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Supporting Information

Stimuli-responsive Janus peptide dendron-drug conjugate as a safe nanoscale drug delivery vehicle for breast cancer therapy

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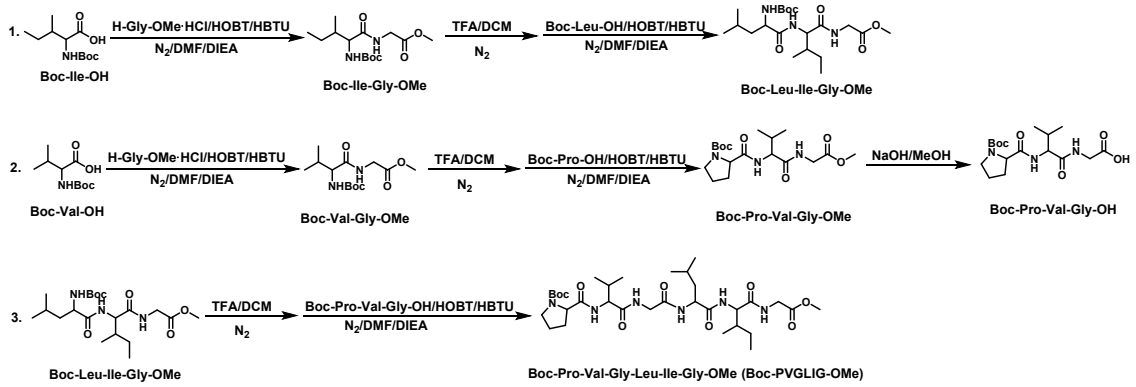
1. Synthesis of enzyme-responsive peptide



Synthesis of Boc-Ile-Gly-OMe. Under a nitrogen atmosphere, Boc-Ile-OH·1/2 H_2O (9.2 g, 40 mmol), H-Gly-OMe·HCl (7.5 g, 60 mmol), HBTU (18.4 g, 56 mmol) and HOBT (7.6 g, 56 mmol) were dissolved in anhydrous DMF (100 mL). The solution was stirred for 5 min in ice bath, and DIPEA (40 mL, 240 mmol) was added. The solution was stirred in ice bath for 30 min and at room temperature for 24 h. After reaction, the solvents were removed. EtOAc (300 mL) was added and the organic solution was washed with $NaHCO_3$ aq. (satd.), HCl (1 M) and NaCl aq. The solution was dried ($MgSO_4$) and concentrated to 20 mL by rotary evaporation. The final product was obtained by recrystallization from EtOAc and ether, giving white solid (10.9 g, 36 mmol) in 90% yield. ESI-MS: m/z =303.37 [$(M + H)^+$, $C_{14}H_{26}N_2O_5^+$].

Synthesis of Boc-Leu-Ile-Gly-OMe. Under a nitrogen atmosphere, product *Boc-Ile-Gly-OMe* (6.2 g, 20 mmol) was dissolved in anhydrous DCM (15 mL). The solution was stirred for 5 min in ice bath, and 15 mL TFA was added. The solution was stirred for 4 h and the solvents were removed. Then anhydrous ether was added, giving white solid. Under a nitrogen atmosphere, the white solid, Boc-Leu-OH (3.1 g, 13.3 mmol), HOBT (2.7 g, 20 mmol) and HBTU (7.6 g, 20 mmol) were dissolved in anhydrous DMF (80 mL). The DIPEA (13 mL, 80 mmol) was added by dropwise under ice bath. The solution was stirred in ice bath for 30 min and at room temperature for 24 h. After reaction, the solvents were removed. EtOAc (400 mL) was added and the organic solution was washed with $NaHCO_3$ aq. (satd.), HCl (1 M) and NaCl aq. The solution was dried ($MgSO_4$) and concentrated to 15 mL by rotary evaporation. The final product was obtained by recrystallization from EtOAc and ether, giving white solid (4.4 g, 11 mmol) in 80% yield. ESI-MS: m/z = 438.53 [$(M + Na)^+$, $C_{20}H_{37}N_3O_6Na^+$].

Synthesis of Boc-Val-Gly-OMe. Under a nitrogen atmosphere, Boc-Val-OH (5.2 g, 24 mmol), H-Gly-OMe·HCl (2.0 g, 16 mmol), HOBT (3.2 g, 24 mmol) and HBTU (9.1 g, 24 mmol) were dissolved in anhydrous DMF (100 mL). The solution was stirred for 5 min in ice bath, and DIPEA

(17 mL, 96 mmol) was added. The solution was stirred in ice bath for 30 min and at room temperature for 24 h. After reaction, the solvents were removed. EtOAc (500 mL) was added and the organic solution was washed with $NaHCO_3$ aq. (satd.), HCl (1 M) and NaCl aq. The solution was dried ($MgSO_4$) and concentrated to 15 mL by rotary evaporation. The final product was obtained by recrystallization with EtOAc and ether, giving white solid (5.9 g, 21 mmol) in 86% yield. ESI-MS: m/z = 311.34 [$(M + Na)^+$, $C_{13}H_{24}N_2O_5Na^+$].

Synthesis of Boc-Pro-Val-Gly-OMe. Under a nitrogen atmosphere, the Boc group on the product Boc-Val-Gly-OMe (4.0 g, 14 mmol) was deprotected as description in "*synthesis of Boc-Leu-Ile-Gly-OMe*", giving white solid. Under a nitrogen atmosphere, the white solid, Boc-Pro-OH (2.2 g, 10 mmol), HBTU (5.7 g, 15 mmol) and HOBT (2.0 g, 15 mmol) were dissolved in anhydrous DMF (50 mL). The DIPEA (10 mL, 60 mmol) was added under ice bath. The solution was stirred in ice bath for 30 min and at room temperature for 24 h. After reaction, the solvents were removed, EtOAc (300 mL) was added and the organic solution was washed with $NaHCO_3$ aq. (satd.), HCl (1 M) and NaCl aq. The solution was dried ($MgSO_4$) and concentrated to 15 mL by rotary evaporation. The final product was obtained by recrystallization from EtOAc and ether, giving white solid (2.3 g, 6 mmol) in 60% yield. ESI-MS: m/z = 386.46 [$(M + H)^+$, $C_{18}H_{31}N_3O_6^+$].

Synthesis of Boc-Pro-Val-Gly-OH. The product *Boc-Pro-Val-Gly-OMe* (7.2 g, 19.4 mmol) was first dissolved in methanol and NaOH aq. (1 M, 60 mL) was added under ice bath. The solution was stirring for 24 h at 4 ^oC. After reaction, the methanol was removed and solution was added into EtOAc (500 mL).The pH of mixture was adjusted to 2-3 by 0.5 M HCl aq. The solution was washed by NaCl aq. (satd.) for 3 times, and the solvent was removed. White solid was collected and waited for further use.

Synthesis of Boc-Pro-Val-Gly-Leu-Ile-Gly-OMe. Under a nitrogen atmosphere, product *Boc-Leu-Ile-Gly-OMe* (10.4 g, 25 mmol) was dissolved in anhydrous DCM (19 mL). The solution was stirred for 5 min in ice bath, and TFA (19 mL) was added. The solution was stirred for 4 h and the solvents were removed. Then anhydrous ether was added, giving white solid.

Under a nitrogen atmosphere, the white solid, product *Boc-Pro-Val-Gly-OH* (7.5 g, 20 mmol), HOBT (3.4 g, 22 mmol) and HBTU (8.3 g, 22 mmol) were dissolved in anhydrous DMF (100 mL). The DIPEA (21 mL, 120 mmol) was added under ice bath. The solution was stirred in ice bath for 30 min, and at room temperature for 48 h. After reaction, the solvents were removed. EtOAc (500 mL) was added and the organic solution was washed with $NaHCO_3$ aq. (satd.), HCl aq (1 M) and NaCl aq. White solid was precipitated in organic phase, and the precipitate was collected via centrifugation. ESI-MS: m/z = 691.41 [$(M + Na)^+$, $C_{32}H_{56}N_6O_9Na^+$].

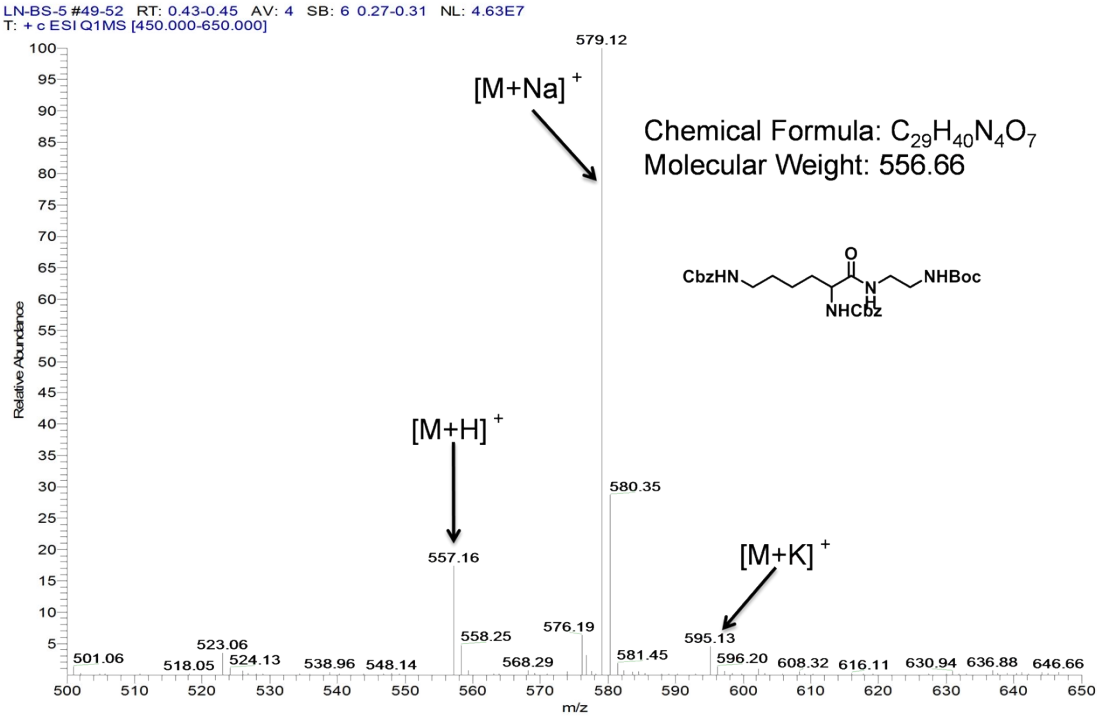


Fig. S1. The ESI-MS of Dendron "G1L". The structure, chemical formula and the molecular weight of the "G1L" were presented. The observed mass were 557.16 $[M+H]^+$, 579.12 $[M+Na]^+$ and 595.13 $[M+K]^+$.

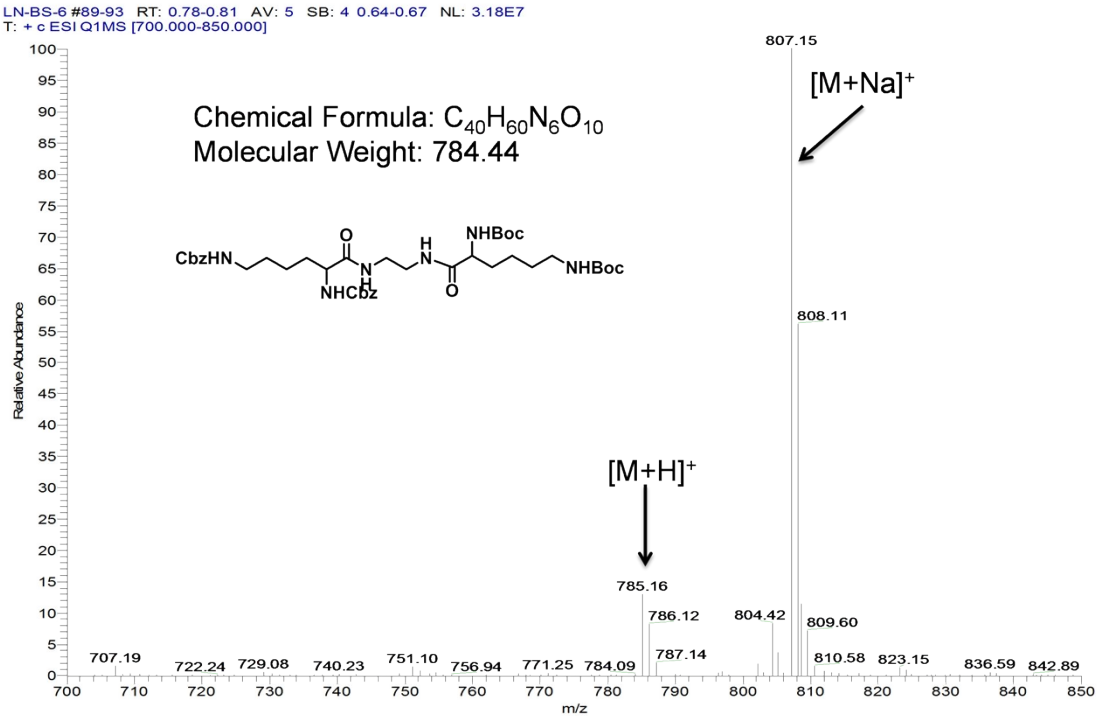


Fig. S2. The ESI-MS of Dendron (G1L-G1L). The structure, chemical formula and the molecular weight of the "G1L-G1L" were presented. The observed mass were 785.16 $[M+H]^+$ and 807.15 $[M+Na]^+$.

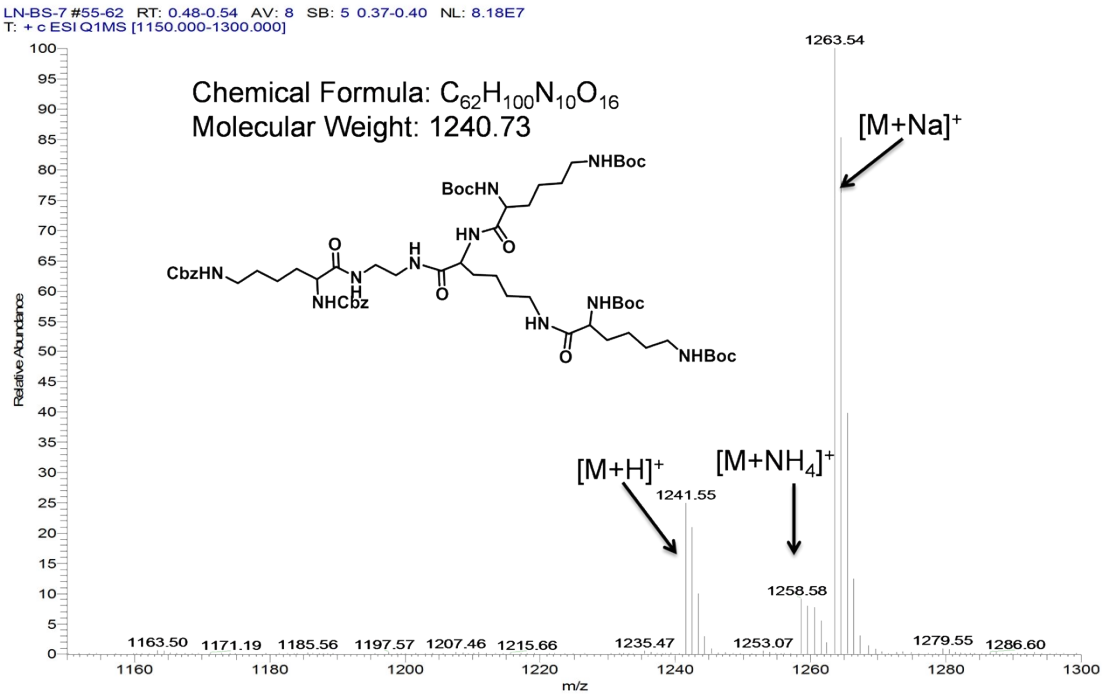


Fig. S3. The ESI-MS of Dendron (G1L-G2L). The structure, chemical formula and the molecular weight of the "G1L-G2L" were presented. The observed mass were 1241.55 $[M+H]^+$, 1258.58 $[M+NH_4]^+$ and 1263.54 $[M+Na]^+$.

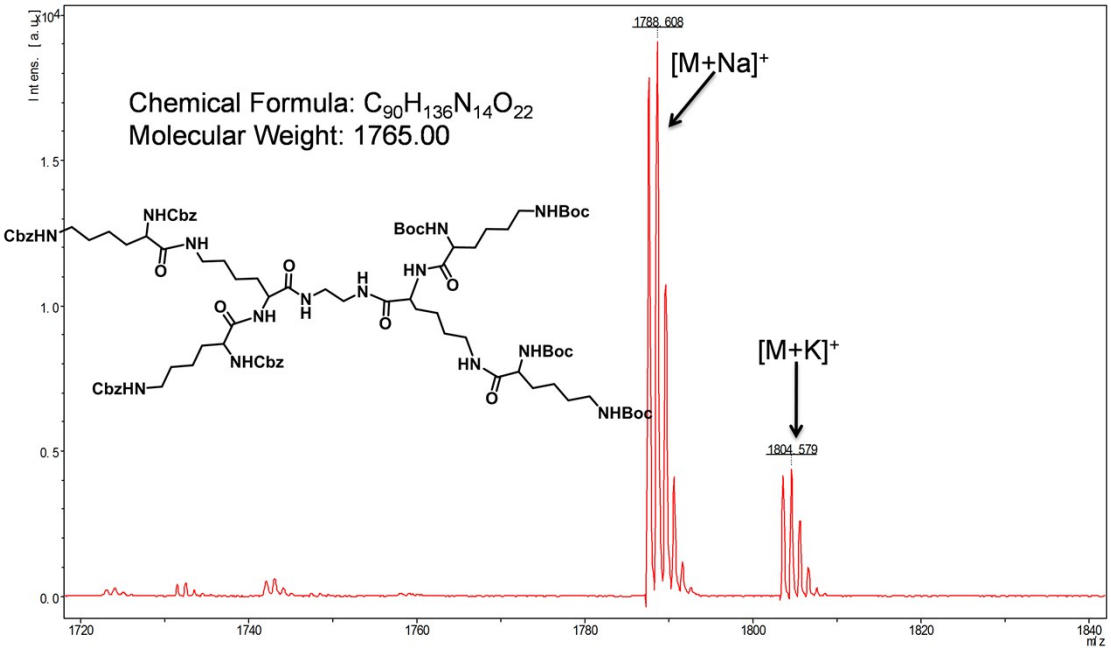


Fig. S4. The MALDI-TOF mass spectrum of Dendron (G2L-G2L). The structure, chemical formula and the molecular weight of the "G2L-G2L" were presented. The observed mass were 1788.608 $[M+Na]^+$ and 1804.579 $[M+K]^+$.

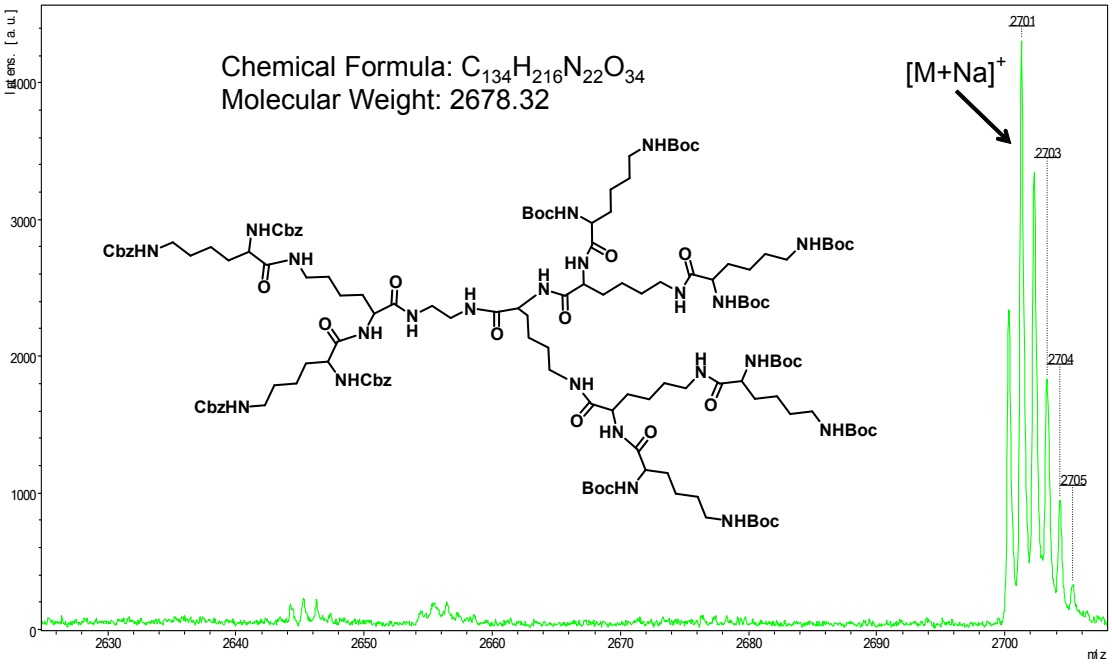


Fig. S5. The MALDI-TOF mass spectrum of Dendron (G2L-G3L). The structure, chemical formula and the molecular weight of the "G2L-G3L" were presented. The observed mass was 2701 $[M+Na]^+$.

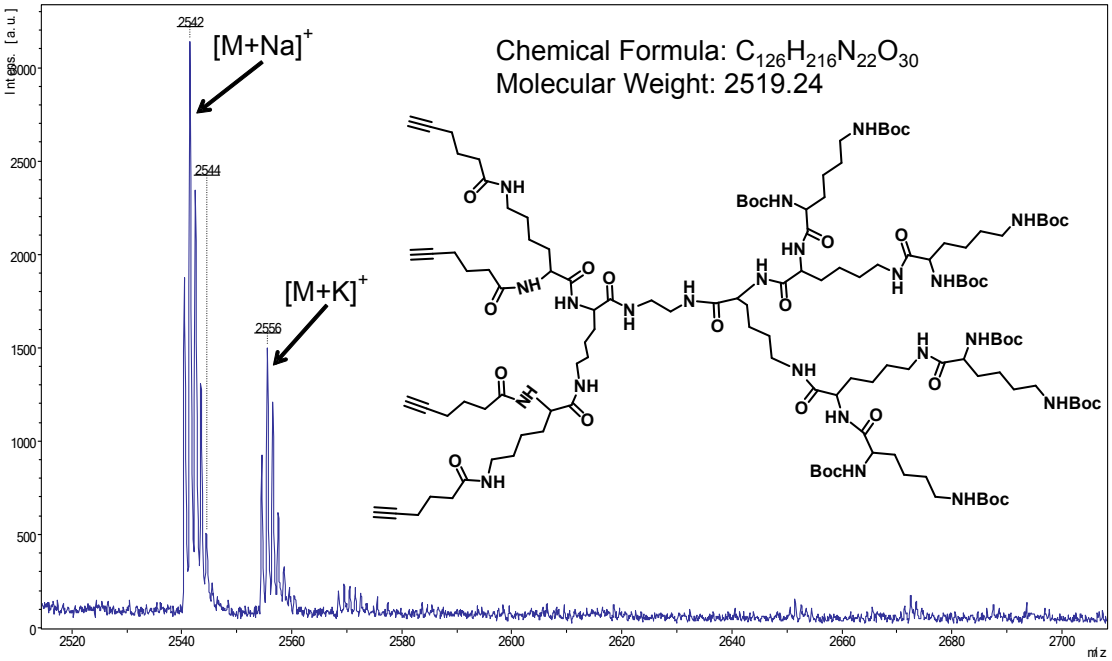


Fig. S6. The MALDI-TOF mass spectrum of Dendron (Alkyne-G2L-G3L). The structure, chemical formula and the molecular weight of the "Alkyne-G2L-G3L" were presented. The observed mass was 2542 $[M+Na]^+$ and 2556 $[M+K]^+$.

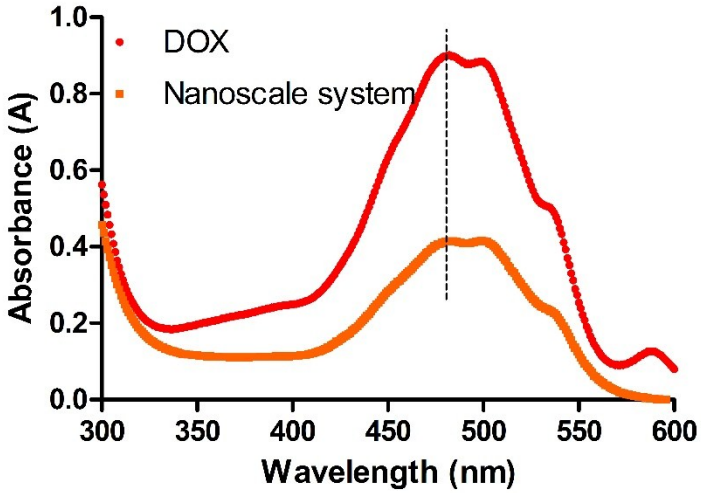


Fig. S7 UV-Vis spectra of free drug DOX and mPEGylated dendron-PVGLIG-DOX conjugate nanoscale system (Nanoscale system) in DMSO.

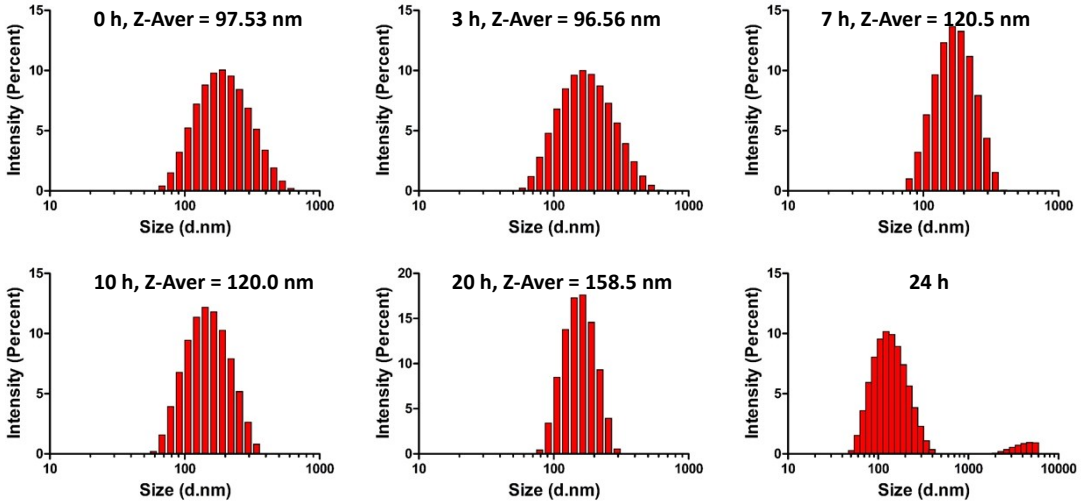


Fig. S8 Stability of mPEGylated dendron-PVGLIG-DOX nanoscale system in the PBS at different time points, as measured by DLS. The real-time hydrodynamic size of the particle incubated in buffer (pH 7.4) for 24 h were recorded.