Electronic Supplementary Material (ESI) for Polymer Chemistry.
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Supporting Information

A Poly (amidoamine) Dendrimer-Based Nanocarrier Conjugated with Angiopep-2 for Dual-Targeting Function in Treating Gliomas Cells

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Experimental

Synthesis of thiolated doxorubicin (DOX-SH)

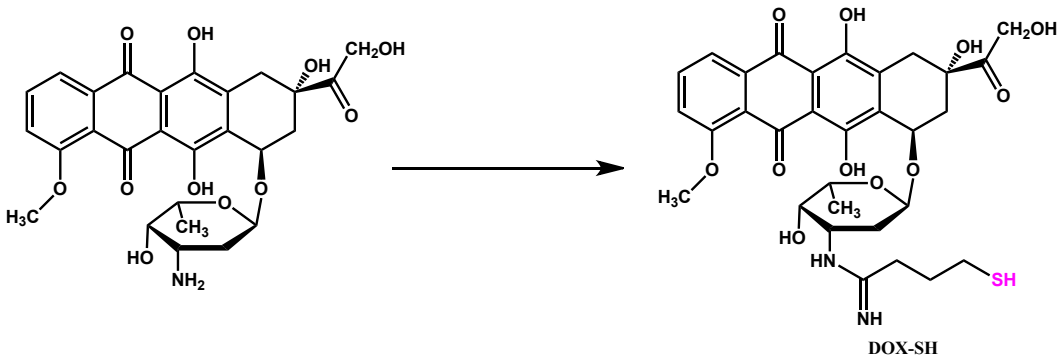
Thiolated doxorubicin (DOX-SH) was prepared according to the literature.[1] DOX-SH was synthesized by mixing DOX and 2-iminothiolane under argon protection (Scheme S1). The solution was stirred for 48 h at dark to ensure the high conversion efficiency of DOX to DOX-SH (ESI-MS calculated $[M+1]^+$ for DOX-SH: 645.6, observed $[M+1]^+$: 645.2, Fig. S1).

Synthesis of G4-DOX-PEG

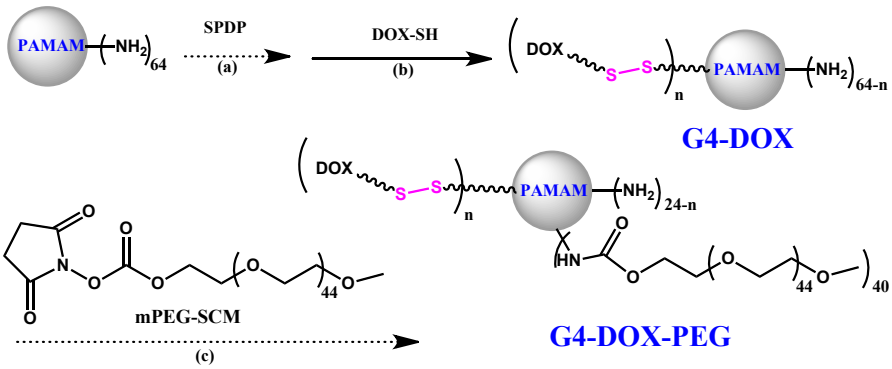
G4-DOX-PEG was synthesized by the reactions described in Scheme S2. The activated DOX was coupled to the exterior of G4 dendrimers via a disulfide linkage. ^{1}H NMR was employed to characterize the obtained compounds. Then PEG chains were grafted onto G4-DOX.

Synthesis of G4-Angiopep-PEG

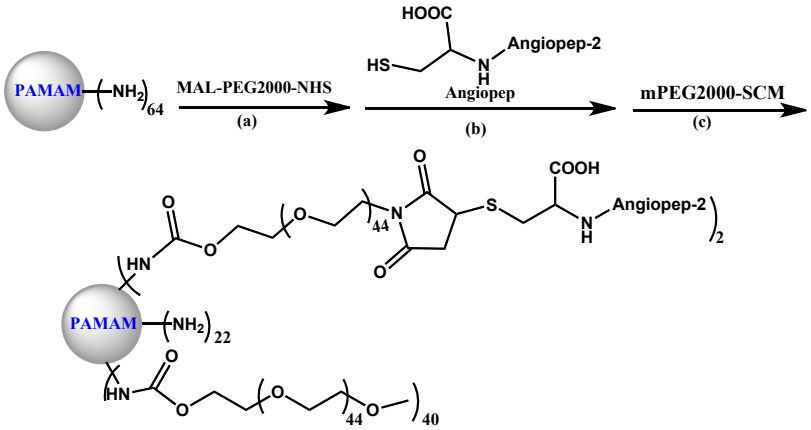
G4-Angiopep-PEG (Scheme S3) was synthesized by reacting G4 PAMAM with MAL-PEG2000-NHS, and next Angiopep was coupled to G4-MAL. The linking of target ligands was performed subsequently in the aqueous solution within a short reaction time to reduce the possibility of protein denaturation. Finally mPEG2000-SCM chains were grafted onto G4-Angiopep. The number of Angiopep-2 moieties on per G4 PAMAM was determined by ^{1}H NMR measurements (Fig. S2).



Scheme S1. Synthesis of thiolated doxorubicin (DOX-SH)**.**



Scheme S2. The synthetic route of G4-DOX-PEG. Reagents and conditions: (a) H_2O, DMF, rt, 2 h, (b) N_2, H_2O, DMF, rt, 2 h, (c) H_2O, DMSO, rt, over night. (rt = room temperature)



G4-Angiopep-PEG

Scheme S3. The synthetic route of dual-targeting drug carrier of G4-Angiopep-PEG. Reagents and conditions: (a) H_2O, DMSO, rt, 15 min, (b) H_2O, DMSO, rt, 30 min, (c) H_2O, DMSO, rt, over night. (rt = room temperature)

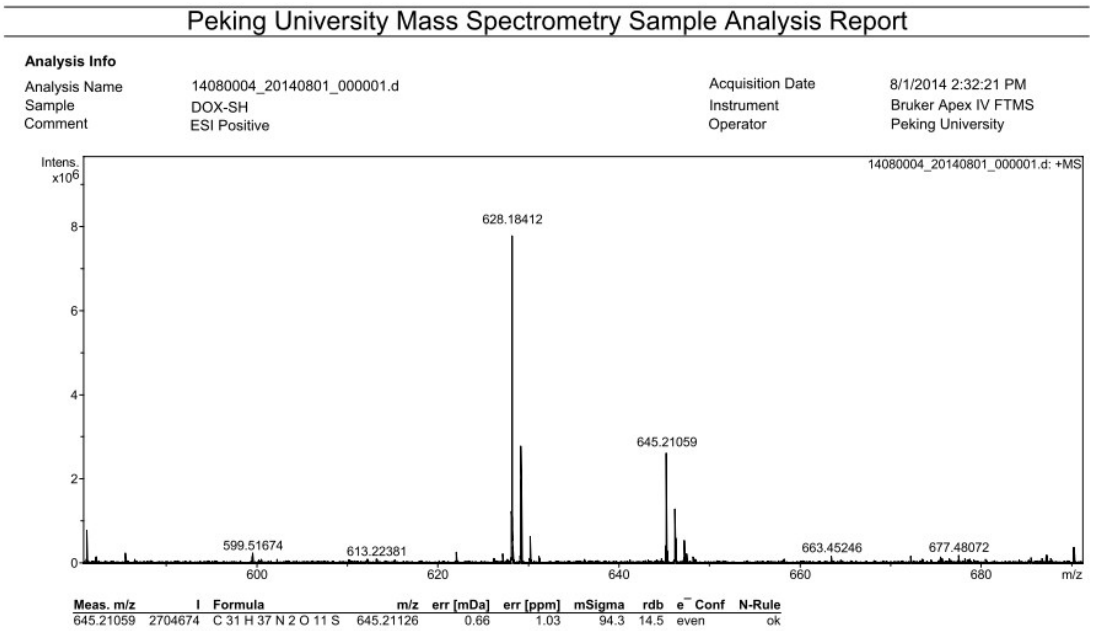


Fig. S1 ESI-MS spectra of DOX-SH.

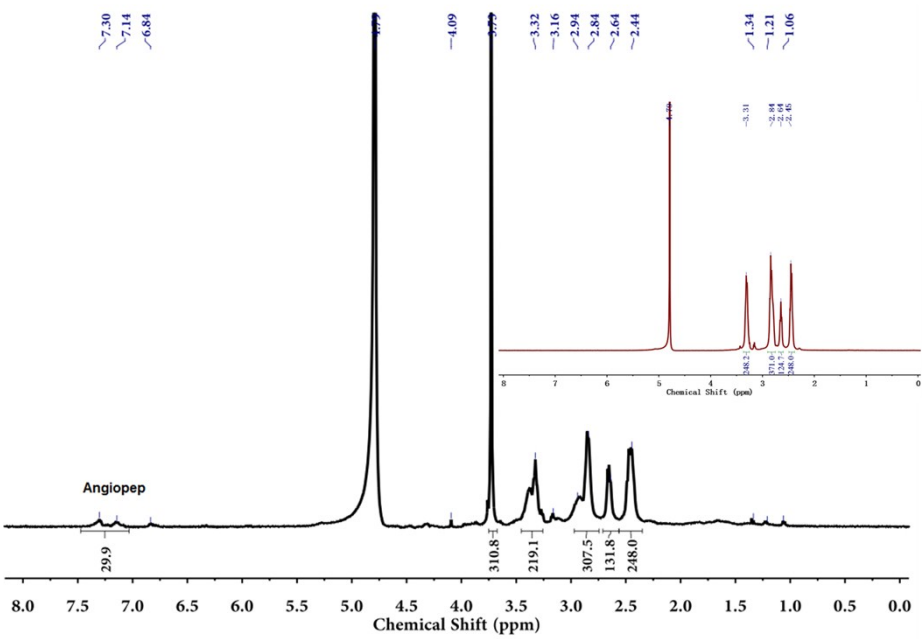


Fig. S2 ^{1}H NMR spectrum of G4-Angiopep-PEG and G4 (inset).

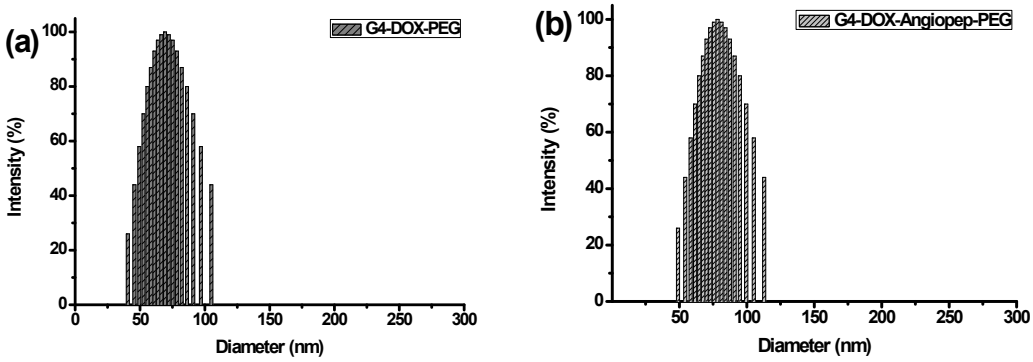


Fig. S3 DLS graphs of (a) G4-DOX-PEG and (b) G4-DOX-Angiopep -PEG. Data were presented as the mean ± standard deviation (n = 3).

Table S1. The zeta potential data of the drug carriers in water.

Drug carriers	Zeta potential (mV)
G4-DOX-Angiopep	16.22±1.37
G4-DOX-PEG	2.61±1.90

G4-DOX-Angiopep-PEG	3.56±2.23

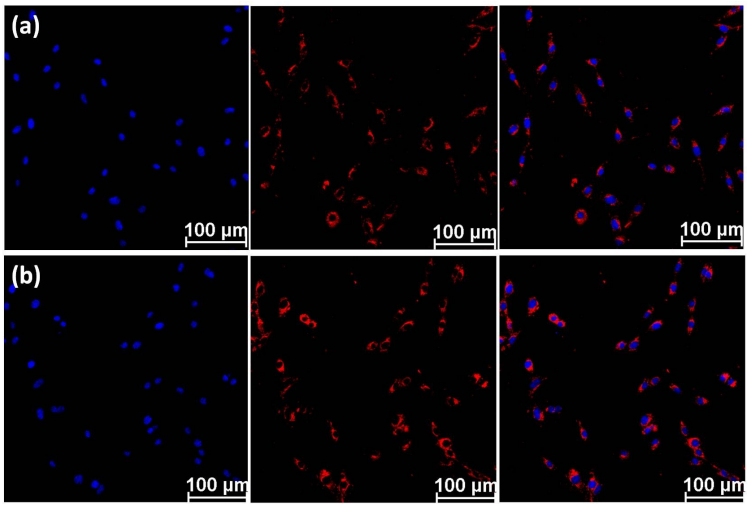


Fig. S4. Confocal images of the C6 cells incubated 6 h in DMEM medium with (a) G4-DOX-PEG and (b) G4-DOX-Angiopep-PEG, respectively. For each panel, images from left to right showed the cells with nuclear staining by Hoechst 33258, with DOX fluorescence, and overlays of both images (scale bar, 100 μm).

References

1 X. Wang, X. Cai, J. Hu, N. Shao, F. Wang, Q. Zhang, J. Xiao and Y. Cheng, *J. Am. Chem. Soc.*, 2013, **135**, 9805.