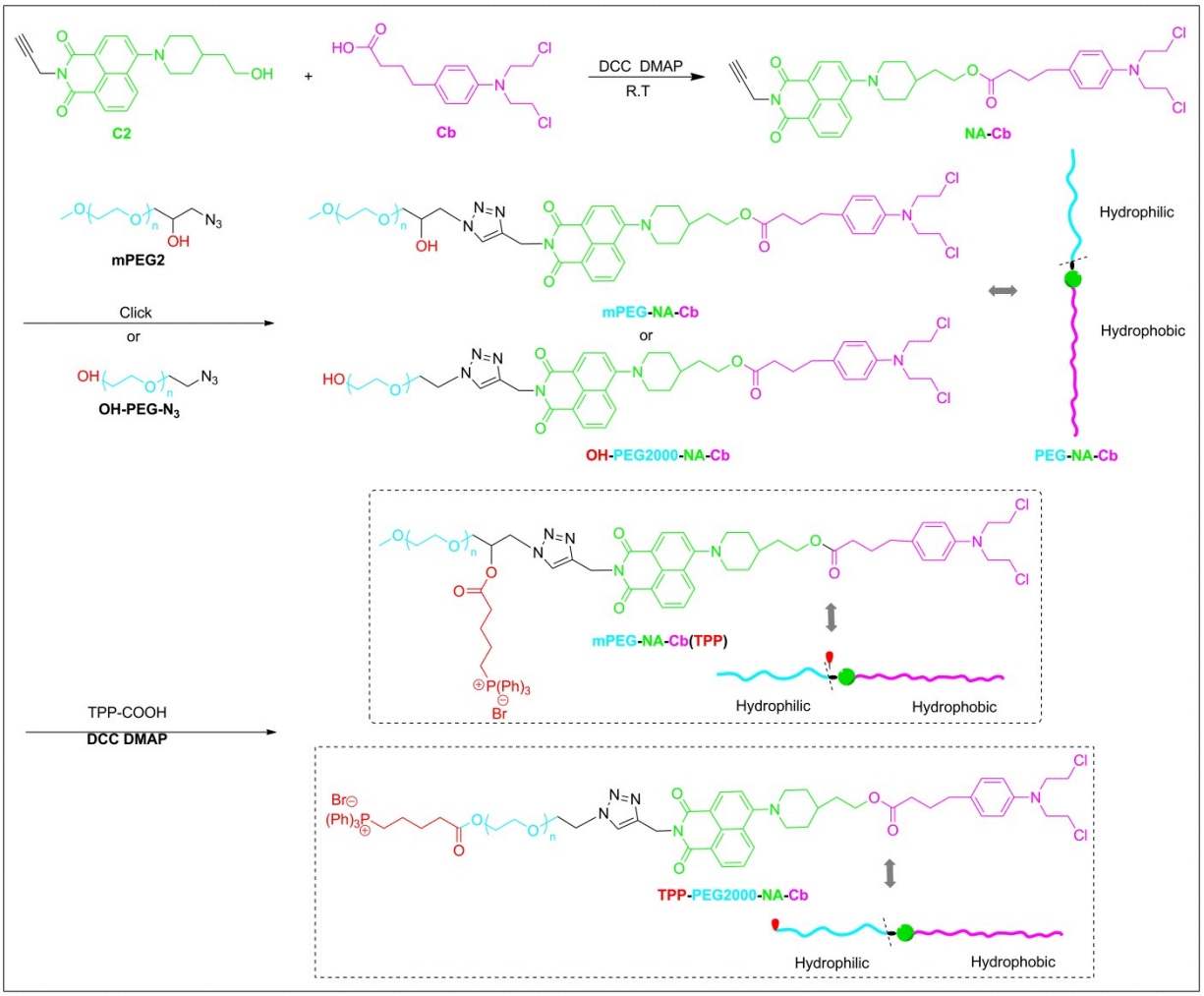
1. Materials and Measurement

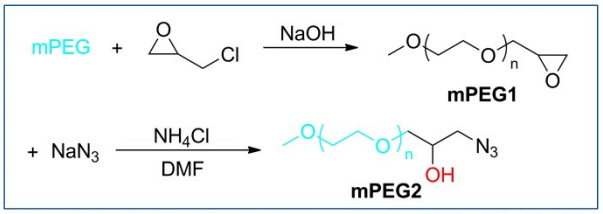
MTS 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium was purchased from Sigma-Aldrich (St. Louis, MO), **mPEG**, CuBr (99%), and *N, N′, N′, N″, N″*-pentamethyldietylenetriamine (PMDETA, 99%) were purchased from Aladdin reagent (Shanghai, China), chlorambucil (98%) and 4-piperidineethanol (96%) was purchased form J & K Scientific respectively, and used as received. Dichloromethane (DCM) and Dimethylformamide (DMF), tetrahydrofuran (THF) were dried and distilled first with purification. Ultra-pure water was used in the experiments. All other agents and solvents were purchased from commercial sources and used directly without further purification. **C2** were synthesized according to literature reported. [1]

^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker DMX400 Spectrometer in $CDCl_3$ and given in relative to internals reference TMS standard. HRMS spectral data were measured on a Bruker Daltonics Bio TOF mass spectrometer. Gel permeation chromatography (GPC) to comfirm the polymer molecular weights and their distributions was carried out on a Waters HPLC system equipped with a model 1515 isocratic pump, a 717 plus autosampler, and a 2424 refractive index (RI) detector with Waters Styragel® HT3 and HT4 columns in series. The eluting solvent was THF at a flow rate of 1.0mL/min at 45℃. The retention times were calibrated against poly (ethylene glycol) standard with the molecular weight range 600 – 80000 Da. Transmission electrom microscopy (TEM, Hitachi H-600) at an acceleration voltage of 100kV was performed to investigate the micelle morphology. The samples were prepared by dropping 2.5 mg/mL of micellar solution onto a copper grid followed by negatively staining with a 1 wt% aqueous solution of phosphotungstic acid, respectively. The size of the micelles was determined using dynamic light scattering (DLS) with the micellar solutions (1 mg mL^{-1}) filtered through a 0.45 μm syringe filter prior to measurement. The measurements were carried out at 25 ℃ using a Zetasizer Nano-ZS90 system from Malvern Instruments equipped with a 633 nm He-Ne laser using backscattering detection with a fixed detector angle of 90°. The critical micelle concentration

(CMC) was determined *via* fluorescence spectrometer. Fluorescence emission spectra were recorded on a F-7000 FL Spectrofluorophotometer (HITACHI) at 298 K. Absorption spectra were recorded on a PERSEE TU-1901UV-Visible Spectrophotometer. MTS method was used for testing the cell viability instead of MTT. Cells were obtained from Shanghai Institute of Biochemistry and Cell Biochemistry and Cell Biology, Chinese Academy of Science.



Scheme S1 The route of the synthesis of amphiphilic prodrugs.



Scheme S2 Convenient synthesis of heterobifunctional clickable mPEG with narrow polydispersity for the further modification

2. Synthesis of various compound

2.1 Convenient synthesis of heterobifunctional clickable mPEG as shown in Schme S2

2.1.1 The epoxidation of mPEG

mPEG (5 mM) and NaOH (250 mmol) were added in 30 mL epichlorohydrin. Under magnetic stirring at room temperature for 24 h with an anhydrous $CaCl_2$ tube for protection from moisture in the air, the reaction completed and subsequently with vacuum filtration to obtain the filtrate. Then product (**mPEG1-2000**) precipitated via excess adding of diethyl ether, which finally dried at room temperature in a vacuum oven overnight after filtration. Yield of **mPEG1-1000** and **mPEG1-2000** are 45.6% and 65.2%. **^{1}H NMR (400 MHz, $CDCl_3$, δ)** displayed in Fig S10 and S12.

^{1}H NMR (400 MHz, $CDCl_3$, δ) of **mPEG1-1000**: 3.65(m, 98H, methylene in mPEG unit), 3.38 (s, 3H, mEPG-terminal methyl), 3.17 (m, 1H, CH in epoxy ring), 2.80 (m, 1H, CH_2 in epoxy ring), 2.62 (m, 1H, CH_2 in epoxy ring).

^{1}H NMR (400 MHz, $CDCl_3$, δ) of **mPEG1-2000**: 3.65(m, 178H, methylene in mPEG unit), 3.38 (s, 3H, mEPG-terminal methyl), 3.17 (m, 1H, CH in epoxy ring), 2.80 (m, 1H, CH_2 in epoxy ring), 2.62 (m, 1H, CH_2 in epoxy ring).

2.1.2 Ring-Opening of mPEG1 with Sodium Azide

mPEG1 (2.9 mM) was dissolved in 30 mL DMF. Sodium azide (19.2 mM) and ammonium choride (19.2 mM) were then added. The reaction was stirred at 50 ℃ for 24 h. Then DMF was removed via vacuum evaporation, and 100 mL dichloromethane was added with subsequently filtration to remove undissolved solid. The dichloromethane solution was extracted with water (50 mL × 3), and dried over anhydrous magnesium sulfate, and filtered. Then product

precipitated by excess adding of diethyl ether, which finally dried at room temperature in a vacuum oven overnight. Yield of **mPEG2-1000** and **mPEG2-2000** are 66.3% and 78.3%. **^{1}H NMR (400 MHz, $CDCl_3$, δ)** displayed in Fig S11 and S13.

^{1}H NMR (400 MHz, $CDCl_3$, δ) of **mPEG1-1000**: 3.65(m, 89H, methylene in mPEG unit), 3.38 (s, 3H, mEPG-terminal methyl).

^{1}H NMR (400 MHz, $CDCl_3$, δ) of **mPEG1-2000**: 3.65(m, 183H, methylene in mPEG unit), 3.38 (s, 3H, mEPG-terminal methyl).

2.2 Synthesis of naphthalimide-labblled Chlorambucil (NA-Cb)

C2 (253.7 mg, 0.7 mM) was dissolved in 3 mL dry DCM. Under magnetic stirring in ice bath, DCC (213 mg, 0.7 mM) was added, then DMAP (158.9 mg, 0.7 mM), Chlorambucil (213 mg, 0.7 mM) put into this reaction mixture. About 30 minutes, the ice bath was removed and reaction stirred overnight at room temperature. After removing the DCU by filtering, the final product was obtained via column chromatography on silica gel using PE / EA (v / v = 1 : 1). Yield : 0.433g, 97.5%. **^{1}H NMR (400 MHz, $CDCl_3$, δ)** displayed in Fig. S14.

^{1}H NMR (400 MHz, $CDCl_3$, δ): 8.61 (d, *J* = 4 Hz, 1H, Ar-*H*), 8.54 (d, *J* = 8 Hz, 1H, Ar-*H*), 8.38 (d, *J* = 8 Hz, 1H, Ar-*H*), 7.69 (t, 6 Hz, Ar-*H*), 7.19 (d, *J* = 8 Hz, 1H, Ar-*H*), 7.08 (d, *J* = 8Hz, 2H, Ar-*H*), 6.63 (d, *J* = 8Hz, 2H, Ar-*H*), 4.95 (s, 2H, NCH_2), 4.21 (t, *J* = 6 Hz, 2H, piperadin-CH_2CH_2), 3.70 (m, 4H, CH_2CH_2Cl), 3.62 (m, 6H, CH_2CH_2Cl and piperadin), 2.91 (t, *J* = 10 Hz, 2H, piperadin), 2.57 (t, *J* = 2 Hz, $COCH_2CH_2CH_2$), 2.35 (d, *J* = 8 Hz, 2H, $COCH_2$), 2.17 (s, 1H, C≡CH), 1.93 (m, 4H, piperadin and $COCH_2CH_2$), 1.73 (m, 2H, piperadin-CH_2), 1.65(m, 3H, piperadin).

HRMS (ESI): Calcd for $[M+NA]^+$, 670.2215; Found, 670.2180.

2.3.1 Synthesis of non-targeted polymeric prodrugs (mPEGX-NA-Cb)

NA-Cb (0.3 mmol) and **mPEG2-X** (0.3 mmol) added in the flask. After replacing the air with argon, 3 mL dry THF was added *via* injection. Subsquently, CuBr (0.12 mmol) and PMDETA (0.12 mmol) put into the reaction mixture. The reaction was carried out at room temperature for 24 h under argon. The final product (bright yellow solid) was obtained after puring by column chromatography on silica gel using DCM / MeOH (v / v = 20 : 1). Micelles were

prepared by directly dissolved. Yield of **mPEG1000-NA-Cb**, **mPEG2000-NA-Cb** and **OH-PEG2000-NA-Cb** are 94.6%, 89% and 80% . **^{1}H NMR (400 MHz, $CDCl_3$, δ)** displayed in Fig S16, S18 and S20.

^{1}H NMR (400 MHz, $CDCl_3$, δ) of **mPEG1000-NA-Cb**: 8.58 (d, *J* = 8 Hz, 1H, Ar-*H*), 8.50 (d, *J* = 8 Hz, 1H, Ar-*H*), 8.36 (d, *J* = 8 Hz, 1H, Ar-*H*), 7.80 (s, 1H, C*H*=C- N=N-), 7.67 (t, *J* = 8 Hz, Ar-*H*), 7.15 (d, *J* = 12 Hz, 1H, Ar-*H*), 7.08 (d, *J* = 8Hz, 2H, Ar-*H*), 6.43 (d, *J* = 8Hz, 2H, Ar-*H*), 5.49 (s, 2H, NCH_2), 4.47 （m, 1H, OH-CH-CH_2）, 4.35 （m, 1H, OH-CH-CH_2）, 4.21 (t, *J* = 6 Hz, 2H, piperadin-$CH_2C$$H_2$), 3.65 (m, 98H, methylene in mPEG unit, piperadin, and CH_2CH_2Cl), 3.38 (s, 3H, mEPG-terminal methyl), 2.90 (t, *J* = 10 Hz, 2H, piperadin), 2.58 (t, *J* = 2 Hz, $COCH_2CH_2C$$H_2$), 2.35 (t, *J* = 8 Hz, 2H, COCH_2), 1.93 (m, 4H, piperadin and $COCH_2C$$H_2$), 1.73 (m, 2H, piperadin-CH_2), 1.65(m, 3H, piperadin).

^{1}H NMR (400 MHz, $CDCl_3$, δ) of **mPEG2000-NA-Cb**: 8.58 (d, *J* = 8 Hz, 1H, Ar-*H*), 8.50 (d, *J* = 8 Hz, 1H, Ar-*H*), 8.36 (d, *J* = 8 Hz, 1H, Ar-*H*), 7.79 (s, 1H, C*H*=C- N=N-), 7.67 (t, *J* = 8 Hz, Ar-*H*), 7.17 (d, *J* = 12 Hz, 1H, Ar-*H*), 7.08 (d, *J* = 8Hz, 2H, Ar-*H*), 6.43 (d, *J* = 8Hz, 2H, Ar-*H*), 5.49 (s, 2H, NCH_2), 4.47 （m, 1H, OH-CH-CH_2）, 4.35 （m, 1H, OH-CH-CH_2）, 4.20 (t, *J* = 6 Hz, 2H, piperadin-$CH_2C$$H_2$), 3.65 (m, 135H, methylene in mPEG unit, piperadin, and CH_2CH_2Cl), 3.38 (s, 3H, mEPG-terminal methyl), 2.90 (t, *J* = 12 Hz, 2H, piperadin), 2.58 (t, *J* = 6 Hz, $COCH_2CH_2C$$H_2$), 2.35 (t, *J* = 8 Hz, 2H, COCH_2), 1.93 (m, 4H, piperadin and $COCH_2C$$H_2$), 1.73 (m, 2H, piperadin-CH_2), 1.65(m, 3H, piperadin).

^{1}H NMR (400 MHz, $CDCl_3$, δ) of **OH-PEG2000-NA-Cb**: 8.58 (d, *J* = 8 Hz, 1H, Ar-*H*), 8.50 (d, *J* = 8 Hz, 1H, Ar-*H*), 8.36 (d, *J* = 8 Hz, 1H, Ar-*H*), 7.79 (s, 1H, C*H*=C- N=N-), 7.66 (t, *J* = 8 Hz, Ar-*H*), 7.17 (d, *J* = 8 Hz, 1H, Ar-*H*), 7.07 (d, *J* = 8Hz, 2H, Ar-*H*), 6.62 (d, *J* = 8Hz, 2H, Ar-*H*), 5.48 (s, 2H, NCH_2), 4.47 （m, 2H, CH_2-CH_2-N）, 4.21 (t, *J* = 6 Hz, 2H, piperadin-$CH_2C$$H_2$), 3.65 (m, 182H, methylene in mPEG unit, piperadin, and CH_2CH_2Cl), 2.92 (m, 2H, piperadin), 2.57 (t, *J* = 8 Hz, $COCH_2CH_2C$$H_2$), 2.34 (t, *J* = 6 Hz, 2H, COCH_2), 1.93 (m, 4H, piperadin and $COCH_2C$$H_2$), 1.73 (m, 2H, piperadin-CH_2), 1.65(m, 3H, piperadin).

2.3.2 Synthesis of mitochondria-targeting polymeric prodrugs

The synthesis of targeted ones was obtained according to the similar way like **NA-Cb**. Briefly, **mPEGX-NA-Cb(**0.11 mM**)** and **TPP-COOH**(0.33 mM) were coupled using DCC (0.33 mM) and DMAP (0.33 mM) in ice bath for about 30 minutes and then stirred overnight. After filtering, the final product was obtained after puring by column chromatography on silica gel

using DCM / MeOH (v / v = 10 : 1).. Micelles were prepared by directly dissolved. Yield of **mPEG1000-NA-Cb(TPP)**, **mPEG2000-NA-Cb** and **TPP-PEG2000-NA-Cb** are 83.4%, 95% and 76%. **^{1}H NMR (400 MHz, $CDCl_3$, δ)** displayed in Fig S17, S19 and S21.

^{1}H NMR (400 MHz, $CDCl_3$, δ) of **mPEG1000-NA-Cb(TPP)**: 8.53 (d, *J* = 8 Hz, 1H, Ar-*H*), 8.47 (d, *J* = 8 Hz, 1H, Ar-*H*), 8.37 (d, *J* = 8 Hz, 1H, Ar-*H*), 7.90-7.69 (m, 17H, Ar-*H* and C*H*=C- N=N-), 7.20 (d, J = 8 Hz, Ar-*H*), 7.08 (d, *J* = 8Hz, 2H, Ar-*H*), 6.63 (d, *J* = 8Hz, 2H, Ar-*H*), 5.41 (q, *J* = 6Hz, 2H, NCH_2), 4.55 (m, 1H, C*H*--CH_2-N-N=N-), 4.47 （m, 1H, -CH_2-N-N=N-）, 4.42 （m, 1H, -CH_2-N-N=N-）, 4.21 (t, *J* = 6 Hz, 2H, piperadin-CH_2CH_2), 3.65 (m, 87H, methylene in mPEG unit, TPP, piperadin, and C$H_2$$CH_2$Cl), 3.38 (s, 3H, mEPG-terminal methyl), 2.92 (t, *J* = 10 Hz, 2H, piperadin), 2.58 (t, *J* = 8 Hz, $COCH_2CH_2$CH_2), 2.35 (m, 2H, COCH_2), 1.93 (m, 6H, piperadin and $COCH_2$CH_2), 1.73-1.66 (m, 7H, piperadin, piperadin-CH_2 and $COCH_2CH_2$CH_2 in TPP).

^{1}H NMR (400 MHz, $CDCl_3$, δ) of **mPEG2000-NA-Cb(TPP)**: 8.53 (d, *J* = 8 Hz, 1H, Ar-*H*), 8.47 (d, *J* = 8 Hz, 1H, Ar-*H*), 8.37 (d, *J* = 8 Hz, 1H, Ar-*H*), 7.90-7.69 (m, 17H, Ar-*H* and C*H*=C- N=N-), 7.20 (d, J = 8 Hz, Ar-*H*), 7.08 (d, *J* = 8Hz, 2H, Ar-*H*), 6.63 (d, *J* = 8Hz, 2H, Ar-*H*), 5.40 (m, 2H, NCH_2), 4.55 (m, 1H, C*H*--CH_2-N-N=N-), 4.47 （m, 1H, -CH_2-N-N=N-）, 4.43 （m, 1H, -CH_2-N-N=N-）, 4.21 (t, *J* = 6 Hz, 2H, piperadin-CH_2CH_2), 3.65 (m, 139H, methylene in mPEG unit, TPP, piperadin, and C$H_2$$CH_2$Cl), 3.38 (s, 3H, mEPG-terminal methyl), 2.92 (t, *J* = 10 Hz, 2H, piperadin), 2.58 (t, *J* = 8 Hz, $COCH_2CH_2$CH_2), 2.35 (m, 2H, COCH_2), 1.93 (m, 6H, piperadin and $COCH_2$CH_2), 1.73-1.66 (m, 7H, piperadin, piperadin-CH_2 and $COCH_2CH_2$CH_2 in TPP).

^{1}H NMR (400 MHz, $CDCl_3$, δ) of **TPP-PEG2000-NA-Cb**: 8.58 (d, *J* = 8 Hz, 1H, Ar-*H*), 8.51 (d, *J* = 8 Hz, 1H, Ar-*H*), 8.37 (d, *J* = 8 Hz, 1H, Ar-*H*), 7.88-7.670 (m, 17H, Ar-*H* and C*H*=C- N=N-), 7.18 (d, J = 8 Hz, Ar-*H*), 7.08 (d, *J* = 8Hz, 2H, Ar-*H*), 6.63 (d, *J* = 8Hz, 2H, Ar-*H*), 5.49 (s, 2H, NCH_2), 4.47 （m, 2H, -CH_2-N-N=N-）, 4.20 (m, 2H, piperadin-CH_2CH_2), 3.64 (m, 152H, methylene in mPEG unit, TPP, piperadin, and C$H_2$$CH_2$Cl), 2.90 (t, *J* = 8 Hz, 2H, piperadin), 2.58 (t, *J* = 8 Hz, $COCH_2CH_2$CH_2), 2.35 (t, *J* = 8Hz, 2H, COCH_2), 1.93 (m, 6H, piperadin and $COCH_2$CH_2), 1.73-1.64 (m, 7H, piperadin, piperadin-CH_2 and $COCH_2CH_2$CH_2 in TPP).

Cell culture were carried out following previously reported procedures.[1]

2.4 CLSM, MTS method and flow cytometry

2.4.1 Cell imaging and mitochondrial target HeLa cells were pre-washed twice and then incubated with 25 or 50 μg mL^{-1} prodrugs and **MT DeepRed** at 37 °C for separately 30 minutes. Then the cells were washed to remove unbounded probes before in situ imaging by confocal laser scanning microscopy analysis (CLSM, ZEISS LSM 780) at excitation wavelengths of 405 nm for **NA** and 633 nm for **MT DeepRed**, respectively.

2.4.2 Cytotoxicity assay Toxicity was determined by MTS method. About 8000 cells per well were seeded in 96-well plates and cultured overnight for 70-80% cell confluence. The medium was replaced with 100 μL of fresh medium with 10% FBS, to which also with different tested concentrations of prodrugs. 48 hours later, 20% MTS solution in PBS was added to each well for additional incubation. The absorbance was measured using an ELISA plate reader (model 680, BioRad) at a wavelength of 490 nm. The metabolic activity of the prodrugs treated cells was expressed as a relative to untreated cell controls taken as 100% metabolic activity. The concentrations of prodrugs were calculated by their theoretical value of molecular weights of prodrugs.

2.4.3 Celluar uptake Celluar uptake of prdrugs was analyzed by flow cytometry. About 100000 cells per well were seeded in 24-well plates. HeLa Cells were incubated with 50 μg mL^{-1} prodrugs for 0.5 h or 2 h. Then the cells were washed with PBS and harvested with trypsin, and resuspended in PBS. The samples were measured in the FL2 channel. Data from 10000 events were gated and tested for 3 times.

2.5 Cb release from micelles

mPEG1000-NA-Cb(TPP) micelles were obtained in PBS at three different pH values(pH 5.00/ 7.40/ 8.00) and were placed at 37 °C in incubator. The samples were freezed in liquid nitrogen and then lyophilized at predetermined time, finally tested via HPLC against free **Cb** standard which was also placed at 37 °C in incubator at same pH.

3. Results

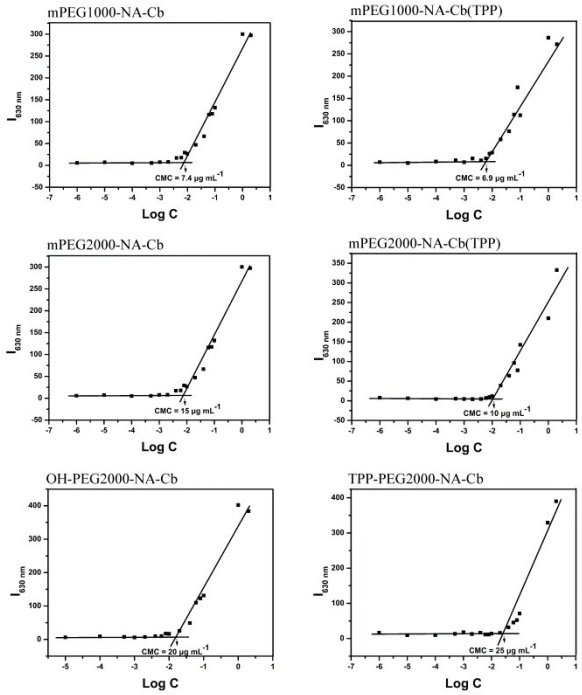


Fig. S1 Critical micelle concentration determination of prodrugs: Fluorescence intensity at λ_{em} = 630 nm of Nile red as function of logarithm of the polymer concentrations in **PBS** buffer solution (10 mM, pH = 7.40).

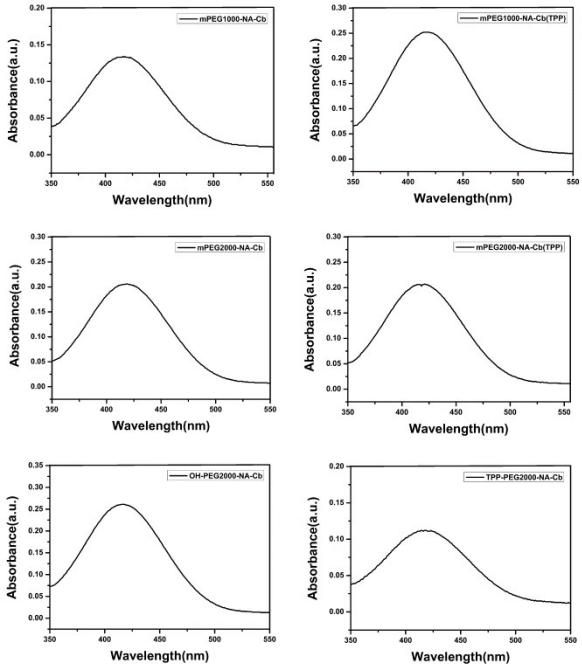


Fig. S2 UV-vis absorption spectra of prodrugs (50 μg mL^{-1}) in **PBS** buffer solution (10 mM, pH = 7.40).

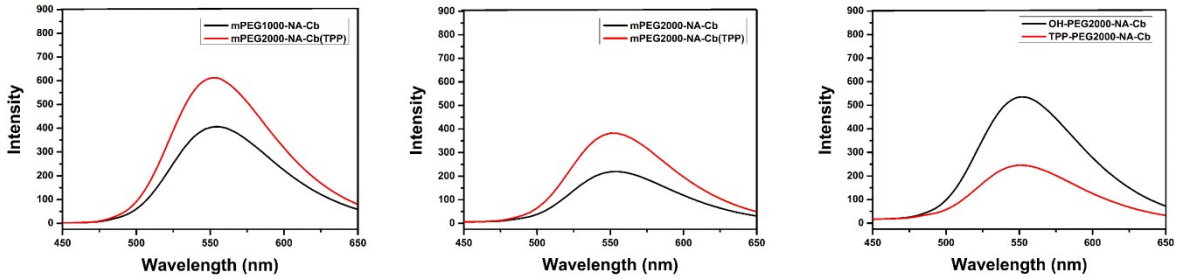


Fig. S3 Fluorescence spectra of prodrugs (50 μg mL^{-1}) in **PBS** buffer solution (10 mM, pH = 7.40).

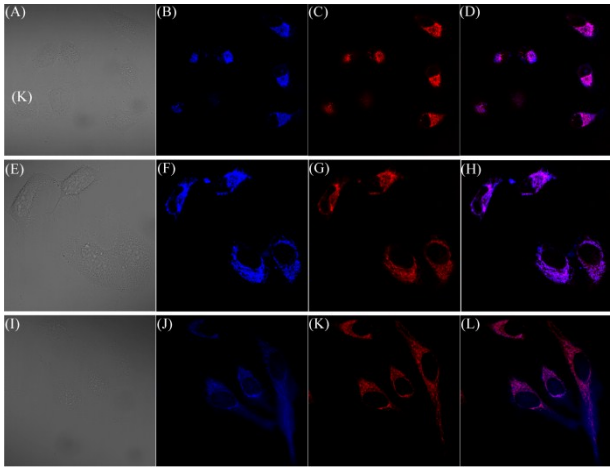


Fig. S4 Confocal fluorescence images of prodrugs (25 μg mL^{-1}) in HeLa cells: (A-D) **mPEG2000-NA-Cb(TPP)**, **P** = 0.88, (E-H) **mPEG1000-NA-Cb(TPP)**, **P** = 0.89 (I-L) **TPP-PEG2000-NA-Cb**, **P** = 0.59; (A, E, I) bright field, (B, F, J) 405 nm excitations, (C, G, K) 633 nm excitations, (D, H, L) merged images (scale bar = 5 μm)

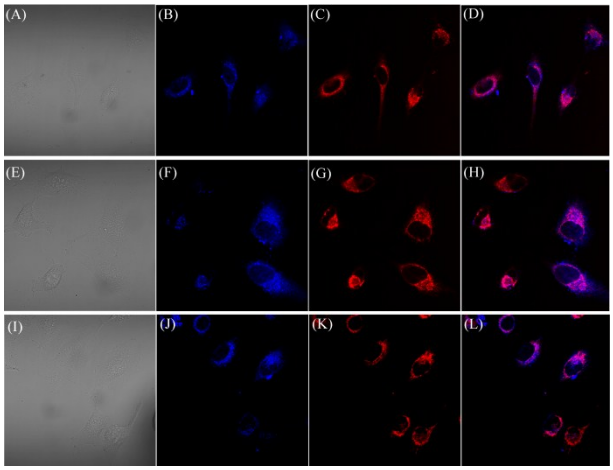


Fig. S5 Confocal fluorescence images of prodrugs (25 μg mL^{-1}) in HeLa cells: (A-D) **mPEG2000-NA-Cb**, **P** = 0.66, (E-H) **mPEG1000-NA-Cb**, **P** = 0.63, (I-L) **OH-PEG2000-NA-Cb**, **P** = 0.51; (A, E, I) bright field, (B, F, J) 405 nm excitations, (C,

G, K) 633 nm excitations, (D, H, L) merged images (scale bar = 5 μm)

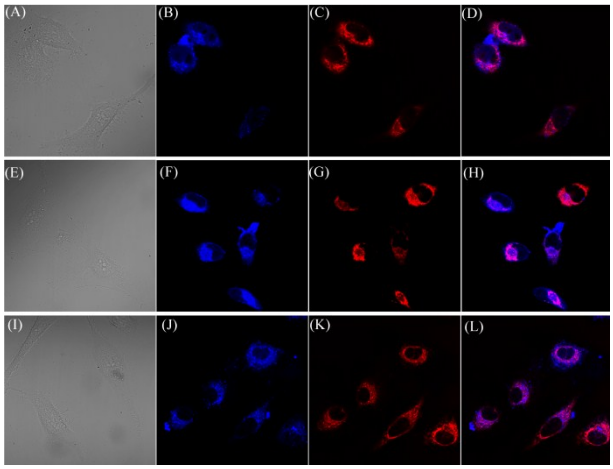


Fig. S6 Confocal fluorescence images of prodrugs (50 μg mL-1) in HeLa cells: (A-D) **mPEG2000-NA-Cb**, **P** = 0.66, (E-H) **mPEG1000-NA-Cb**, **P** = 0.60, (I-L) **OH-PEG2000-NA-Cb**, **P** = 0.59; (A, E, I) bright field, (B, F, J) 405 nm excitations, (C, G, K) 633 nm excitations, (D, H, L) merged images (scale bar = 5 μm)

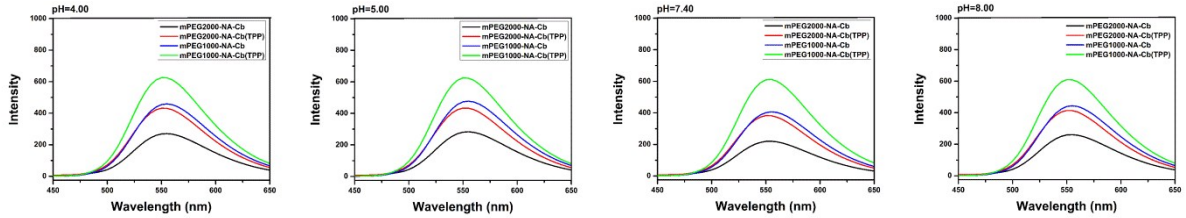


Fig. S7 Fluorescence spectra of prodrugs (50 μg mL^{-1}) in **PBS** buffer solution at different pH values (10 mM, pH =4.00& 5.00&7.40&8.00).

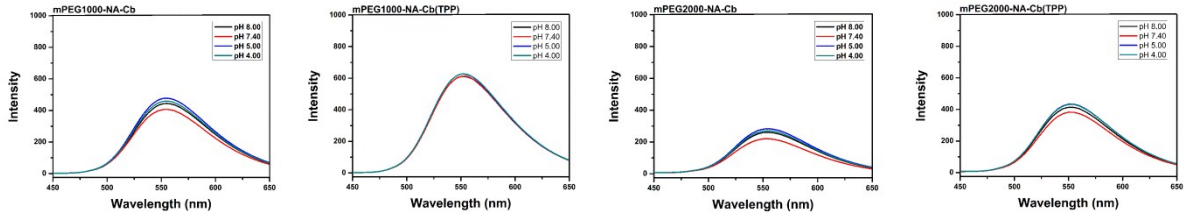


Fig. S8 Fluorescence of prodrugs (50 μg mL^{-1}) in **PBS** buffer solution is immune to pH changes (10 mM, pH =4.00& 5.00&7.40&8.00).

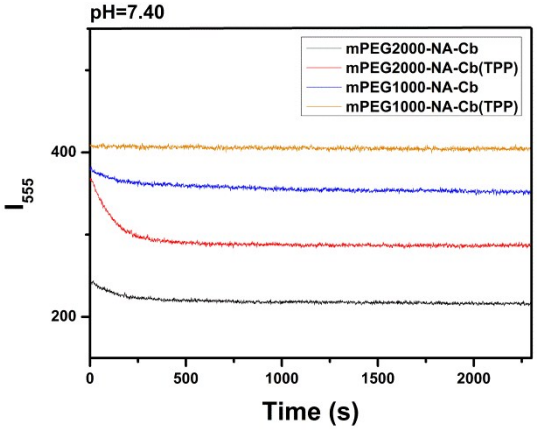


Fig. S9 Temporal profile of prodrugs at λ_{555nm} in **PBS** buffer solution (10 mM, pH = 7.40).

Table S1 GPC .

Polymer	Mn	Mw/Mn
mPEG1000	870	1.06
mPEG1-1000	1030	1.04
mPEG2-1000	1100	1.04
mPEG2000	1870	1.04
mPEG1-2000	1910	1.04
mPEG2-2000	1990	1.04
OH-PEG2000-N_3	1530	1.07

NMR of various compounds

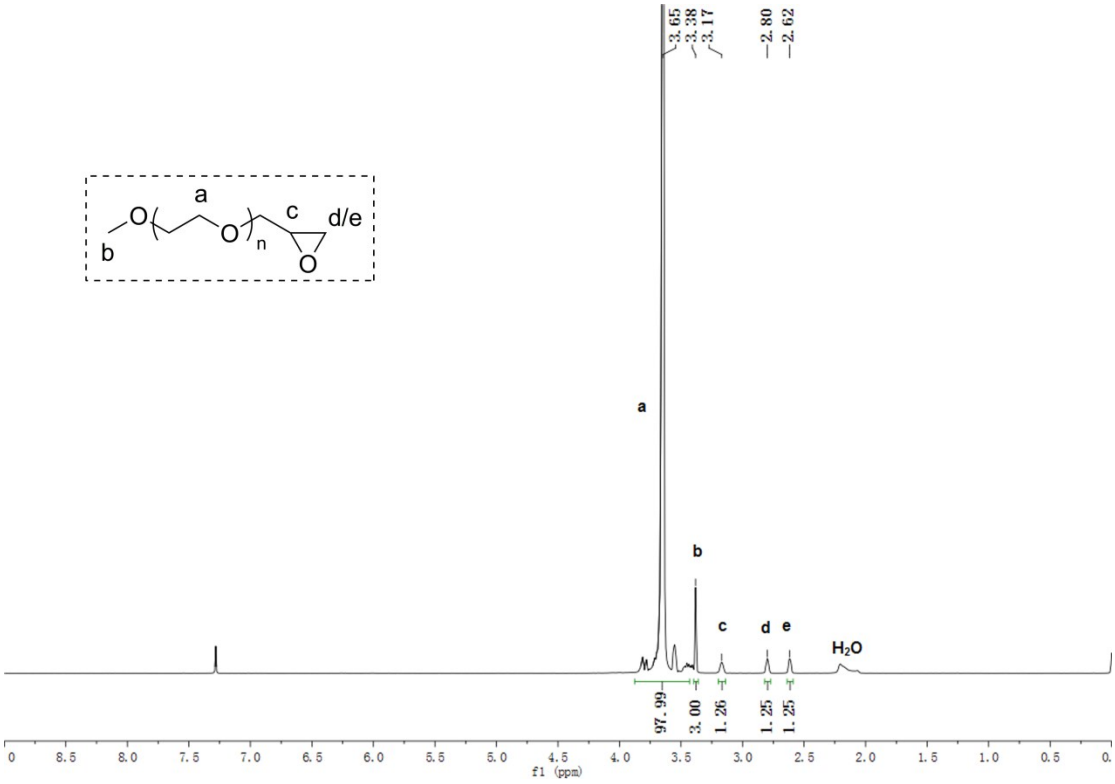


Fig. S10 ^{1}H NMR spectrum of **mPEG1-1000** in $CDCl_3$

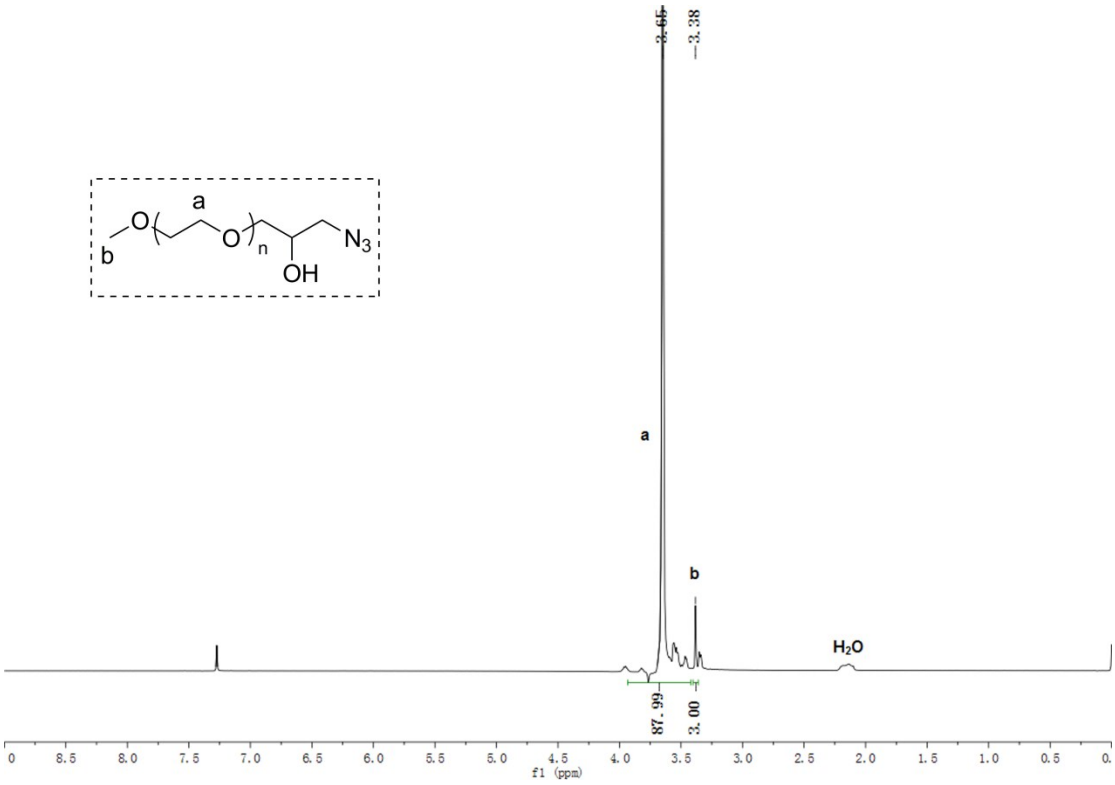


Fig. S11 ^{1}H NMR spectrum of **mPEG2-1000** in $CDCl_3$

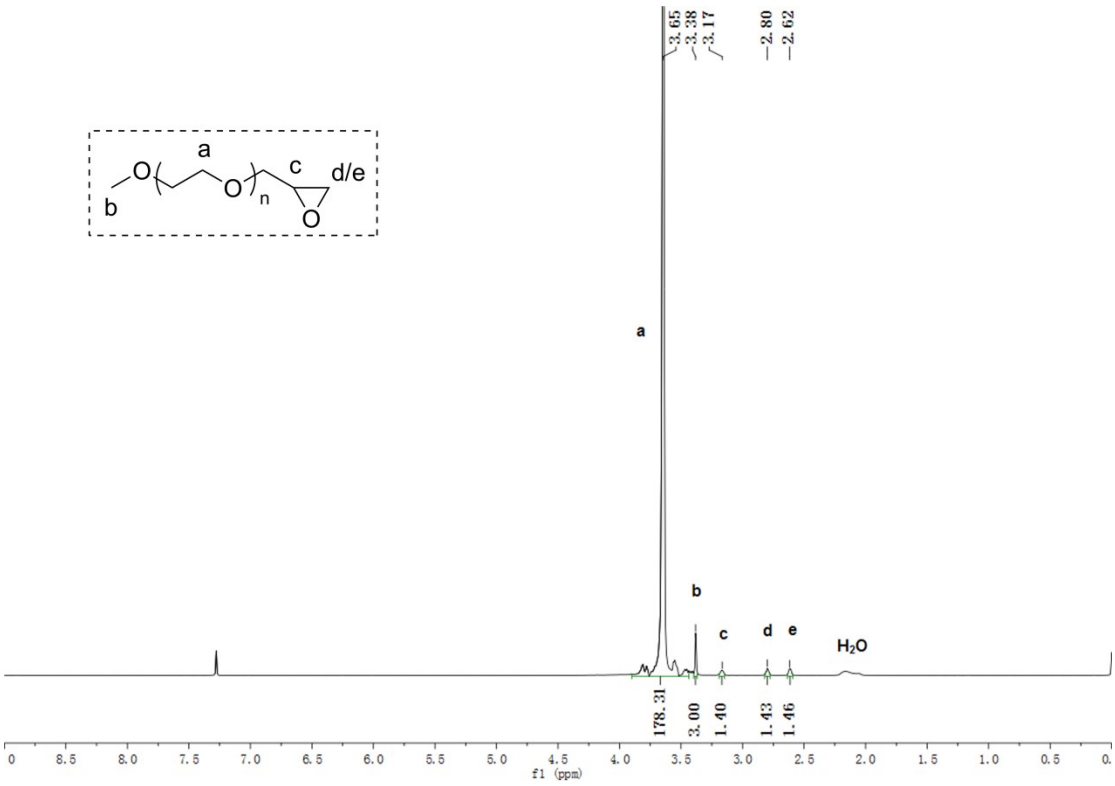


Fig. S12 ^{1}H NMR spectrum of **mPEG1-2000** in $CDCl_3$

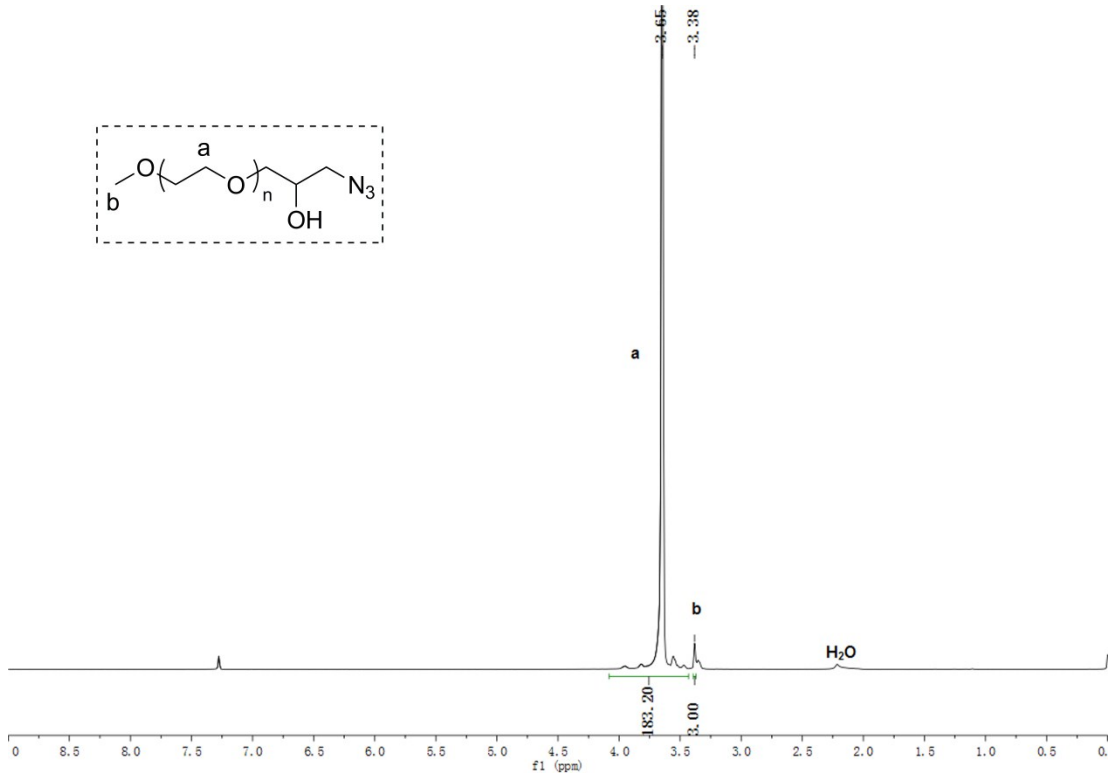


Fig. S13 ^{1}H NMR spectrum of **mPEG2-2000** in $CDCl_3$

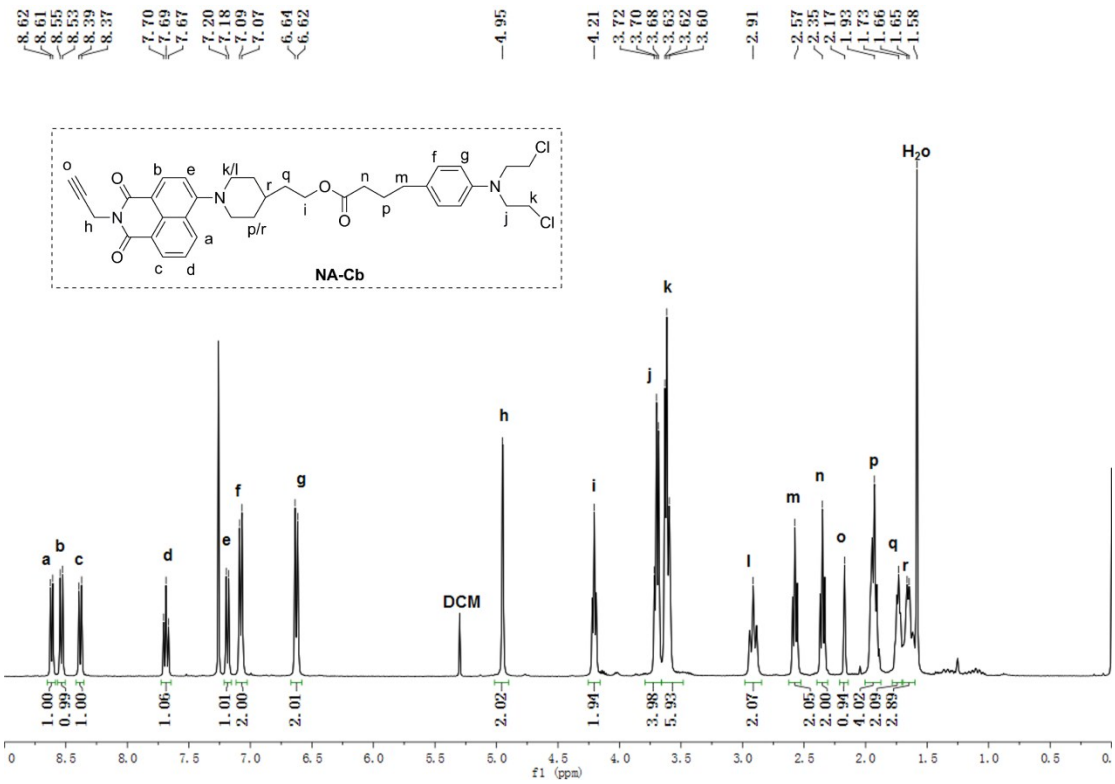


Fig. S14 ^{1}H NMR spectrum of **NA-Cb** in $CDCl_3$

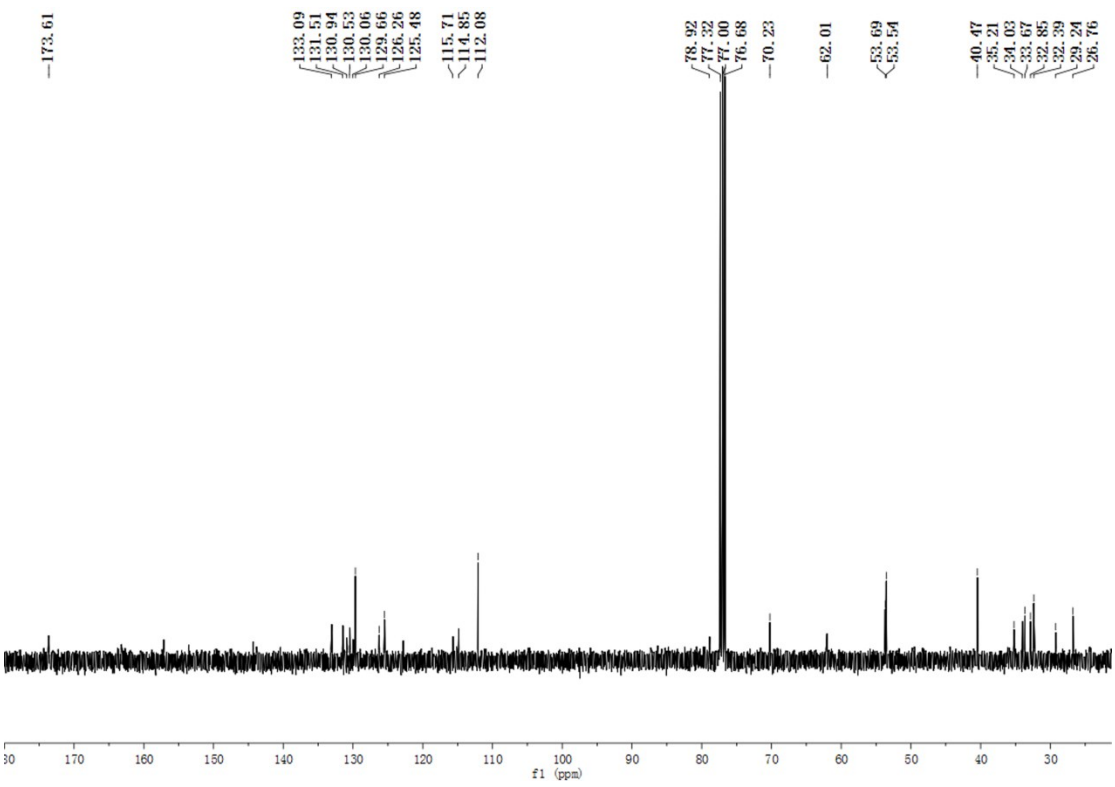


Fig. S15 ^{13}C NMR spectrum of **NA-Cb** in $CDCl_3$

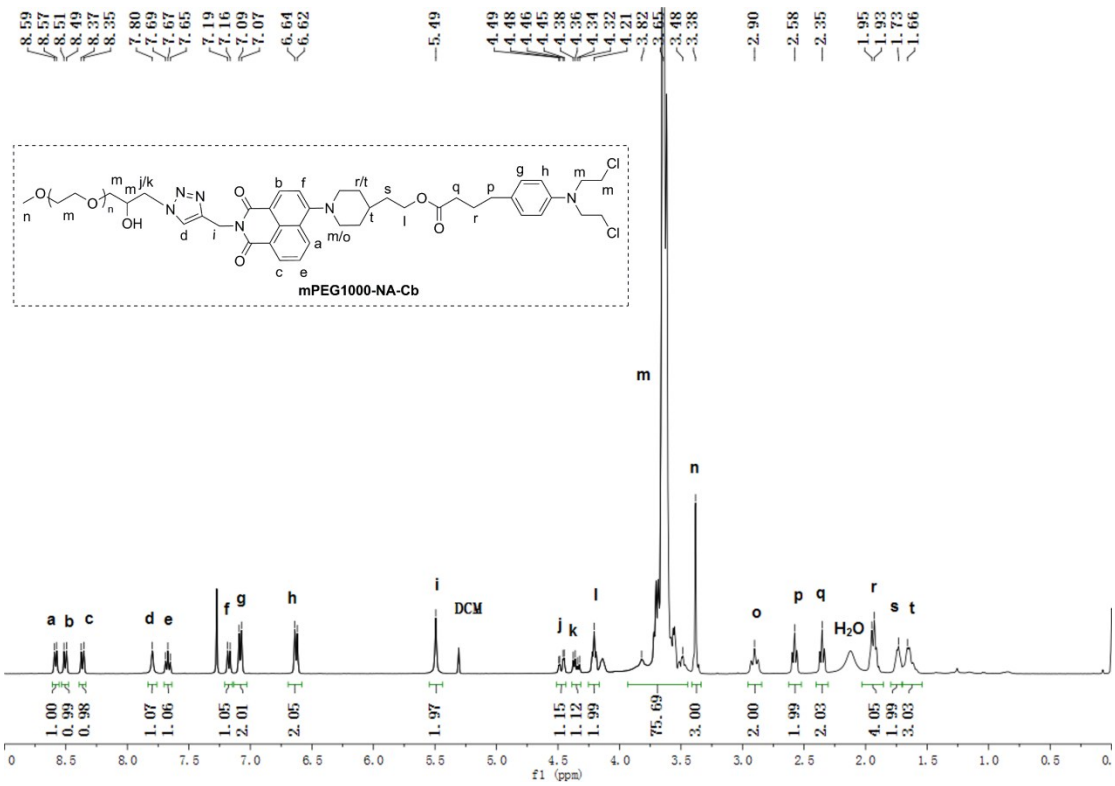


Fig. S16 ^{1}H NMR spectrum of **mPEG1000-NA-Cb** in $CDCl_3$

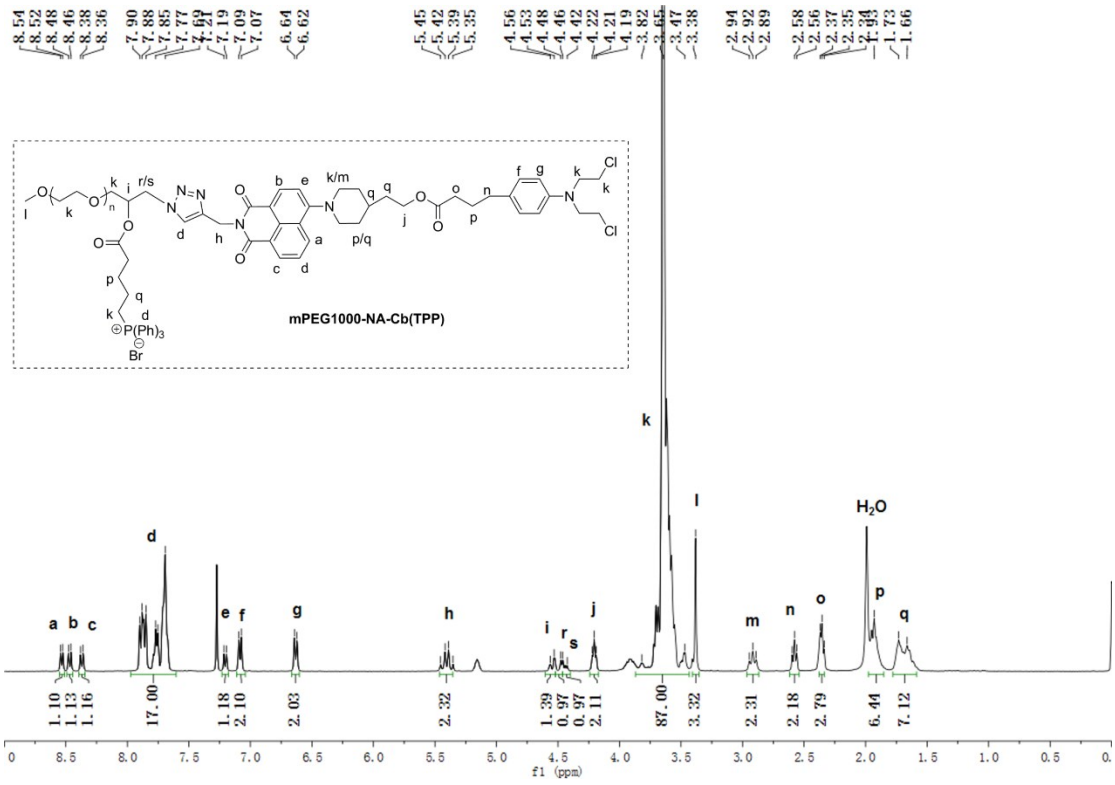


Fig. S17 ^{1}H NMR spectrum of **mPEG1000-NA-Cb(TPP)** in $CDCl_3$

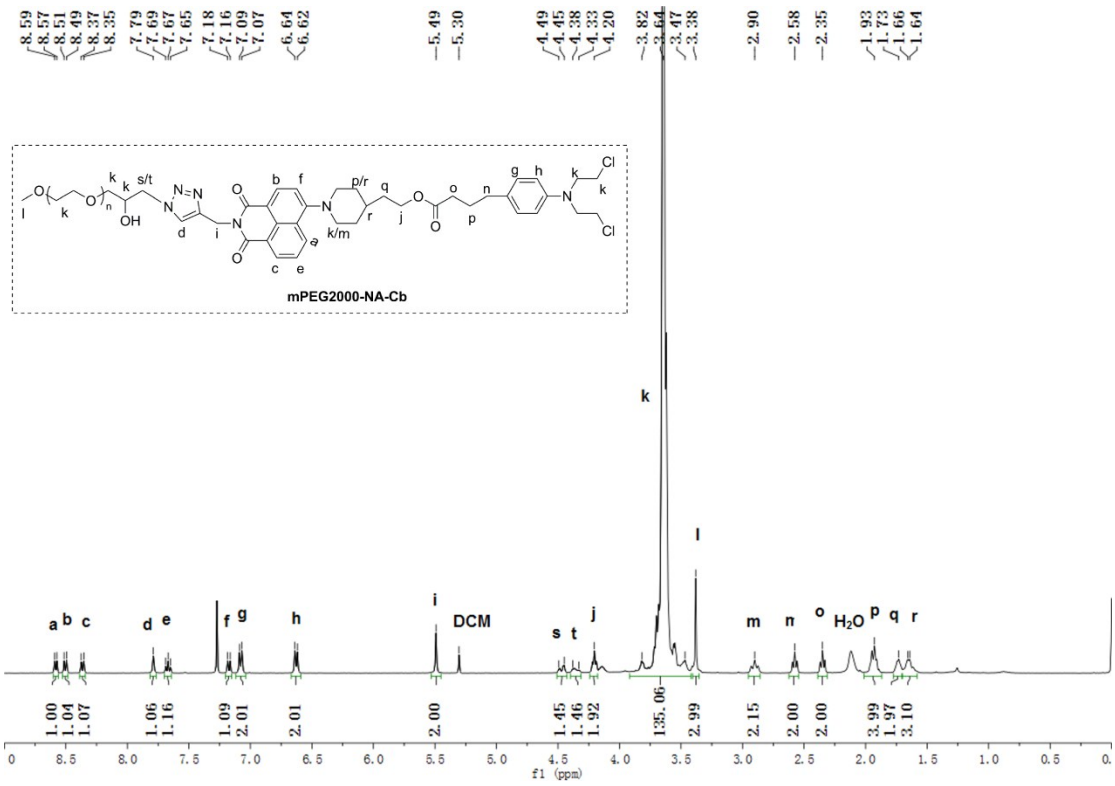


Fig. S18 ^{1}H NMR spectrum of **mPEG2000-NA-Cb** in $CDCl_3$

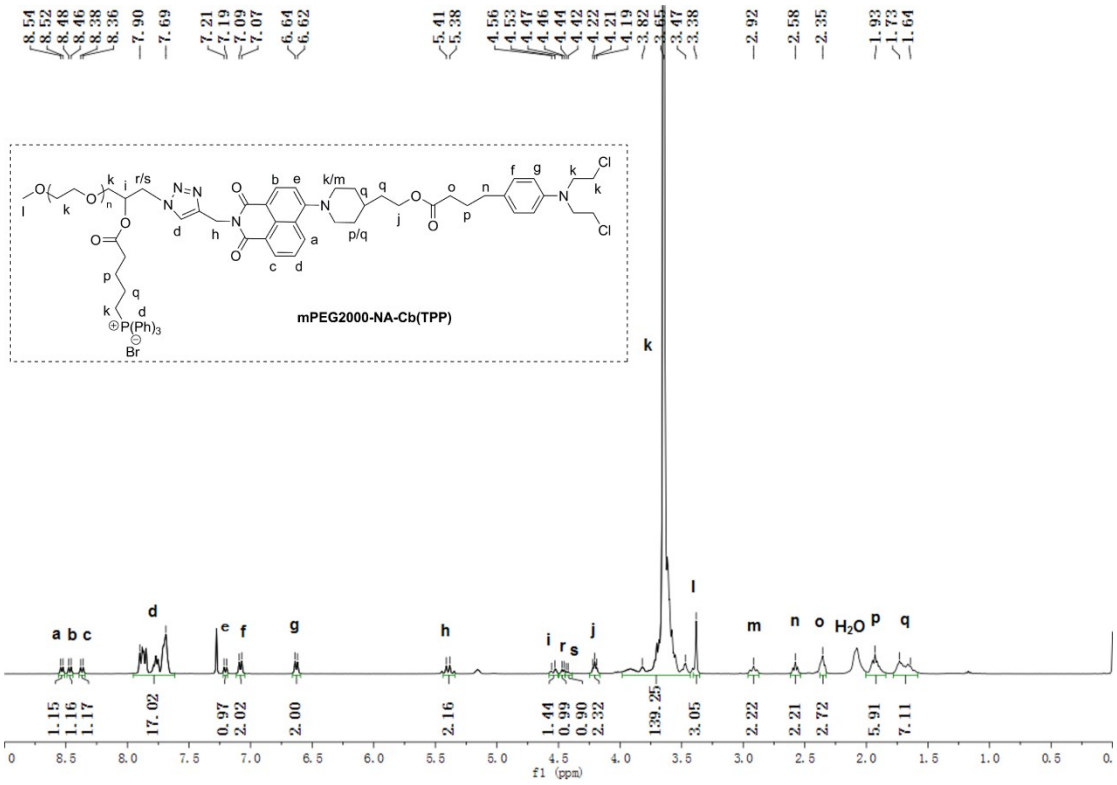


Fig. S19 ^{1}H NMR spectrum of **mPEG2000-NA-Cb(TPP)** in $CDCl_3$

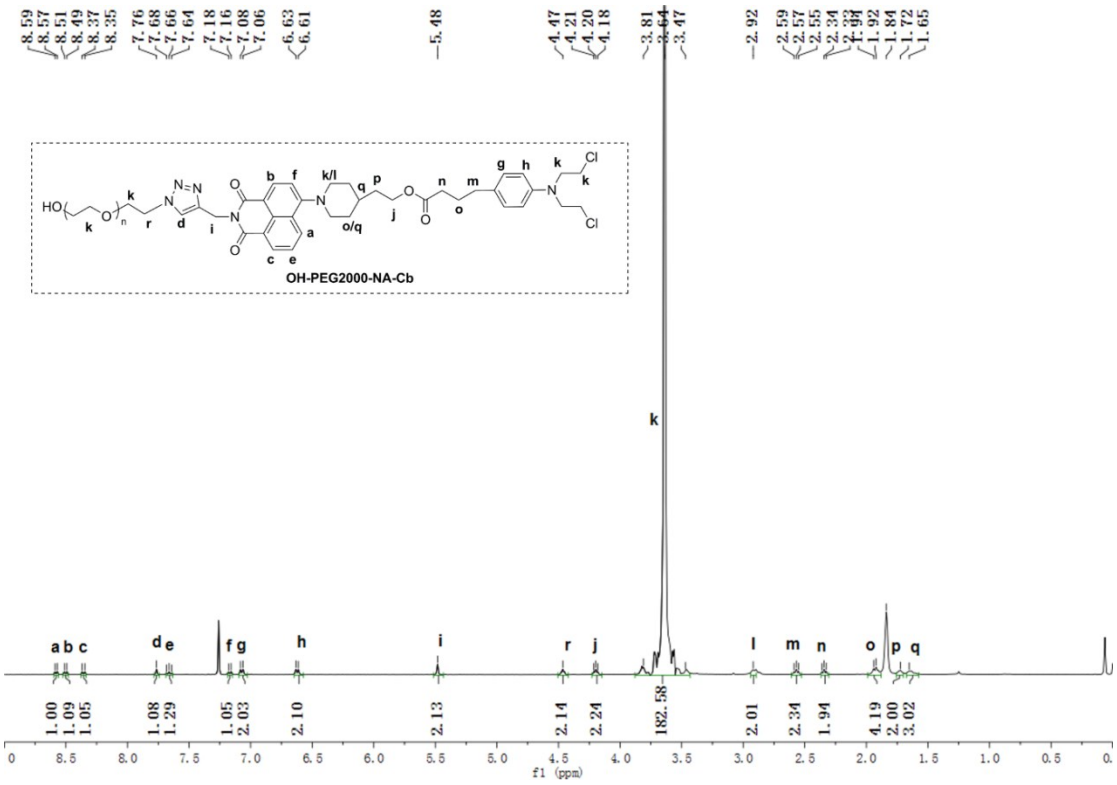


Fig. S20 ^{1}H NMR spectrum of **OH-PEG2000-NA-Cb** in $CDCl_3$

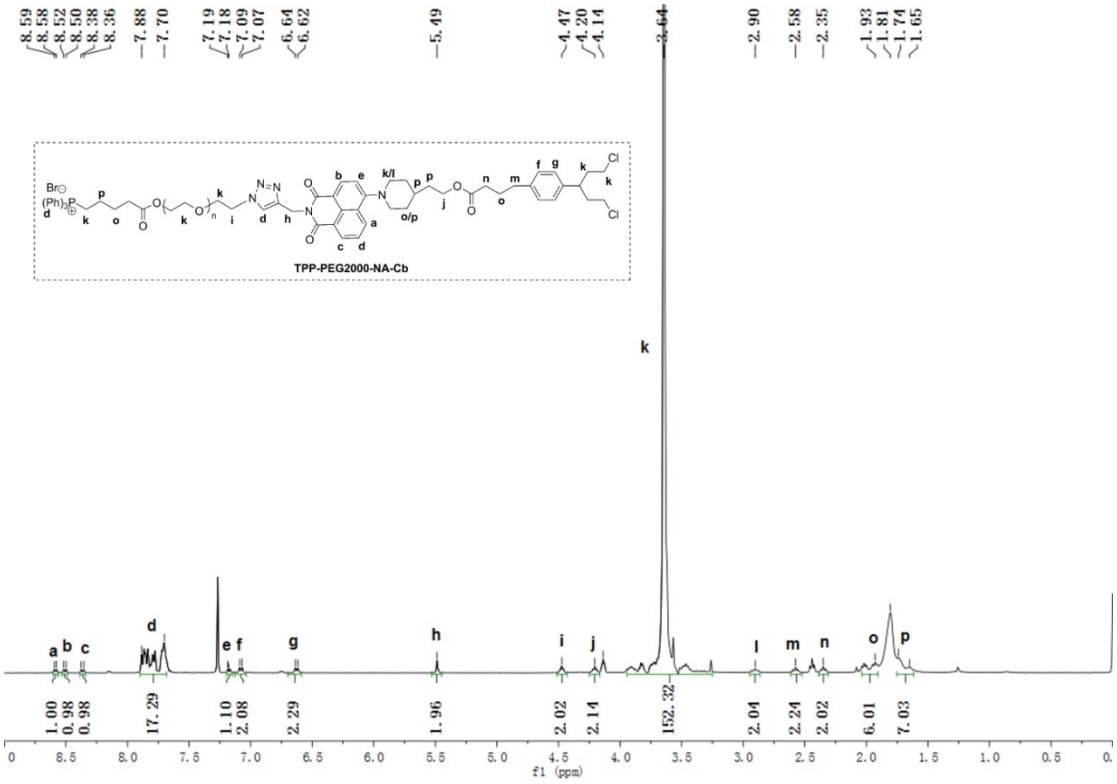


Fig. S21 ^{1}H NMR spectrum of **TPP-PEG2000-NA-Cb** in $CDCl_3$

Reference:

1 B.-Y. Liu, W.–X. Wu, N. Wang, X.-Q. Yu, Polym. Chem., 2015, 6, 364-368.