Supporting Information

Supramolecular PEGylated Dendritic Systems as pH/Redox Dual-Responsive Theranostic Nanoplatforms for Platinum Drug Delivery and NIR Imaging

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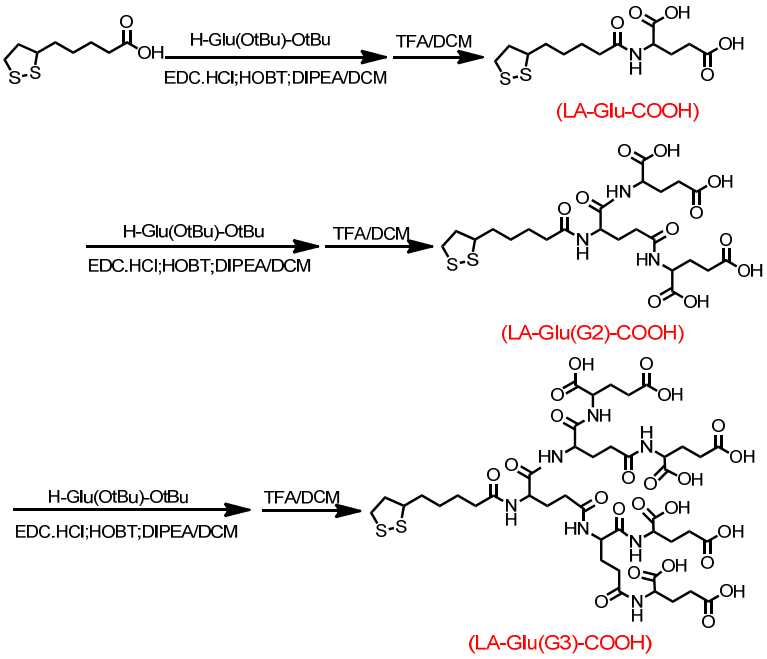
1 Experimental Detail

1.1 Materials

H-Glu(OtBu)-OtBu, Boc-Lys(Boc)-OH, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC.HCl) and 1-hydroxybenzotriazole hydrate (HOBT) were purchased from GL Biochem (Shanghai, China). N,N-diisopropylethylamine (DIPEA), trifluoroacetic acid (TFA) and triethylamine (TEA) were purchased from Asta Tech Pharmaceutical (Chengdu, China). Lipoic acid (LA), methoxypolyethylene glycol (mPEG, Mw=1000), bovine serum albumin (BSA), sodium azide (NaN_3), 2-deoxy-d-glucose (2-DG), chlorpromazine, cytochalasin D, nystatin and methyl-β-cyclodextrin (M-β-CD) were obtained from Aladdin (Shanghai, China). Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) and dithiothreitol (DTT) were obtained from TCI Development Co., Ltd. (Shanghai, China). Cystamine dihydrochloride, silver nitrate ($AgNO_3$), potassium tetrachloroplatinate (II) (K_2PtCl_4), 2,6-dihydroxyacetophenone (DHAP), chloroform-d ($CDCl_3$) and glutathione reduced ethyl ester (GSH-OEt) were purchased from Sigma Aldrich (USA). Hydroxysuccinimide ester of Cy5.5 (Cy5.5-NHS) was obtained from Fanbo Biochemicals (Beijing, China). Cis-diamminedichloroplatinum (cisplatin) for injection was purchased from Qilu Pharmaceutical Co., Ltd (Shandong, China). Doxorubicin hydrochloride (DOX.HCl) was purchased from Hisun Pharmaceutical (Zhejiang, China). All the above reagents were used as received. All the organic solvents were purified using the standard method and distilled before use. RPMI 1640 medium, fetal bovine serum (FBS) and NE-PER nuclear and cytoplasmic extraction reagent were purchased from Thermo Fisher Scientific (USA). Penicillin and streptomycin were purchased from Hyclone (USA). LysoTracker Blue DND-22 was purchased from Invitrogen (Carlsbad, CA, USA). Hoechst 33342 and cell counter kit-8 (CCK-8) were obtained from Dojindo Laboratories (Kumamoto, Japan). Cell cycle and apoptosis analysis kit and 3,3'-dioctadecyloxacarbocyanine perchlorate (DiO) were purchased from Beyotime Institute of Biotechnology (Beijing, China). BALB/c nude mice and BALB/c mice were purchased from Dashuo Experimental Animal Center (Chengdu, China).

1.2 Synthesis of lipoic acid (LA) functionalized peptide dendrons (LA-Glu(G3)-COOH)



Scheme S1 Synthetic route of LA functionalized poly-glutamine dendrimers (LA-Glu(G3)-COOH).

Synthesis of LA functionalized glutamine (LA-Glu-COOH)

LA (1.00 g, 4.8 mmol), H-Glu(OtBu)-OtBu (1.51 g, 5.8 mmol), EDC.HCl (1.12 g, 5.8 mmol) and HOBT (0.79 g, 5.8 mmol) were dissolved in 50 mL dichloromethane (DCM) and cooled to 0 °C in an ice-bath. DIPEA (3.2 mL, 19.2 mmol) was added under N_2. The mixture was kept at 0 °C for 30 min and slowly warmed to room temperature. After 24 h, the mixture was washed with brine, saturated $NaHCO_3$ and HCl (1 mol L^{-1}). The crude product was purified by silica gel chromatography eluted with DCM/methanol (MeOH) (15:1) to give LA-Glu(OtBu)-OtBu (1.98 g, yield 91.7%) as light yellow solid. LA-Glu(OtBu)-OtBu (1.50 g, 3.4 mmol) was reacted with trifluoroacetic acid (TFA) (5.2 mL, 68.0 mmol) to remove the tert-butoxy (OtBu) groups. With vigorous stirring for 4 h, the solvent was evaporated and diethyl ether was added to precipitate LA-Glu-COOH (1.11 g, yield 98.2%) as white powder.

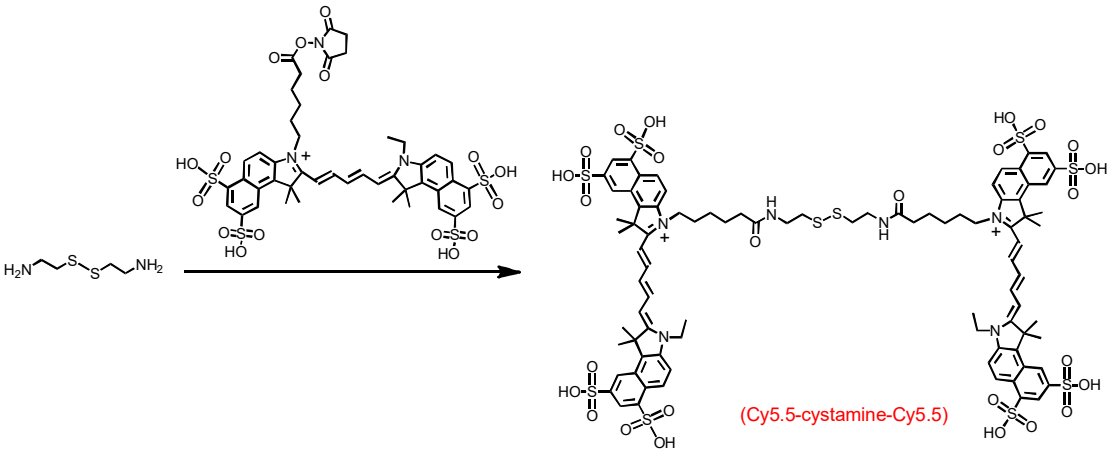
Synthesis of LA functionalized Generation 2 poly-glutamine dendrimers (LA-Glu(G2)-COOH)

LA-Glu-COOH (1.00 g, 3.0 mmol), H-Glu(OtBu)-OtBu (1.87 g, 7.2 mmol), EDC.HCl (1.39 g, 7.2 mmol) and HOBT (0.98 g, 7.2 mmol) were dissolved in 50 mL DCM and cooled to 0 °C in an ice-bath. DIPEA (4.0 mL, 24.0 mmol) was added under N_2. The mixture was kept at 0 °C for 30 min and slowly warmed to room temperature. After 24 h, the mixture was washed with brine, saturated $NaHCO_3$ and HCl (1 mol L^{-1}). The crude product was purified by silica gel chromatography eluted with DCM/MeOH (15:1) to give LA-Glu(G2)-(OtBu)-OtBu (2.15 g, yield 88.5%) as light yellow solid. LA-Glu(G2)(OtBu)-OtBu (1.00g, 1.2 mmol) was reacted with TFA (3.7 mL, 48.0 mmol) to remove OtBu groups. With vigorous stirring for 4 h, the solvent was evaporated and diethyl ether was added to precipitate LA-Glu(G2)-COOH (0.70 g, yield 97.2%) as white powder.

Synthesis of LA functionalized Generation 3 poly-glutamine dendrimers (LA-Glu(G3)-COOH)

LA-Glu(G2)-COOH (0.50 g, 0.8 mmol), H-Glu(OtBu)-OtBu (1.00 g, 3.8 mmol), EDC.HCl (0.73 g, 3.8 mmol) and HOBT (0.52 g, 3.8 mmol) were dissolved in 50 mL DCM and cooled to 0 °C in an ice-bath. DIPEA (2.1 mL, 12.8 mmol) was added under N_2. The mixture was kept at 0 °C for 30 min and slowly warmed to room temperature. After stirring for 48 h, the solvent was removed in vacuum and the residue was dissolved in chloroform. The mixture was washed with brine, saturated $NaHCO_3$ and HCl (1 mol L^{-1}). The crude product was purified by silica gel chromatography eluted with DCM/MeOH (15:1) to give LA-Glu(G3)-(OtBu)-OtBu (1.11 g, yield 85.4%) as yellow solid. LA-Glu(G3)-(OtBu)-OtBu (1.00 g, 0.6 mmol) was reacted with TFA (3.7 mL, 48.0 mmol) to remove OtBu groups. With vigorous stirring for 8 h, the solvent was evaporated and diethyl ether was added to precipitate LA-Glu(G3)-COOH (0.67 g, yield 94.4%) as light yellow powder.

1.3 Synthesis of fluorescence initiator (Cy5.5-cystamine-Cy5.5)

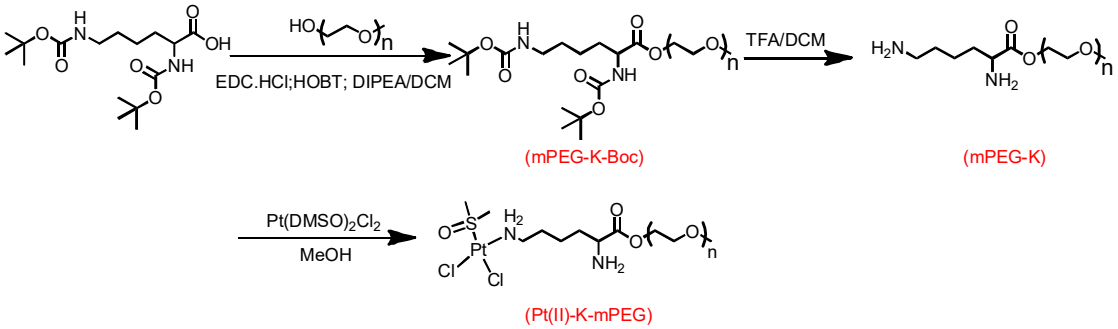


Scheme S2 Synthetic route of Cy5.5-cystamine-Cy5.5.

Synthesis of Cy5.5-cystamine-Cy5.5

Cy5.5-NHS (1.10 mg, 0.1 μmol) dissolved in 200 μL dimethyl sulfoxide (DMSO) was slowly added to 1 mL of cystamine dihydrochloride (0.10 mg, 0.4 μmol) aqueous solution with vigorous stirring at room temperature for 24 h. The organic solvent and unreacted Cy5.5 were removed by dialysis (MWCO 1000) against deionized water at 4 °C. After freeze-drying, the resulting Cy5.5-cystamine-Cy5.5 conjugates (CCs) were stored at -20 °C before use.

1.4 Synthesis of PEGylated platinum drug precursor



Scheme S3 Synthetic route of Pt(II)-K-mPEG.

Synthesis of NH_2-Lys-mPEG (mPEG-K)

mPEG (1.00 g, 1.0 mmol), Boc-Lys(Boc)-OH (0.35 g, 1.0 mmol), EDC.HCl (0.23 g, 1.2 mmol) and HOBT (0.16 g, 1.2 mmol) were dissolved in DCM, and cooled to 0 °C in an ice-bath with stirring. DIPEA (0.7 mL, 4.2 mmol) was added under N_2. The mixture was kept at 0 °C for 30 min and slowly warmed to room temperature. After stirring for 24 h, the mixture was washed with brine, saturated $NaHCO_3$ and HCl (1 mol L^{-1}). The crude product was purified by silica gel chromatography eluted with DCM/MeOH (20:1) to give Boc-Lys(Boc)-mPEG (mPEG-K-Boc) (1.21 g, yield 90.2%). mPEG-K-Boc (1.00 g, 0.7 mmol) was reacted with TFA (1.1 mL, 14.0 mmol) to remove N-tert-butoxycarbonyl (Boc) groups. With vigorous stirring for 4 h, the solvent was evaporated and diethyl ether was added to precipitate NH_2-Lys-mPEG (mPEG-K) (0.85 g, yield 94.2%).

Synthesis of $Pt(DMSO)_2Cl_2$

K_2PtCl_4 (100.00 mg, 0.2 mmol) was dissolved in water and DMSO (68 μL, 0.1 mmol) was added slowly. The mixture was reacted for 4 h with vigorous stirring. The resulting light yellow powder was washed with ice cold water, ethanol and diethyl ether and dried in vacuum as $Pt(DMSO)_2Cl_2$ (80.21 mg, yield 79.4%).

Synthesis of Pt(II)-K-mPEG

A dispersion of $Pt(DMSO)_2Cl_2$ (50.00 mg, 0.1 mmol) in MeOH was added to a solution of mPEG-K (133.89 mg, 0.1 mmol). The mixture was stirred at room temperature for 24 h and the unreacted solid was removed via filtration. The platinum drug precursor was obtained after concentration as Pt(II)-K-mPEG.

1.5 Characterization of amphiphilic dendrons and platinum precursor

Purified amphiphilic dendrimers, platinum precursor and intermediate products were accurately weight and dissolved in $CDCl_3$ with tetramethylsilane (TMS) as internal reference. ^1H spectra were recorded using Bruker Avance II NMR spectrometer (Bruker, Switzerland) at 400 MHz. Mass spectra were measured using electrospray ionization (ESI) mass spectrometry (TSQ Quantum Ultra, Thermo Fisher Scientific, USA) and matrix-assisted laser desorption/ionization–time-of-flight mass

spectrometry (MALDI-TOF MS, Bruker Autoflex III, Germany).

1.6 Supramolecular engineering of theranostic supramolecular PEGylated dendritic systems (TSPDSs)

When dissolving LA-Glu(G3)-COOH dendrimers (100.00 mg, 0.1 mmol) above critical aggregation concentration (CAC) in water, the dendrimers self-assembled into nanoparticle with LA as hydrophobic core and carboxyl group as the hydrophilic peripheral residues. Catalytic amount of CCs (8.79 mg, 4.5 μmol) was *in-situ* reduced by treating with TCEP (0.90 mg, 3.1 μmol) in water for 45 min to cleave the disulfide bonds and fluorescent initiator was obtained as Cy5.5-SH. Subsequently, the fluorescent initiator was added to initiate cross-linking of the disulfide bond in self-assembly dendritic systems (SDSs) into poly-(disulfide)s. The mixture was reacted at room temperature for 24 h and dialyzed against deionized water. The disulfide-stabilized supramolecular dendritic systems (DSDSs) were obtained after freeze-drying.

Before conjugating platinum to DSDSs, PEGylated platinum drug precursor (100.00 mg, 80.0 μmol) was dissolved in water and reacted with silver nitrate ($AgNO_3$, 25.84 mg, 150.0 μmol) to form aqueous complex. The solution was stirred at room temperature in the dark for 4 h. White precipitate of silver chloride (AgCl) was observed indicating the reaction process. The mixture was centrifuged at 10000 rpm for 15 min to remove the precipitate and the supernatant was further purified by passing through a 0.22 μm filter. DSDSs (88.70 mg, 10.0 μmol) were dissolved in NaOH (3.20 mg, 80.0 μmol) aqueous solution to change carboxylic group in deprotonated form and was added to the above PEGylated platinum aqueous solution. The mixture was reacted at 37 °C with gentle shaking to result in platinum-DSDSs conjugates. The prepared conjugates were dialyzed (MWCO 3500) against deionized water for 2 days and freeze-dried as theranostic supramolecular PEGylated dendritic systems (TSPDSs).

1.7 Critical aggregation concentration (CAC) determination

The critical aggregation concentration (CAC) of LA-Glu(G3)-COOH was determined by fluorescence spectrometer (F-7000, Japan) with pyrene as a probe. The dendrimers were dissolved

in 1 mL water with various concentrations ranging from 1.0×10^{-6} to 1.0 mg mL^{-1}. Pyrene in acetone (10 μL, 6.0×10^{-5} mol L^{-1}) was added. After sonication for 30 min, acetone was evaporated and final pyrene concentration was fixed at 6×10^{-7} mol L^{-1} in water. The excitation spectra were recorded from 300 to 360 with an emission wavelength of 395 nm. The fluorescence intensity ratios of I_{338}/I_{334} of pyrene were plotted as a function of logarithm of dendrimer concentrations. The CAC value was calculated as the intersection of low and high concentration regions.

1.8 Characterization of TSPDSs

Size distributions and morphologies

Freeze-dried TSPDSs were accurately weighed and dissolved with deionized water at a concentration of 100 μg mL^{-1}. The sample was observed by dynamic light scattering (DLS, Malvern Zetasizer Nano ZS, UK) for size distributions and zeta potential. Transmission electron microscopy (TEM, Tecnai GF20S-TWIN, USA) and scanning electron microscopy (SEM, JSM-5900LV, Japan) were used for morphology observation. TEM sample was prepared by dropping fresh solution onto copper grid and SEM sample was prepared by dropping fresh solution onto silicon slice.

Energy-dispersive spectroscopy (EDS) and X-ray photoelectron spectroscopy (XPS)

The platinum content in TSPDSs was confirmed by energy-dispersive spectroscopy (EDS). And the chemical nature of platinum in TSPDSs was observed by X-ray photoelectron spectroscopy (XPS, XSAM800, UK) with C_{1s} peak at 284.6 eV for energy calibration.

Thermo gravimetric analysis (TGA)

The platinum amount in TSPDSs was determined by thermo gravimetric analysis (TGA, NETZSCH STA 449C, Germany). Analyses were conducted over a temperature range of 40 °C to 900 °C in air with a programmed temperature increase of 10 °C/min. The amount of platinum loaded in TSPDSs was further confirmed by inductively coupled plasma mass spectrometry (ICP-MS, Perkin ELAN DRC-e, Canada).

Responsiveness of TSPDSs in particle size

TSPDSs were dissolved in different aqueous solutions (pH 7.4, pH 6.8, pH 5.0, 10 μM DTT and 10 mM DTT, respectively) at a concentration of 100 μg mL^{-1}. The changes in particle size were monitored with DLS over different incubation time (0 h, 0.5 h, 1.0 h, 6.0 h and 12.0 h).

In vitro dual-responsive drug release

TSPDSs (1mL, 1 mg mL^{-1}) were dissolved in phosphate buffered solution (PBS, pH 7.4 without DTT, pH 6.8 with 10 μM DTT, pH 5.0 without DTT and pH 5.0 with 10 mM DTT, respectively) and sealed into dialysis tubes (MWCO 2000). The dialysis tubes were incubated in 20 mL buffer solution at 37 °C with gentle shaking. 0.5 mL sample solution was taken out from the incubation buffer at predetermined time intervals and the same amount of corresponding buffer was added. The platinum amount in taken out dialysis medium was measured by ICP-MS.

1.9 Cell culture

Human lung epithelial tumor cell line A549 was obtained from Chinese Academy of Science Cell Bank for Type Culture Collection (Shanghai, China). Cells were cultured in RPMI 1640 medium supplemented with 10% (v/v) FBS and 1% (v/v) penicillin/streptomycin in a humidified atmosphere containing 5% CO_2 at 37°C.

1.10 *In vitro* cytotoxicity

To evaluate the effects of TSPDSs on A549 cells, cell counter kit-8 (CCK-8) assay was employed to evaluate the cell proliferation quantitatively. A549 cells were seeded in 96-well plate at a density of 8×10^3/well for 24 h. LA-Glu(G3)-COOH (D), mPEG-K, D+mPEG-K complex and TSPDSs were added in various concentrations followed by incubation for another 48 h. The medium was refreshed with PBS (pH 7.4) twice, and 10 μL of CCK-8 was added into each well for 2 h incubation at 37 ºC. The absorbance of each sample was measured using a microplate reader (Model 550, Bio-Rad, USA) at 450 nm. Relative cell viability (n=6) was expressed as: $(OD_{sample}-OD_{blank})/(OD_{control}-OD_{blank}) \times 100\%$.

1.11 Cellular internalization of TSPDSs

The cellular uptake and intracellular behavior of TSPDSs were determined by confocal laser scanning microscopy (CLSM, LEICA TCS SP5, Germany) and fluorescence activated cell sorting (FACS, Beckman Coulter Cytomics FC-500, USA) using A549 cells.

A549 cells were seeded into 35 mm glass bottom culture dish for 24 h and incubated with TSPDSs for 1 h, 3 h, 6 h and 24 h, respectively. After being rinsed twice with PBS, the cells were fixed with 4% paraformaldehyde for 5 min and then treated with DiO or Hoechst 33342 for 25 min for cell membrane and nuclei staining. To observe endosomal escape of TSPDSs, cells were incubated with TSPDSs for 1 h, 3 h, 6 h and 24 h. LysoTracker Blue was added and incubated with cells for 1 h for lysosome staining. Cells pretreated with GSH-OEt for 2 h and further incubated with TSPDSs for another 3 h was used for comparison. The cells were imaged by CLSM.

A549 cells were seeded into 6-well plates for 24 h and incubated with TSPDSs in RPMI 1640 medium for various time (1 h, 3 h, 6 h and 24 h). Thereafter, cells were harvested and centrifuged at 1000 rpm for 5 min. Then the cells were resuspended in 0.5 mL PBS and analyzed by FACS for quantitative internalization of TSPDSs.

1.12 Endocytotic pathways

A549 cells were seeded in 6-well plate (2×10^6 cells/well) and incubate for 24 h. Cells without any treatment was used as negative control and cells treated with TSPDSs (10 μg mL^{-1}) at 37 ºC for 3 h in serum-free medium was used as positive control. Sodium azide (NaN_3) in 2-deoxy-d-glucose (2-DG) (an inhibition of ATP synthesis), chlorpromazine (an inhibitor of clathrin-mediated endocytosis), cytochalasin D for micropinocytosis inhibition, nystatin or M-β-CD (an inhibitor of caveolae-mediated endocytosis) was separately added in serum-free medium. After 1 h incubation at 37 ºC, TSPDSs were added and incubated for another 3 h. Cells cooled at 4 ºC for 1 h and further incubated with TSPDSs for 3 h was also conducted. The medium was removed and rinsed with PBS for three times. Cells were harvested and centrifuged at 1000 rpm for 5 min. Then the cells were resuspended in 0.5 mL PBS and analyzed by FACS for quantitative internalization of TSPDSs.

1.13 Intracellular platinum distribution

A549 cells were seeded into 6-well plates and incubated with cisplatin and TSPDSs (1.5 μg mL^{-1} platinum) for 24 h. The cells were harvested, counted and treated with NE-PER nuclear and cytoplasmic extraction reagent to separate nucleus and cytoplasm following the manufacturer's protocol. Generally, harvested cells were fully suspended with 200 μL ice-cold CER I and incubated on ice for 10 min followed by adding 11 μL ice-cold CER II and incubation for another 1 min. The cell suspension was centrifuged at 16000 g for 5 min and the supernatant was obtained as cytoplasmic extract. The insoluble fraction was resuspended in 100 μL ice-cold NER with vigorous vortex for 15 s. The sample was placed on ice and continued vortexing for 15 s every 10 min for a total of 40 min. The suspension was centrifuged at 16000 g for 10 min and the supernatant was collected as nucleus extract. The separated cytoplasmic extract and nucleus extract were digested with H_2O_2 and HNO_3 and the platinum amount in cytoplasm and nucleus was measured by ICP-MS.

1.14 Cell cycle analysis

Cell cycle distribution was analyzed by flow cytometry. After treatment with D+mPEG-K complex, cisplatin and TSPDSs (platinum concentration of 1.5 μg mL^{-1}) for 24 h, the cells were harvested, washed with PBS and fixed with pre-cooled 70% ethanol at 4 °C for 2 h. After washing with ice-cold PBS, the fixed cells were stained with propidium iodide (PI) at 37 °C for 0.5 h. The stained cells were analyzed by FACS for cell cycle distribution.

1.15 *In vivo* antitumor efficacy of TSPDSs

The animal experiments were conducted in accordance with the institutional regulations established by Sichuan University. A549 cells (2×10^6 cells) were subcutaneously injected into the right flanks of nude male mice in 100 μL PBS. When the tumor volume reached approximately 100 mm^3, mice were randomly divided into four groups (n=6) and intravenously injected with 200 μL physiological saline, D+mPEG-K complex, cisplatin and TSPDSs at an equivalent dose of 3.25 mg platinum kg^{-1} per mouse on day 0, 3, 6 and 9. The tumor size was measured using a caliper and tumor volume was calculated according to the formula of $V=AB^2/2$ (where A and B were the length

and width of tumor, respectively). Simultaneously, the body weights of mice were recorded every 3 days for 21 days.

1.16 Histopathology and immunohistochemical evaluation

After a treating course for 21 days, tumors and major organs were excised from the mice and fixed in 10% formalin saline for 24 h. The tissues were embedded in paraffin, sectioned and stained with haematoxylin-eosin (H&E) for histological analysis, platelet endothelial cell adhesion molecule-1 (CD31) and Ki-67 for immunohistochemistry analysis and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) for tumor apoptotic analysis.

1.17 *In vivo* and *ex vivo* NIR imaging

A549 cells (2×10^6 cells) were subcutaneously injected into the right flanks of the nude male mice in 100 μL PBS to obtain A549-tumor bearing mice. Until the tumor volume reached approximately 100 mm^3, TSPDSs were intravenously injected at a dose of 3.25 mg platinum kg^{-1} per mouse. And A549-tumor bearing mouse treated with physiological saline was used as control. Then the mice were imaged by Maestro in vivo fluorescence imaging system (Maestro CRi, Inc., USA) for the NIR signal of Cy5.5 at 1 h, 3 h and 6 h postinjection. After 24 h, mice were sacrificed and tumors and major organs were separated for *ex vivo* imaging.

1.18 Protein adsorption

To determine the protein adsorption of TSPDSs, bovine serum albumin (BSA) was used as a model protein. Both cisplatin and TSPDSs (100 μg mL^{-1}, 1 mL) were incubated with a solution of BSA (100 μg mL^{-1}, 1 mL, pH 7.4) with a final concentration of cisplatin or TSPDSs and BSA at 50 μg mL^{-1}. After incubation at 37 °C for a determined time (1 h, 2 h, 4 h and 6 h), 1.0 mL of each sample was centrifuged at 8000 rpm for 15 min to precipitate the protein-absorbed particles. The BSA concentration of the supernatant was determined via UV-Vis spectrometer (Perkin Elmer Lambda 650, USA) by the absorbance at 280 nm and the absorbed protein was calculated with a standard curve of BSA. All tests were run in triplicate.

1.19 Pharmacokinetic studies

The pharmacokinetic properties of TSPDSs after a single injection were studied in 4-week old BALB/c male mice. Cisplatin and TSPDSs were intravenously injected at a dose of 3.25 mg platinum kg^{-1} (3 mice per group). Mice were sacrificed at defined time points (0.05 h, 0.5 h, 1 h, 6 h and 12 h) after injection, and blood samples were collected and digested with H_2O_2 and HNO_3. Then 5 mL H_2O was added to each sample to dissolve the residue and the platinum contents were measured by ICP-MS.

1.20 *In vivo* platinum biodistribution

The biodistribution of platinum in A549-tumor bearing mice after a single injection of cisplatin and TSPDSs were determined. After 2 h and 12 h intravenous injection of cisplatin and TSPDSs at a dose of 3.25 mg platinum kg^{-1} (3 mice per group), mice were sacrificed to separate kidney and tumors. After weighing, the tissues were digested with H_2O_2 and HNO_3. Once the digestion was accomplished, the residue was dissolved in 5 mL water and measured by ICP-MS.

1.21 Peripheral blood analysis

After treatment for 21 days, the mice were sacrificed and blood was immediately collected in a heparinized tube. After centrifugation at 3000 rpm for 15 min, serum was separated for clinical chemical parameter analysis, including blood urea nitrogen (BUN) and creatinine to determine the function of kidney.

1.22 Guest molecule loading

The potential of the amphiphilic dendrimers for guest molecule loading was determined by fluorescence spectrometer using doxorubicin (DOX) as model antitumor drug. First, doxorubicin hydrochloride (DOX.HCl, 1.0 equiv) was treated with excess triethylamine (TEA, 3.0 equiv) in aqueous solution with vigorous stirring to produce hydrophobic DOX. DOX and LA-Glu(G3)-COOH were dissolved in DMSO under ultrasonic conditions. Then the mixture was added dropwise

into water under ultrasonic condition to prepare DOX-loaded dendritic self-assembly. The fluorescence spectra of DOX loaded in dendrimers of various DOX concentrations were obtained with an excitation wavelength of 480 nm in water. Fluorescence spectra of DOX at corresponding concentrations were observed for comparison.

1.23 Statistical analysis

Differences between two groups were evaluated by one-way ANOVA followed by Student's-test. All results were averaged and values were reported as mean ± standard deviation (SD) if not indicated. $P < 0.05$ was considered statistically significant and $p < 0.01$ was considered highly significant.

2 Supplementary Results

2.1 Characterizations of LA-functionalized peptide dendrons

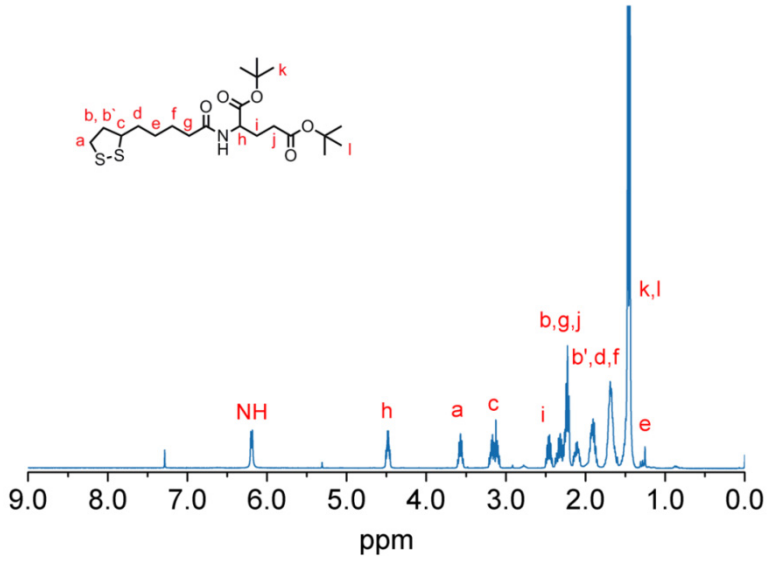


Figure S1. ^{1}H NMR spectrum of LA-Glu(OtBu)-OtBu in $CDCl_3$.

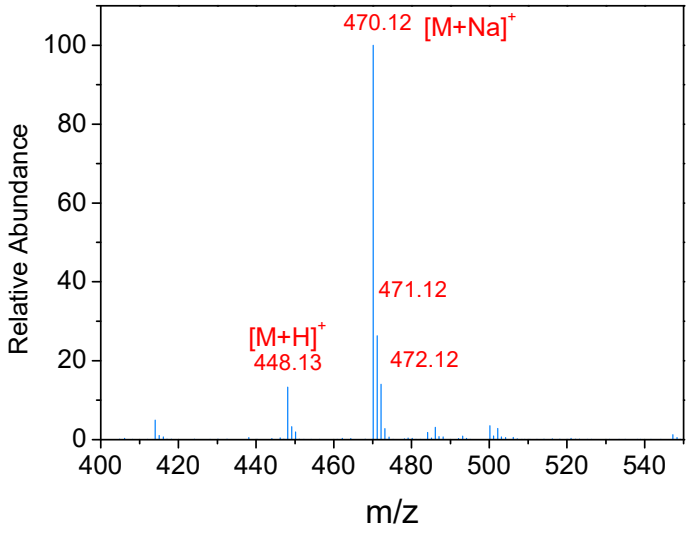


Figure S2. ESI-MS spectrum of LA-Glu(OtBu)-OtBu. MS (m/z, $[M+H]^+$): 448.13 (observed), 448.21 (calculated). MS (m/z, $[M+Na]^+$): 470.12 (observed), 470.20 (calculated).

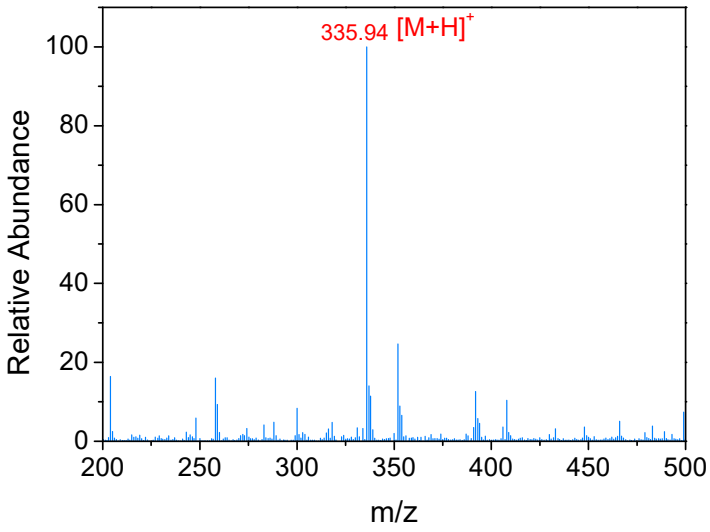


Figure S3. ESI-MS spectrum of LA-Glu-COOH. MS (m/z, $[M+H]^+$): 335.94 (observed), 336.09 (calculated).

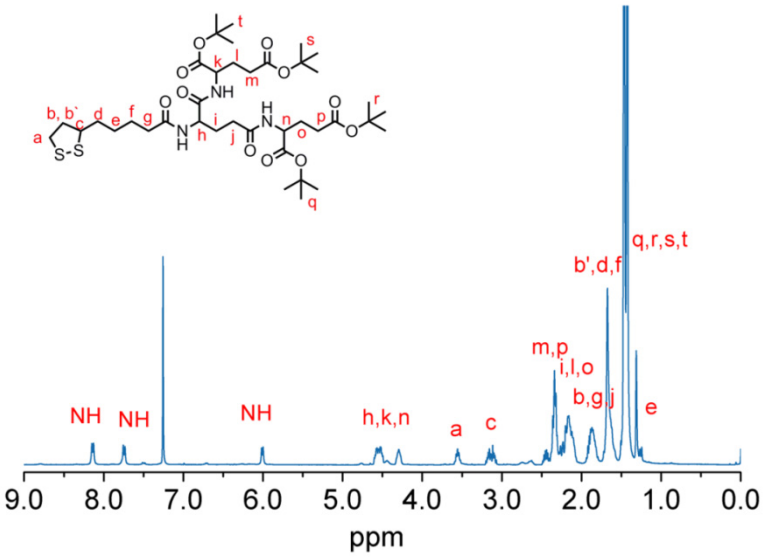


Figure S4. ^{1}H NMR spectrum of LA-Glu(G2)-OtBu in $CDCl_3$.

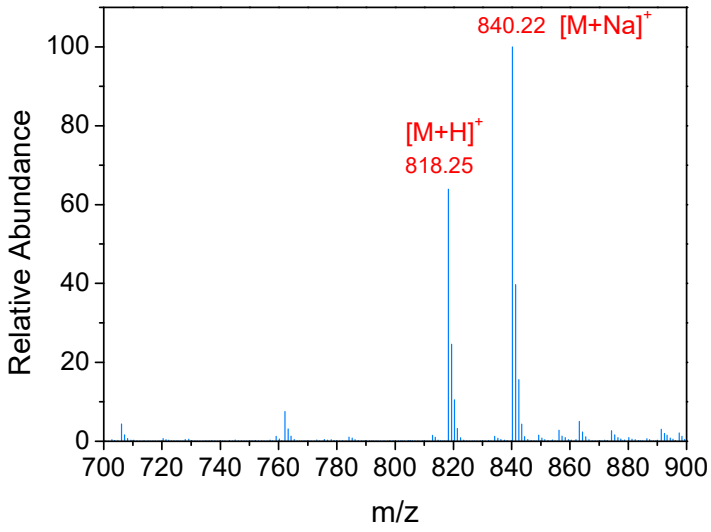


Figure S5. ESI-MS spectrum of LA-Glu(G2)-OtBu. MS (m/z, $[M+H]^+$): 818.25 (observed), 818.42 (calculated). MS (m/z, $[M+Na]^+$): 840.22 (observed), 840.41 (calculated).

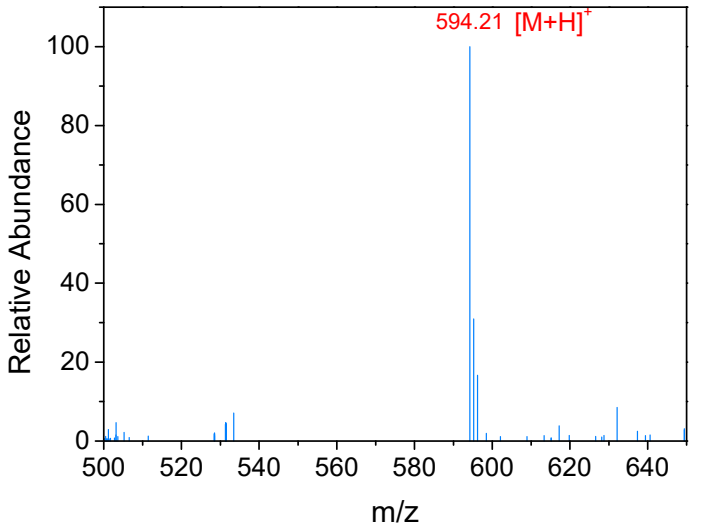


Figure S6. ESI-MS spectrum of LA-Glu(G2)-COOH. MS (m/z, $[M+H]^+$): 594.21 (observed), 594.17 (calculated).

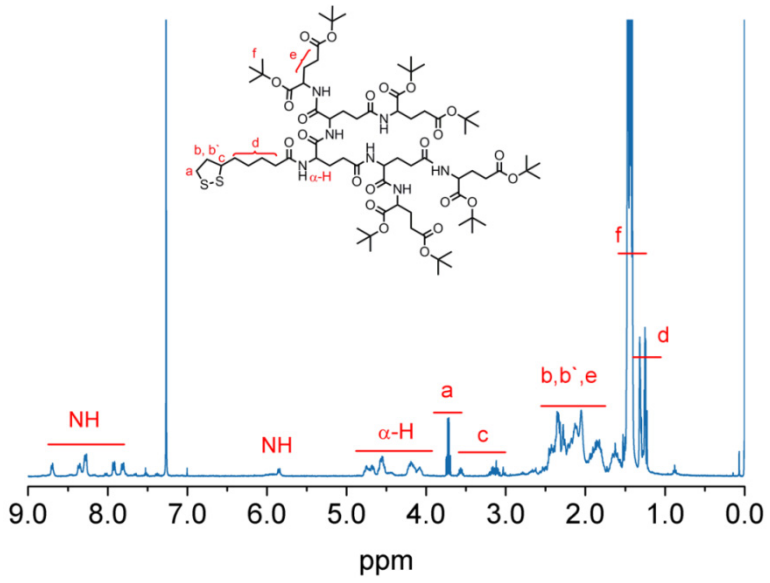


Figure S7. ^{1}H NMR spectrum of LA-Glu(G3)-OtBu in $CDCl_3$.

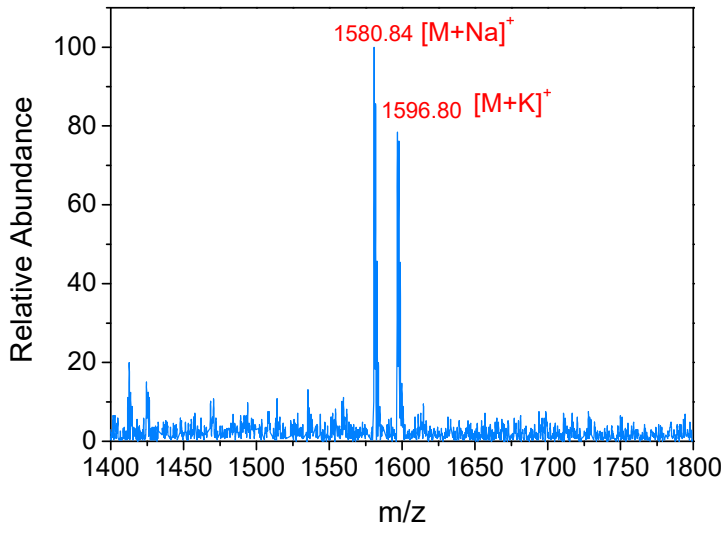


Figure S8. MALDI-TOF-MS spectrum of LA-Glu(G3)-OtBu with DHAP as matrix. MS (m/z, $[M+Na]^+$): 1580.84 (observed), 1580.83 (calculated). MS (m/z, $[M+K]^+$): 1596.80 (observed), 1596.94 (calculated).

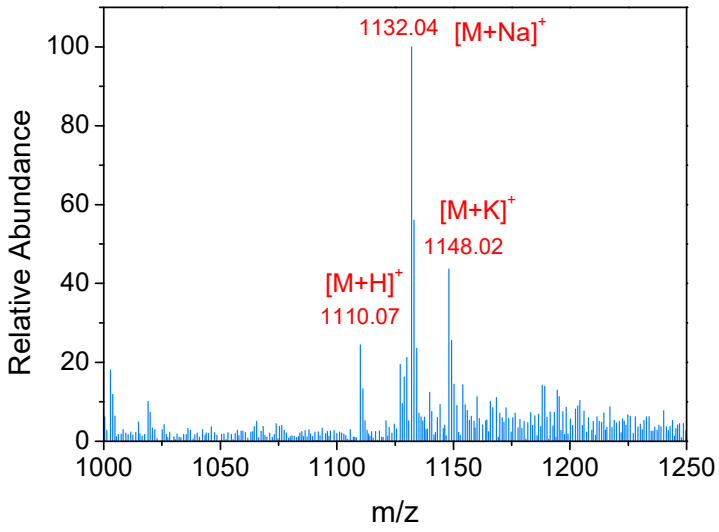


Figure S9. ESI-MS spectrum of LA-Glu(G3)-COOH. MS (m/z, $[M+H]^+$): 1110.07 (observed), 1110.34 (calculated). MS (m/z, $[M+Na]^+$): 1132.04 (observed), 1132.33 (calculated). MS (m/z, $[M+K]^+$): 1148.02 (observed), 1148.44 (calculated).

2.2 Characterizations of mPEG-K

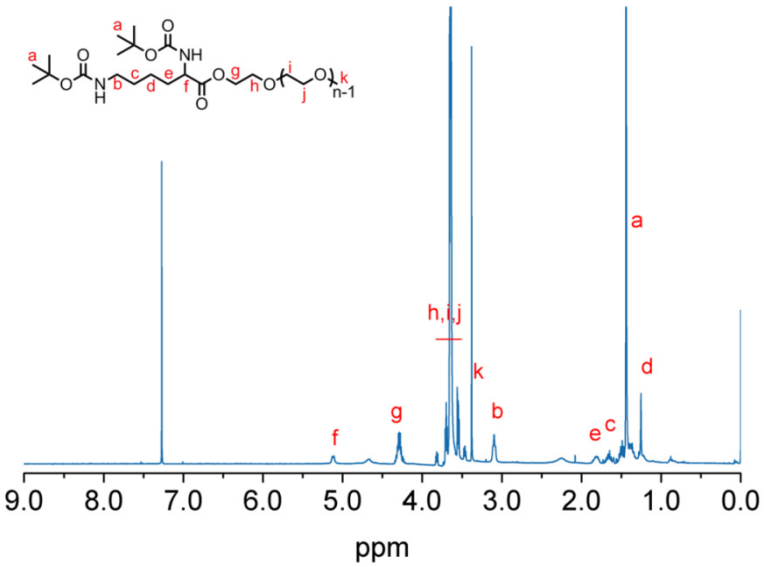


Figure S10. ^{1}H NMR spectrum of mPEG-K-Boc in $CDCl_3$.

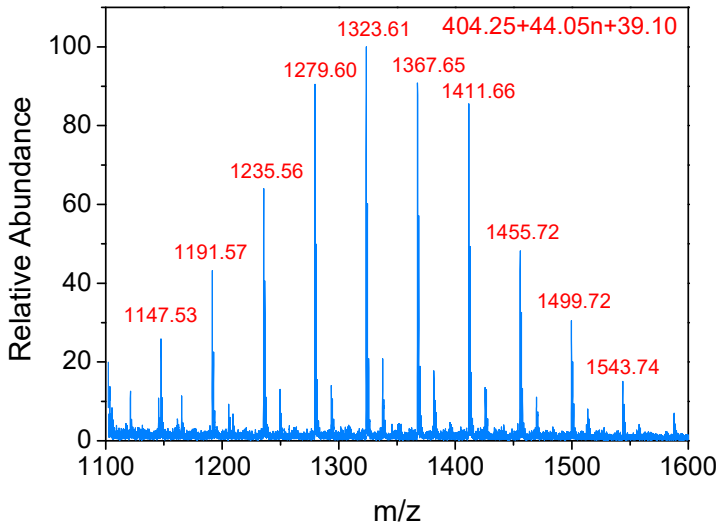


Figure S11. MALDI-TOF-MS spectrum of mPEG-K-Boc with DHAP as matrix.

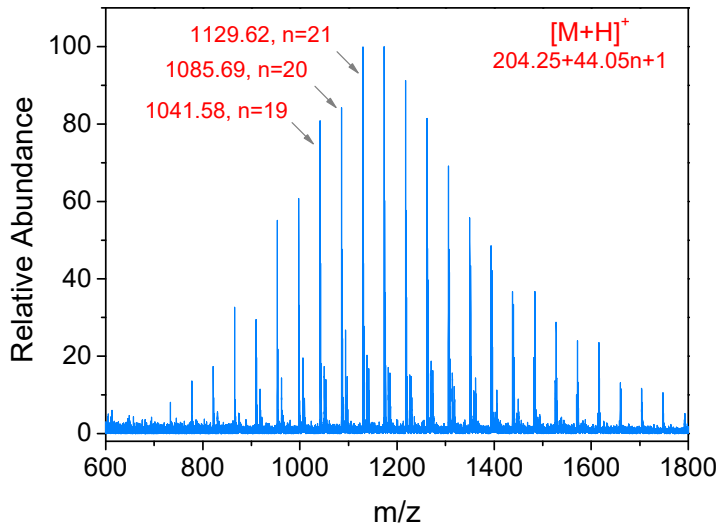


Figure S12. MALDI-TOF-MS spectrum of mPEG-K with DHAP as matrix.

2.3 Characterizations of Pt(II)-K-mPEG

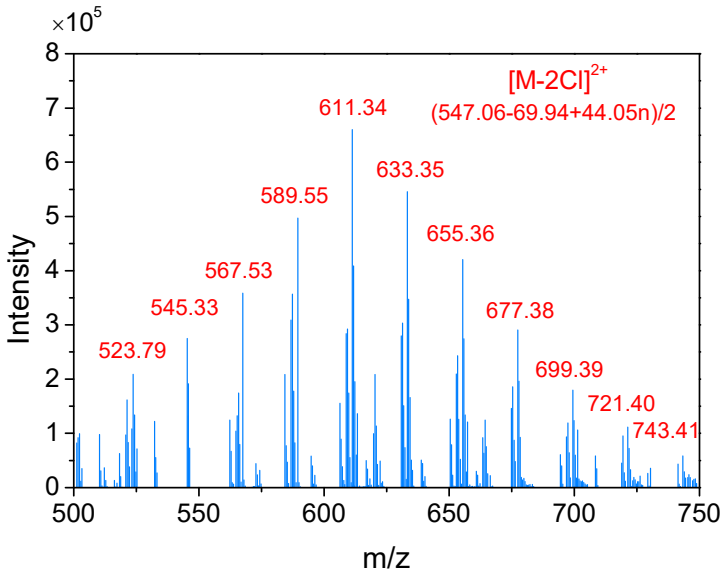


Figure S13. ESI-MS spectrum of Pt(II)-K-mPEG.

2.4 CAC determination

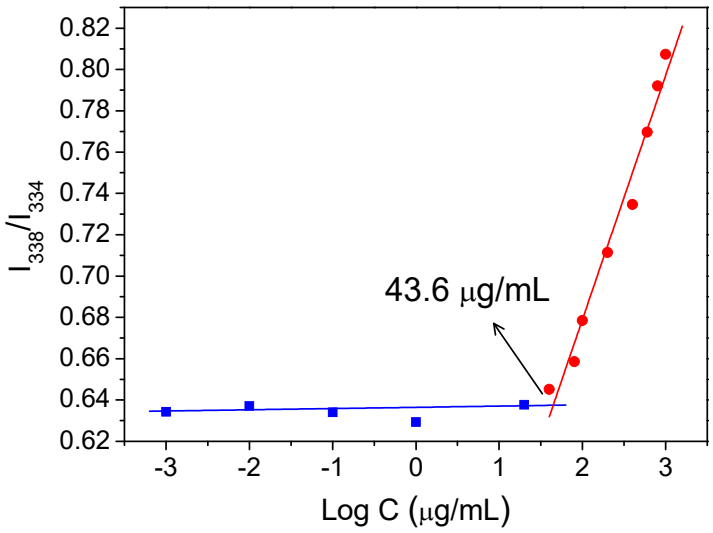


Figure S14. CAC of LA-Glu(G3)-COOH in aqueous medium which was determined by fluorescence spectroscopy with pyrene as a polarity probe.

2.5 UV-Vis spectra

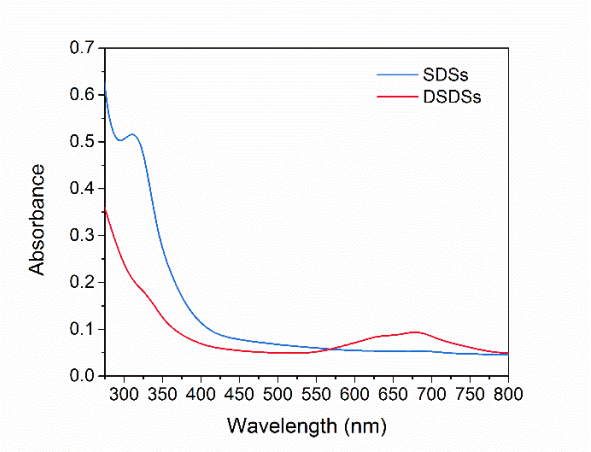


Figure S15. UV-Vis spectral changes of SDSs (blue) and DSDSs (red) in water.

2.6 Viscosity measurement

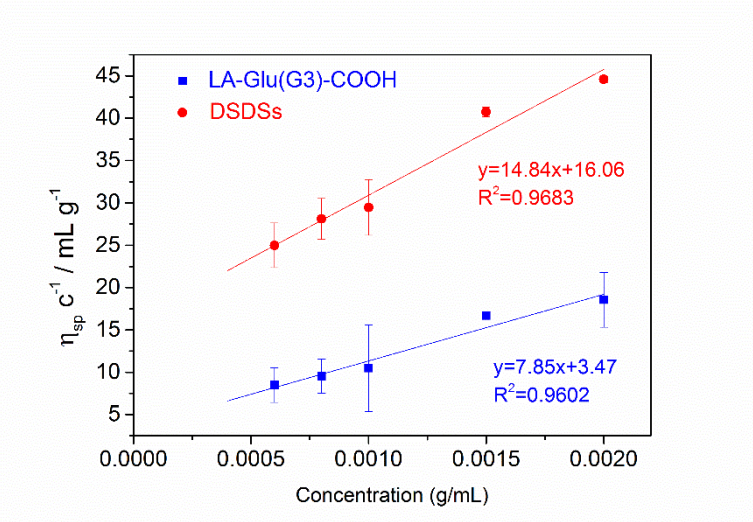


Figure S16. Viscosity measurement of LA-Glu(G3)-COOH (blue) and DSDSs (red) with different concentrations in DMF at 298K.

Viscosity measurements were carried out in N, N-dimethylformamide (DMF) at 298 K using a Cannon Ubbelohde semi-microdilution viscometer. DSDSs always exhibited higher viscosity value than that of LA-Glu(G3)-COOH with the same concentration. And this result indicated the transition from LA-functionalized dendrimers to disulfide-stabilized supramolecular dendritic systems with increasing molecular weight after polymerization [1,2].

2.7 Morphologies and size distributions

SEM images

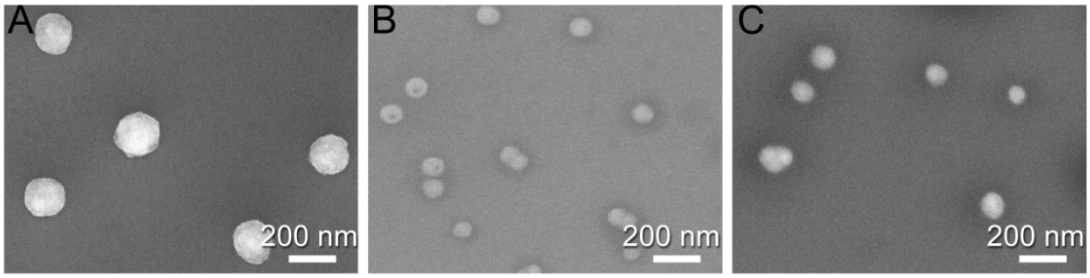


Figure S17. SEM images for (A) SDSs, (B) DSDSs and (C) TSPDSs.

Stabilities of TSPDSs

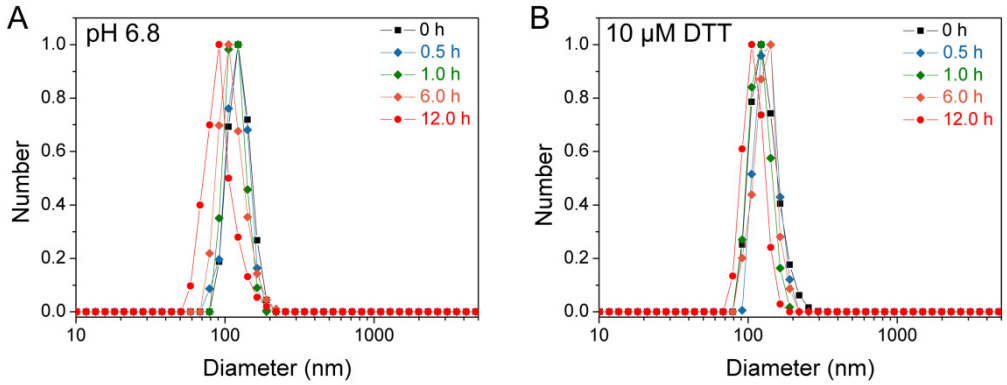


Figure S18. Particle size distributions of TSPDSs at (A) pH 6.8 and (B) 10 μM DTT for various incubation time (0 h, 0.5 h, 1.0 h, 6.0 h and 12.0 h).

2.8 *In vitro* platinum release from TSPDSs

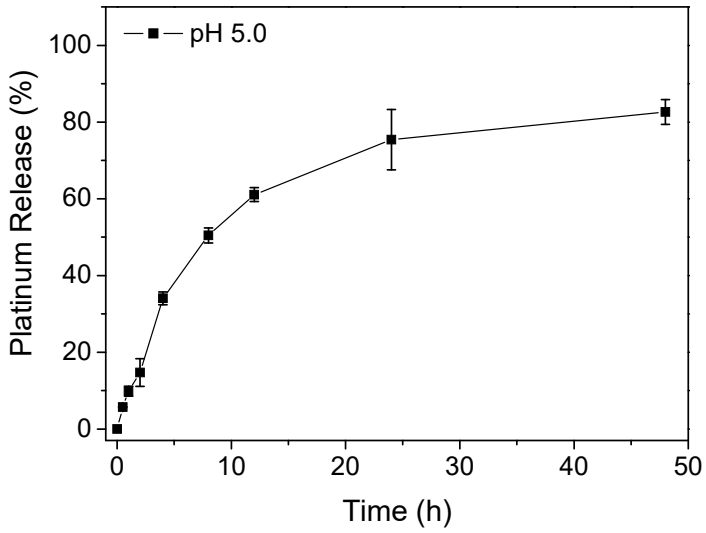


Figure S19. Platinum release profile of TSPDSs in PBS (pH 5.0) for 48 h at 37 °C. The platinum amount was determined by ICP-MS.

2.9 Internalization of TSPDSs

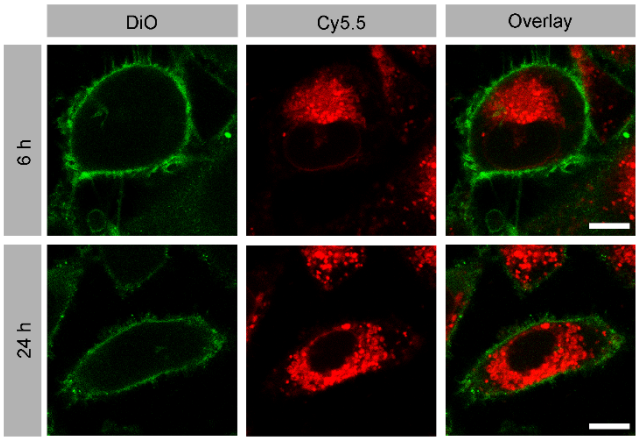


Figure S20. CLSM images for cellular uptake of TSPDSs for 6 h and 24 h with Cy 5.5 channel of TSPDSs (red), DiO channel of cell membrane (green) and overlay. Scale bar corresponds to 10 μm.

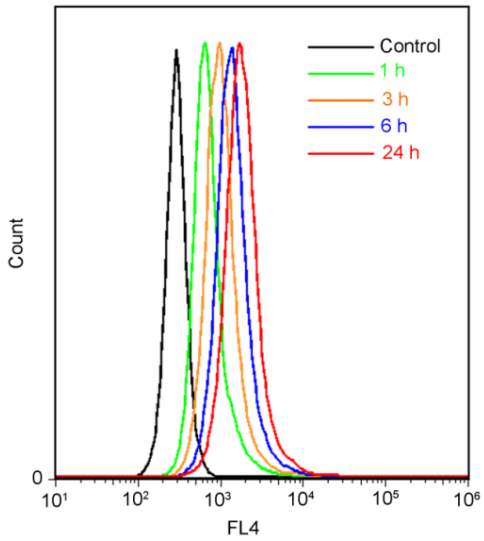


Figure S21. Cellular uptake of TSPDSs by A549 cells determined for different incubation time (1 h, 3 h, 6 h and 24 h) by FACS analysis.

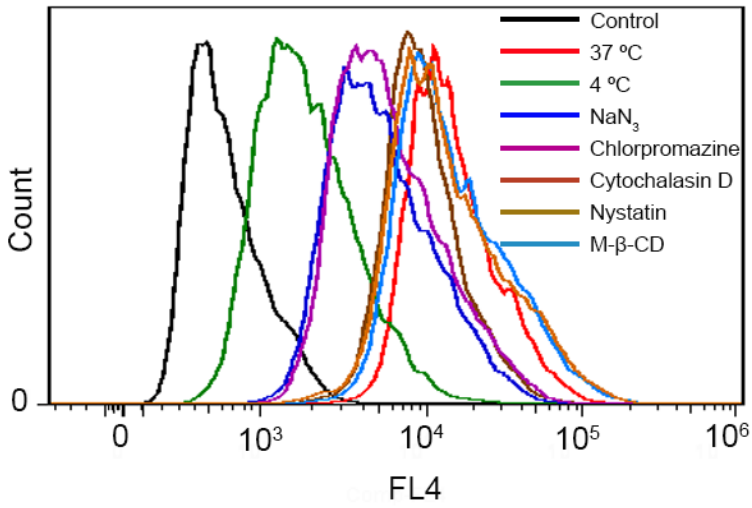


Figure S22. Quantitative analysis of the internalized amount of TSPDSs by A549 cells by flow cytometry. The cells were cooled at 4 °C or pretreated with different inhibitors at 37 °C in serum-free medium for 1 h and further incubated with TSPDSs for 3 h.

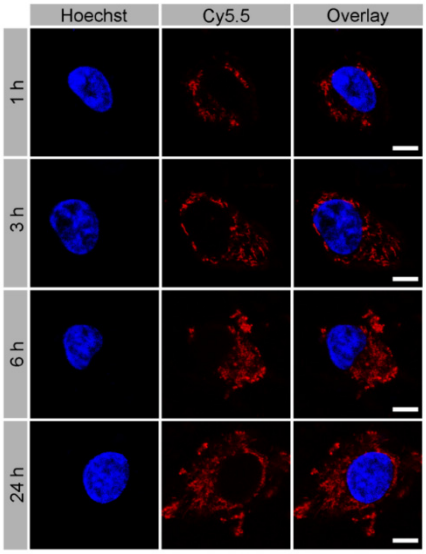


Figure S23. CLSM images of cellular internalization of TSPDSs (red) for 1 h, 3 h, 6 h and 24 h incubation with Hoechst 33342 (blue) for nuclei staining. Scale bar corresponds to 10 μm.

2.10 Histological analysis

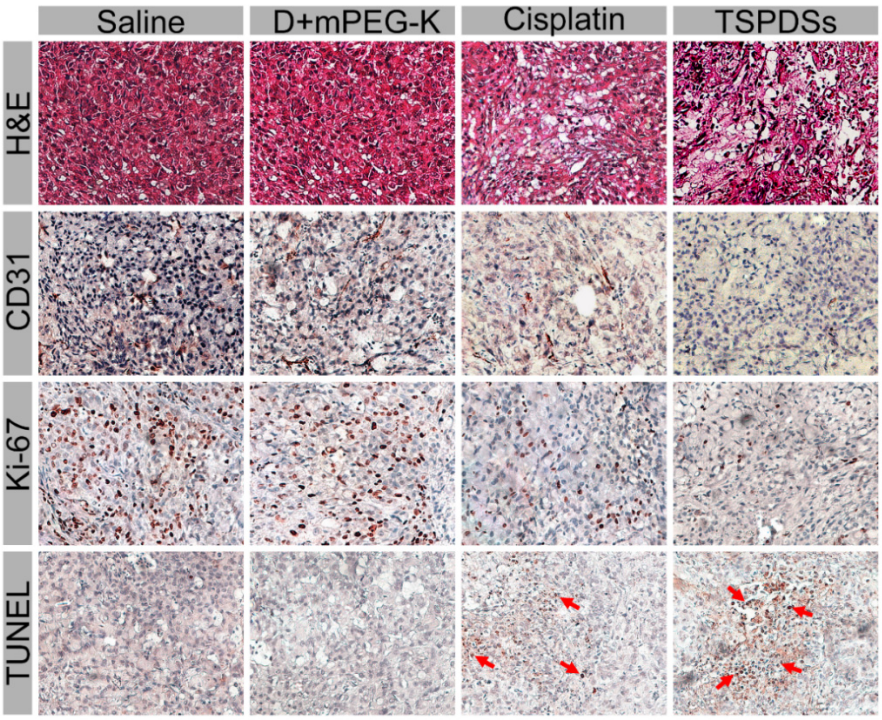


Figure S24. Histological and immunohistochemical analysis of H&E, CD31, Ki-67, and TUNEL assays for A549 tumor xenografts. CD31-positive vessels, Ki-67-positive cells and TUNEL-positive are stained brown. Arrows indicated the examples of TUNEL-positive cells with apoptotic morphology.

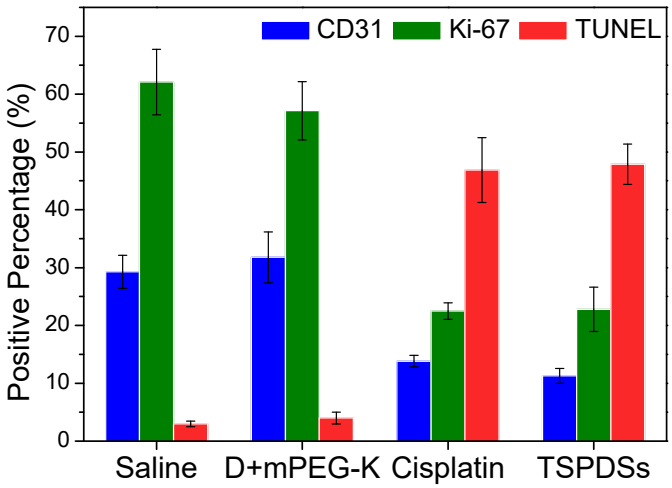


Figure S25. Integrated optical density (IOD) measurement of CD31, Ki-67 and TUNEL images of the tumor section for different treated groups (means ±SD, n=4).

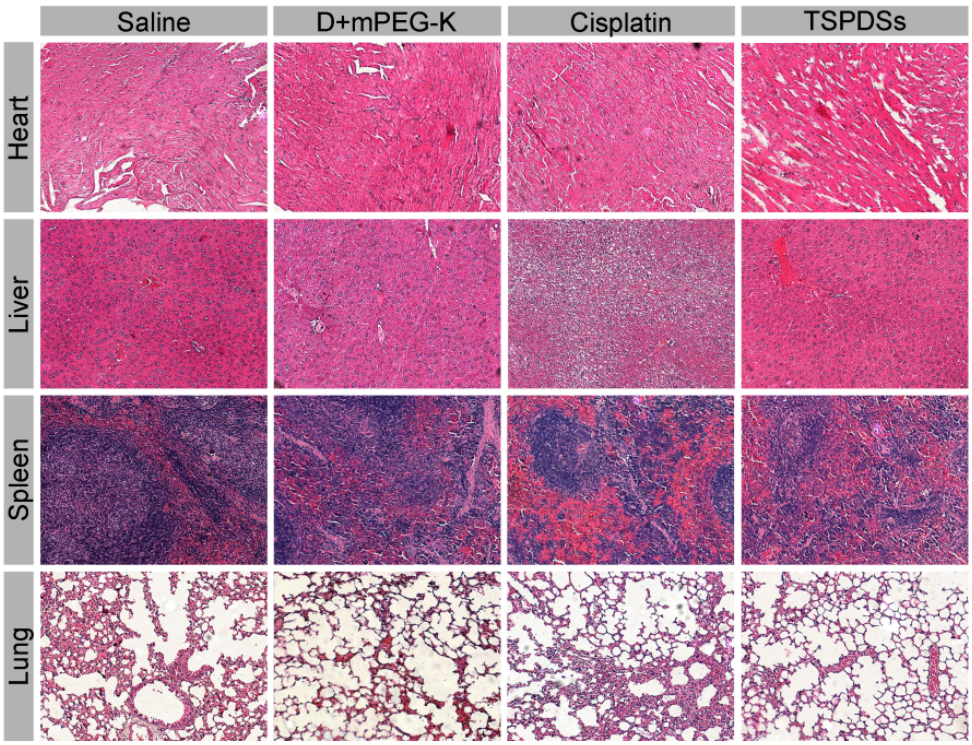


Figure S26. Histological analyses of H&E assay for the major organs including heart, liver, spleen and lung 21 days after different treatments.

2.11 Protein adsorption

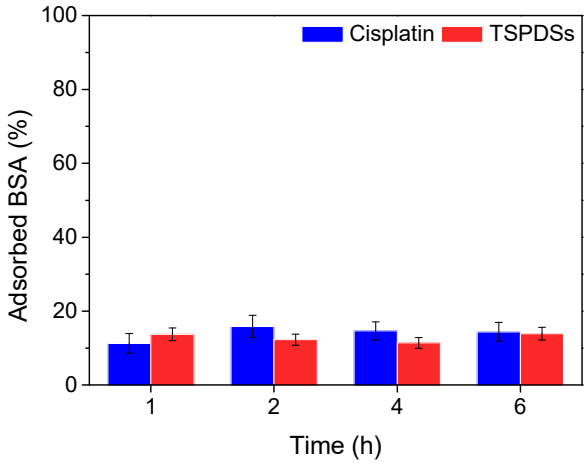


Figure S27. BSA adsorption on cisplatin and TSPDSs after incubation with BSA at 37 °C for different periods of time (means ± SD, n=3).

2.12 DOX fluorescence spectra

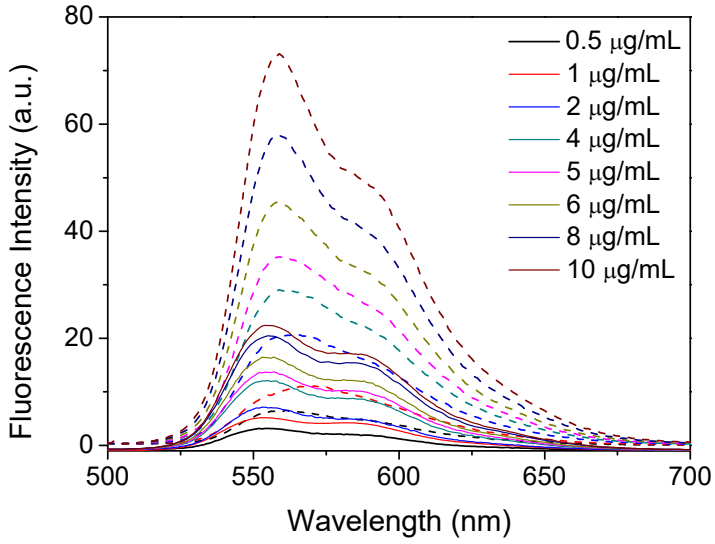


Figure S28. Fluorescence emission spectra of doxorubicin (DOX) in DMSO (dot) and DOX loaded in LA-Glu(G3)-COOH in water (line) with an excitation wavelength of 480 nm at various concentrations from 0.5 to 10 μg mL^{-1}.

Remarkable attenuation in fluorescence intensity was observed for the encapsulated DOX in water compared with the equivalent DOX concentration in DMSO. The fluorescence attenuation, which was attributed to the self-quenching behavior resulting from increased local concentrations of drug molecules, indicated that DOX was loaded inside the hydrophobic core of self-assembled dendrimers. This result demonstrated that amphiphilic dendrimers provided hydrophobic core for other antitumor drug encapsulation [3].

Table S1 Quantitive result of the internalized amount of TSPDSs by A549 cells treated with different endocytotic inhibitors using flow cytometry.

	Control	37 °C	4 °C	NaN_3	Chlorpromazine	Cytochalasin D	Nystatin	M-β-CD
Relative cellular uptake percentage (%)	0.3	99.9	23.2	79.0	83.5	99.2	98.9	99.6

4 °C , for all active energy-dependent endocytotic process inhibition; NaN_3, for ATP depletion; chlorpromazine, for inhibition of clathrin-mediated endocytosis; cytochalasin D, for micropinocytosis inhibition; nystatin and M-β-CD, for caveolae-mediated endocytosis inhibition.

Table S2 Pharmacokinetic parameters of platinum in plasma of mice after a single intravenous injection of cisplatin and TSPDSs at a platinum dose of 3.25 mg kg^{-1} per mouse (means ± SD, n=3).

Parameters	Unit	Cisplatin	TSPDSs
C_{max}	mg/L	12.12 ± 1.43	102.04 ± 4.78
AUC	mg/L·h	12.10 ± 3.67	262.26 ± 23.15
$t_{1/2}$	h	1.00 ± 0.32	3.17 ± 0.20
CL	L/h	0.80 ± 0.03	0.04 ±0.004

C_{max}, maximal platinum concentration in blood; AUC, area under the curve; $t_{1/2}$, half life time; CL, clearance.

3 References

1. Yan X, Xu D, Chi X, Chen J, Dong S, Ding X, Yu Y, Huang F. A multiresponsive, shape-persistent, and elastic supramolecular polymer network gel constructed by orthogonal self-assembly. Adv Mater. 2012, 24: 362-9.

2. Ding Y, Wang P, Tian YK, Tian YJ, Wang F. Formation of stimuli-responsive supramolecular polymeric assemblies via orthogonal metal–ligand and host–guest interactions. Chem Commun. 2013, 49: 5951-3.

3. Gillies E R, Fréchet J M J. pH-responsive copolymer assemblies for controlled release of doxorubicin. Bioconjugate Chem. 2005, 16: 361-8.