Supporting Information for

Poly (glycerol) used for constructing Mixed Polymeric Micelles as T_1 MRI Contrast Agent for Tumor-targeted Imaging

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Materials and measurements

Cyclen was purchased from Nanjing Chemlin Chemical Industry Co., Ltd. Bromoacetyl bromide, t-butylbromoacetate and *N*-hydroxysuccinimide (NHS) were obtained from TCI. Dicyclohexylcarbodiimide (DCC) and propargyl amine were obtained from J&K Chemical. CH_3CN was purchased from Sinopharm Chemical Reagent Co., Ltd and stored over calcium hydride before distillation. Other reagents and solvents were obtained from Sinopharm Chemical Reagent Co., Ltd and used as received.Mass spectra were taken using the Agilent Accurate Mass Q-TOF spectrometer.

Synthesis and characterization of alkynyl-DOTA(Gd)

Alkynyl-DOTA(Gd) was synthesized by the modified methods reported in published literatures[1-2]. The synthetic route was shown in Figure S1.

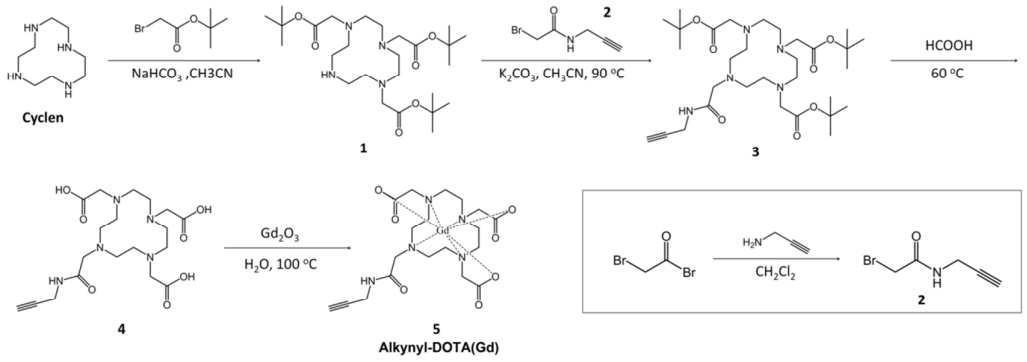

Figure S1. Schematic illustration on synthetic route of alkynyl-DOTA(Gd)

Compound 1 A suspension of cyclen (4.1 g, 23.7 mmol) and $NaHCO_3$ (6.22 g, 74.0 mmol) in anhydrous CH_3CN (400 mL) was stirred under nitrogen at 0 ^oC for 10 minutes. After that, t-butylbromoacetate (10.5 mL, 71.7 mmol) was added dropwise. The resulting mixture was allowed to rise to room temperature and continue to react for another 48 hours. After removing the

insoluble salt through filtration, the filtrate was concentrated by rotary evaporation and the residual mixture was purified via recrystallization with toluene to give a white solid (4.2 g, 30%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 10.0 (br, H, NH), 3.38 (s, 4H, CH_2), 3.30 (s, 2H, CH_2), 3.11 (s, 4H, CH_2), 2.90 (m,12H, CH_2), 1.47 (m, 27H, CH_3); **ESI-MS** (9:1 CH_3CN/H_2O) m/z 516($M+H^+$, 100%), 538 ($M+Na^+$, 10).

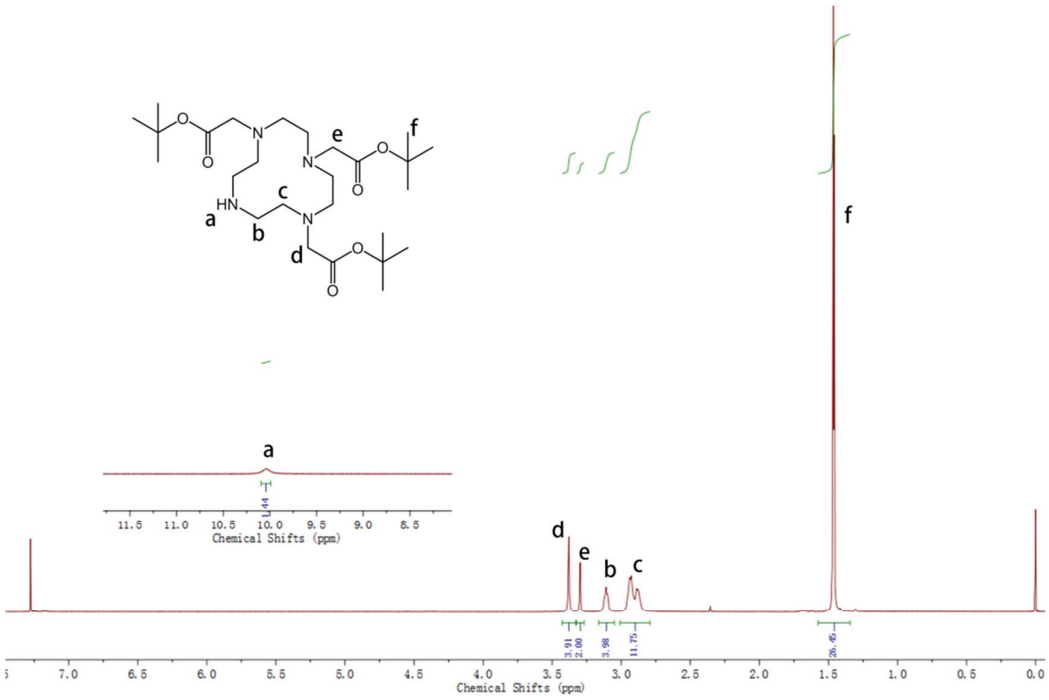

Figure S2. ^{1}H NMR spectrum of compound 1 (recorded in $CDCl_3$).

Compound 2 A solution of propargyl amine (4 mL, 58.3 mmol) in CH_2Cl_2 (200 mL) was covered with aqueous NaOH (0.1 M, 40 mL). To this bi-layer liquid, bromoacetyl bromide (16.3 mL, 187 mmol) was slowly added with a syringe to the below layer under 0 ^oC, causing the immediate formation of white precipitation. The resulting mixture was stirred vigorously at room temperature for 2.5 hours, after which, CH_2Cl_2 (100 mL) and H_2O (100 mL) was added and the whole solution was transferred to the separatory funnel. The organic phase was isolated and washed with Na_2CO_3

solution (50 mM, 3×100 ml). The resulting solution was dried with $MgSO_4$ over night and the solvent was removed by rotary evaporation to yield brown oil, which was further dried in vacuum for 24 hours to obtain brown solid (2.9 g, 28%). **^{1}HNMR** (400 MHz, $CDCl_3$) δ 6.69 (br, H, NH), 4.10 (m, 2H,CH_2), 3.90 (s, 2H, CH_2), 2.30 (t, H, CH); **ESI-MS** (9:1 CH_3CN/H_2O) m/z 176 ($M+H^+$, 100%), 198 ($M+Na^+$, 62).

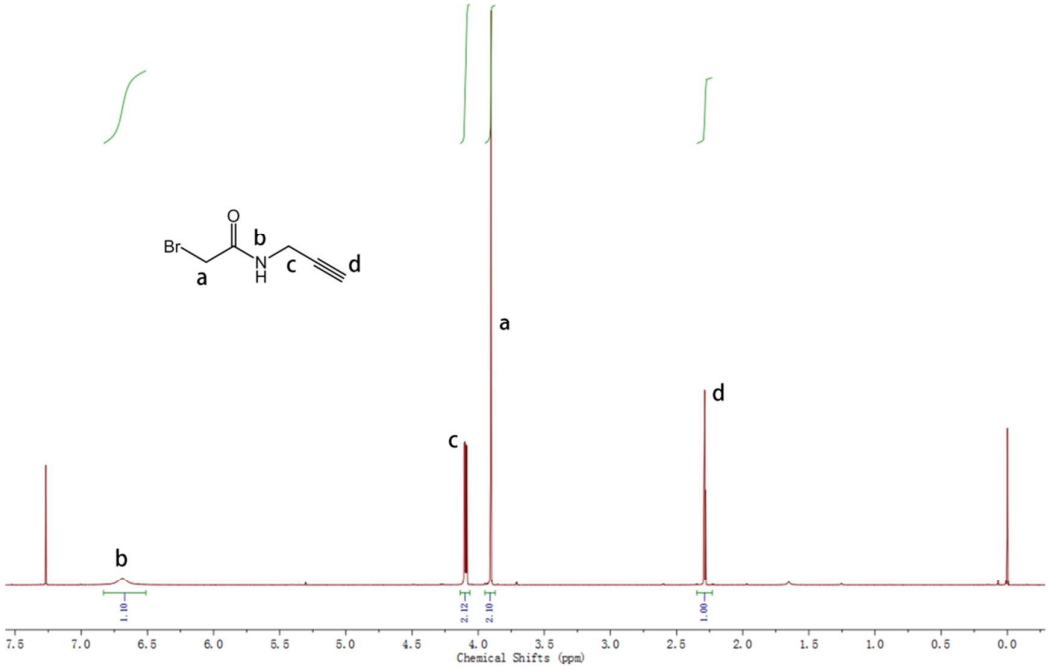

Figure S3. ^{1}H NMR spectrum of compound 2 (recorded in $CDCl_3$).

Compound 3 A solution of **compound 1** (1.48 g, 2.48 mmol), **compound 2** (1.29 g, 7.33 mmol) and K_2CO_3 (2.78 g, 20.1 mmol) in 150 mL anhydrous CH_3CN was refluxed under nitrogen at 90 ^oC for 48 hours. After removal the insoluble solid by filtration and reducing the solvent to dryness through rotary evaporation, the residual mixture was purified by silica gel column using chloroform-methanol (6:1) to give a faint yellow solid (0.8 g, 41%). **^{1}HNMR** (400 MHz, $(CD_3)_2SO$) δ 8.62 (t, H, NH), 3.88 (m, 2H, CH_2), 3.33 (s, 2H, CH_2), 3.10 (t, H, CH), 1.90-3.06 (br,

16H, CH_2), 1.43 (m, 27H, CH_3); **ESI-MS** (9:1 CH_3CN/H_2O) m/z 610 ($M+H^+$, 100%), 632 ($M+Na^+$, 90).

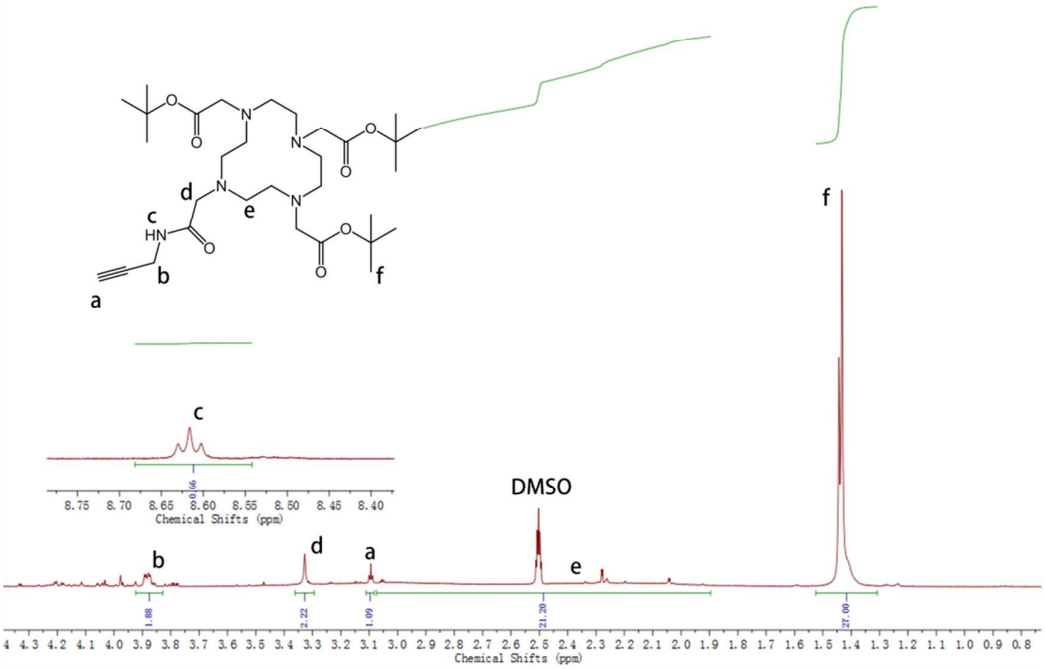

Figure S4. ^{1}H NMR spectrum of compound 3 (recorded in DMSO-d_6).

Compound 4 Compound 3 (750 mg, 1.23 mmol) was dissolved in 30 mL formic acid and the reaction solution was stirred at 60 ^oC over night. After removal of the solvent via forming azeotrope with methanol, the residual brown solid was dissolved with a minimal volume of methanol and then precipitated in excess cold diethyl ether. The precipitation treatment was repeated twice. The final product was dried in vacuum at 40 ^oC to give a brown solid (0.5 g, 94%). **^{1}HNMR** (400 MHz, D_2O) δ 8.43 (s, H, NH), 3.95 (d, 2H, CH_2), 3.85 (d, 4H, CH_2), 3.49 (m, 12H, CH_2), 3.10 (m, 8H, CH_2), 2.60 (s, H, CH); **ESI-MS** (9:1 CH_3CN/H_2O) m/z 442 ($M+H^+$, 100%), 221.5 ($M+2H^+$, 85).

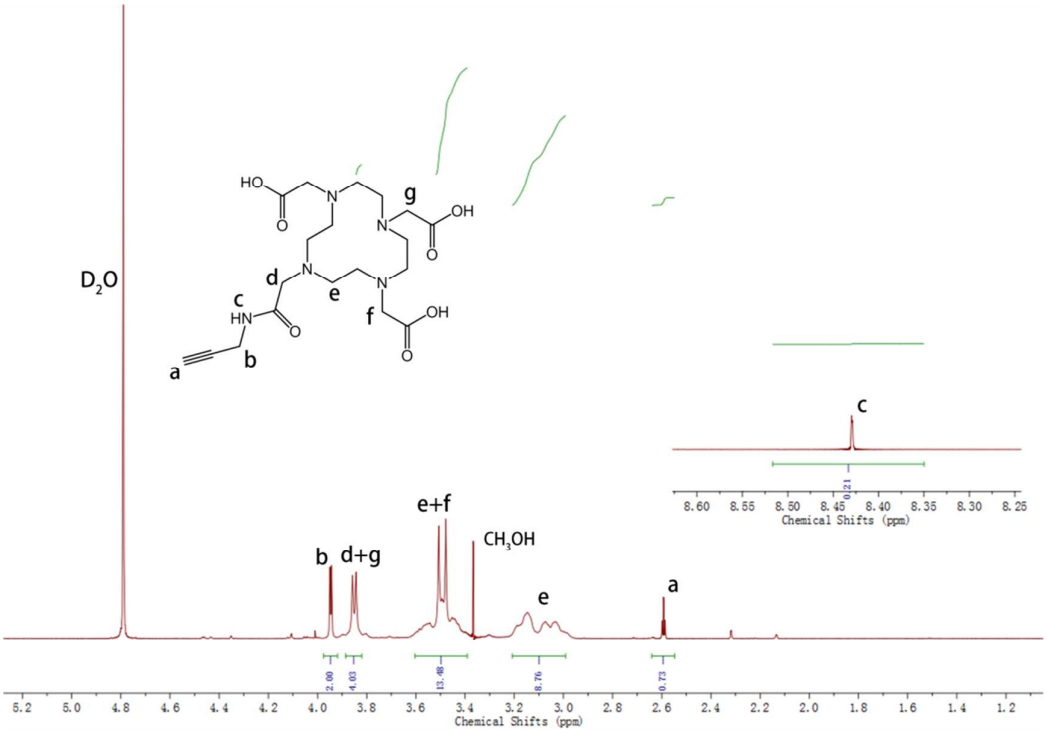

Figure S5. ^{1}H NMR spectrum of compound 4 (recorded in D_2O).

Compound 5 A mixture of compound 4 (500 mg, 1.13 mmol) and Gd_2O_3 (275 mg, 0.76 mmol) in 20 mL H_2O was refluxed at 100 ^{o}C for 24 hours. The reaction mixture was cooled to room temperature and filtrated with a filter covered by a layer of celite. The resulting solution was evaporated to dryness through rotary evaporation and then further dried under vacuum at 40 ^{o}C for 24 hours to yield a product of faint yellow solid (0.6 g, 66%). **ESI-MS** (9:1 CH_3CN/H_2O) m/z 597 ($M+H^+$, 100%), 299 ($M+2H^+$, 85).

Synthesis and characterization of azido functionalized folic acid (N_3-FA)

N_3-FA was prepared by the following method[3]: folic acid (0.5 g, 1.1 mmol) and triethylamine (0.25 mL) were dissolved in 20 mL DMSO. Then, NHS (0.26 g, 2.2 mmol) and DCC (0.25 g, 1.2 mmol) were added, and the mixture reacted at room temperature in the dark for 24 h. Afterward, 1-azido-3-aminopropane (0.24 g, 2.4 mmol) was added, and the reaction was continued for 24 h.

The resulting solution was precipitated in diethyl ether and dried in vacuum. The product was further purified by dissolving in 1M NaOH solution and precipitation by addition of 1M HCl solution. The precipitate was collected through centrifugation, washed with ethanol/H_2O (1:1) three times, and finally dried in vacuum to obtain yellow product (345 mg, 60%). The successful conjugation of 1-azido-3-aminopropane was verified by ^{1}H NMR characterization.

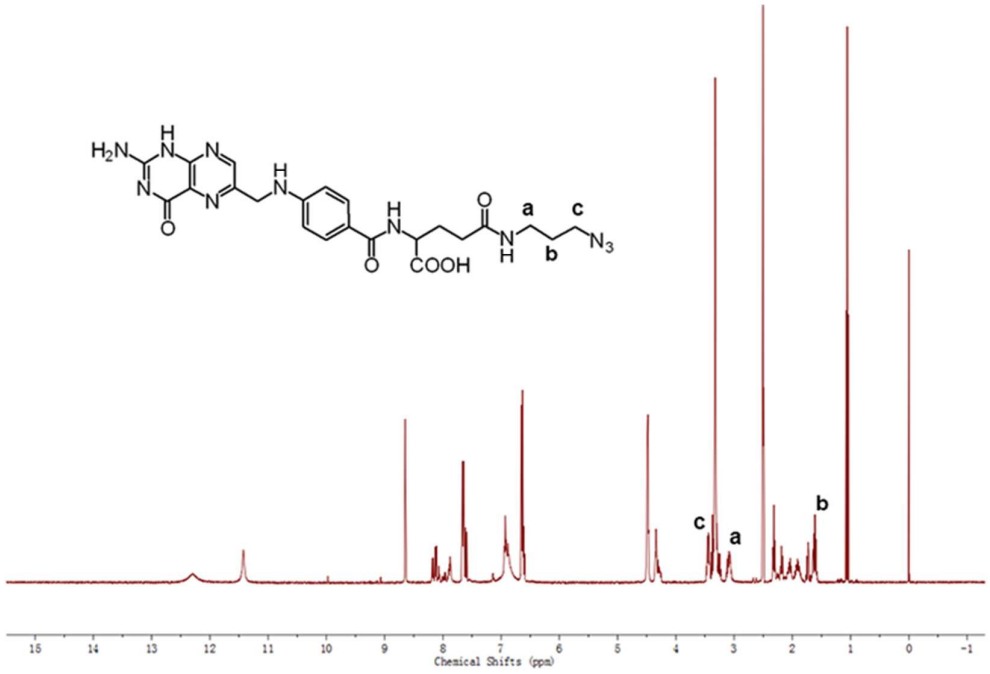

Figure S6. ^{1}H NMR spectrum of azido-folic acid (recorded in DMSO-d_6).

Characterization of chemical structures and analysis for polymers

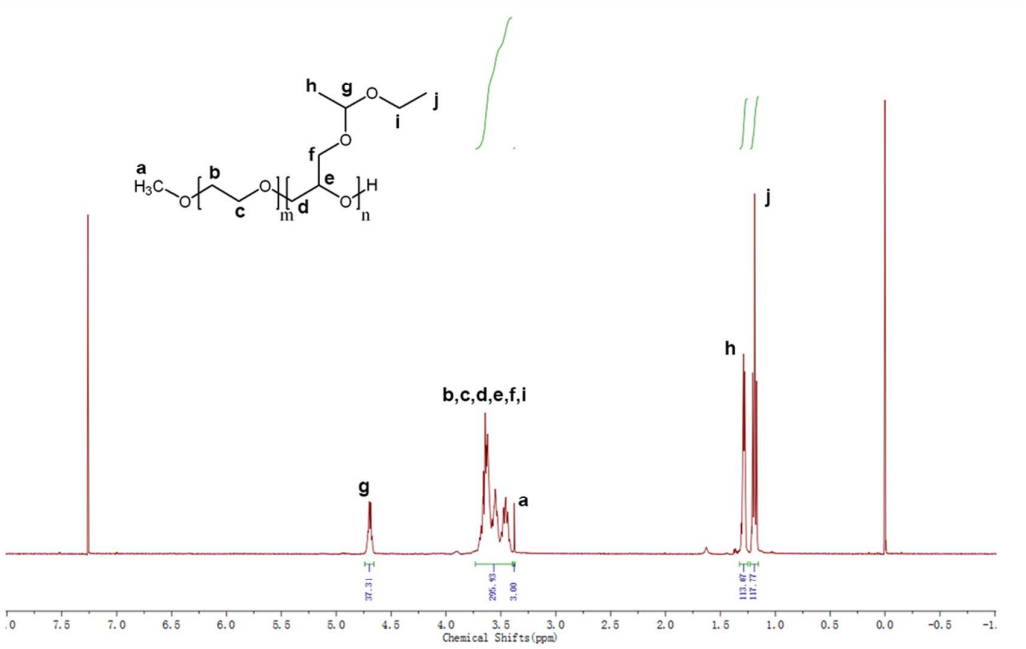

Figure S7. ^{1}H NMR spectrum of mPEG-PEEGE (recorded in $CDCl_3$).

The degree of polymerization (DP) of mPEG- PEEGE is calculated according to the areas of peak (a) and peak (g), and the calculation formula is shown in Equation 1, which turns out to be 37. Therefore, the molecular weight of mPEG- PEEGE calculated by (M_n) is 5.75×10^3 g/mol.

$$DP = \frac{A_g / 1}{A_a / 3} \quad \text{(Equation 1)}$$

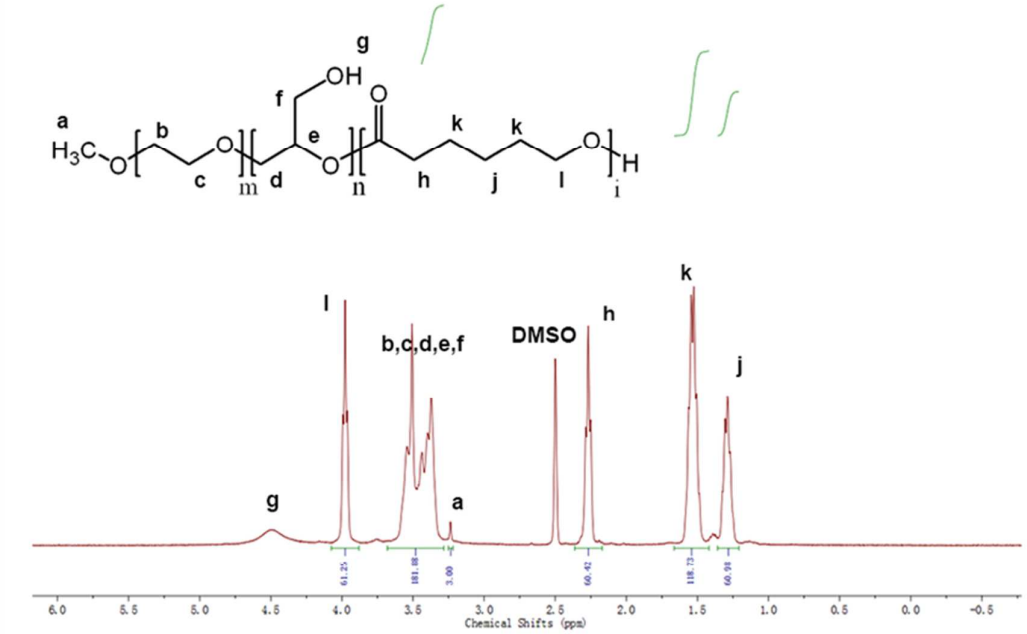

Figure S8. ^{1}H NMR spectrum of mPEG-PG-*b*-PCL (recorded in DMSO-d_6).

The degree of polymerization (DP) of mPEG-PG-*b*-PCL is calculated according to the areas of peak (a) and peak (h), and the calculation formula is shown in Equation 2, which turns out to be 30. Therefore, the molecular weight of mPEG-PG-*b*-PCL calculated by (M_n) is 6.51×10^3 g/mol.

$$DP = \frac{A_h / 2}{A_a / 3} \quad \text{(Equation 2)}$$

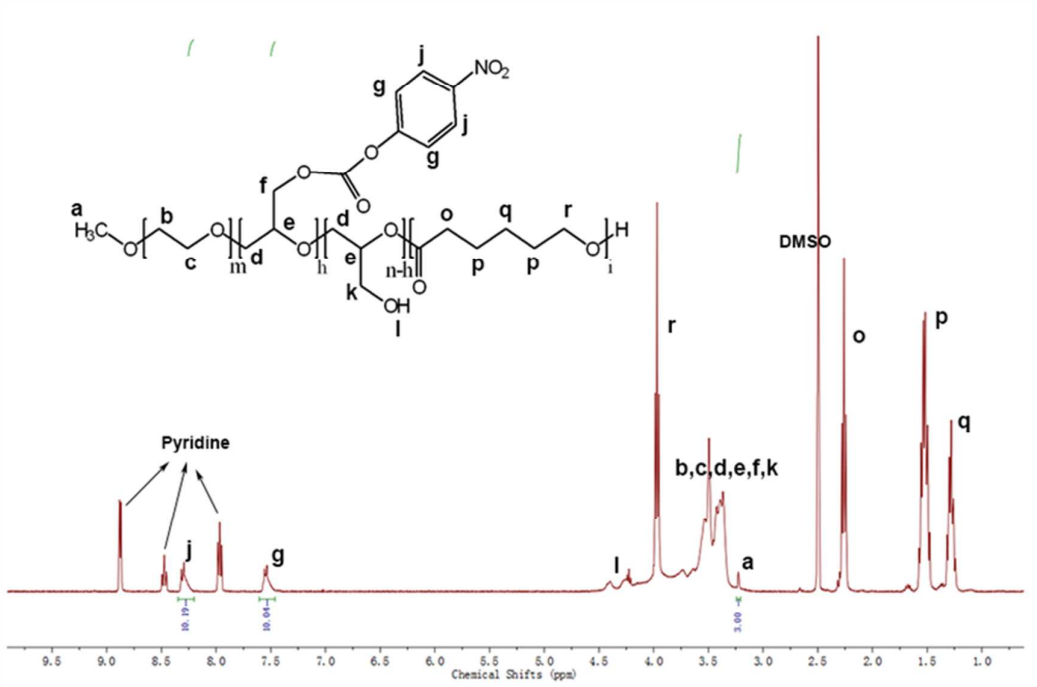

Figure S9. ^{1}H NMR spectrum of mPEG-PG(NPC)-*b*-PCL (recorded in DMSO-d_6).

The quantitative analysis of reaction ratio of hydroxyl groups in mPEG-PG-*b*-PCL with *p*-NPC is calculated according to the areas of peak (a) and peak (g), and the calculation formula is shown in Equation 3, which reveals that 5 hydroxyl groups are modified with *p*-NPC.

$$Number = \frac{A_g / 2}{A_a / 3} \quad \text{(Equation 3)}$$

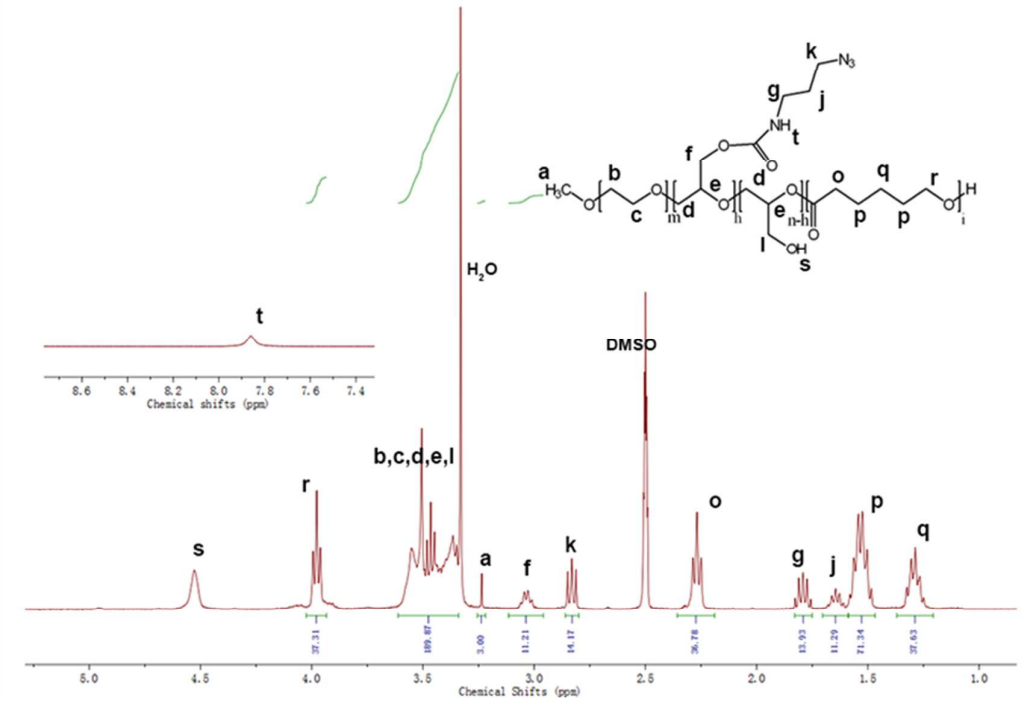

Figure S10. ^{1}H NMR spectrum of mPEG-PG(N_3)-*b*-PCL (recorded in DMSO-d_6).

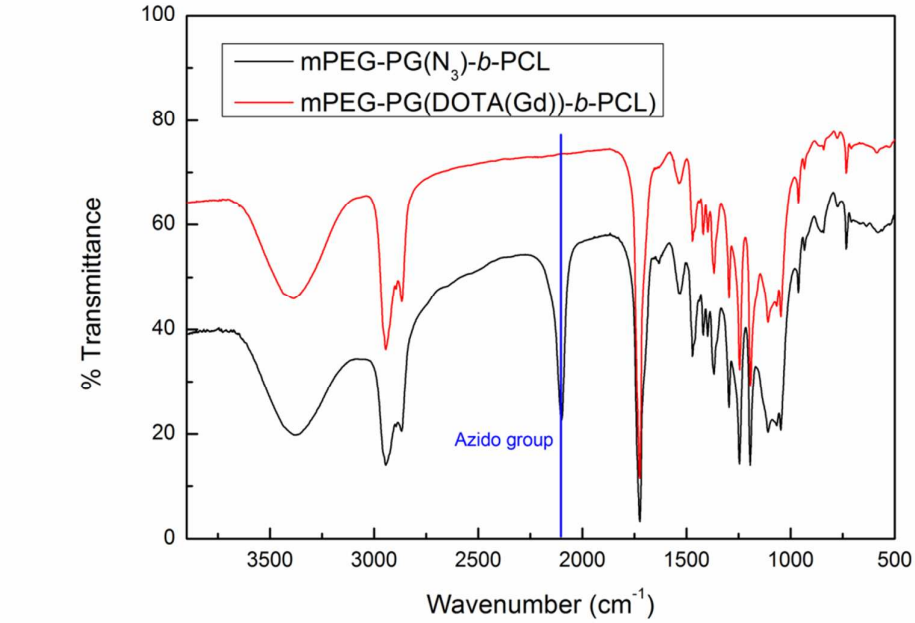

Figure S11. FTIR absorption spectra of mPEG-PG(N_3)-*b*-PCL and mPEG-PG(DOTA(Gd))-*b*-PCL.

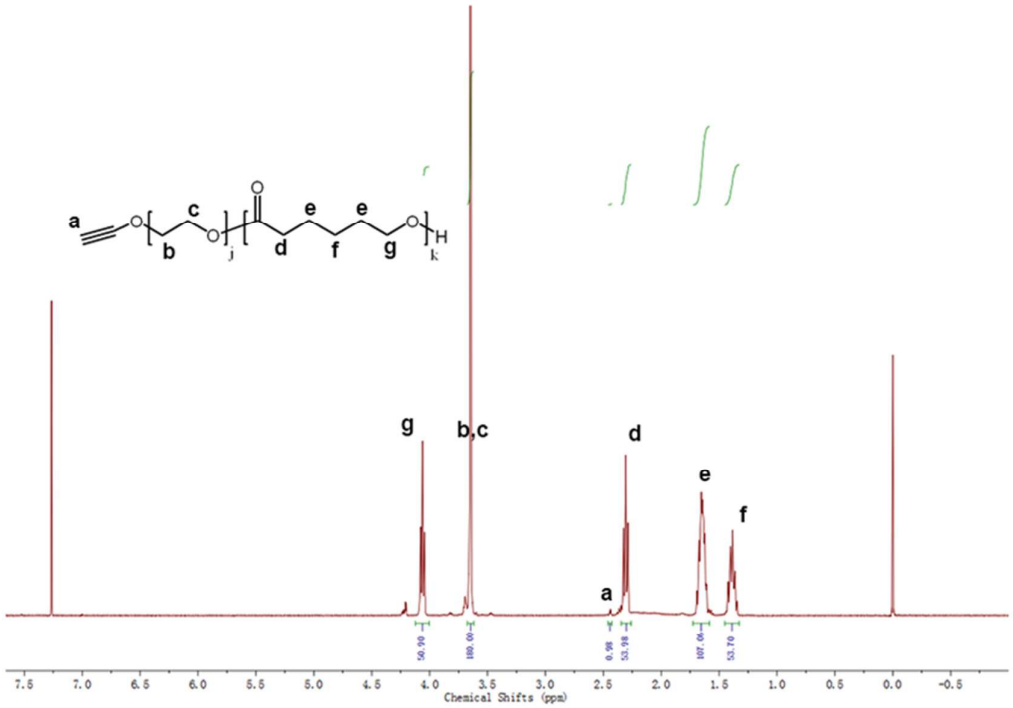

Figure S12. ^{1}H NMR spectrum of alkynyl-PEG-*b*-PCL (recorded in $CDCl_3$).

The degree of polymerization (DP) of alkynyl-PEG-*b*-PCL is calculated according to the areas of peak (a) and peak (d), and the calculation formula is shown in Equation 4, which turns out to be 27. Therefore, the molecular weight of alkynyl-PEG-*b*-PCL calculated by (M_n) is 5.08×10^3 g/mol.

$$DP = \frac{A_d / 2}{A_a / 1} \quad \text{(Equation 4)}$$

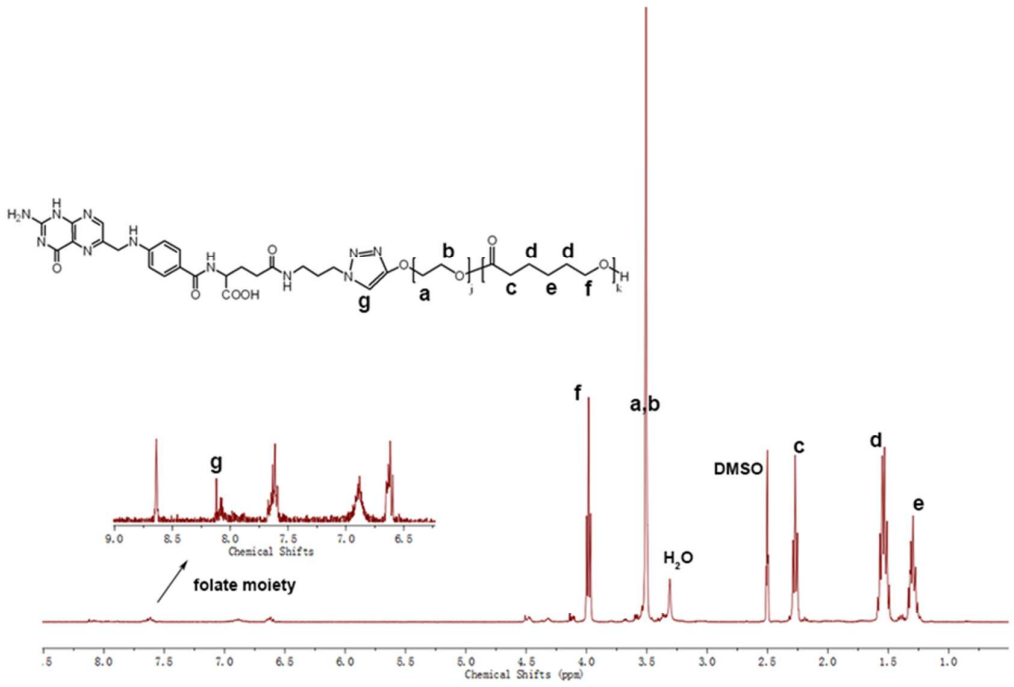

Figure S13. ^{1}H NMR spectrum of FA-PEG-*b*-PCL (recorded in DMSO-d_6).

References

(1) Viguier, R. F.; Hulme, A. N. A sensitized europium complex generated by micromolar concentrations of copper (I): toward the detection of copper (I) in biology. *J. Am. Chem. Soc.* **2006,** *128*, 11370-11371.

(2) Prasuhn Jr, D. E.; Yeh, R. M.; Obenaus, A.; Manchester, M.; Finn, M. Viral MRI contrast agents: coordination of Gd by native virions and attachment of Gd complexes by azide–alkyne cycloaddition. *Chem. Commun.* **2007**, 1269-1271.

(3) Liu, L.; Zheng, M.; Renette, T.; Kissel, T. Modular synthesis of folate conjugated ternary copolymers: polyethylenimine-graft-polycaprolactone-block-poly (ethylene glycol)-folate for targeted gene delivery. *Bioconjugate chem.***2012,** *23*, 1211-1220.