Supporting Information

Glutathione-Activatable Bola Dendrimers Mediate Tumor-Specific Cytosolic siRNA Delivery via Dynamic Thiol-Disulfide Exchange

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1. Materials

N, N, N', N'-tetramethyl-(1H-benzotriazol-1-yl)-uranium hexafluorophosphate (HBTU), Boc-L-Lys(Boc)-OH, 1-hydroxybenzotriazole (HOBT) were purchased from GL Biochem Ltd. (Shanghai, China). Hydrogen chloride (HCl), propargylamine, 1-bromooctadecane, N, N-diisopropylethylamine (DIPEA), 1,8-Diazabicyclo[5.4.0] undec-7-ene (DBU) and pyrene were purchased from Energy Chemical Ltd. (Shanghai, China). Cystamine dihydrochloride, 6-Chlorohexanol, 8-Bromo-1-octanol, 11-Bromo-1-undecanol, 8-Bromooctanoicacid, Azidotrimethylsilane, 4-Dimethylaminopyridine (DMAP), 1,6-Hexanediamine, Trifluoroacetic acid (TFA) were purchased from Aladdin. (Shanghai, China). 4-Nitrophenyl chloroformate was purchased from TCI Shanghai (Shanghai, China). Cesium fluoride was purchased from J&K Scientific (Beijing, China). Cuprous iodide (CuI) was purchased from Sigma-Aldrich (Shanghai, China). Sodium hydroxide (NaOH), sodium shloride (NaCl), triethylamine was purchased from China National Medicines Corporation Ltd. (Shanghai, China). Petroleum ether (PE), ethyl acetate (EA), dichloromethane (CH_2Cl_2), methanol (MeOH), dimethyl sulfoxide (DMSO) and dimethylformamide (DMF) were distilled before use. All the other reagents and solvents were used any further purification from commercial sources. Analytical thin layer chromatography (TLC) was performed using silica gel60 F254 plates 0.2 mm thick with UV light (254 and 364 nm) as revelator. Chromatography was prepared on silica gel (Merck 200-300 mesh). Dialysis tubing was purchased from Sigma Aldrich (St. Quentin Fallavier, France) and Yuanye Bio-Technology Co., Ltd. (Shanghai, China).

The human AKT2 siRNA (sense: 5'-GCUCCUUCAUUGGGUACAAdTdT-3'; antisense: 5'-UUGUACCCAAUGAAGGAGCTdTd-3'), PLK1 siRNA (sense: 5'-UGA AGAAGAUCACCCUCCUUAdTdT-3'; antisense: 5'-UAAGGAGGGUGAUCUUCU UCAdTdT-3'), PLK1 siRNA (sense: 5'-UGAAGAAGAUCACCCUCCUUAdTdT-3'; antisense: 5'-UAAGGAGGGUGAUCUUCUUCAdTdT-3') and scramble siRNA (sense: 5'-CUU ACG CUG AGU ACU UCG A dTdT-3'; antisense: 5'-UCG AAG UAC UCA GCG UAA G dTdT-3') was purchase from Guangzhou Ruibo (Guangzhou, China). RNase A and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were supplied by Sigma-Aldrich (Merck Life Science, Shanghai, China). Hochest33342 and Lyso-Tracker Red were purchased from Beyotime Biotechnology (Shanghai, China). All the biochemical reagents are suitable for cell culture and were

purchased from Sigma-Aldrich (Shanghai, China), Servicebio (Wuhan, China), (Cell signaling Technology (Massachusetts, USA), Vazyme Biotech Co. Ltd. (Nanjing, China) and Thermo Fisher Scientific Inc. (Carlsbad, USA). All other reagents and solvents were used without any further purification from commercial sources.

High-performance liquid chromatography (HPLC) was performed on a Waters Empower system (Waters 1525, binary HPLC pump) equipped with a photodiode array detector (Waters 2998) and a SinoChrom C8 column (5 μm, 4.6 mm × 250 mm), using a gradient elution mode (10–100% acetonitrile in water) over 30 minutes with acetonitrile and water as the mobile phases (both containing 0.04% TFA). The flow rate was 0.8 mL/min, and the injection volume was 20 μL. Peaks were detected at 210 nm. The retention times (RTs) were in minutes.

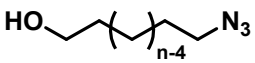
A Bruker AC (300 MHz) spectrometer was employed to determine the ^{1}H NMR and ^{13}C NMR spectra of compounds. The temperature for all NMR data collection is 300 K if not specified. Chemical shifts were recorded in parts per million (δ, ppm) with reference to tetramethylsilane (TMS). The HRMS were measured by using the Agilent 6230 time-of-flight LC/MS (LC/TOF) system. The infrared spectra were recorded by using a Bruker ALPHA FT-IR spectrophotometer (Bruker, Ettlingen, Germany) in the range of 400-4000 cm.

2. Method

Synthesis and characterization of bola amphiphile peptide dendrimers

Compound peptide dendron was synthesized according to the literature.[1][4]

Synthesis of hydrophobic bola lipid chain



HO-C_6-N_3: Azide trimethylsilane (403 mg, 3.5 mmol, 2.5 eq), Cesium fluoride (532 mg, 3.5 mmol, 2.5 eq) were dissolved in anhydrous DMF (2.0 mL) under nitrogen atmosphere, stirring for 30 min at room temperature. A solution of 6-Chlorohexanol (190 mg, 1.4 mmol, 1.0 eq) in anhydrous DMF (3.0 mL) were added to the mixture. The reaction mixture was stirred under nitrogen for 12 h at 65℃ until the reaction was complete as indicated by TLC. After solvent evaporation, the reaction solution was diluted with 20 mL EA and washed with water (3 × 10.0 mL). The organic phase was washed by saturated NaCl solution (15.0 mL), dried with Na_2SO_4, filtered, and concentrated. The crude product was further purified by silica gel column

chromatography (PE/EA = 6/1→4/1), yielding **HO-C_6-N_3** as colorless oily liquid (178 mg, 89%).

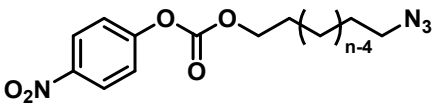
^{1}H NMR (300 MHz, $CDCl_3$) δ 3.65 (t, J = 6.5 Hz, 2H), 3.27 (t, J = 6.9 Hz, 2H), 1.73 - 1.29 (m, 8H).

HO-C_8-N_3: Azide trimethylsilane (437 mg, 3.8 mmol, 2.5 eq), Cesium fluoride (578 mg, 3.8 mmol, 2.5 eq) were dissolved in anhydrous DMF (2.0 mL) under nitrogen atmosphere, stirring for 30 min at room temperature. A solution of 8-Bromo-1-octanol (313 mg, 1.5 mmol, 1.0 eq) in DMF (3.0 mL) were added to the above solution. The rest of the protocol is the same as HO-C_6-N_3, yielding **HO-C_8-N_3** as colorless oily liquid (210 mg, 82%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.64 (t, J = 5.4 Hz, 2H), 3.25 (t, J = 6.9 Hz, 2H), 1.70 - 1.50 (m, 4H), 1.39 - 1.28 (m, 8H).

HO-C_{11}-N_3: Azide trimethylsilane (288 mg, 2.5 mmol, 2.5 eq), Cesium fluoride (380 mg, 2.5 mmol, 2.5 eq) were dissolved in anhydrous DMF (2.0 mL) under nitrogen atmosphere, stirring for 30 min at room temperature. A solution of 11-Bromo-1-undecanol (252 mg, 1.0 mmol, 1.0 eq) in DMF (3.0 mL) were added to the above solution. The rest of the protocol is the same as HO-C_6-N_3, yielding **HO-C_{11}-N_3** as colorless oily liquid (177 mg, 83%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 3.64 (t, J = 5.4 Hz, 2H), 3.25 (t, J = 6.9 Hz, 2H), 1.70 - 1.50 (m, 4H), 1.39 - 1.28 (m, 8H).



PNC-C_6-N_3: HO-C_6-N_3 (143 mg, 1.0 mmol, 1.0 eq), 4-Dimethylaminopyridine (25 mg, 0.20 mmol, 0.20 eq) were dissolved in anhydrous DCM (2.0 mL) under nitrogen atmosphere. Triethylamine (0.20 mL, 1.3 mmol, 1.5 eq) was added to the above solution under stirring for 10 min at 0℃. And then a solution of 4-Nitrophenyl chloroformate (310 mg, 1.5 mmol, 1.0 eq) in anhydrous DCM (3.0 mL) were added to the mixture. The reaction mixture was stirred under nitrogen for 5 h at 30℃ until the reaction was complete as indicated by TLC. After solvent evaporation, the mixed solution was diluted with H_2O (15.0 mL) and extracted with DCM (3 × 10.0 mL). The organic phase was washed by saturated NaCl solution (15.0 mL), dried with Na_2SO_4, filtered, and concentrated to get a crude product. The crude product was purified by

column chromatography (PE/EA = 6/1) to yield **PNC-C_6-N_3** as colorless oily liquid (195 mg, 69%).

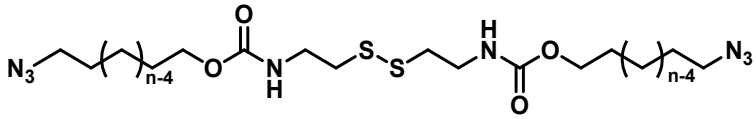
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 - 8.18 (m, 2H), 7.47 - 7.30 (m, 2H) 4.36 (t, J = 6.6 Hz, 2H), 3.29 (t, J = 6.8 Hz, 2H), 1.83 - 1.74 (m, 2H), 1.72 - 1.54 (m, 2H), 1.54 - 1.36 (m, 4H).

PNC-C_8-N_3: HO-C_8-N_3 (172 mg, 1.0 mmol, 1.0 eq) and 4-Dimethylaminopyridine (25 mg, 0.20 mmol, 0.20 eq) were dissolved in anhydrous DCM (2.0 mL) under nitrogen atmosphere. The rest of the protocol is the same as PNC-C_6-N_3, yielding **PNC-C_8-N_3** as colorless oily liquid (285 mg, 85%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 - 8.19 (m, 2H), 7.48 - 7.31 (m, 2H) 4.29 (t, J = 6.7 Hz, 2H), 3.27 (t, J = 6.9 Hz, 2H), 1.88 - 1.70 (m, 2H), 1.65 - 1.56 (m, 2H), 1.50 - 1.24 (m, 8H).

PNC-C_{11}-N_3: HO-C_{11}-N_3 (212 mg, 1.0 mmol, 1.0 eq) and 4-Dimethylaminopyridine (25 mg, 0.20 mmol, 0.20 eq) were dissolved in anhydrous DCM (2.0 mL) under nitrogen atmosphere. The rest of the protocol is the same as PNC-C_6-N_3, yielding **PNC-C_{11}-N_3** as colorless oily liquid (302 mg, 80%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38 - 8.19 (m, 2H), 7.47 - 7.32 (m, 2H) 4.29 (t, J = 6.7 Hz, 2H), 3.26 (t, J = 6.9 Hz, 2H), 1.80 - 1.71 (m, 2H), 1.64 - 1.55 (m, 2H), 1.50 - 1.19 (m, 14H).



n=8, bola DS-C_6-N_3: PNC-C_6-N_3 (100 mg, 0.32 mmol, 2.0 eq) and 4-Dimethylaminopyridine (3.6 mg, 0.030 mmol, 0.20 eq) were dissolved in anhydrous DCM (2.0 mL) under nitrogen atmosphere. Triethylamine (0.10 mL, 0.64 mmol, 4.0 eq) was added to the above solution under stirring for 10 min at 0°C. And then a solution of cystamine (24 mg, 0.16 mmol, 1.0 eq) in anhydrous DCM (1.0 mL) was added to the mixture. The reaction mixture was stirred under nitrogen for 12 h at 30°C until the reaction was complete as indicated by TLC. After solvent evaporation, the mixed solution was diluted with 15 mL H_2O and extracted with DCM (3 × 10.0 mL). The organic phase was washed by saturated NaCl solution (15.0 mL), dried with Na_2SO_4, filtered, and concentrated to get a crude product. The crude product was purified by column chromatography (PE/EA = 10/1) to yield **bola DS-C_6-N_3** as a white solid (66 mg, 85%).

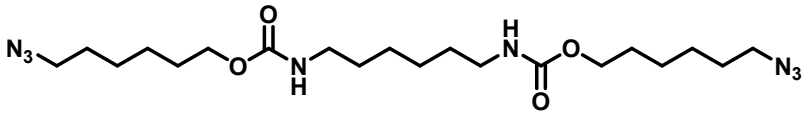
^{1}H NMR (300 MHz, $CDCl_3$) δ 4.06 (t, J = 6.5 Hz, 4H), 3.50 (q, J = 6.2 Hz, 4H), 3.27 (t, J = 6.8 Hz, 4H), 2.81 (t, J = 6.3 Hz, 4H), 1.73 - 1.50 (m, 8H), 1.50 - 1.30 (m, 8H).

n=8, bola DS-C_8-N_3: PNC-C_8-N_3 (108 mg, 0.32 mmol, 2.0 eq) and 4-Dimethylaminopyridine (3.6 mg, 0.03 mmol, 0.2 eq) were dissolved in anhydrous DCM (2.0 mL) under nitrogen atmosphere. The rest of the protocol is the same as bola DS-C_6-N_3, yielding bola **DS-C_8-N_3** as a white solid (73 mg, 74%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.05 (t, J = 6.6 Hz, 4H), 3.49 (q, J = 6.1 Hz, 4H), 3.25 (t, J = 6.9 Hz, 4H), 2.8 (t, J = 6.3 Hz, 4H), 1.74 - 1.48 (m, 8H), 1.42 - 1.33 (m, 16H).

n=11, bola DS-C_{11}-N_3: PNC-C_{11}-N_3 (124 mg, 0.32 mmol, 2.0 eq) and 4-Dimethylaminopyridine (3.6 mg, 0.030 mmol, 0.20 eq) were dissolved in anhydrous DCM (2.0 mL) under nitrogen atmosphere. The rest of the protocol is the same as DS C_6-N_3, yielding bola **DS-C_{11}-N_3** as a white solid (85 mg, 85%)

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.05 (t, J = 6.6 Hz, 4H), 3.50 (q, J = 6.2 Hz, 4H), 3.25 (t, J = 6.9 Hz, 4H), 2.8 (t, J = 6.3 Hz, 4H), 1.62 - 1.55 (m, 8H), 1.38 - 1.27 (m, 28H).

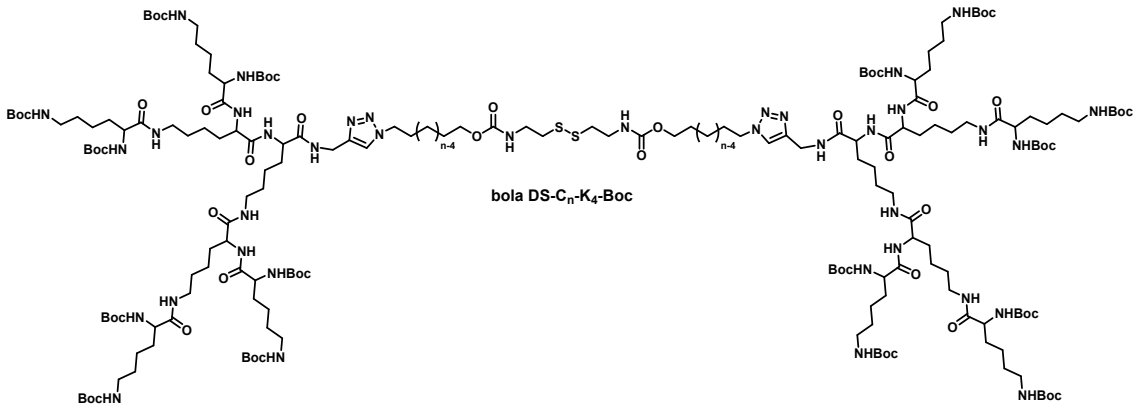


2C_6-N_3: PNC-C_6-N_3 (200 mg, 0.65 mmol, 2.0 eq) and 4-Dimethylaminopyridine (7.9 mg, 0.060 mmol, 0.20 eq) were dissolved in anhydrous DCM (2.0 mL) under nitrogen atmosphere. Trimethylamine (0.2 mL, 1.3 mmol, 4.0 eq) was added to the above solution under stirring for 10 min at 0℃. And then a solution of 1,6-Hexanediamine (38 mg, 0.32 mmol, 1.0 eq) in anhydrous DCM (3.0 mL) was added to the above solution. The reaction mixture was stirred under nitrogen for 12 h at 30℃ until the reaction was complete as indicated by TLC. After solvent evaporation, the mixed solution was diluted with 15.0 mL H_2O and extracted with DCM (3 × 10.0 mL). The organic phase was washed by saturated NaCl solution (15.0 mL), dried with Na_2SO_4, filtered, and concentrated to get a crude product. The crude product was purified by column chromatography (PE/EA = 5/1→3/1) to yield **2C_6-N_3** as a white solid (108 mg, 73%).

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.07 (t, J = 6.5 Hz, 4H), 3.29 (t, J = 6.8 Hz, 4H), 3.18 (q, J = 6.5 Hz, 4H), 1.62 (d, J = 20.4 Hz, 8 H), 1.58 - 1.46 (m, 4H), 1.46 - 1.39 (m,

8H), 1.35 (dt, J = 7.1, 3.3 Hz, 4H).

Synthesis and characterization of bola DS-C_n-K_4 (n = 6, 8, 11)



bola DS C_6-K_4-Boc: The azide precursor bola DS-C_6-N_3 (23 mg, 0.047 mmol, 1.0 eq), Copper(1) iodide (4.7 mg, 0.024 mmol, 0.50 eq) and PD 2-5 (173 mg, 0.098 mmol, 2.1 eq) were dissolved in 5.0 mL anhydrous DMF under nitrogen atmosphere. DBU (54 mg, 0.35 mmol, 7.5 eq) was added to the above solution under stirring at 0℃. The reaction mixture was stirred under nitrogen for 3 h at 50℃ until the reaction was complete as indicated by TLC and FT-IR. After solvent evaporation, the mixed solution was diluted with 25.0 mL $CHCl_3$ and washed with saturated NH_4Cl solution (3 × 10.0 mL). The organic phase was washed by saturated NaCl solution (15 mL), dried with Na_2SO_4, filtered, and concentrated to get a crude product. The crude product was purified by column chromatography on silica gel (DCM/MeOH = 20/1→10/1) to yield **bola DS-C_6-K_4-Boc** as a white solid (134 mg, 72%).

^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$ = 1/1) δ 7.78 (s, 2H), 4.48 (d, J = 4.7, 4H), 4.43 - 4.23 (m, 10H), 4.03 (t, J = 6.1 Hz, 12H), 3.42 (t, J = 6.7 Hz, 4H) ,3.23 (t, J = 31.4 Hz, 12H), 3.04 (t, J = 6.2 Hz, 16H), 2.81 (t, J = 6.7 Hz, 4H), 2.03 - 1.05 (m, 248H).

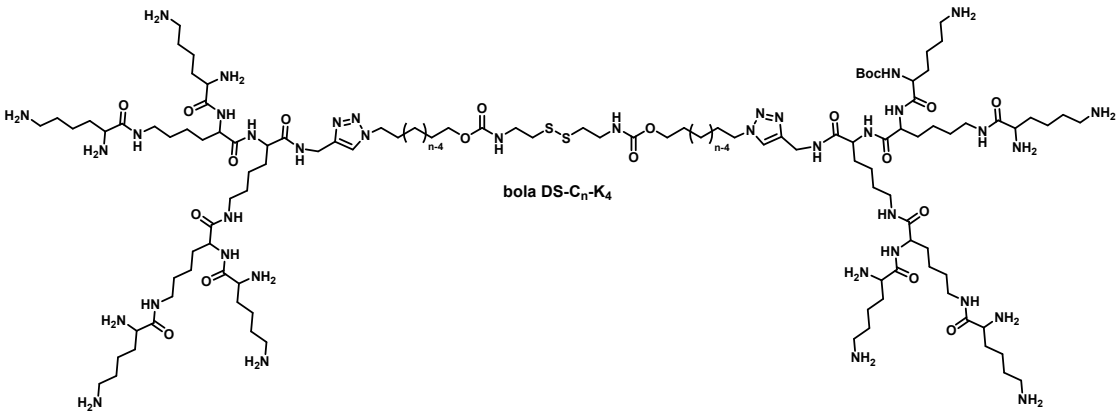
bola DS-C_8-K_4-Boc: The azide precursor bola DS-C_8-N_3 (22 mg, 0.041 mmol, 1.0 eq), Copper(1) iodide (4.0 mg, 0.021 mmol, 0.50 eq) and PD 2-5 (152 mg, 0.087 mmol, 2.1 eq) were dissolved in 5.0 mL anhydrous DMF under nitrogen atmosphere. DBU (47 mg, 0.31 mmol, 7.5 eq) was added to the above solution under stirring at 0℃. The rest of the protocol is the same as bola DS-C_6-K_4-Boc, yielding **bola DS-C_8-K_4-Boc** as a white solid (121 mg, 73%).

^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$ = 1/1) δ 7.79 (s, 2H), 4.48 (d, J = 3.6 Hz, 4H), 4.43 - 4.22 (m, 10H), 4.12 - 3.92 (m, 12H), 3.42 (t, J = 6.8 Hz, 4H), 3.17 (d, J = 6.3 Hz, 12H), 3.04 (t, J = 6.4 Hz, 16H), 2.81 (t, J = 6.8 Hz, 4H), 2.02 - 1.13 (m, 256H).

bola DS-C_{11}--K_4-Boc: The azide precursor bola DS-C_8-N_3 (20 mg, 0.032 mmol, 1.0 eq), Copper (1) iodide (3.0 mg, 0.016 mmol, 0.50 eq) and PD 2-5 (117 mg, 0.066 mmol, 2.1 eq) were dissolved in 5.0 mL anhydrous DMF under nitrogen atmosphere. DBU (36 mg, 0.24 mmol, 7.5 eq) was added to the above solution under stirring at 0℃. The rest of the protocol is the same as bola DS-C_6-K_4-Boc, yielding **bola DS-C_{11}-K_4-Boc** as a white solid (90 mg, 69%).

^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$= 1/1) δ 7.74 (s, 2H), 4.49 (d, J = 5.1, 4H), 4.34 (dd, J = 16.6, 9.4 Hz, 12H), 4.17 - 3.94 (m, 12H), 3.44 (t, J = 6.7 Hz, 4H) ,3.29 - 3.12 (m, 12H), 3.06 (t, J = 6.7 Hz, 16H), 2.81 (t, J = 6.7 Hz, 4H), 2.05 - 0.98 (m, 268H).

Synthesis of bola DS-C_n-K_4 (n = 6, 8, 11)



bola DS-C_6-K_4: Bola DS-C_6-K_4-Boc (64 mg, 0.016 mmol, 1.0 eq) was dissolved in anhydrous $CHCl_3$ (1.2 mL). Then 4 N HCl/EA (1.2 mL, 2.6 mmol, 160 eq) was added to the above solution under stirring at 0℃. The reaction mixture was stirred under nitrogen for 12 h at 30℃ until the reaction was complete as indicated by TLC. After solvent evaporation, and the residue was washed with anhydrous diethyl ether (3 × 10 mL). The product was further purified by dialysis using dialysis tube of MWCO 500, followed by lyophilization to give **bola DS-C_6-K_4** (38 mg, 80%) as a white solid.

^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$ = 1/1) δ 7.88 (s, 2H), 4.49 - 4.27 (m, 14H), 4.12 - 3.88 (m, 12H), 3.42 (t, J = 6.7 Hz, 4H), 3.32 - 3.08 (m, 12H), 3.00 (dt, J = 14.4, 7.2 Hz, 16H), 2.81 (t, J = 6.7 Hz, 4H), 2.17 - 1.09 (m, 104H). ^{13}C NMR (126 MHz, Methanol-d_4) δ 172.62, 169.02, 168.84, 77.81, 77.55, 77.29, 54.11, 52.98, 52.73, 50.30, 39.16, 30.71, 30.52, 30.00, 28.69, 28.20, 26.47, 26.34, 25.94, 25.17, 21.71, 21.22. ESI-

HRMS: calcd. for $C_{108}H_{214}N_{38}O_{18}S_2$ $[M+2H]^{2+}$ 1198.3231, found 1198.3195. HPLC (RT = 13.9 min).

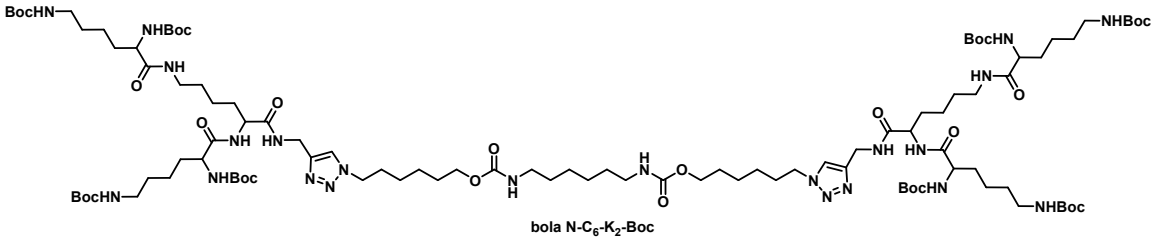
n=8, bola DS-C_8-K_4: Bola DS-C_8-K_4-Boc (65 mg, 0.016 mmol, 1.0 eq) was dissolved in $CHCl_3$ (1.2 mL). Then 4 N HCl/EA (1.2 mL, 2.6 mmol, 160 eq) was added to the above solution under stirring at 0℃. The rest of the protocol is the same as bola DS-C_6-K_4, yielding **bola DS-C_8-K_4** (58 mg, 85%) as a white solid.

^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$ = 1/1) δ 7.77 (s, 2H), 4.45 (s, 4H),4.41 - 4.22 (m, 10H), 4.02 (t, J = 6.6 Hz, 4H), 4.83 - 4.59 (m, 8H), 3.43 (t, J = 6.8 Hz, 4H), 3.31 - 3.07 (m, 12H), 3.03 - 2.86 (m, 16H), 2.82 (t, J = 6.7 Hz, 4H), 1.96 - 1.23 (m, 112H). ^{13}C NMR (126 MHz, Methanol-d_4) δ 172.74, 172.61, 169.00, 168.90, 168.74, 144.54, 123.18, 78.19, 77.93, 77.67, 64.72, 54.10, 53.53, 52.96, 52.68, 50.25, 47.82, 47.65, 47.48, 39.78, 39.12, 39.05, 38.90, 38.76, 37.91, 34.38, 31.30, 31.04, 30.77, 30.58, 30.04, 28.84, 28.71, 28.32, 26.59, 26.45, 26.15, 25.54, 22.98, 22.86, 22.64, 21.73, 21.25, 21.20. ESI-HRMS: calcd. for $C_{112}H_{222}N_{38}O_{18}S_2$ $[M+2H]^{2+}$ 1226.3572, found 1226.3537. HPLC (RT = 15.0 min).

n=11, bola DS-C_{11}-K_4: Bola DS-C_{11}-K_4-Boc (66 mg, 0.016 mmol, 1.0 eq) was dissolved in $CHCl_3$ (1.2 mL). Then 4 N HCl/EA (1.2 mL, 2.6 mmol, 160 eq) was added to the above solution under stirring at 0℃. The rest of the protocol is the same as bola DS-C_6-K_4, yielding **bola DS-C_{11}-K_4** (36 mg, 74%) as a white solid.

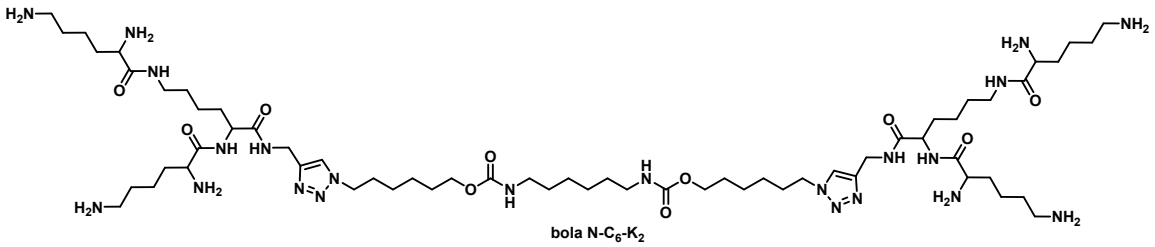
^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$ = 1/1) δ 7.3 (s, 2H), 4.47 (s, 4H),4.43 - 4.29 (m, 10H), 4.12 - 3.84 (m, 12H), 3.44 (t, J = 6.8 Hz, 4H), 3.31 - 3.07 (m, 12H), 3.05 - 2.97 (m, 16H), 2.82 (t, J = 6.7 Hz, 4H), 2.00 - 1.19 (m, 124H). ^{13}C NMR (126 MHz, $CD_3OD/CDCl_3$ = 1/1) δ 172.68, 170.06, 123.02, 77.82, 77.56, 77.30, 64.98, 53.48, 53.18, 52.99, 50.44, 39.79, 39.14, 38.90, 30.17, 29.37, 29.34, 29.30, 29.14, 28.92, 28.24, 26.55, 26.42, 26.37, 25.71, 22.90, 22.54, 21.79, 21.37. ESI-HRMS: calcd. for $C_{118}H_{234}N_{38}O_{18}S_2$ $[M+2H]^{2+}$ 1268.4039, found 1268.3993. HPLC (RT = 16.6 min).

Synthesis of bola N-C_6-K_4



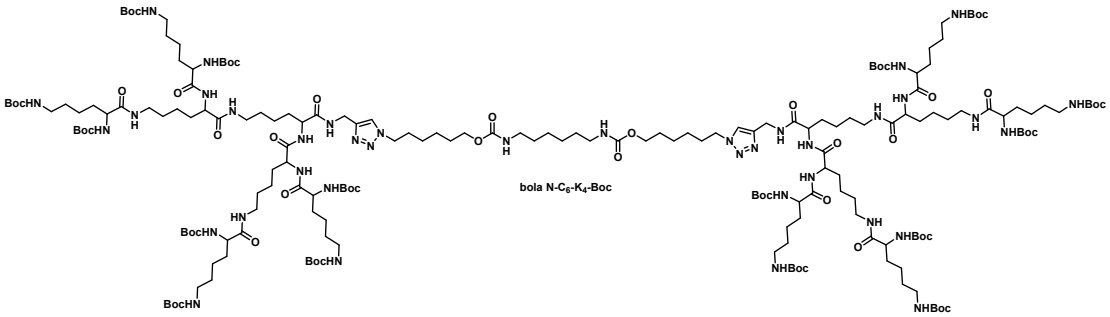
bola N-C_6-K_2-Boc: The azide precursor $2C_6$-N_3 (39 mg, 0.085 mmol, 1.0 eq), Copper(1) iodide (16 mg, 0.085 mmol, 1.0 eq) and PD 2-3 (150 mg, 0.18 mmol, 2.1 eq) were dissolved in 3.0 mL anhydrous DMF under nitrogen atmosphere. DBU (97 mg, 0.64 mmol, 7.5 eq) was added to the above solution under stirring at 0°C. The reaction mixture was stirred under nitrogen for 3 h at 50℃ until the reaction was complete as indicated by TLC and FT-IR. After solvent evaporation, the mixed solution was diluted with 40.0 mL DCM and washed with saturated NH_4Cl solution (3 × 20.0 mL). The organic phase was washed by saturated NaCl solution (20.0 mL), dried with Na_2SO_4, filtered, and concentrated to get a crude product. The crude product was purified by column chromatography on silica gel (DCM/MeOH = 20/1→10/1) to yield **bola N-C_6-K_2-Boc** as a white solid (128 mg, 71%).

^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$ = 3/1) δ 7.79 (s, 2H), 4.46 (s, 4H), 4.37 (t, J = 7.1 Hz, 4H), 4.29 (dd, J = 8.7, 5.4 Hz, 2H), 4.00 (t, *J* = 6.6 Hz, 8H), 3.22 - 3.00 (m, 16H), 1.97 - 1.27(m, 132H).



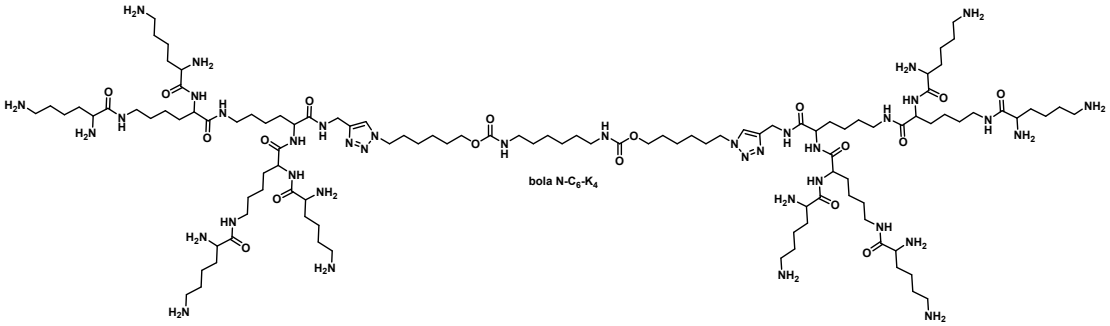
bola N-C_6-K_2: Bola N-C_6-K_2-Boc (152 mg, 0.071 mmol, 1.0 eq) was dissolved in anhydrous DCM (0.5 mL). Then 3.48N HCl/EA（1.7 mL, 5.7 mmol, 80 eq）was added to the above solution under stirring at 0℃. The reaction mixture was stirred under nitrogen for 12 h at 30℃ until the reaction was complete as indicated by TLC. After solvent evaporation, and the residue was washed with anhydrous diethyl ether (3 × 10.0 mL). The product was further purified by dialysis using dialysis tube of MWCO 500-1000, followed by lyophilization to give **bola N-C_6-K_2** as a white solid (71 mg, 61%).

^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$ = 3/1) δ 7.89 (s, 2H), 4.50 - 4.31 (m, 10H), 4.15 - 3.91 (m, 8H), 3.30 - 2.91 (m, 16H), 2.00 - 1.27 (m, 60H).



bola N-C_6-K_4-Boc: Boc-L-Lys(Boc)-OH (169 mg, 0.49 mmol, 11 eq), N, N, N', N'-tetramethyl-(1H-benzotriazol-1-yl)-uranium hexafluorophosphate (HBTU) (185 mg, 0.49 mmol, 11 eq) and 1-hydroxybenzotriazole (HOBT) (67 mg, 0.49 mmol, 11 eq) were dissolved in anhydrous DMF (3.0 mL). N, N-diisopropylethylamine (DIPEA) (0.3 mL, 1.64 mmol, 38 eq) was added to the above solution under stirring for 30 min at 0°C under nitrogen atmosphere. And then bola N-C_6-K_2 (71 mg, 0.044 mmol, 1.0 eq) were added to the above solution. The reaction mixture was stirred under nitrogen for 48 h at 30°C until the reaction was complete as indicated by TLC. After solvent evaporation, the mixed solution was diluted with $CHCl_3$ (40.0 mL) and washed by saturated $NaHCO_3$ solution (3 × 20 mL), 0.5N HCl solution (3 × 20 mL) and saturated NaCl solution (20.0 mL). The organic phase was dried with Na_2SO_4, filtered, and concentrated to get a crude product. The crude product was purified by column chromatography (DCM/CH_3OH = 50/1→10/1) to yield **bola N-C_6-K_4-Boc** as a white solid (97 mg, 56%).

^{1}H NMR (300 MHz, CD_3OD/$CDCl_3$ = 3/1) δ 7.81 (s, 2H), 4.51 (d, J = 13.2 Hz, 4H), 4.38 (t, J = 7.1 Hz, 4H), 4.28 (t, J = 7.2 Hz, 6H), 4.12 - 3.91 (m, 12H), 3.27 - 2.96 (m, 32H), 1.97 - 1.28 (m, 252H).



bola N-C_6-K_4: Bola N-C_6-K_4-Boc (90 mg, 0.023 mmol, 1.0 eq) was dissolved in anhydrous $CHCl_3$ (1.0 mL). Then 3.48 N HCl/EA (1.1 mL, 3.6 mmol, 160 eq) was added to the above solution under stirring at 0°C. The reaction mixture was stirred under nitrogen for 12 h at 30°C until the reaction was complete as indicated by TLC. After

solvent evaporation, and the residue was washed with anhydrous diethyl ether (3 × 10 mL). The product was further purified by dialysis using dialysis tube of MWCO 500-1000, followed by lyophilization to give **bola N-C_6-K_4** as a white solid (26 mg, 40%).

^{1}H NMR (300 MHz, $CD_3OD/CDCl_3$ = 3/1) δ 7.89 (s, 2H), 4.49 - 4.29 (m, 14H), 4.06 - 3.79 (m, 12H), 3.31 - 2.92 (m, 32H), 1.97 - 1.27 (m, 108H). HPLC (RT = 14.1 min).

HPLC of bola amphiphile peptide dendrimer

High-performance liquid chromatography (HPLC) analysis was performed using a Waters Empower system (Waters 1525 binary HPLC pump, Waters Corp., The Capricorn, Singapore) equipped with a photodiode array detector (Waters 2998, Waters Corp., The Capricorn, Singapore) and a SinoChrom C8 column (5 μm, 4.6 mm × 250 mm). The mobile phase consisted of acetonitrile [containing 0.04% trifluoroacetic acid (TFA)] and water (also containing 0.04% TFA). A gradient elution from 10% to 100% acetonitrile in water was applied over 30 minutes. The flow rate was set to 0.8 mL/min, and the injection volume was 20 μL. Detection was carried out at a wavelength of 210 nm, and retention times (RTs) were recorded in minutes. All reagents were HPLC-grade and were obtained from Anhui Tedia High Purity Solvents Co., Ltd. (Anqing, China) and Aladdin Ltd. (Shanghai, China).

Critical aggregation concentration (CAC) values

Pyrene was employed as a fluorescence probe to determine the CAC. The concentration of bola amphiphile peptide dendrimer solutions was varied from 1.0 × 10^{-7} to 1.0 × 10^{-4} M, and the final pyrene concentration was adjusted to 3.0 × 10^{-7} M in water. After sonicated for 30 min and rest at room temperature for 2 h to form the micelle formation, the solution was then detected using a FL8500 fluorescence spectrophotometer. Fluorescence measurements of solution were carried out using an FL8500 fluorescence spectrophotometer. Pyrene fluorescence spectra were recorded at an excitation wavelength of 334 nm. The excitation and emission bandwidths were set to 20 nm and 1 nm, respectively. The fluorescence intensity ratio of I_{373}/I_{384} was analyzed.

Ellman reagent method

5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB) is a widely employed reagent for the quantification of free thiol groups. When DTNB reacts with thiols, its colorless disulfide bonds are cleaved, resulting in the formation of mixed disulfides and the release of 5-thio-2-nitrobenzoic acid (TNB). The liberated TNB exhibits a distinct absorbance peak at 412 nm, enabling accurate determination of thiol concentrations through spectrophotometric measurement at this wavelength.

To evaluate the glutathione (GSH)-responsive degradation behavior of the bola amphiphilic peptide dendrimers, solutions of the dendrimers (2.0×10^{-4} M) and dithiothreitol (DTT, 2.0×10^{-4} M) were incubated at 37°C under shaking for varying time intervals (0, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 h). At each time point, 10 μL of the reaction mixture was aliquoted and mixed with 90 μL of buffer and 500 μL of DTNB solution (2.0×10^{-3} M). Following a 10 minutes incubation, the absorbance at 412 nm was recorded, and the thiol concentration was calculated using a pre-established standard calibration curve. The degradation rates of the carriers were assessed by monitoring the temporal changes in thiol content. All experiments were performed in triplicate to ensure reproducibility.

Dynamic light scattering (DLS)

The siRNA solution or H_2O was mixed with an appropriate volume of dendrimer solution at N/P ratio of 10, and incubated at room temperature for 30 min. The final siRNA concentration was adjusted to 1.0 μM. After filtering by 220 nm membrane (Pall, Port Washington, NY, USA), sizes and ζ-potentials of the resulting siRNA/bola amphiphilic peptide dendrimers complexes solutions and bola amphiphilic peptide dendrimers were measured at 25°C using a NanoBrookOmni (Brookhaven, Long Island, N.Y.) equipped with a 633 nm laser. All measurements were performed in triplicate.

GSH-triggered disassembly of siRNA/bola DS-C_n-K_4 complexes: the siRNA/bola DS-C_n-K_4 complexes (N/P = 10, 1 μM siRNA) were treated with 10 mM GSH, while the control group received no treatment. All samples were incubated at 37 °C, and hydrodynamic size was measured at different time points (0, 1, 2, 4, 6, 8, 12, and 24 h).

Transmission electron microscopy (TEM)

A 20 μL aliquot of siRNA/bola DS-C_n-K_4 complexes, with an N/P ratio of 10 and containing 200 ng of siRNA, was deposited onto weighing paper. A copper grid was placed on the droplet for 10 minutes. Excess liquid was then removed by blotting with filter paper. The sample was stained with 2% (w/v) phosphomolybdic acid for 5 minutes and air-dried at room temperature. Imaging was conducted using a transmission electron microscope (TEM) HT7700.

Circular dichroism (CD) analysis

The CD spectra were recorded using Chirascan spectrometer (Applied Photophysics Ltd., Leatherhead, UK). The samples of bola amphiphile peptide dendrimer solutions were prepared at concentrations of 0.05 mg/mL in H_2O and incubated at room temperature for 30 min to measurement. Spectral analysis and secondary structure estimation were performed using CDNN software.

Gel retardation analysis

The bola amphiphilic peptide dendrimers were diluted to appropriate concentration, and siRNA was dissolved in H_2O. Both solutions were mixed with at various N/P ratios ranging from 0.1 to 50 and incubated at 37 °C for 30 min. The final concentration of siRNA in each mixture was 200 ng/well. The resulting siRNA/bola DS-C_n-K_4 complexes were evaluated via electrophoretic mobility–shift assays conducted using 2% agarose gels in standard TAE buffer for 20 min. After staining with GoodView™ nucleic acid dyes (Solarbio), the siRNA bands were visualized using an automatic chemiluminescence imaging system (5200 Multi) (Tanon, Shanghai, China).

For GSH responsive siRNA release assays, the siRNA/bola DS-C_n-K_4 complexes were prepared at an N/P ratio of 10 (containing 200 ng siRNA). A freshly prepared GSH solution was added to achieve a final concentration of 10 mM. The mixture was incubated at 37 °C for 2 h, followed by analysis via agarose gel electrophoresis as described above. siRNA release was visualized using the same chemiluminescence imaging system.

RNase A assay

An aliquot containing 100 μM of bola DS-C_n-K_4 and the indicated amounts of siRNA at N/P ratio of 10 were kept at 37 °C for 30 min. Then, the siRNA/bola-dendrimer complexes were incubated with RNase A (1 μg/mL) at 37°C for various durations (0, 10, 30, 60, 90, and 120 min). After incubation, 0.05% (mg/mL) SDS solution was added to each sample and incubated on ice for 10 min. The resulting mixtures were electrophoresed through a 1% agarose gel in standard TAE buffer. The siRNA bands were stained with a nucleic acid dye and visualized using Automatic chemiluminescence imaging system (5200Muti) (Tanon). Naked siRNA was served as a control.

Cell culture

Human ovarian cancer SKOV-3 cells, human non-small cell lung cancer cell A549 cells, human cervical cancer HeLa cell, human hepatocellular carcinomas HepG2 cells, canine kidney cell MDCK cell, and mouse fibroblast L929 cells were purchased from Tongpai Biotechnology Co., Ltd. (Shanghai, China).

SKOV-3 cells were cultured in McCoy's 5A (Hyclone, Logan, UT, USA), supplemented with 10% fetal bovine serum (FBS) (Gibco). HeLa cells were cultured in RPMI-1640 (Hyclone, Logan, UT, USA) supplemented with 10% FBS. HepG2 cells and A549 cells were maintained in DMEM (Hyclone, Logan, UT, USA) supplemented with 15% FBS. L929 cells were maintained in DMEM (Hyclone, Logan, UT, USA), supplemented with 10% FBS. MDCK cells were cultured in MEM (Hyclone, Logan, UT, USA) with 15% FBS. Cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

SKOV-3, HeLa, L929 and MDCK cells were respectively seeded in 96-well plates at a density of 1.0×10^4 cells/well and incubated overnight to allow adherence. The cells were then treated with bola DS-C_n-K_4 and siRNA/bola DS-C_n-K_4 complexes (N/P = 10, 50 nM siRNA) for 8 h. After transfection, the medium was replaced with the complete medium containing 10% FBS, and cells were maintained under standard culture conditions for 24, 48, 72 h for metabolic toxicity assessment. Subsequently, 10 μL of MTT solution (10 mg/mL) was added to each well and incubated for 2 h. The medium was then replaced with 100 μL of DMSO. The optical density (OD) of each

well at 570 nm were measured by Cytation5 Microplate Reader (BioTek). Untreated cells served as positive control. The difference of OD values between treated and non-treated cells reflects the viability of cells after treatments and thus stands for the metabolite toxicity. All experiments were performed in triplicate.

Lactate dehydrogenase (LDH) assay

L929 and MDCK cells were seeded at a density of 1.0×10^4 cells per well in 96-well plates and incubated overnight. Cells were then treated with either bola DS-C_6-K_4 alone or siRNA/bola DS-C_6-K_4 complexes (N/P = 10, 50 nM siRNA) for 7.5 h. Lactate dehydrogenase (LDH) release was assessed to evaluate membrane cytotoxicity using a commercial LDH assay kit (Dojindo Laboratories, Shanghai, China) according to the manufacturer's protocol. The optical density (OD) of these solutions were measured at 490 nm via Cytation5 Microplate Reader (BioTek, Winooski, VT, USA). Cells was performed with lysis buffer and culture medium as positive and negative control, and set as 100% and 0% LDH release, respectively. Each assay was performed in triplicate. The percentage of LDH is calculated according to the following:

LDH% = [(the absorbance of sample – the absorbance of negative control) / (the absorbance of positive control – the absorbance of negative control)] × 100%.

Hemolysis experiment

Red blood cells (RBCs) were isolated from 1 mL mouse blood and diluted to 4% (v/v) with PBS. Bola DS-C_n-K_4 and siRNA/bola DS-C_6-K_4 were prepared at final concentrations of 0.1, 0.5, 1.0, 2.5, 5.0, 7.5, 10, 20, 50 μM. Then the solution was mixed with an equal volume of the 4% RBC suspension. The mixtures were incubated at 37 °C for 2 h and then centrifuged for 5 min. A volume of 150 uL supernatant was carefully removed into a 96-well plate, and the absorbance of 540 nm was measured using a Cytation5 Microplate Reader (BioTek). 1.0% TritonX-100 solution (Beyotime Biotechnology, Shanghai, China) and PBS buffer served as positive (100% hemolysis) and negative (0% hemolysis) controls, respectively. The percentage of hemolysis was calculated as follows:

Hemolysis% = [(the absorbance of sample - the absorbance of negative control) / (the absorbance of positive control - the absorbance of negative control)] ×100%.

GSH level measurement

A549 and BSO-treated A549 cells were seeded in 6-well plates at a density of 3.0 × 10^5 cells/well and incubated for 24 h. Cells were then harvested by centrifugation and resuspended in 1.0 mL of complete growth medium. The GSH/GSSG assay kit (Beyotime Biotechnology, Shanghai, China) was added at a final concentration of 0.50 μM, and the cells were incubated at 37 °C for 15 minutes in the dark. Following incubation, cells were washed PBS with three times, and mean fluorescence intensity was quantified by flow cytometry (Attune NxT, Thermo Fisher Scientific, USA). All experiments were performed in triplicate.

In vitro transfection

SKOV-3, A549, HeLa and HepG2 cells were seeded in 6-well plates at a density of 8.0 × 10^4 cells/well and cultured overnight in 2.0 mL of fresh complete medium containing 10% FBS. The solutions of siRNA/bola DS-C_n-K_4 complexes (N/P ratio of 10, 50 nM siRNA) were prepared immediately prior to transfection. The desired amount of siRNA and bola DS-C_n-K_4 was diluted in 0.10 mL of Opti-MEM transfection medium, mixed gently by pipetting, and incubated at room temperature for 10 min. The bola DS-C_n-K_4 solution was added to the siRNA solution, homogenized for 10 s and incubated for an additional 30 min at room temperature to allow complex formation. Subsequently, 0.80 mL of Opti-MEM medium was added to the mixture to bring the final volume to 1.0 mL. After removal of the culture medium, cells were washed once with PBS, followed by the addition of 1.0 mL of the transfection complex solution. Cells were incubated at 37 °C for 8 h. The transfection mixture was replaced with the fresh medium containing 10% FBS, and cells were cultured under standard conditions for an additional 48 h for the qRT-PCR assay and 72 h for western blot assay.

Effect of dioleoylphosphatidylethanolamine (DOPE): The transfection experiments involving DOPE were performed in SKOV-3 and A549 cells as described above. Before transfection, the desired amount of bola DS-C_n-K_4 was pre-mixed with DOPE at mole ratio of 1 (dendrimer/DOPE) and equilibrated at room temperature for 10 min. Then, the mixture solution was diluted in 0.1 mL of Opti-MEM transfection medium for complexation with PLK1 siRNA (N/P ratio of 10, 50 nM siRNA).

Effect of BSO: The transfection experiments involving BSO were performed as described above, except that A549 cells were pre-incubated with 200 μM BSO at 37ºC for 36 h.

Flow cytometry

Cell uptake: A549 cells were seeded into 24-well plates at a density of 1.2×10^5 cells per well supplemented with 10% FBS and incubated for 24 h. The cells were incubated with Cy5-labeled siRNA/bola DS-C_n-K_4 complexes (N/P ratio of 10, 20 nM Cy5–siRNA) was added to cells and incubated for 0.5, 1, 2, 4 h. After incubation, the cells were washed three times with cold PBS and analyzed by flow cytometry (Attune NxT, Thermo Fisher Scientific). Each assay was performed in triplicate.

Cell uptake mechanism: A549 cells were incubated with cytochalasin D, genistein, chlorpromazine, and DTNB for 1 hours. After 1 hour, the supernatant was removed, and 250 μL of Cy5 labeled siRNA/bola DS-C_6-K_4 complexes solution (N/P = 10, 20 nM Cy5 siRNA) was added to each well, which was placed in the incubator to incubate 30 min. After that, the cells were collected by the above methods, and the inhibitory effects of different inhibitors on cell uptake were determined by flow cytometry. Each assay was performed in triplicate

Apoptosis analysis: After 4 days of transfection, the cells of each group were collected. The cells were stained with an apoptosis detection kit (Vazyme Biotech Co. Ltd, Nanjing, China) and analyzed by flow cytometry.

Confocal microscopy

A549 cells were seeded into 2.5 dishes (1.2×10^5 cells/dish) one day before transfection. Cells were then incubated with Cy5-labeled siRNA/bola DS-C_6-K_4 complex (N/P ratio of 10, 20 nM Cy5-siRNA) for 0.5, 1, 2, 4 h at 37ºC. After incubation, cells with washed with cold PBS, followed by staining with PBS containing Hoechst33342 (10 μg/mL) and Lyso-Tracker green (0.10 μM) for 15 min at 37ºC. Images were captured with a Zeiss LSM880 Meta laser scanning confocal microscope (Carl Zeiss, Jena, Germany) using ZEN2.3 pro software (Carl Zeiss GmbH).

Western blots analysis

Equal amounts of total protein extracted from cultured cells (A549, HeLa, SKOV-3 or HepG2 cells) or tumors (A549 xenograft tumors) were separated by SDS-PAGE gradient gel, and transferred to the PVDF membrane after electrophoresis. Membranes were blocked with 5% (w/v) skimmed milk and incubated overnight at 4 °C with anti-human PLK1 rabbit polyclonal antibody (Cell Signaling Technology, Danvers, MA, USA; 1:1000), or anti-human AKT2 rabbit polyclonal antibody (Cell Signaling Technology; 1:1000), or anti-human vinculin rabbit polyclonal antibody (Sigma-Aldrich, Shanghai, China; 1:10,000). After three washes, membranes were incubated for 2 h at 25 °C with anti-rabbit or anti-mouse secondary antibodies (Invitrogen, Waltham, MA, USA; 1:5000). Protein bands were visualized using an enhanced chemiluminescence Western blot detection system (Tanon). The housekeeping protein (vinculin) used for normalization.

Quantitative Real-Time (qRT)–PCR Analysis

Total RNA was extracted from cultured A549 cells or A549 tumor tissues using the TRIzol method (Vazyme Biotech Co., Ltd., Nanjing, China), and RNA purity and concentration were quantified using a Cytation 5 microplate reader (BioTek Instruments, Winooski, VT, USA). One microgram of RNA was reverse transcribed into complementary DNA (cDNA) using the HiScript® II QRT SuperMix Kit (Vazyme Biotech Co., Ltd., Nanjing, China). Quantitative real-time PCR (qRT-PCR) was performed on the QuantStudio3™ Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific) with 2 × SYBR Green Master Mix (Vazyme Biotech Co. Ltd). The relative expression of PLK1 mRNA was normalized to that of Vinculin as the internal control.

The primers (GenScript Biotech Corp., Nanjing, China) were used as follows:

PLK1-forward primer: 5'-AGCCTGAGGCCCGATACTACCTAC-3';

PLK1-reverse primer: 5'-ATTAGGAGTCCCACACAGGGTCTTC-3';

vinculin-forward primer: 5’-CTCGTCCGGGTTGGAAAAGAG-3;

vinculin-reverse primer: 5’-AGTAAGGGTCTGACTGAAGCAT-3’.

Cell wound healing assay

The A549 cells were transfected with PBS buffer, bola DS-C_6-K_4, scramble/bola DS-C_6-K_4 and siPLK1/bola DS-C_6-K_4 (N/P ratio of 10, 50 nM siRNA) for 72 hours and

cultured to 6-well plate. After transfection, a scratch wound was generated using a 200 μL pipette tip. The cells were washed with 1 mL PBS and 2 mL fresh culture medium was added. Cell migration was monitored at 0, 6, 12, and 24 h post-wounding using an inverted light microscope. Wound closure was quantified by measuring the difference in the wound width, and greyscale intensity values (G) of the scratch area were analyzed using ImageJ software (NIH, USA). The percentage of wound healing was calculated using the following formula:

Wound healing (%) = $((G_{\text{at different time}} - G_{\text{at initial time point}})/G_{\text{at initial time point}}) \times 100\%$.

Cell invasion experiment

Cell invasion was assessed using Transwell invasion chambers (Corning Inc., Corning, NY, USA) pre-coated with Matrigel (Corning Inc., Corning, NY, USA) following the manufacturer's protocol. A549 cells were transfected with PBS buffer, bola DS-C_6 K_4, scramble/bola DS-C_6 K_4 and siPLK1/bola DS-C_6-K_4 (N/P ratio of 10, 50 nM siRNA) for 72 hours. After transfection, cells were digested and suspended in serum-free medium, then seeded at a density of 2×10^5 cells/well in the upper chamber of Transwell. The lower chamber was filled with complete medium containing 20% FBS. Then the upper chamber containing inoculated cells was then placed in the lower chamber and cultured for 24 hours. Following incubation, the medium in both chambers was discarded, and non-invading cells along with residual Matrigel on the upper membrane surface were gently removed using a cotton swab. The membrane was washed three times with PBS for 5 min each to ensure thorough cleaning. Cells that had invaded to the lower surface of the membrane were fixed by immersing the assembled chambers in 600 μL of methanol for 30 min. After fixation, the methanol was removed and the chambers were air-dried in a ventilated hood. The membranes were then stained with 0.1% crystal violet solution for 1 h. Excess dye was removed by rinsing with distilled water, and stained cells were imaged using an inverted microscope. Subsequently, the dye was solubilized with DMSO, and absorbance was measured at 590 nm using a Cytation 5 microplate reader (BioTek). Each assay was performed in triplicate.

Toxicity assessment in healthy mice

All experimental protocols were approved by the Institutional Animal Care and Use Committee of China Pharmaceutical University, and performed in accordance with the guidelines and policies for in vivo toxicity evaluation (Approval No. YSL-202509065). Female 5-week-old ICR mice were obtained from Sino-British SIPPR/BK Lab Animal Ltd. (Shanghai, China). Mice were randomized into five groups (3 mice per group), and were then intravenously injected with, normal saline, bola DS-C_6-K_4, scramble/bola DS-C_6-K_4 (N/P ratio 5, 1.0 mg/kg siRNA), and LPS, respectively. After 24 h, mice were sacrificed. The serum specimens were collected to measure the inflammatory factors and biochemical parameters. Tissue specimens of different organs (hearts, lungs, livers, kidneys, and spleens) were fixed in 10% neutral-buffered formalin and embedded in paraffin. Sections of 4 μm thickness were stained with haematoxylin and eosin (H&E) staining.

In vivo siRNA delivery in lung cancer A549 xenograft mouse model

All animals involved in this work were maintained in the Institutional Animal Care and Use Committee of China Pharmaceutical University. All procedures were approved by the Institutional Animal Care and Use Committee of China Pharmaceutical University and performed in accordance with the guidelines and policies. The approval number is "2022-12-027". BALB/c nude mice (6 weeks old) were purchased from Weitong Lihua Laboratory Animal Technology Co., Ltd (Shanghai, China). 1×10^7 A549 cells were inoculated subcutaneously in the flank region of 6-week-old female BALB/c nude mice. When A549 tumors grew to about 50 mm^3, the mice were randomly divided into four groups (5 animals each), and were then intravenously treated with normal saline, bola DS-C_6-K_4, scramble/bola DS-C_6-K_4, siPLK1/bola DS-C_6-K_4 (N/P ratio 5, 1.0 mg/kg siRNA) every 2 days, respectively. All the mice were sacrificed after five treatments. The tumors were collected and the weight of tumors were recorded. Each collected tumor was divided into two portions. One portion was snap-frozen in liquid nitrogen and stored at -80 °C for RNA and protein extraction. PLK1 mRNA expression was quantified using qRT-PCR, and PLK1 protein levels were evaluated by Western blot, as previously described. The other portion was fixed in 4% paraformaldehyde and used for terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) and immunohistochemical (IHC) analyses. TUNEL staining was performed to assess apoptosis, while Ki67 staining was used to evaluate in vivo cell

proliferation. Serum samples were collected for biochemical analysis. Major organs, including the heart, lungs, liver, kidneys, and spleen, were harvested, were fixed in 10% neutral-buffered formalin and embedded in paraffin, and sliced for histological evaluations. Hematoxylin and eosin (H&E) staining, Ki67 immunostaining, and TUNEL assays were carried out to assess tissue morphology and systemic effects.

Statistical methods

Quantitative data are expressed as mean ± standard deviation (SD) from at least three independent experiments. Group comparisons were analyzed using two-tailed Student's t-test (two groups) or one-way ANOVA with Tukey post-hoc test (multiple groups) in GraphPad Prism 8.0 (GraphPad Software, San Diego, CA). Significance thresholds were defined as *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

3. Result

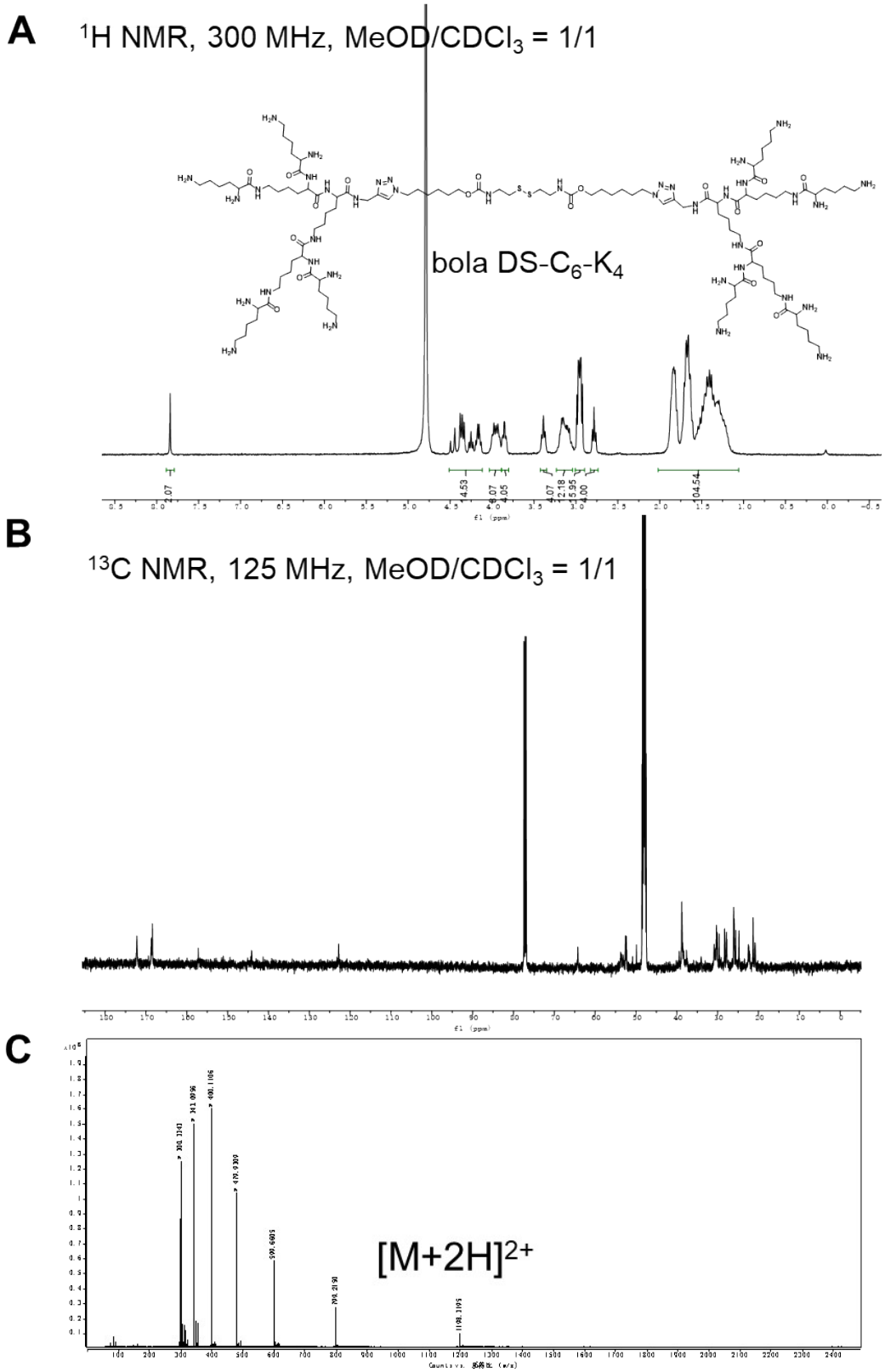


Figure S1. ^{1}H NMR (A), ^{13}C NMR (B) and HRMS (C) characterization of bola DS- C_6-K_4.

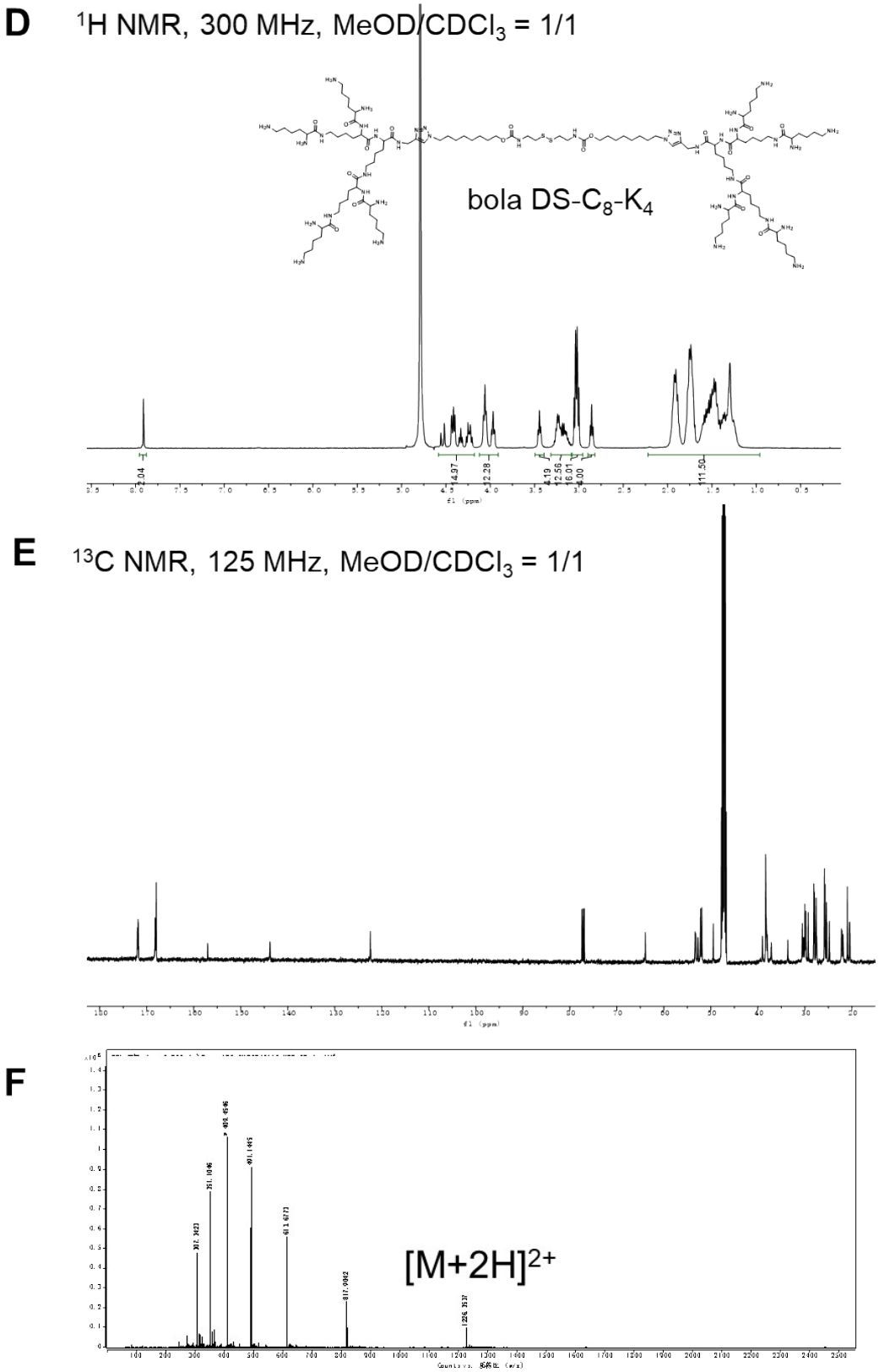


Figure S2. ^{1}H NMR (A), ^{13}C NMR (B) and HRMS (C) characterization of bola DS- C_8-K_4.

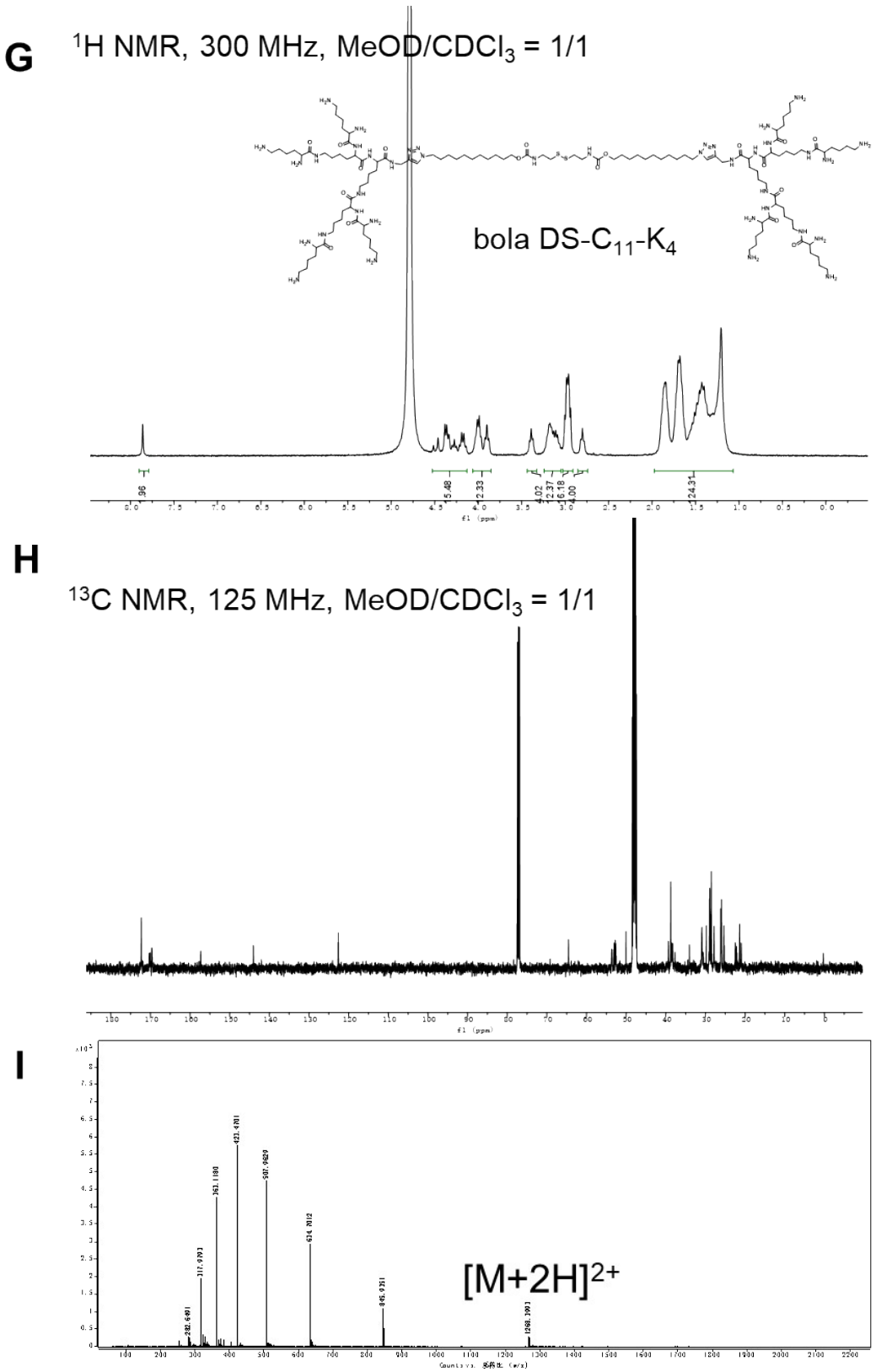


Figure S3. ^{1}H NMR (A), ^{13}C NMR (B) and HRMS (C) characterization of bola DS- C_{11}-K_4.

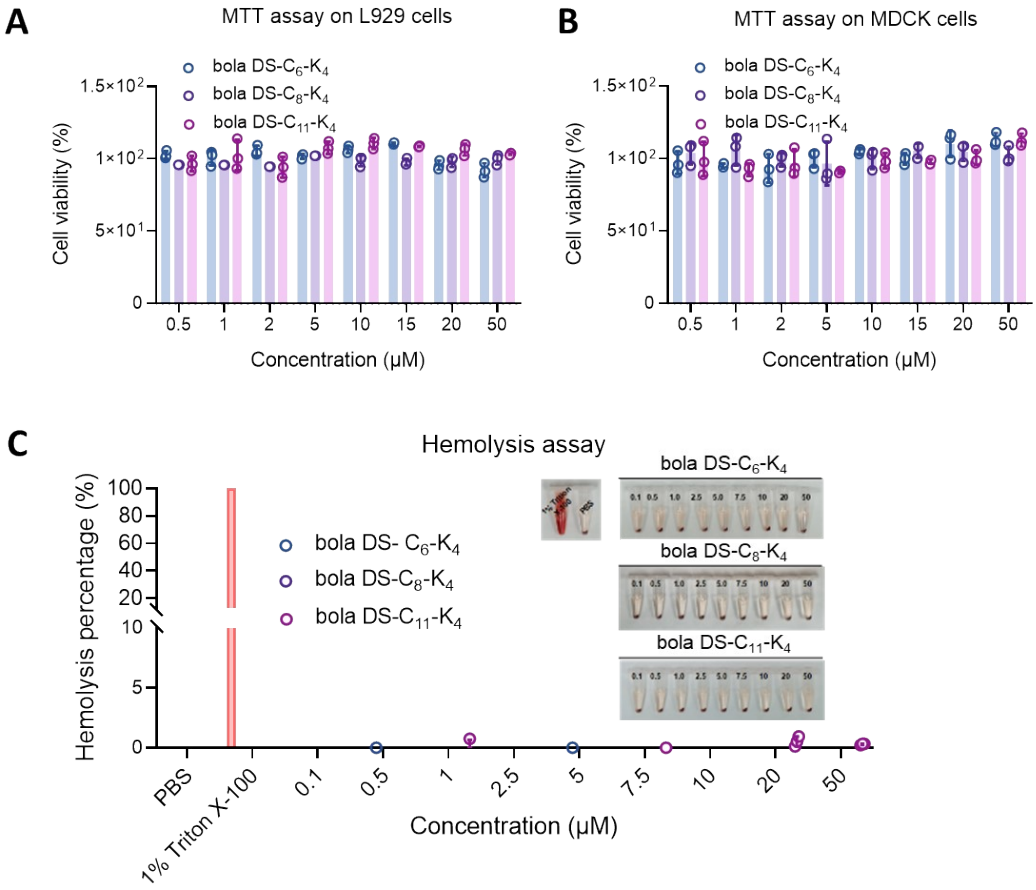


Figure S4. Cell viability assay of L929 cells (A), and MDCK cells (B) upon treatment with bola-amphiphilic peptide dendrimers using MTT assay. (C) Hemolytic toxicity evaluation of bola-amphiphilic peptide dendrimers using mouse red blood cells. Quantitative analysis of hemolysis determined by UV absorption at 540 nm. Triton was used positive control. (mean ± SD, n = 3).

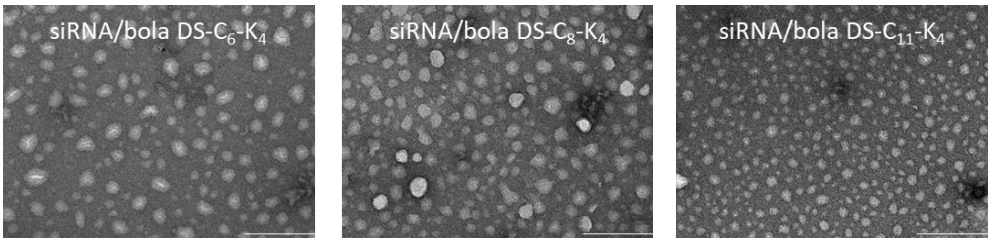


Figure S5. Transmission electron microscopic (TEM) imaging of the siRNA/bola DS-Cn-K4 complex (200 ng siRNA, N/P ratio of 10). Scar bar: 200 nm.

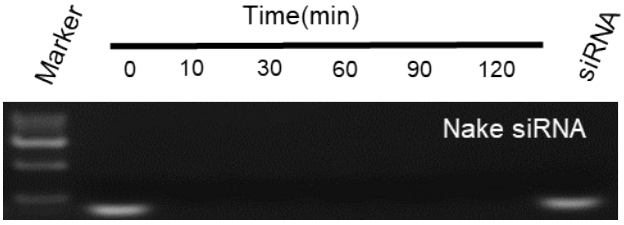


Figure S6. The siRNA stability against RNase at different time (siRNA 200 ng per well).

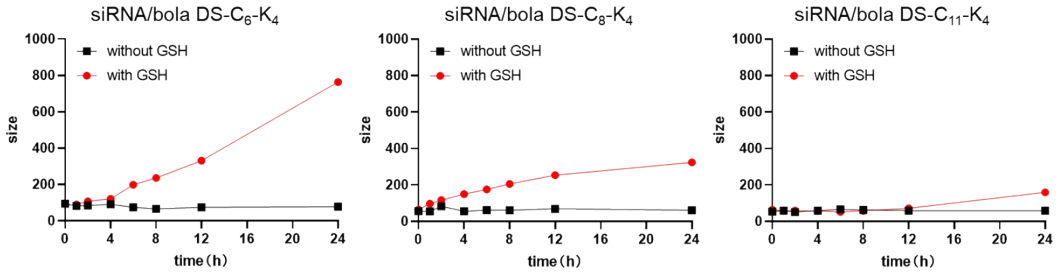


Figure S7. GSH-responsive disassembly of the siRNA/bola DS-C_n-K_4 complexes obtained with siRNA (1.0 μM) at N/P ratio of 10 using DLS.

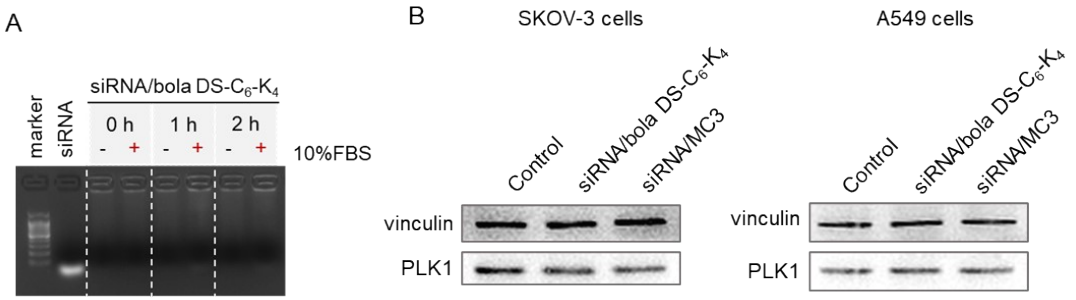


Figure S8. (A) Serum stability of siRNA/bola DS-C6-K4 complexes (siRNA 200 ng per well, N/P ratio of 10). (B) The siRNA delivery efficiency of bola DS-C_6-K_4 (50 nM siRNA, N/P ratio of 10) and DLin-MC3-DMA in SKOV-3 cells and A549 cells.

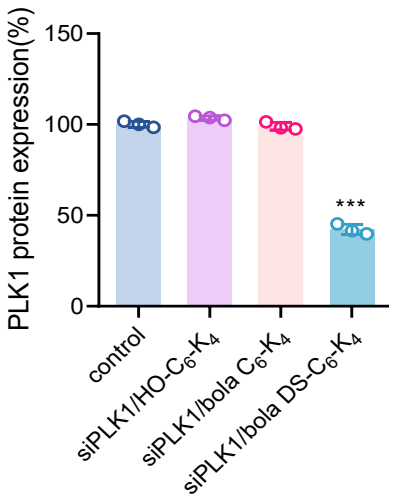


Figure S9. PLK1 protein expression in A549 cells assessed using Western blotting, following treatment with siPLK1/HO-C_6-K_4, siPLK1/bola C_6-K_4, and siPLK1/bola DS-C_6-K_4 (N/P = 10, 50 nM siRNA). siPLK1: siRNA targeting PLK1. Quantitative analysis of western blotting determined by Image J software. (mean ± SD, n = 3)

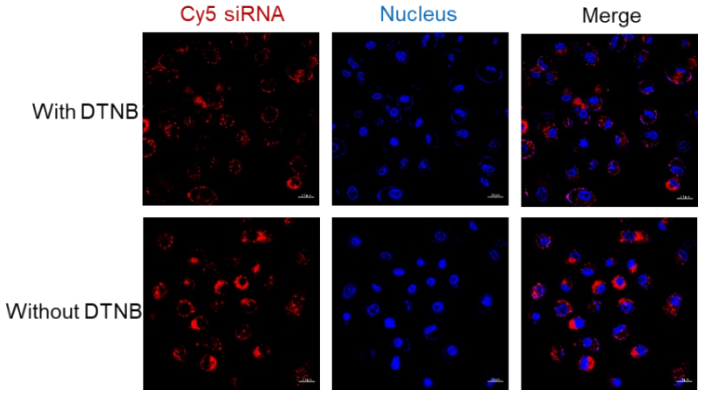


Figure S10. Cellular uptake of the siRNA/bola DS-C_6-K_4 complexes in present of DTNB. (N/P = 10, 20 nM Cy5-labeled siRNA) The red channel image shows the Cy5-labeled siRNA (red), the blue channel image shows the nuclei of the A549 cells stained by Hoechst33342 (blue). Scale bar, 20 μm.

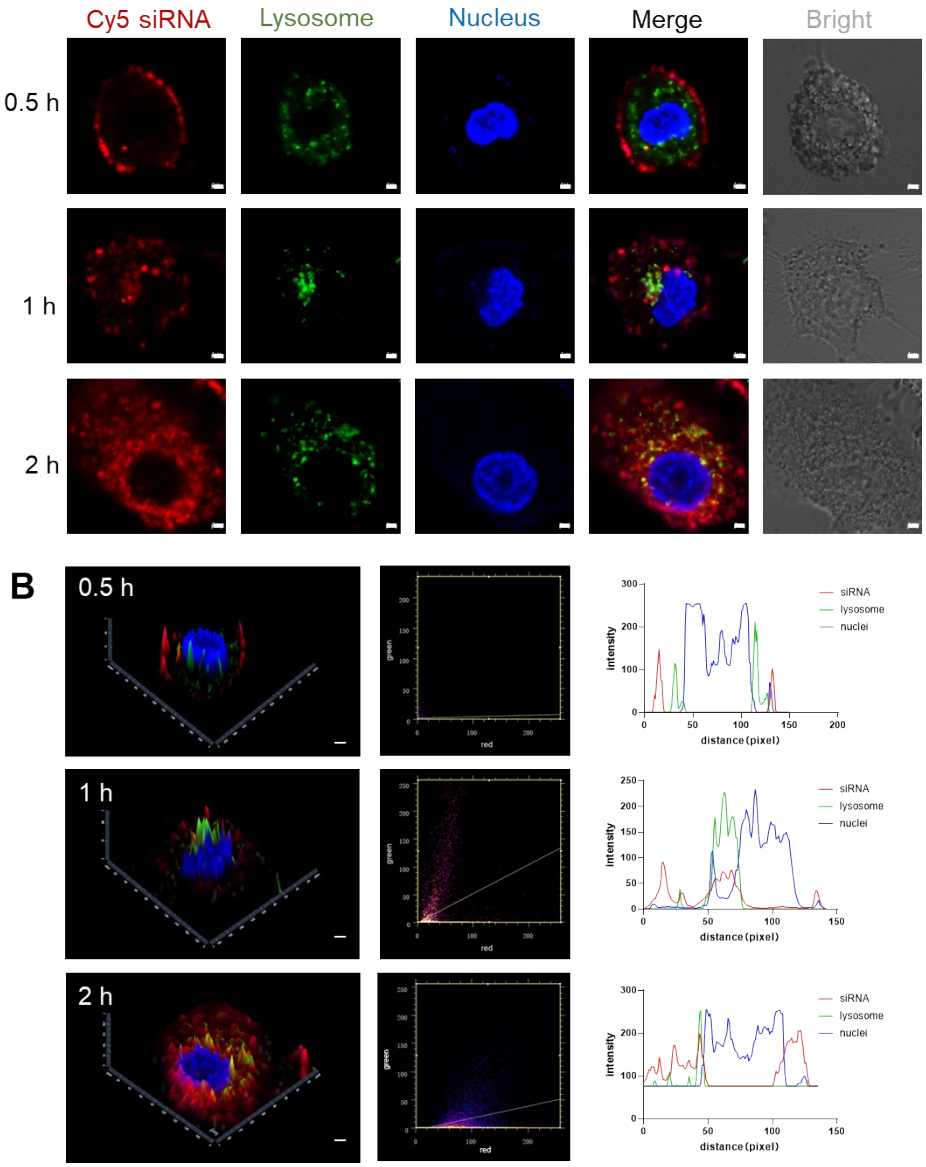


Figure S11. (A) Confocal imaging of the endosomal escape of the siRNA/bola DS-C_6-K_4 complexes in A549 cells (N/P ratio of 10, 20 nM Cy5-labeled siRNA). (B) 3-dimensional images and co-localization analysis of fluorescence intensity between siRNA/bola DS-C_6-K_4 complexes and lysosomes in A549 cells. The red channel image shows the Cy5-labeled siRNA (red), the green channel image shows the lysosome stained by lysotracker (green), and the blue channel image shows the nuclei of A549 cells stained by Hoechst33342 (blue). Scale bar, 5 μm.

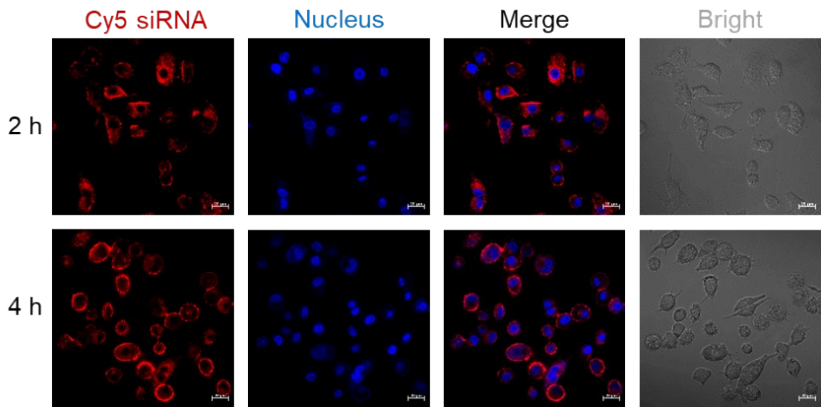


Figure S12. Confocal imaging of the cell uptake of the siRNA/bola DS-C_6-K_4 complexes in A549 cells at 2 h or 4 h (N/P ratio of 10, 20 nM Cy5-labeled siRNA). The red channel image shows the Cy5-labeled siRNA (red), and the blue channel image shows the nuclei of A549 cells stained by Hoechst33342 (blue). Scale bar, 20 μm.

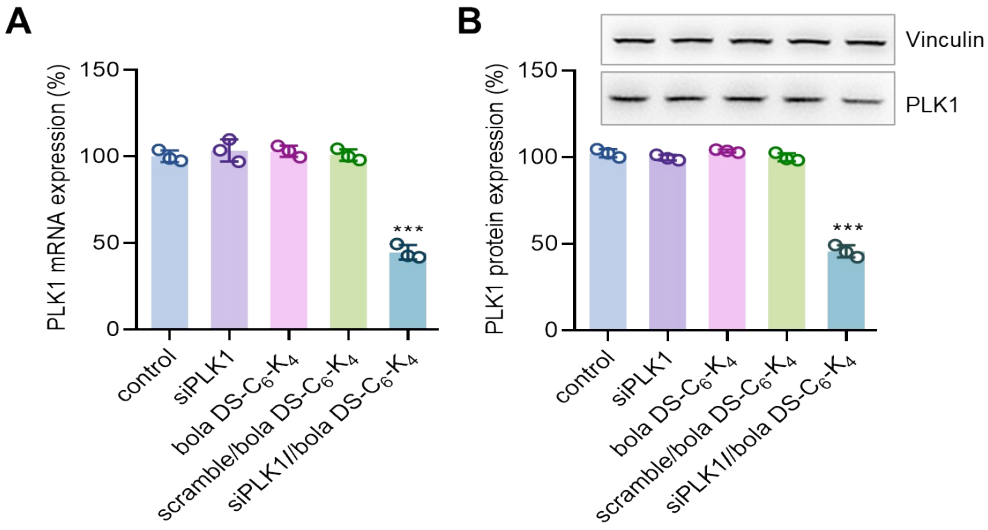


Figure S13. PLK1 mRNA (A) and protein expression (B) in A549 cells following treatment with siPLK1, bola DS-C_6-K_4, scramble/ bola DS-C_6-K_4, and siPLK1/bola DS-C_6-K_4 (N/P = 10, 50 nM siRNA). siPLK1: siRNA targeting PLK1, scramble siRNA: scramble. Quantitative analysis of western blotting determined by Image J software. (mean ± SD, n = 3)

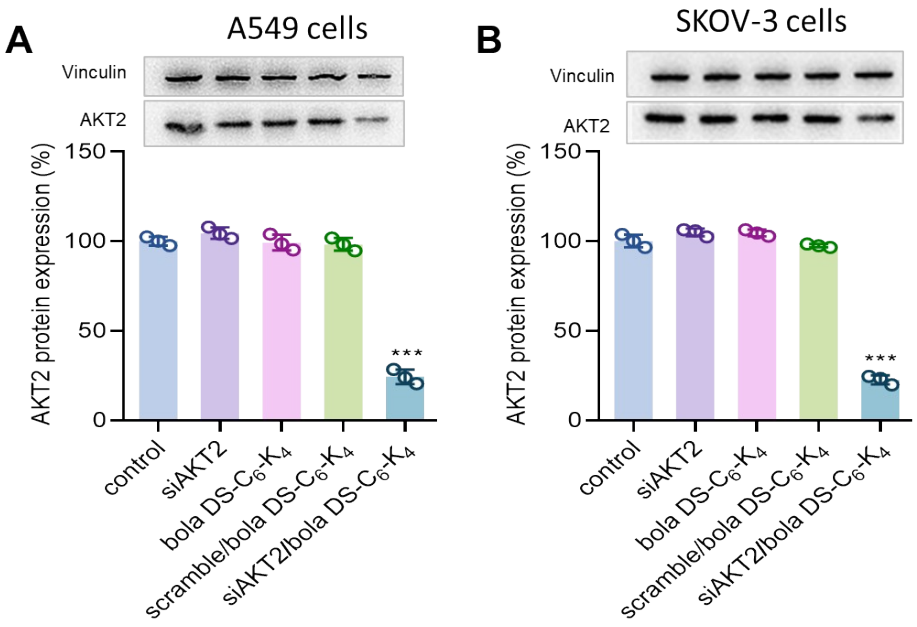


Figure S14. AKT2 protein expression in A549 cells (A) and SKOV-3 cells (B) following treatment with siPLK1, bola DS-C_6-K_4, scramble/bola DS-C_6-K_4, and siAKT2/bola DS-C_6-K_4 (N/P = 10, 50 nM siRNA). siAKT2: siRNA targeting AKT2, scramble siRNA: scramble. Quantitative analysis of western blotting determined by Image J software.

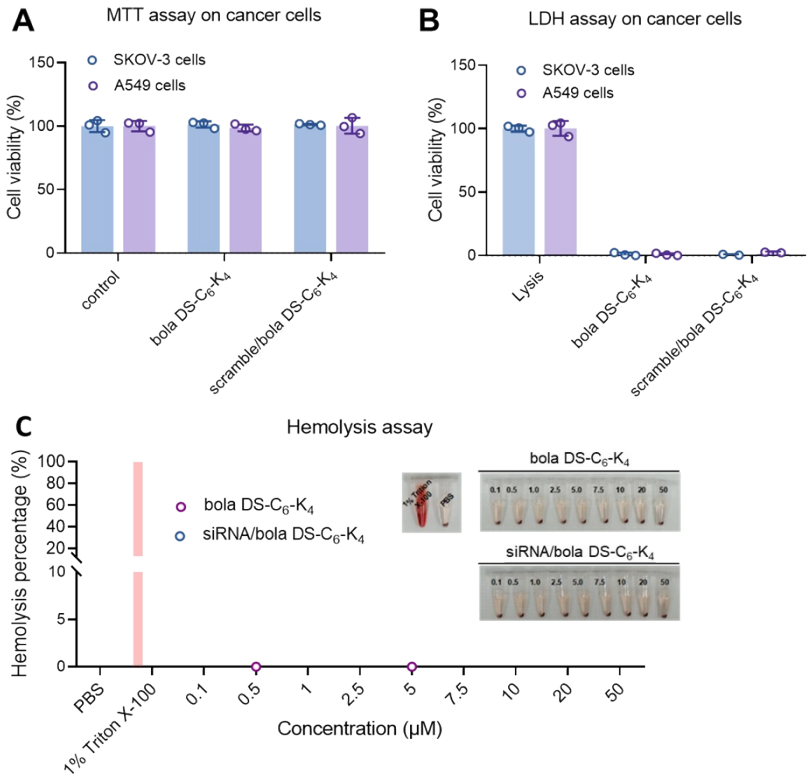


Figure S15. (A) MTT assay of the metabolic toxicity of siRNA/dendrimer complexes formed with bola DS-C_6-K_4 in SKOV-3 cells and A549 cells. (B) LDH assay of the membrane damage toxicity

of siRNA/dendrimer complexes formed with bola DS-C_6-K_4 in SKOV-3 cells and A549 cells. (E) Hemolysis assay of siRNA/bola DS-C_6-K_4. Quantitative analysis of hemolysis was performed by recording UV absorption at 540 nm. scramble: scramble siRNA. (mean ± SD, n = 3). Triton was used positive control for hemolysis assay.

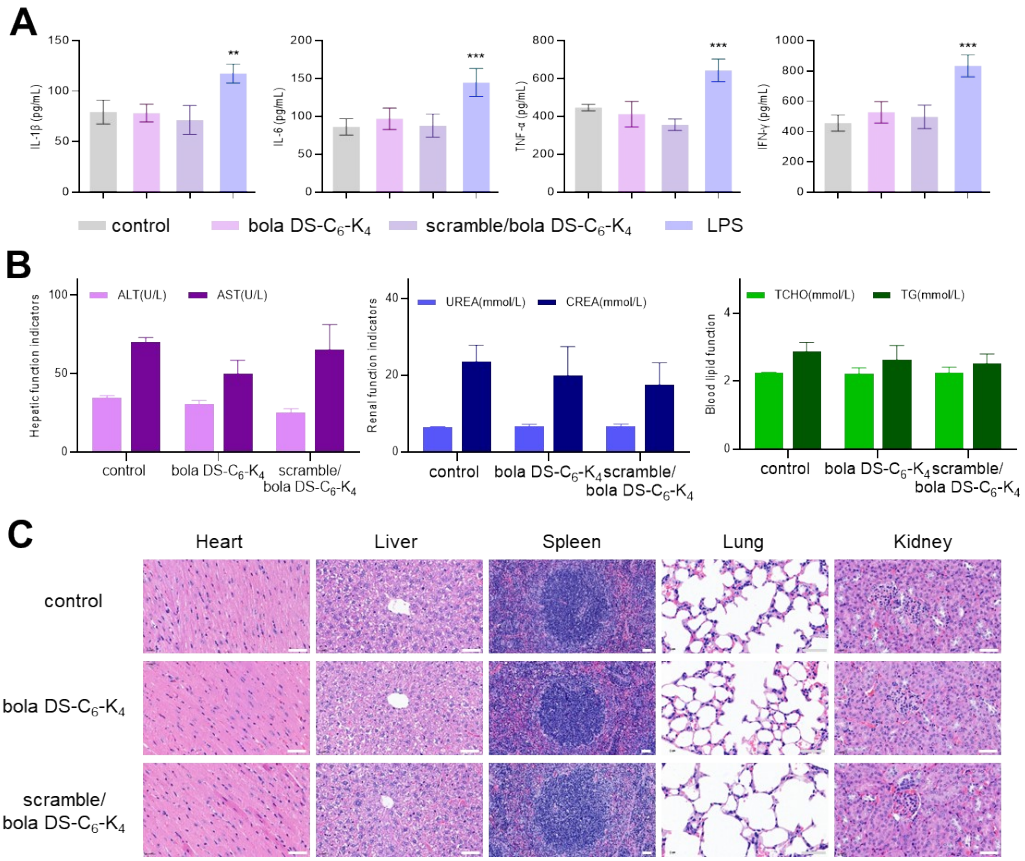


Figure S16. (A) Serum inflammatory cytokines (IL-1β, IL-6, TNF-α, and IFN-γ) in mice treated with PBS (control), bola DS-C_6-K_4, scramble/bola DS-C_6-K_4, and LPS as positive control. (B) Blood biochemistry for liver/kidney function (ALT, AST, BUNA, CREA, TCHO, TP) in serum of mice treated with PBS (control), bola DS-C_6-K_4, scramble/bola DS-C_6-K_4, and LPS. (C) H&E staining of major organs from mice treated with PBS (control), bola DS-C_6-K_4, scramble/bola DS-C_6-K_4, and LPS. (mean ± SD, n = 3, Scale bar: 50 μm).

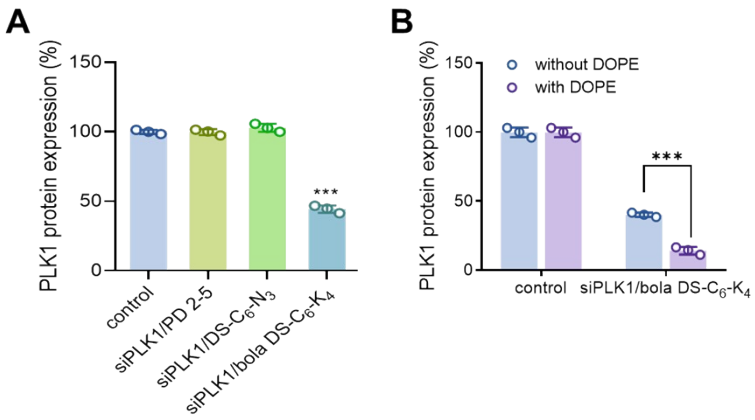


Figure S17. (A) siRNA delivery mediated by PD 2-5 or DS-C_6-N_3 for gene silencing compared with that mediated by bola DS-C_6-K_4 in A549 cells. (B) PLK1 protein protein expression in A549 cells upon treatment with the s siPLK1/bola DS-C_6-K_4 (N/P = 10, 50 nM siRNA) in the presence and absence of the fusogenic helper lipid DOPE. siPLK1: siRNA targeting PLK1. Quantitative analysis of western blotting determined by Image J software. (mean ± SD, n = 3)

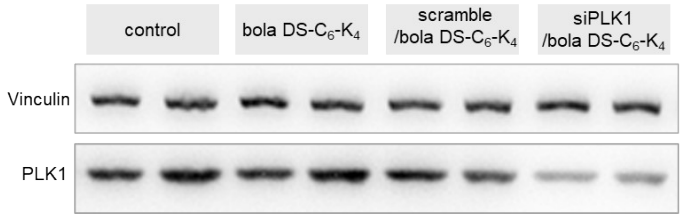


Figure S18. Western blotting analysis of PLK1 protein expression in A549 xenograft mice after intravenously administrated with PBS buffer (control), bola DS-C_6-K_4 alone, scramble/bola DS-C_6-K_4, siPLK1/bola DS-C_6-K_4 (1.0 mg/kg siRNA, 3.8 mg/kg bola DS-C_6-K_4, N/P ratio of 5.0) twice per week for two weeks. siPLK1: siRNA targeting PLK1, scramble: scramble siRNA.

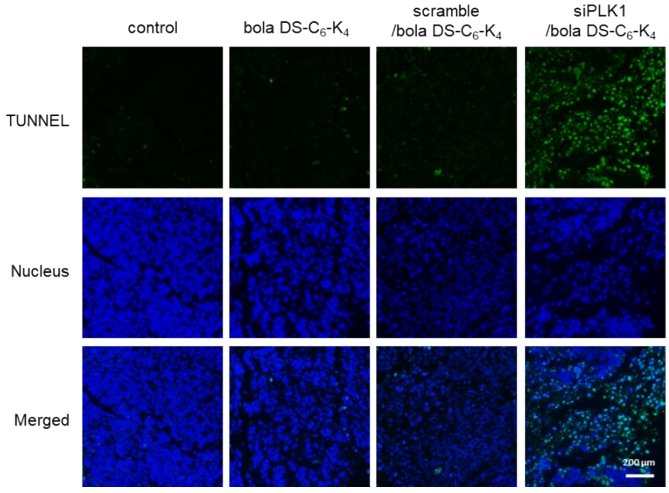


Figure S19. Cell apoptosis detected using the TUNEL assay in resected tumors from A549 xenograft mice after treatment with PBS buffer (control), bola DS-C_6-K_4 alone, scramble/bola DS-

C_6-K_4, siPLK1/bola DS-C_6-K_4 (1.0 mg/kg siRNA, 3.8 mg/kg bola DS-C_6-K_4, N/P ratio of 5.0). siPLK1: siRNA targeting PLK1, scramble: scramble siRNA. Scale bar, 200 μm.

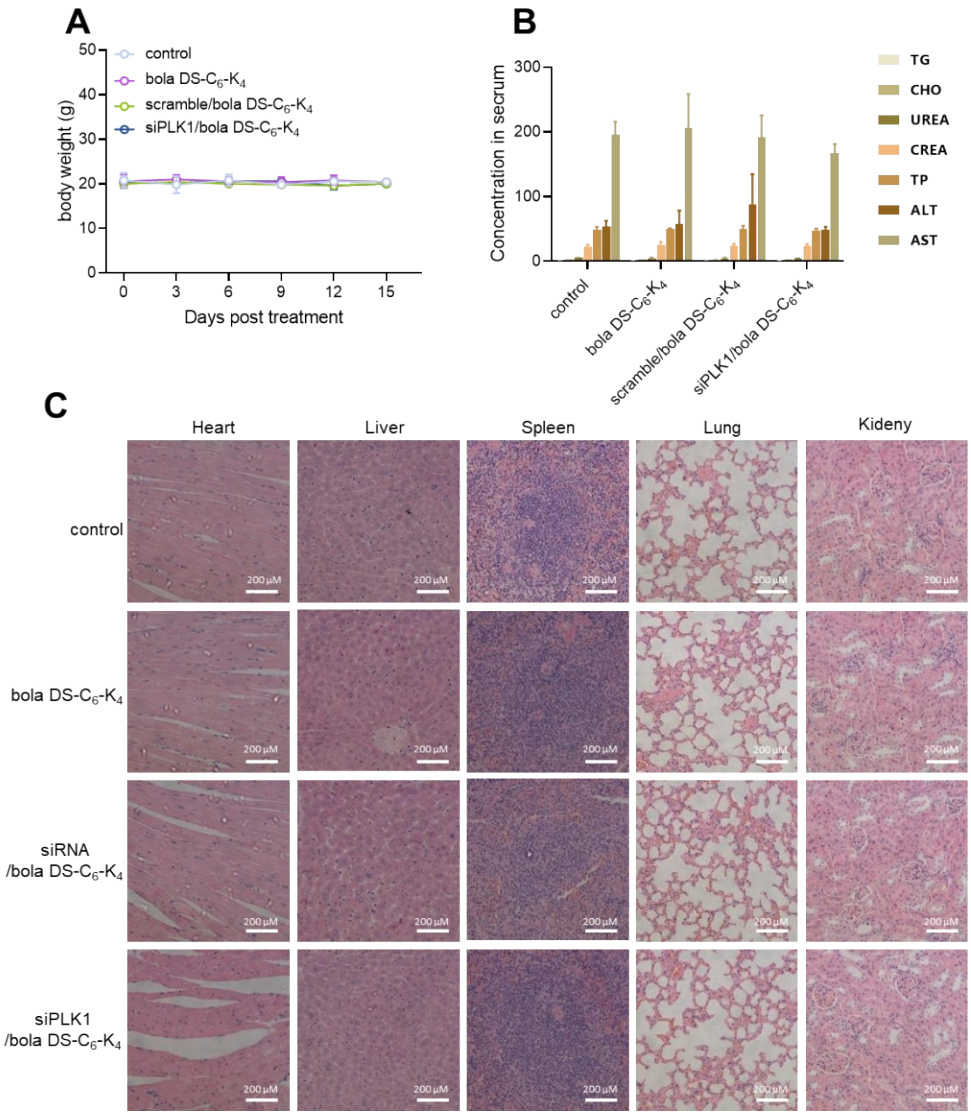


Figure S20. (A) Body weights of A549 xenograft mice were recorded at different time-points during the treatment regimes. (B) Blood biochemistry analysis of liver and kidney function and (C) histological analysis of tissues from major organs of A549 xenograft mice after treatment. siPLK1: siRNA targeting PLK1, scramble: scramble siRNA. Scale bar, 200 μm. (mean ± SD, n =5).

Table S1. CD spectrum of bola DS-C_n-K_4 (n = 6, 8, 11)

Group	bola DS-C_6-K_4	bola DS-C_8-K_4	bola DS-C_{11}-K_4
α-Helix	4.95	5.34	4.39
β-Parallel	45.33	43.43	48.49
β-turn	11.21	11.35	11.17
Rndm.Coil	38.52	39.83	36.16

Table S2. Particles size and zeta potential of bola DS-C_n-K_4 and siRNA/bola DS-C_n-K_4 complexes (n = 6, 8, 11)

Group	**Size (nm)**	**Zeta potential (mV)**
bola DS-C_6-K_4	56.86 ± 2.03	30.55 ± 2.05
bola DS-C_8-K_4	107.06 ± 1.08	28.62 ± 2.50
bola DS-C_{11}-K_4	105.60 ± 1.71	23.46 ± 2.23
siRNA/bola DS-C_6-K_4	77.94 ± 1.12	26.85 ± 3.35
siRNA/bola DS-C_8-K_4	65.53 ± 1.29	23.81± 0.95
siRNA/bola DS-C_{11}-K_4	48.21 ± 1.00	24.63 ± 2.44

References

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[4]. Y. Dong, Y. Chen, D. Zhu, K. Shi, C. Ma, W. Zhang, P. Rocchi, L. Jiang and X. Liu, *J Control Release.*, 2020, **322**, 416-425.