Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Subcellular Co-delivery of Two Different Site-oriented Payloads based on Multistage Targeted Polymeric Nanoparticles for Enhanced Cancer Therapy

1、Synthesis of c,c,t-[$Pt(NH_3)_2Cl_2(O_2CCH_2CH_2O_2H)_2$](Pt(IV)-COOH)

The c,c,t-[Pt(NH3)2Cl2(O2CCH2CH2O2H)2](Pt(IV)-COOH) and the intermediate were synthesized as our previous work described [1].

(1) Synthesis of c,c,t-[$Pt(NH_3)_2Cl_2(OH)_2$] [Pt(IV)]

Pt(IV) molecule was synthesized by hydrogen peroxide at mild reaction conditions. Briefly, to a solution of cisplatin (0.5 g, 1.67 mmol) in H_2O (10 mL) was added hydrogen peroxide (30 wt %, 20 mL) and the reaction mixture was stirred at 70 ^oC for 6 h. The product, c,c,t-[$Pt(NH_3)_2Cl_2(OH)_2$] was collected by filtration and washed with ice cold water. Yield: 74.3% (0.41 g, 1.23 mmol).

(2) Synthesis of c,c,t-[$Pt(NH_3)_2Cl_2(O_2CCH_2CH_2O_2H)_2$]

Succinic anhydride (0.49 g, 4.91 mmol) was added to the suspension of c,c,t-[Pt(NH3)2Cl2(OH)2] (0.4 g, 1.2 mmol) in DMSO (20 mL) and the mixture was stirred 70 ^oC overnight. The solution was concentrated and acetone was added to precipitate a light yellow solid, which was washed with acetone, diethyl ether and then dried. Yield: 65.8% (0.42 g, 0.79 mmol). 1H NMR ($DMSOd_6$), d (ppm) 12.09 (s, 2H), 6.45 (s, 6H), 2.51 (m, 4H), 2.34 (m, 4H). ESI-MS (positive mode, m/z): 535.0 $[M+H]^+$, 556.9 $[M+Na]^+$,573.9 $[M+K]^+$. Elemental analysis found (calcd) for $C_8H_{16}Cl_2N_2O_8Pt$ (%): C, 17.73 (17.99); H, 3.13 (3.02); N, 5.01 (5.24).

[1] Rong Huang, Qiucui Wang, Xiangyang Zhang, Jin Zhu, Baiwang Sun. Trastuzumab-cisplatin conjugates for targeted delivery of cisplatin to HER2-overexpressing cancer cells. *Biomedicine & Pharmacotherapy* 2015, 72, 17–23.

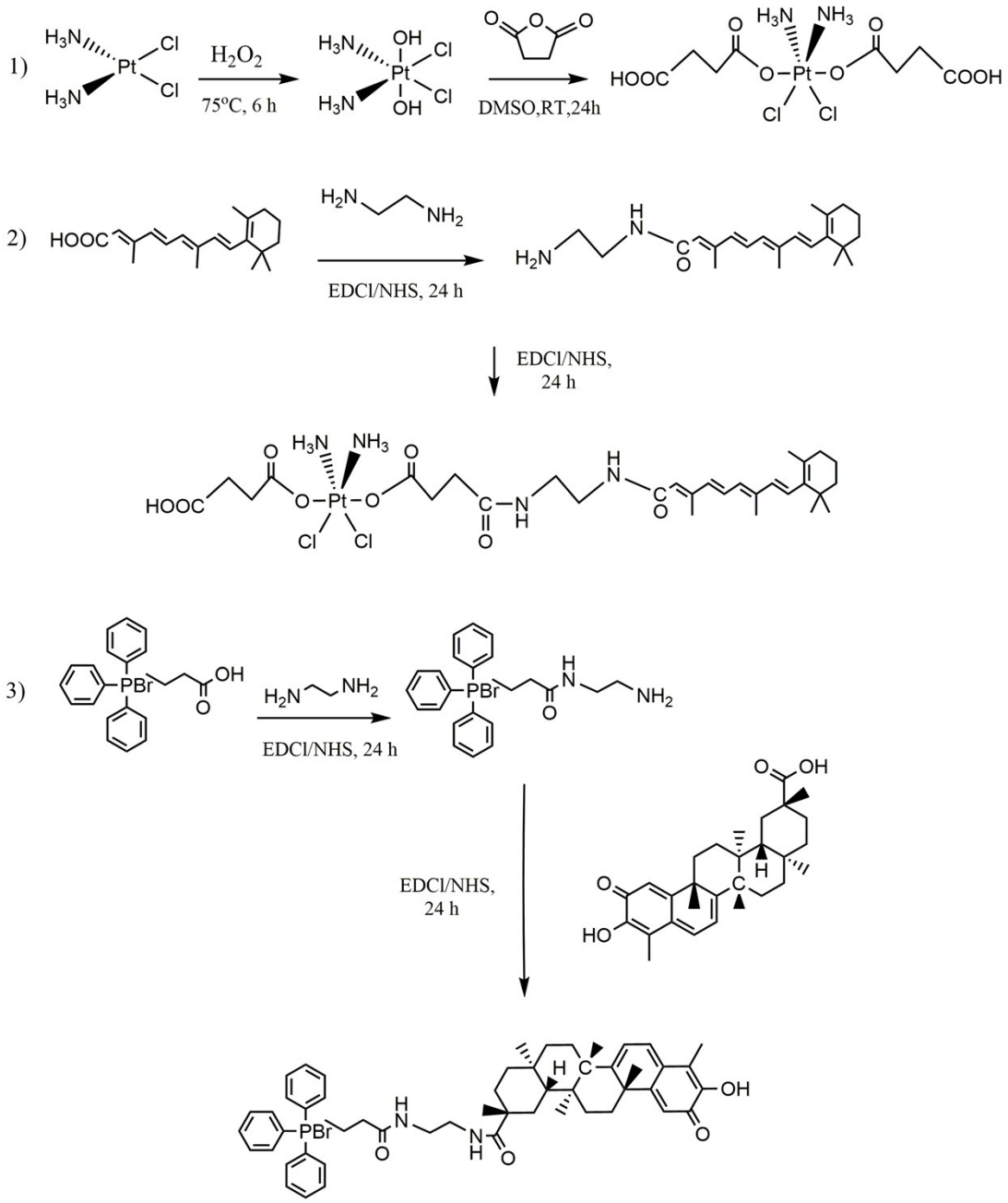

Fig. S1 Overview of the synthetic route for the synthesis of RA-Pt and TPP-Cet.

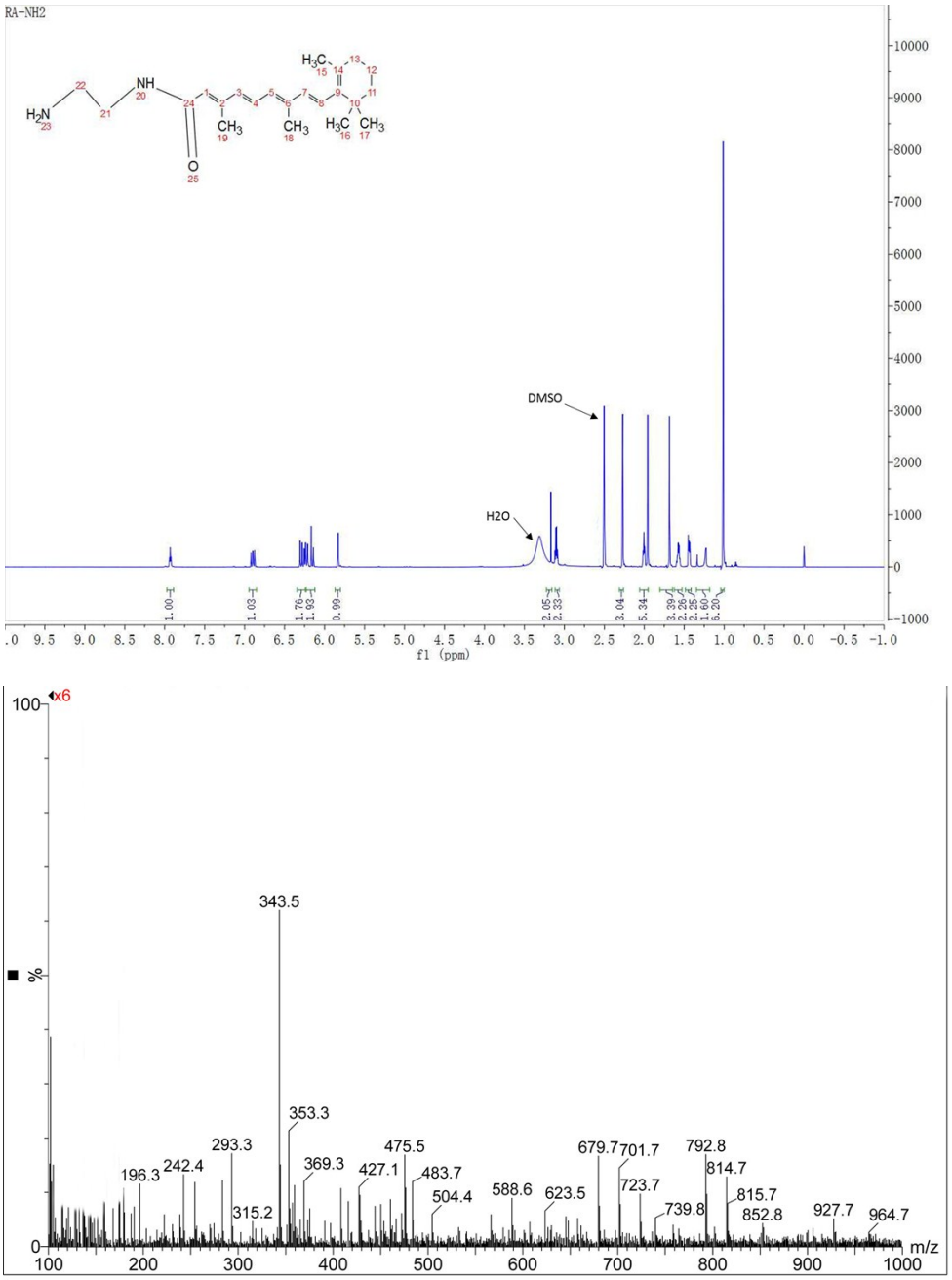

Fig. S2 The ^{1}HNMR spectrum and ESI-MS data of RA-NH_2.

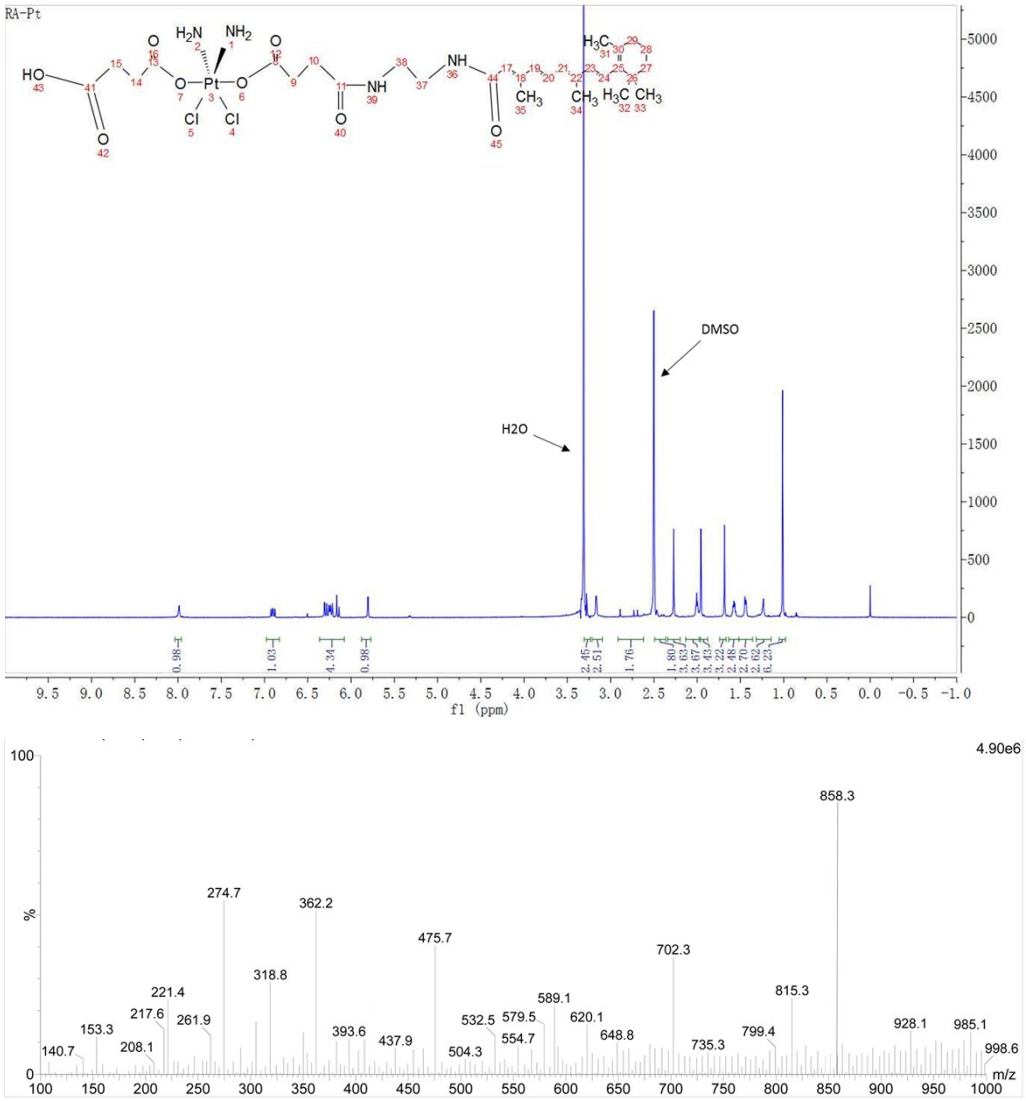

Fig. S3 The ^{1}HNMR spectrum and ESI-MS data of RA-Pt.

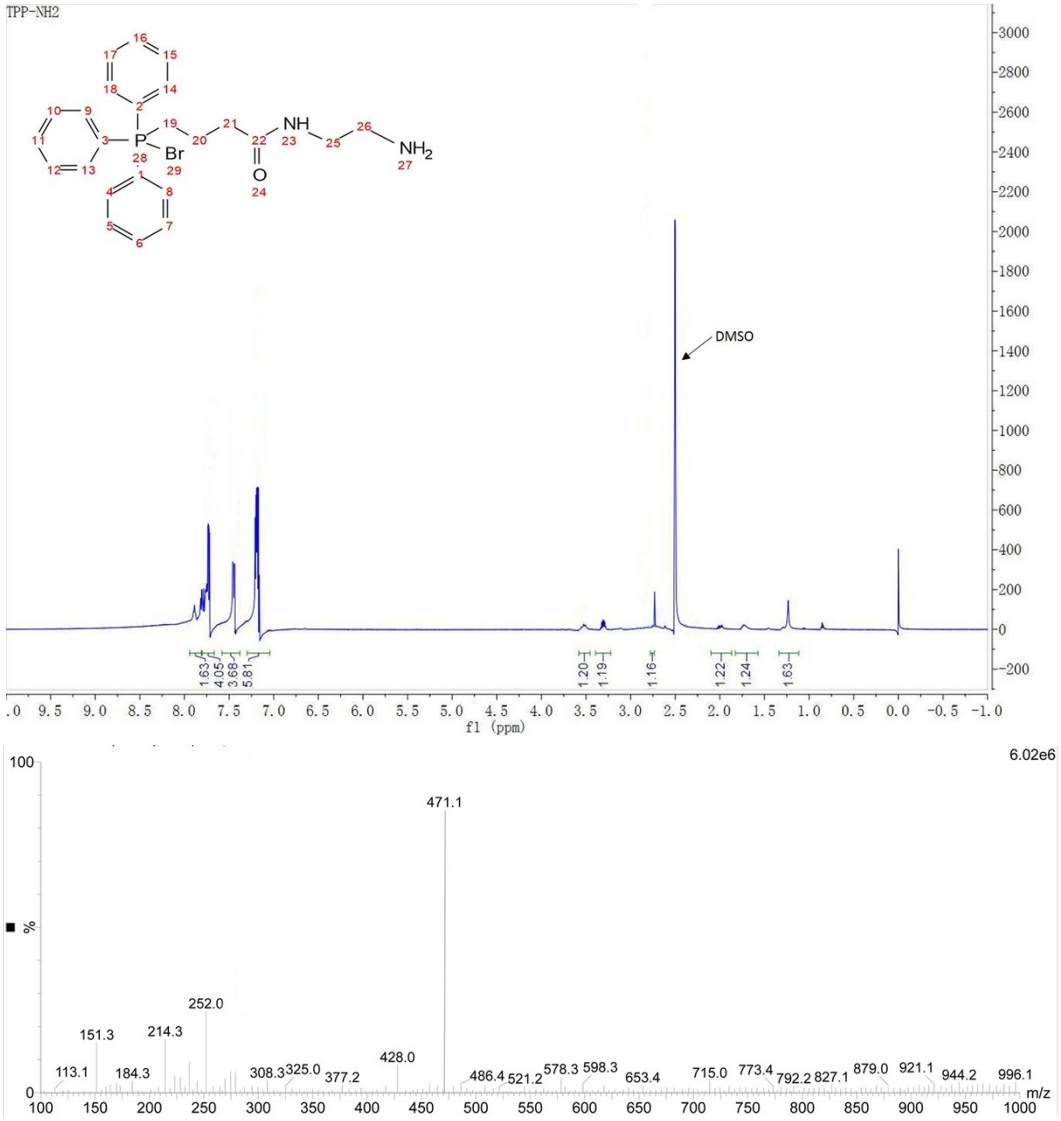

Fig. S4 The ^{1}HNMR spectrum and ESI-MS data of TPP-NH_2.

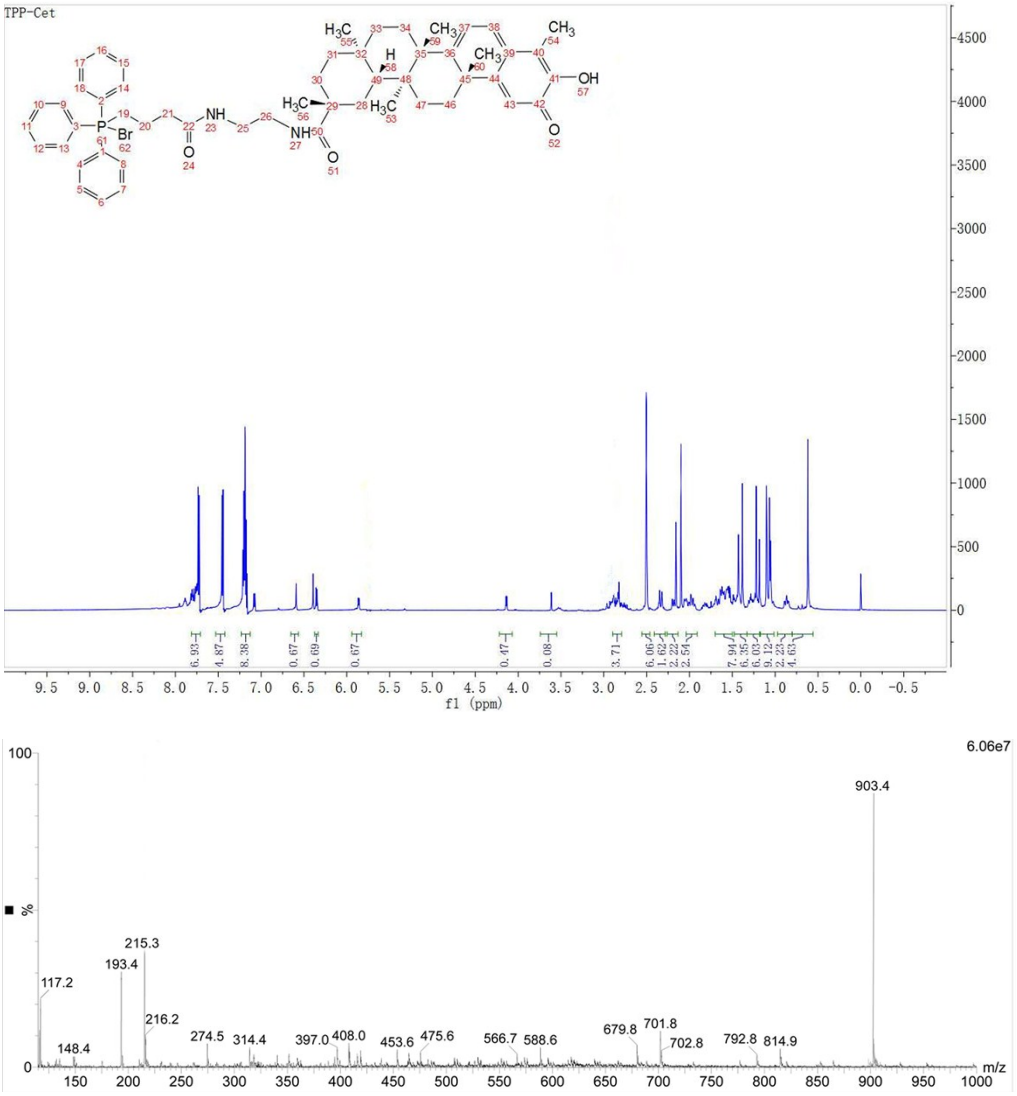

Fig. S5 The ^{1}HNMR spectrum and ESI-MS data of TPP-Cet.

Samples	cRGD-PEG-PCL/FA-PEG-DSPE/PLGA/Lecithin (mg)	Dimeter (nm)	PDI	ζ potential (mV)
NPs		**131.2**	**0.311**	**-24.93**
Fc-NPs	**1:1:10:30**	**79.88**	**0.253**	**-50.60**
RA-Pt/TPP-Cet@Fc-lNPs		**99.65**	**0.261**	**-49.97**

Table S1 Dimeter, Polymer dispersion index (PDI), Zeta potential from blank nanoparticles without targeted ligands (NPs), blank nanoparticles conjugate folate and cRGD (Fc-NPs), RA-Pt and TPP-Cet loaded nanoparticles conjugate folate and

cRGD (RA-Pt/TPP-Cet@Fc-INPs).

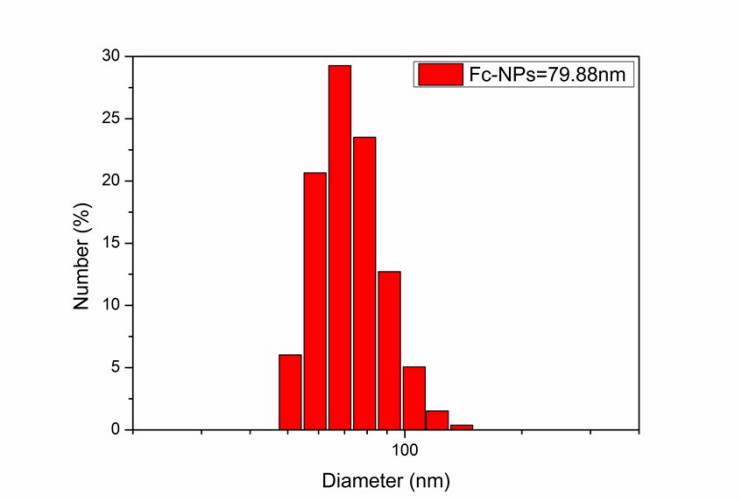

Fig. S6 Size distribution of folate and cRGD conjugate nanoparticles without drugs (Fc-NPs).

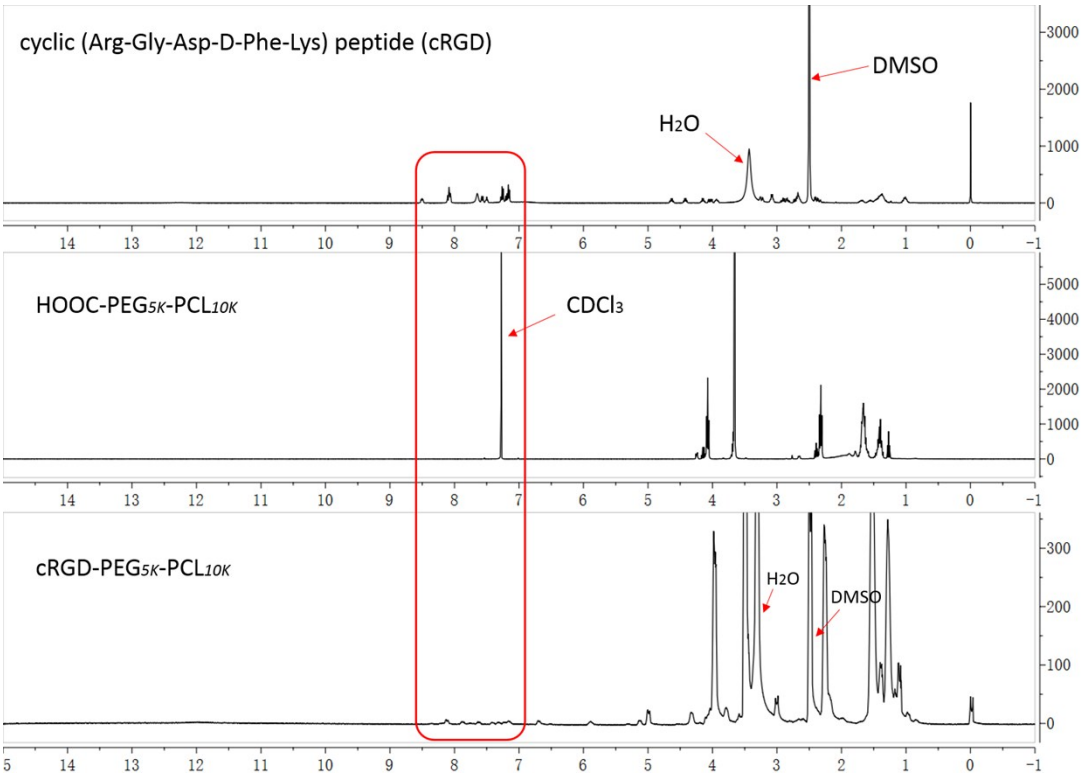

Fig. S7 ^{1}HNMR spectra of cRGD conjugated amphiphilic copolymer. The characteristic peaks of the benzene ring CH on cRGD at δ 7.15-8.11 ppm were obviously appeared in the picture of cRGD-PEG_{5k}-PCL_{10k} at 7.16-7.89 ppm while HOOC-PEG_{5k}-PCL_{10k} showed nothing at this region.

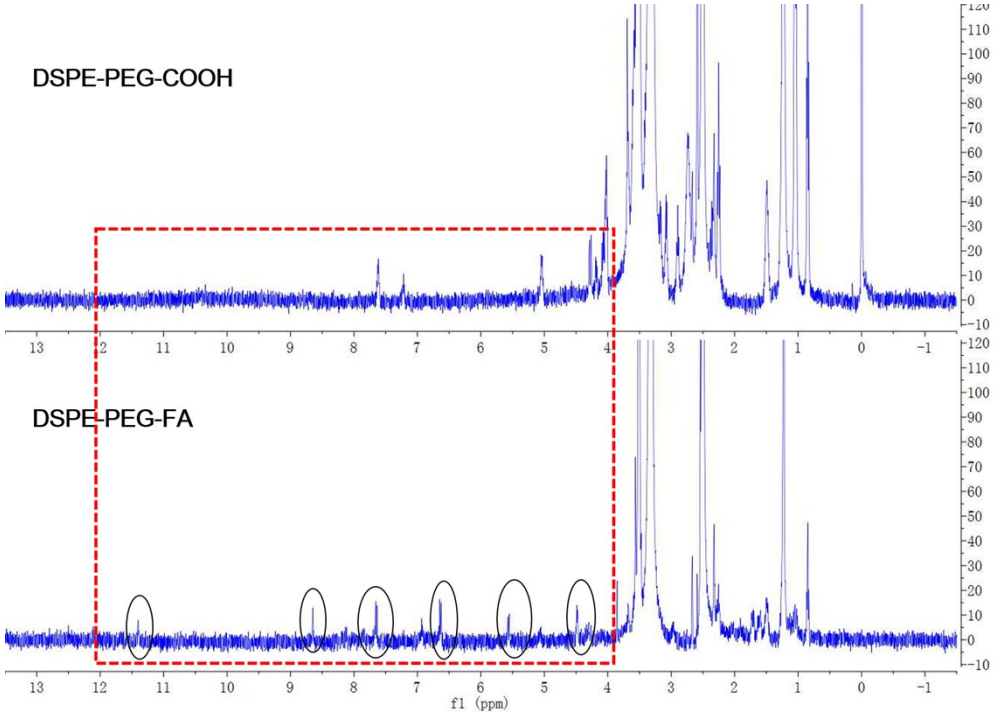

Fig. S8 [1]HNMR spectra of cRGD conjugated amphiphilic copolymer. The characteristic peaks of the folate at δ 4.55-11.48 ppm were obviously appeared in the picture while the HOOC-PEG2k-DSPE showed nothing at this region, which confirmed the conjugation of folate.

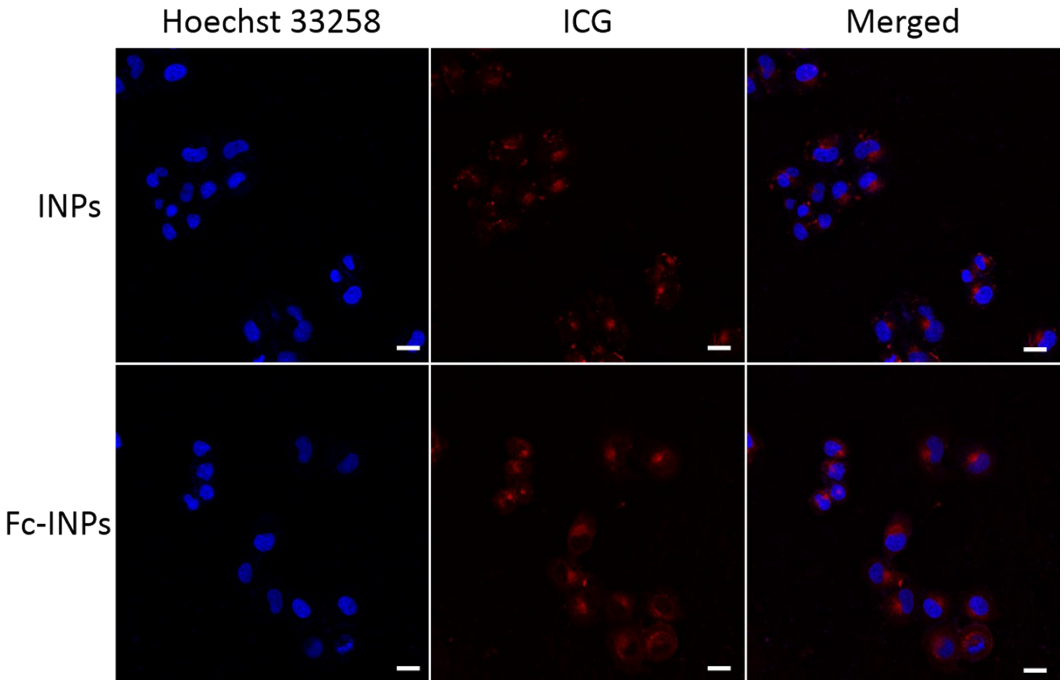

Fig. S9 CLSM images of MCF-7 cells incubated with INPs and Fc-INPs for 6 h. Images

from left panel to right present cell nuclei stained by Hoechst 33258 (blue), ICG fluorescence in cells (red), and merge of the two images, respectively. Scale bar: 20 μm.

IC_{50} (μmol/L)	MDA-MB-231	MCF-7
Cislaptin	17.35±0.22	12.96±0.25
RA-Pt	14.85±0.31	9.06±0.21
Celastrol	6.13±0.12	7.23±0.18
TPP-Cet	4.58±0.32	6.17±0.27

Table S2 IC_{50} [μmol/L] for the 24 h of action of investigated drugs on MDA-MB-231 and MCF-7 determined by MTT assay.