Supporting Information

Molecular Precise and High Molecular Weight Peptide Dendrimers for Drug-Specific Delivery

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Table S1. The polydispersity of interior-functionalized PLL dendrimers

POSS-PLL	G1	G2	G3	G4
M_{theor}	3524	7193	14531	29206
$M_{n,GPC}$	9653	13440	17960	29260
PDI	1.03	1.04	1.07	1.05
$M_{n,Maldi\text{-}TOF}$	1907	3980	8054	16256

POSS-(FK)	G0.5	G1	G1.5	G2	G2.5	G3
M_{theor}	2867	4702	7056	10725	15435	22773
$M_{n,GPC}$	6555	10450	11810	17170	19830	24240
PDI	1.03	1.04	1.05	1.06	1.08	1.07
$M_{n,Maldi\text{-}TOF}$	2059	3106	5435	7485	12190	16302

POSS-(HK)	G0.5	G1	G1.5	G2	G2.5	G3
M_{theor}	5702	8514	14585	20208	32350	43595
$M_{n,GPC}$	5778	8793	9034	14960	20150	25190
PDI	1.05	1.06	1.09	1.10	1.09	1.06
$M_{n,Maldi\text{-}TOF}$	5700	8714	14586	20185	32192	43470

POSS-(YK)	G0.5	G1	G1.5	G2	G2.5	G3
M_{theor}	4414	7217	10726	16332	23349	34562
$M_{n,GPC}$	8463	11930	15520	20680	23580	29600
PDI	1.05	1.06	1.08	1.09	1.16	1.17
$M_{n,Maldi\text{-}TOF}$	4432	7161	10745	16337	23377	34518

Table S2. Number of the encapsulated SN38 molecules of several (POSS)-cored polylysine (PLL) dendrimers

Peptide dendrimer	Number of peripheral groups	Molecular weight	Average number of encapsulated SN38	mass%
POSS-PLL-G3-EG	64	10813	0.5 ± 0.1	1.8%
POSS-PLL-G4-EG	128	21771	1.2 ± 0.2	2.2%
POSS-(FK)G3-EG	64	26613	1.6 ± 0.3	2.3%
POSS-(FK)G4-EG	128	45135	3.9 ± 1.1	3.4%
POSS-(HK)G3-EG	64	39623	2.2 ± 0.6	2.2%
POSS-(HK)G4-EG	128	58142	5.0 ± 0.8	3.4%
POSS-(YK)G3-EG	64	30653	2.1 ± 0.6	2.7%
POSS-(YK)G4-EG	128	49172	3.8 ± 1.1	3.0%

Table S3. Size distributions and zeta potential of the interior-functionalized PLL dendrimers in water.

Sample	Zeta potential (mV)	Radius (nm) in water
POSS-PLL-G3-EG	-1.4 ± 0.9	5.8 ± 0.7
POSS-PLL-G4-EG	-2.5 ± 1.2	6.1 ± 1.2
POSS-(FK)G3-EG	-3.6 ± 0.7	6.2 ± 0.7
POSS-(FK)G4-EG	-2.8 ± 1.8	7.8 ± 1.1
POSS-(HK)G3-EG	1.6 ± 1.5	6.4 ± 1.3
POSS-(HK)G4-EG	4.3 ± 2.6	8.1 ± 1.2
POSS-(YK)G3-EG	2.6 ± 1.3	7.1 ± 0.8
POSS-(YK)G4-EG	3.4 ± 0.7	8.6 ± 1.9

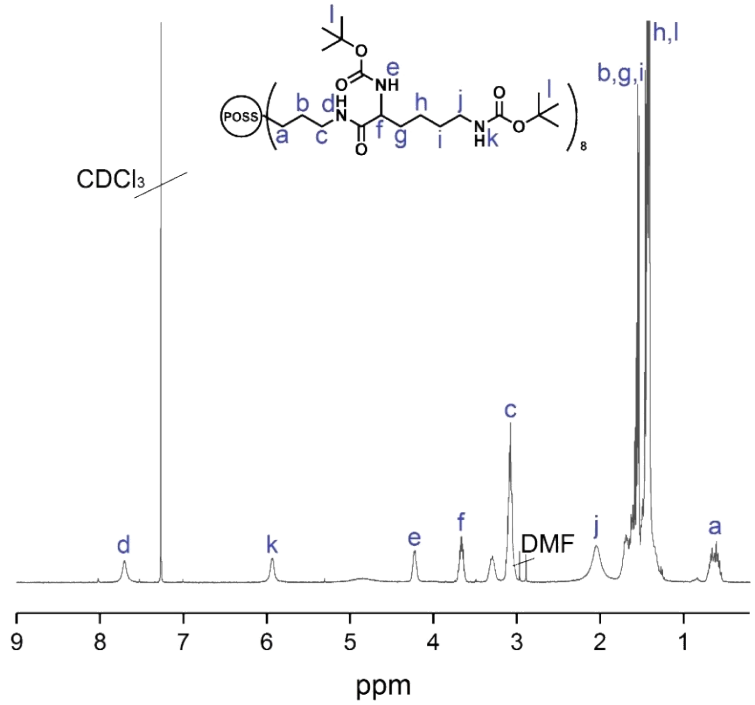

Figure S1. The ^{1}H-NMR spectra of POSS-PLL-G1-Boc.

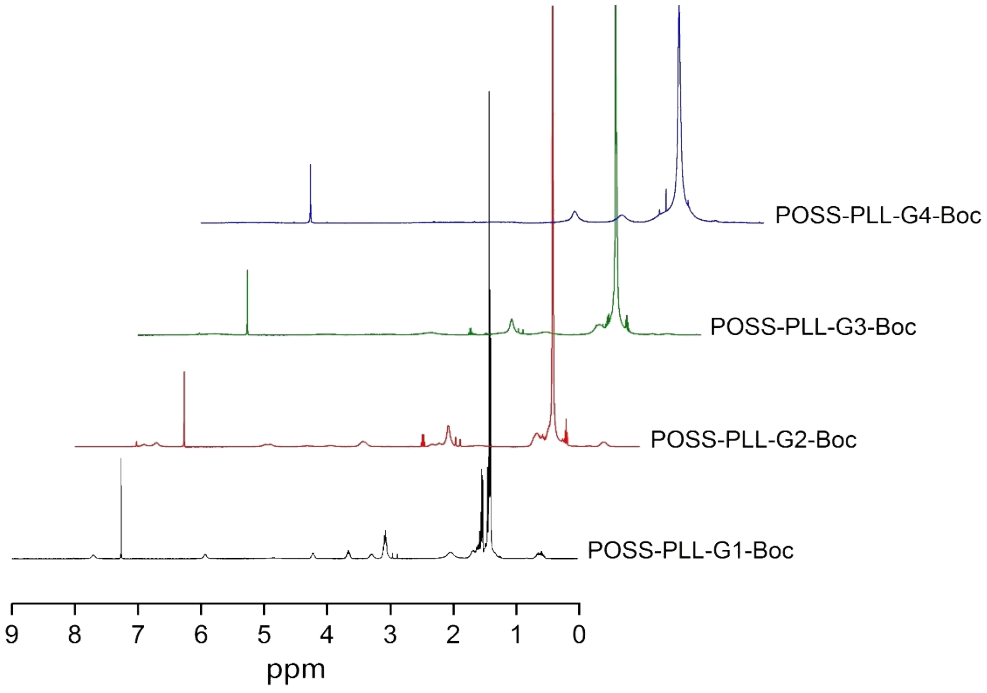

Figure S2. The ^{1}H-NMR spectra of POSS-PLL-G1-Boc, POSS-PLL-G2-Boc, POSS-PLL-G3-Boc, and POSS-PLL-G4-Boc in $CDCl_3$.

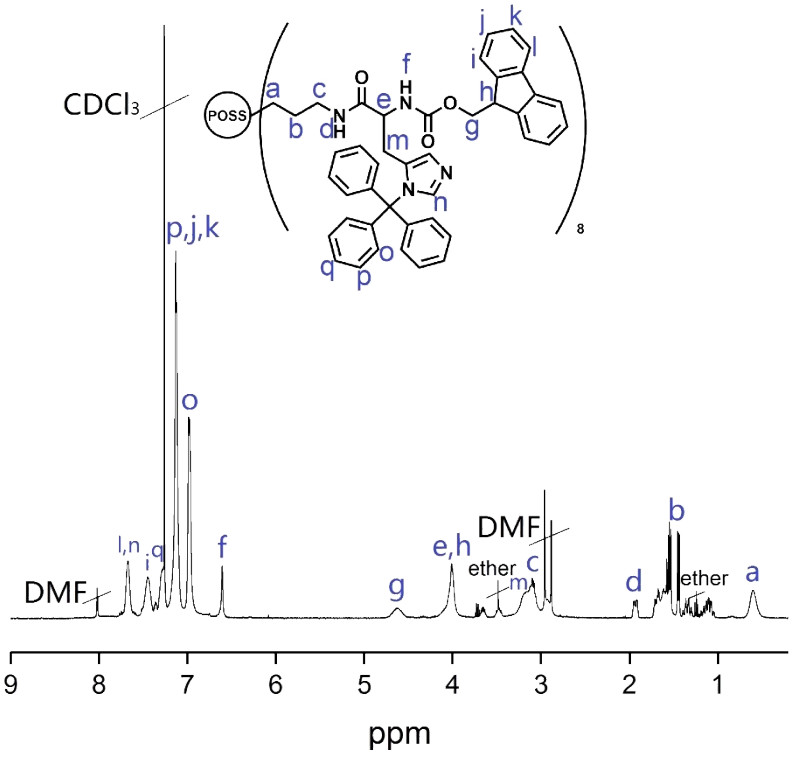

Figure S3. The ^{1}H-NMR spectra of POSS-(HK)-G0.5-Fmoc.

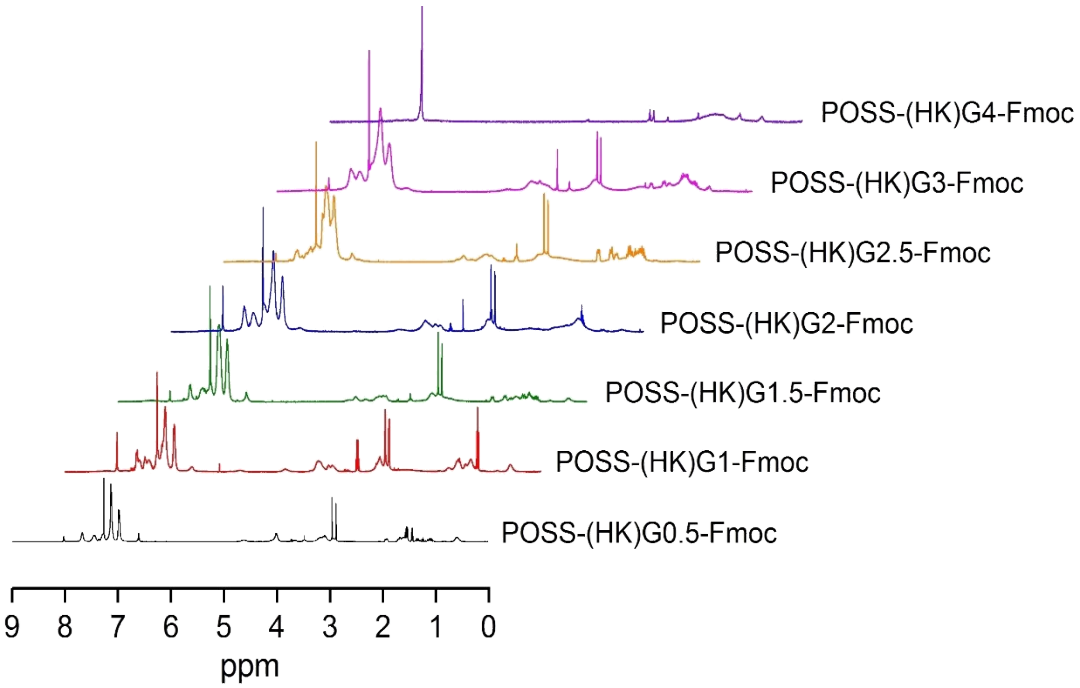

Figure S4. The ^{1}H-NMR spectra of POSS-(HK)-Gn-Fmoc (n = 0.5-4) in $CDCl_3$.

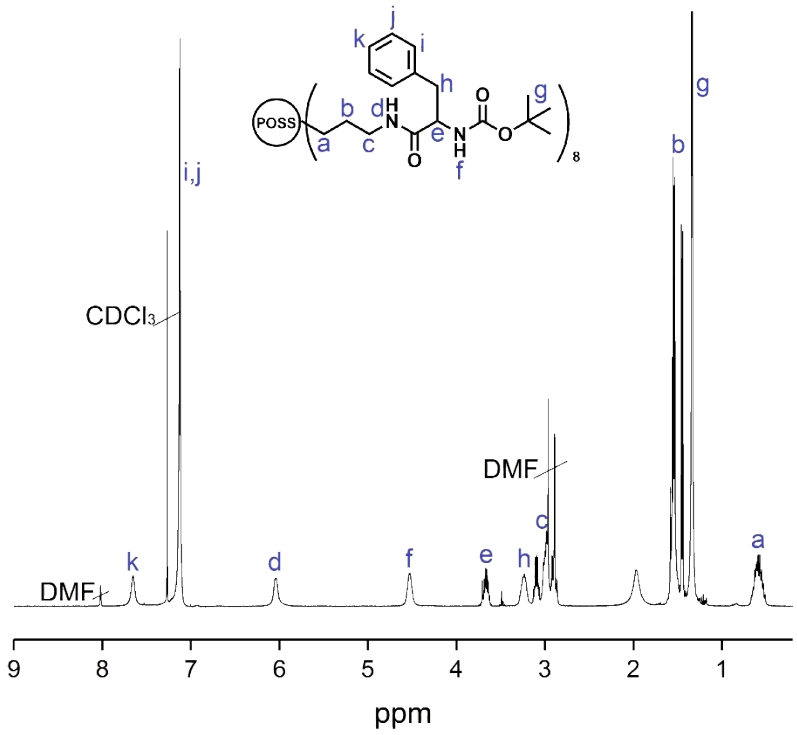

Figure S5. The 1H-NMR spectra of POSS-(FK)-G0.5-Boc in CDCl3.

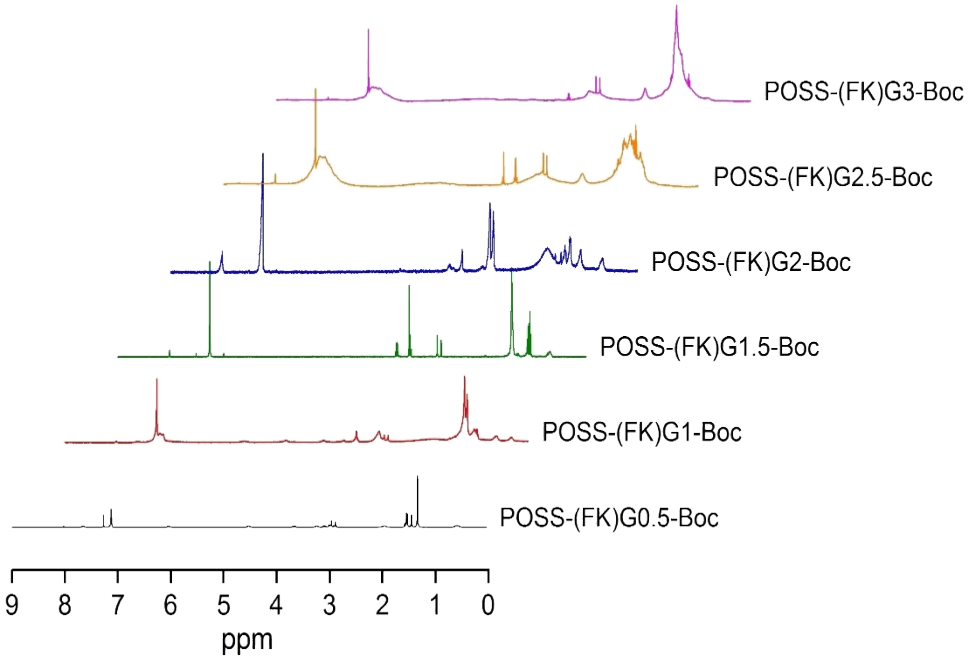

Figure S6. The ^{1}H-NMR spectra of POSS-(FK)-Gn-Fmoc (n = 0.5-4) in $CDCl_3$.

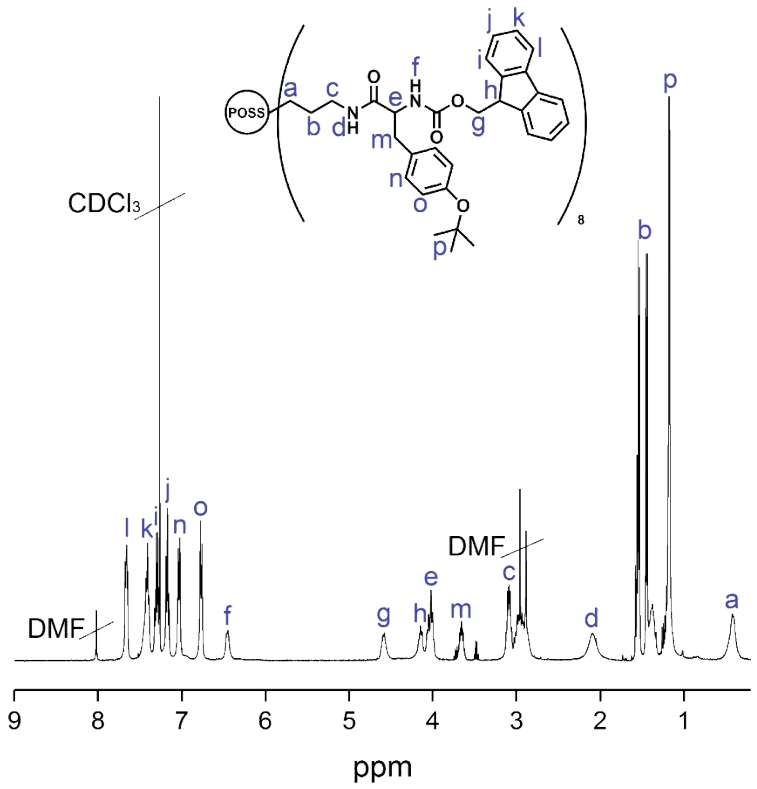

Figure S7. The ^{1}H-NMR spectra of POSS-(YK)-G0.5-Fmoc in $CDCl_3$.

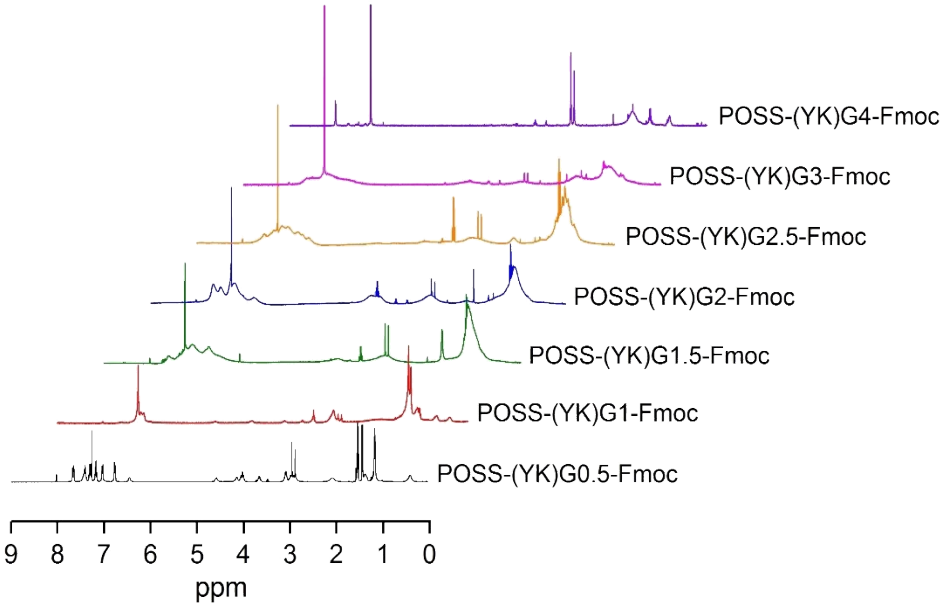

Figure S8. The ^{1}H-NMR spectra of POSS-(YK)- Gn-Fmoc (n = 0.5-4) in $CDCl_3$.

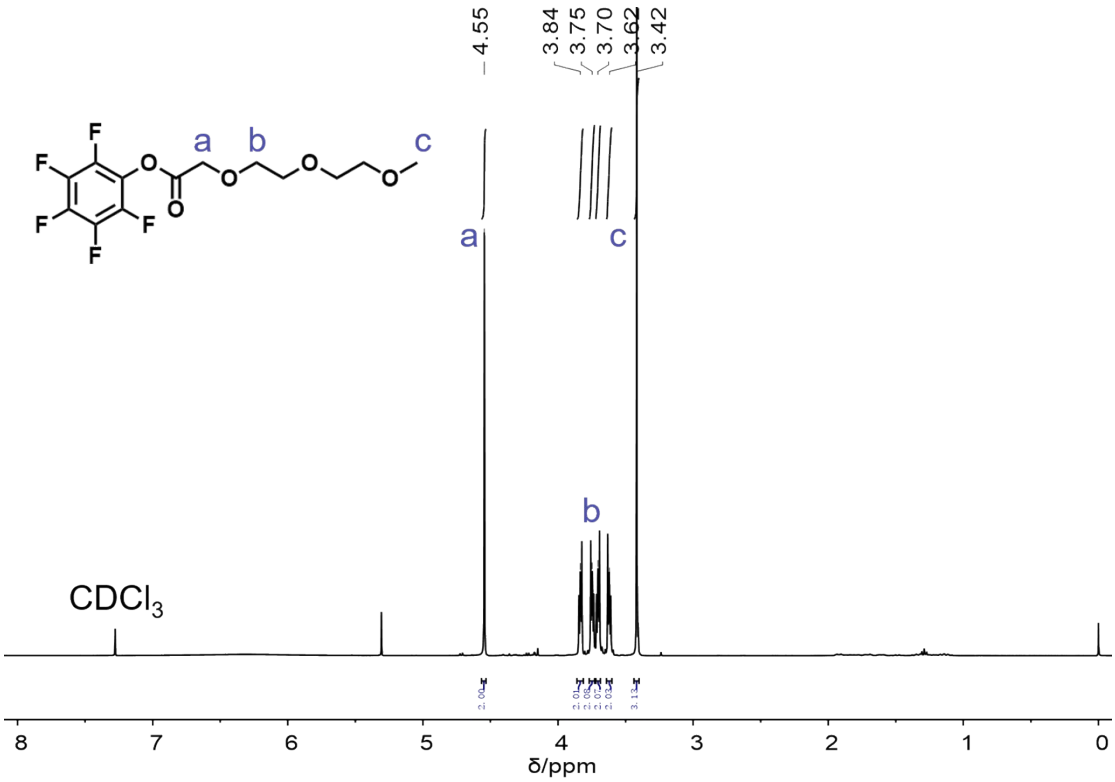

Figure S9. The ^{1}H-NMR spectra of $(EG)_3$-OPfp in $CDCl_3$.

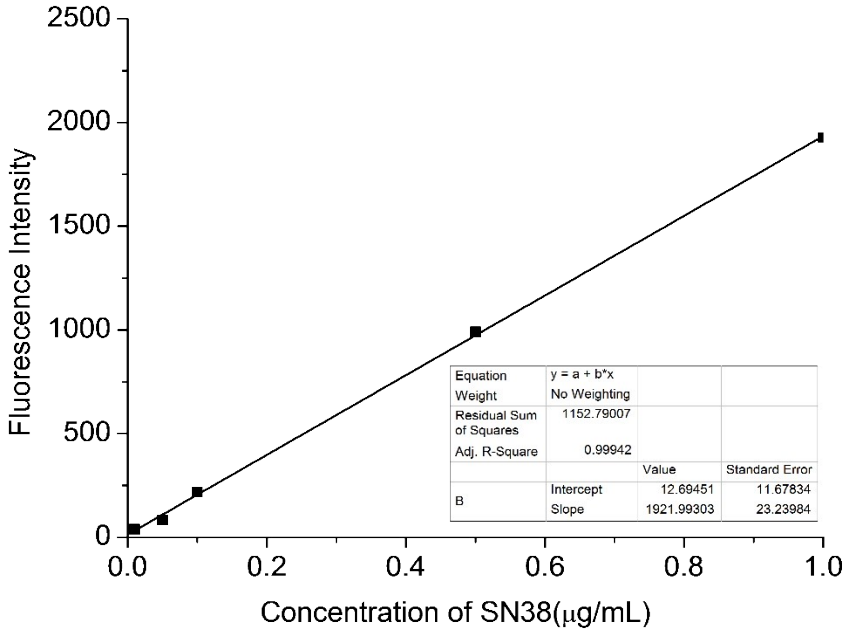

Figure S10. The calibration curve of free SN38.

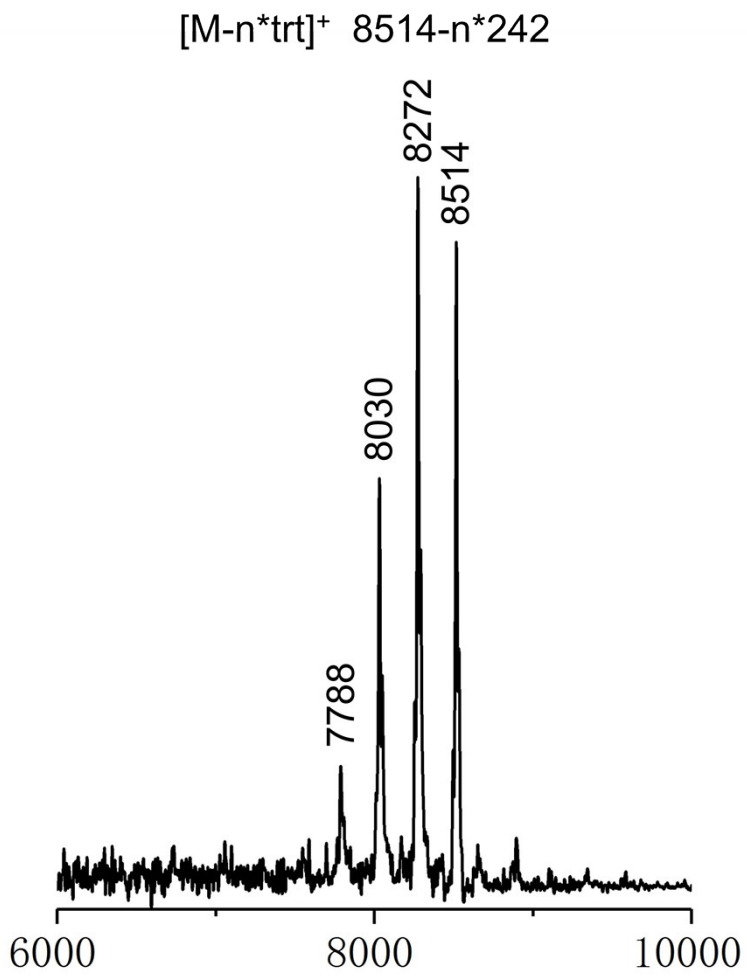

Figure S11. The MALDI-TOF mass spectra of POSS-(HK) G1 with repeat peak spacing of 242 Da.

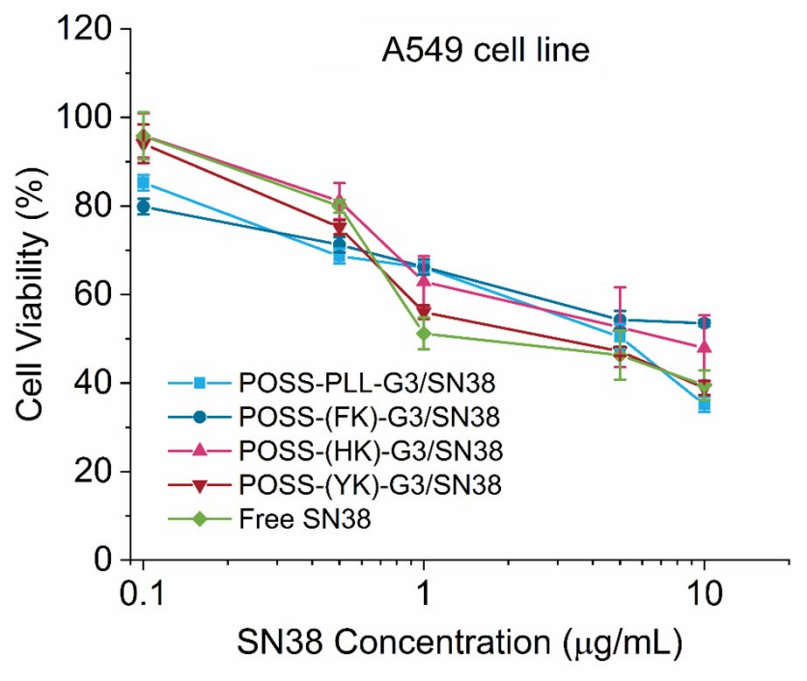

Figure S12. The MTT assay of the G3 dendrimers and their complexes with SN38 against A549.

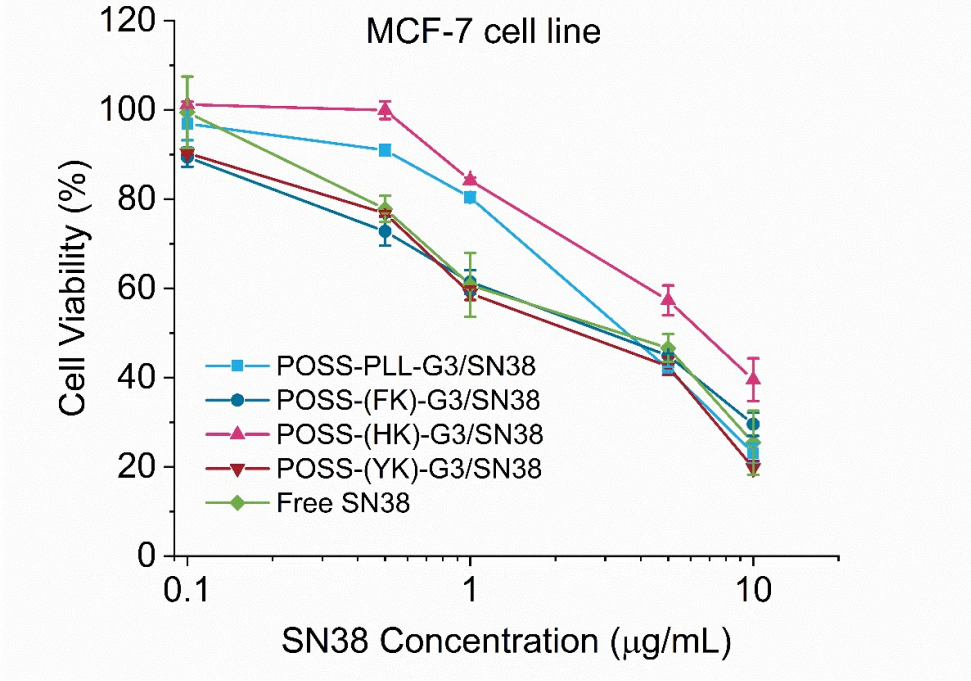

Figure S13. The MTT assay of the G3 dendrimers and their complexes with SN38 against MCF-7.

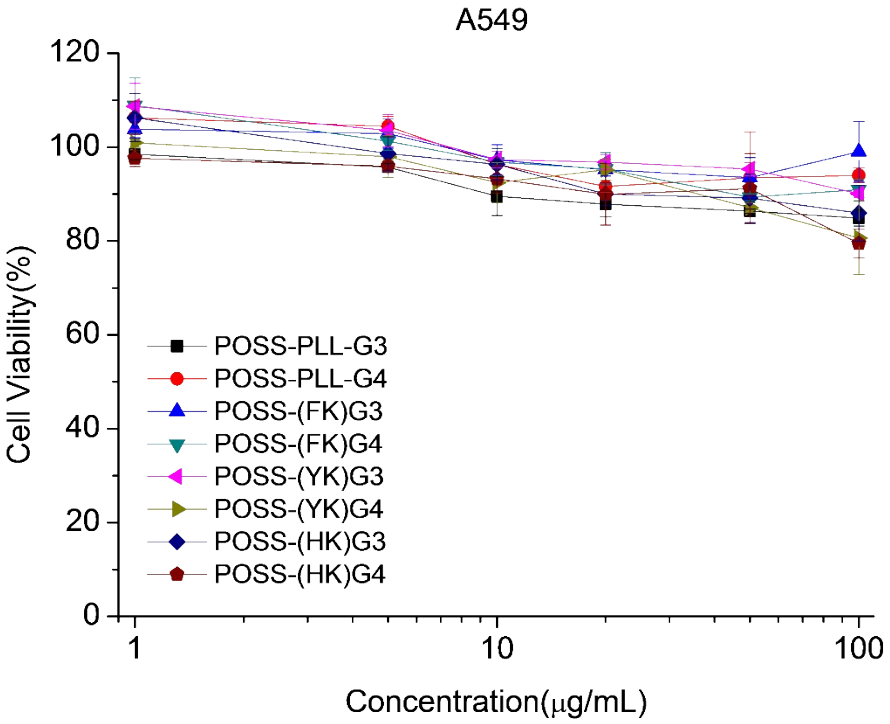

Figure S14. The MTT assay of the G3 dendrimers against A549.

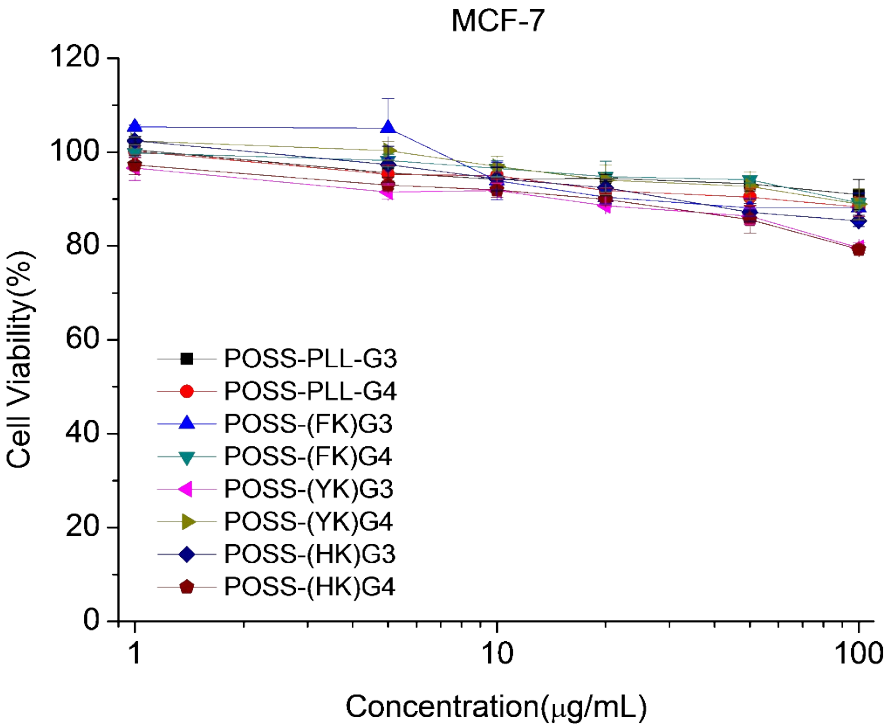

Figure S15. The MTT assay of the G3 dendrimers against MCF-7.

2. Experimental Section

Materials

Polyhedral oligomeric silsesquioxane (POSS) was purchased from Hybrid Plastics, Inc. N-(tert-Butoxycarbonyl)-L-phenylalanine and (2S)-2,6-bis(9H-fluoren-9-ylmethoxycarbonylamino) hexanoic acid respectively were purchased from GL Biochem Ltd. (Shanghai, China). Trifluoroacetic acid (TFA), pentafluorophenol (PFP), N, N-Diisopropylethylamine (DIPEA) and dicyclohexylcarbodiimide (DCC) were purchased from Energy Chemical Reagent Co., Ltd. (Shanghai, China). N, N-Dimethyl formamide (DMF), dichloromethane (DCM), piperidine and ethyl ether were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Pentafluorophenyl *N*-α-*N*-ε-di-Boc-L-lysinate (Boc-Lys(Boc)-OPFP) was synthesized according to the literature.

Instruments

Nuclear magnetic resonance (^{1}H NMR) spectra and two-dimensional nuclear Overhauser enhancement spectroscopy (2D-NOESY) were recorded using a Bruker Avance DRX-400 spectrometer (Bruker BioSpin Corporation, Billerica, USA) with deuterated chloroform ($CDCl^{3}$) or dimethyl sulfoxide ($DMSO\text{-}d^{6}$) as a solvent and tetramethylsilane as the internal standard. Gel permeation chromatography (GPC) was performed on a Wyatt GPC/SEC-MALS (Wyatt Technology Corp., Santa Barbara, USA) system equipped with a DAWN® HELEOS® II 18-angle static light scattering detector and an Optilab® T-rEX™ refractive index detector. Data were recorded and processed using ASTRA v6.0 (Wyatt Technology Corp.) software. DLS measurements of the dendrimer solution were performed using photon correlation spectroscopy (Malvern Zen3600). Matrix-assisted laser-desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) was performed on a Bruker ultrafleXtreme™ MALDI-TOF MS (Bruker Daltonics GmbH, Bremen, Germany) using 2,5-dihydroxybenzoic acid (DHB) or α-cyano-4-hydroxycinnamic acid diethylammonium (CCA) as the matrix. The laser intensity was set to the lowest value possible to acquire high-resolution spectra. The spectra were analyzed using FlexAnalysis v3.4 (Bruker Daltonics GmbH, Bremen, Germany).

Synthesis of (POSS)-cored polylysine (PLL) dendrimers

587 mg POSS•HCl (MW=1173.18, 0.5 mmol) was dissolved in 5 mL DMF, followed by the addition of 1.2 mL DIPEA and 4.10 g Boc-Lys(Boc)-Opfp (MW=512.47, 8.0 mmol). The mixture was stirred at room temperature for 12 h. The solution was turned to clear and the solvent was concentrated to 10 mL by vacuum evaporation. The solution was poured into cold ethyl ether (100 mL) and the precipitation was collected by filtration. POSS-PLL-G1-Boc was obtained after filtration and drying with a yield of 97%. POSS-PLL-G1-Boc was dissolved in DCM/TFA (1:1, 20 mL) with stirring for 2 h at RT. The solution was poured into cold ethyl ether and the precipitation was collected by filtration. POSS-PLL-G1·TFA was obtained after drying in vacuum. POSS-PLL-G2, POSS-PLL-G3 and POSS-PLL-G4 were synthesized by repeating the reaction with Boc-Lys(Boc)-Opfp and deprotection in TFA. All the CPT-dendrimers were obtained in the form of TFA salt and with a yield over 90%. ^{1}H NMR (400 MHz, $CDCl_3$, δ) of POSS-PLL-Boc G1: 7.71 (s, 8H, -NH-), 5,94 (s, 8H, -NH-), 3.67 (m, 16H, -CH-), 3.08 (m, 16H, -CH_2-), 2.06 (s, 16H, -CH_2-), 1.77-1.45 (m, 64H, -CH_2-), 1.43 (s, 144H, -CH_3). The 1H NMR spectra of POSS-PLL-Boc G2, G3 and G4 are similar to that of POSS-PLL-Boc G1 except that the intensity of lysine and t-Butyloxy carbonyl group increased with the generation (Figure S2).

Synthesis of interior-functionalized PLL dendrimers scaffolds with histidine, phenylalanine and tyrosine

469 mg POSS•HCl (MW=1173.18, 0.44 mmol) was dissolved in 5 mL DMF, followed by the addition of 1.0 mL DIPEA and 5.03 g Fmoc-His(Trt)-OPfp (MW=785.76, 8.0 mmol). The mixture was stirred at room temperature for 12 h. The solution was poured into cold ethyl ether (200 mL) and the precipitation was collected by filtration. ^{1}H NMR (400 MHz, $CDCl_3$, δ) of POSS-H-Fmoc G0.5: 7.68 (s, 32H, ArH), 7.45 (s, 16H, ArH), 7.28 (m, 24H, ArH), 7.13 (m, 80H, ArH), 6.89 (d, 48H, ArH), 6.61 (s, 8H, -NH-), 4.63 (s, 16H, -CH_2-), 4.01 (s, 16H, -CH_2-), 3.30-3.01 (m, 32H, -CH_2-), 1.55 (m, 16H, -CH_2-), 0.59 (m, 16H, -CH_2-). The obtained POSS-(HK)-Fmoc G0.5 was then dissolved in the DMF/ Piperidine (1:1, 10 mL) and stirred 5 h at RT for the Fmoc deprotections. The solution was concentrated by evaporation and the solution was poured into cold ethyl ether (200 mL) and drying in vacuum with a yield of 86%. 900 mg POSS-(HK)-Fmoc G0.5 (MW=3916.78, 0.23 mmol) was dissolved in 20 mL DMF, followed by the addition of 2.77 g Fmoc-Lys(Fmoc)-

Opfp (MW=751.71, 3.68 mmol). DIPEA (0.30 mL, 1.73 mmol) was then added to the solution and the mixture was stirred at RT for 12 h. The solution was poured into cold ethyl ether (200 mL) to obtain large quantities of white precipitation. POSS-(HK)-Fmoc G1.0 was isolated and obtained after deprotection in piperidine and drying in vacuum with a yield of 83%. ^{1}H NMR (400 MHz, $CDCl_3$, δ) of POSS-(HK)-Fmoc G1: 7.67-6.85 (m, 264H, ArH), 4.25 (s, 16H, ArH), 3.95 (m, 48H, -CH_2-), 3.06 (m, 32H, -CH_2-), 1.75 (m, 16H, -CH_2-), 1.58 (m, 16H, -CH_2-), 1.35 (m, 16H, -CH_2-), 0.58 (s, 16H, -CH_2-) POSS-(HK)-Fmoc G1.5, G2.0, G2.5, G3.0, G3.5 and G4.0 were synthesized by repeating the reaction with Fmoc-His(Trt)-OPfp and the reaction with Fmoc-Lys(Fmoc)-Opfp. (Figure S4). POSS-(YK)-Fmoc G4 were synthesized similarly, except that in preparation of POSS-(YK)-Fmoc G0.5, G1.5, G2.5 and G3.5 the reaction with Fmoc-His(Trt)-OPfp was replaced by the reaction with Fmoc-Tyr(tBu)-Opfp. (Figure S8).

587 mg POSS•HCl (MW=1173.18, 0.5mmol) was dissolved in 20 mL DMF, followed by the addition of 1.2 mL DIPEA and 3.45 g Boc-Phe-OPfp (MW=431.12, 8.0 mmol). The mixture was stirred at room temperature for 12 h. The solution was poured into cold ethyl ether (200 mL) and the precipitation was collected by filtration. with a yield of 82%. ^{1}H NMR (400 MHz, $CDCl_3$, δ) of POSS-F-Boc G0.5: 7.65 (s, 8H, -NH-), 7.12 (s, 32H, ArH), 6.05 (s, 8H, -NH-), 4.52 (s, 8H, -NH-), 3.67 (m, 8H, -CH-), 3.23 (s, 16H, -CH_2-), 3.09 (m, 16H, -CH_2-), 1.34 (s, 72H, -CH_3), 0.57 (m, 16H, -CH_2-) POSS-(FK)-Boc G1, G1.5, G2.0, G2.5, G3.0, G3.5 and G4.0 were synthesized by repeating the reaction with Boc-Lys(Boc)-OPfp and the reaction with Boc-Phe-OPfp. (Figure S6). POSS-(FK)-Boc G4 and POSS-PLL-Boc G4 were purified by chromatography on Sephadex LH-20 columns because the product become more likely to dissolve in ethyl ether with the increase of the amount of t-butyloxycarboryl groups.

$(EG)_3$-OH (1.782 g, 10 mmol) and pentafluorophenol (PFP) (2.02 g, 11 mmol) were dissolved in DCM (20 mL) at 0 ^{o}C. DCC (11.6 g, 56 mmol) in DCM (10 mL) was slowly added to this solution with stirring. The mixture was kept stirring at 0 ^{o}C for 1 h. The temperature was allowed to rise to RT and kept for 6 h. The solution was filtered and the solvent was removed to obtain a colorless liquid. The crude product was kept at -20 ^{o}C overnight and the precipitate was removed by filtration to obtain pure $(EG)_3$-OPFP with a yield of 74%.^{1}H NMR (400 MHz, $CDCl_3$, δ) $(EG)_3$-OPfp: 4.55 (s, 2H, -CO-CH_2-), 3.84 (d, 2H, -O-CH_2-), 3.75 (d, 2H, O-CH_2-), 3.7 (d, 2H, O-CH_2-), 3.62 (d, 2H, O-CH_2-), 3.42 (s, 3H, O-CH_2-).

POSS-(HK)G3.0·TFA (200 mg, 0.007 mmol) and $(EG)_3$-OPfp (300 mg, 0.87 mmol) was dissolved in DMF (2 mL). DIPEA (70 μL) was added in the solution and the solution was stirred at RT for 12 h. The solution was then poured into cold ethyl ether (40 mL). POSS-(FK)G3-EG was isolated and obtained after drying in vacuum with a yield of 88%. POSS-(FK)G4-EG, POSS-(FK)G3-EG, POSS-(YK)G4-EG, POSS-(YK)G3-EG and POSS-(HK)G3-EG were synthesized similarly by repeating the reaction.

NMR studies

^{1}H NMR experiments and the two-dimensional nuclear Overhauser enhancement spectroscopy (2D-NOESY) experiments were conducted on a Bruker Advance 400 MHz NMR spectrometer at 298.2 K, were used to characterize the synthesized dendrimers and the dendrimer/CPT-11 complexes. The temperature was kept constant within ± 0.2 K by the use of a Bruker temperature control unit. All the data were processed with NMR software Topspin in windows workstation.

Dynamic Laser Light Scattering

DLS measurements of the interior-functionalized PLL dendrimer solution were performed using photon correlation spectroscopy after ultrasonic treatment. The measurements were performed using a He−Ne laser of 633 nm. The measurement time was about 2 min, and each run underwent ∼12 subruns. Each value reported is the average of three measurements.

UV-absorption calibration curves

SN38(5.0 mg) was dissolved in DMSO(5 mL) and configured as 1.0 mg/mL SN38 solution, then it was diluted to 1.0 μg/mL, 0.5 μg/mL, 0.1 μg/mL, 0.05 μg/mL, 0.01 μg/mL with PBS. The fluorescence intensity (λ_{Ex} = 370 nm, λ_{Em} = 550 nm) of each sample was measured at 25℃ with Molecular Device SpectraMax®M2/M2e. The calibration curve of SN38 was created by using the fluorescence intensity of the series of samples having known concentrations of SN38. SN38 in methanol solution or distilled water gives prominent absorbance in UV region at its characteristic wavelength (370 nm for SN38). Since the dendrimers in the diluted solutions give no absorbance at 370 nm, the absorbance obtained from dendrimer/SN38 solution would be solely from SN38. This absorbance was correlated with the calibration curve and amount of SN38 was determined.[1]

Molecular dynamics simulations

The initial three-dimensional structures of Lys, Phe, His and SN38 were built using Avogadro[2].

The molecular docking experiments were performed using Autodock tools and Autodock vina [3]

The initial configurations of PLL-G4, PLL-G5, HIS-G4, PHE-G4, TYR-G4 dendrimers as well as SN38 with General Amber Force Field (GAFF)[4] atom types and Restrained Electrostatic Potential (RESP)[5] charges was generated using Dendrimer Builder Toolkit (DBT)[6] and AMBER[7]. Simulated annealing was performed to mimic the strong stirring during the drug loading experiment. The systems were gradually heated from 300 to 500 K in 2 ns, and cooled down from 500 to 300 K in 2 ns. Fully atomistic MD simulations were performed on PLL-G4/SN38, PLL-G5/SN38, HIS-G4/SN38, PHE-G4/SN38 complexes using GROMACS 5.1.4[8] with GAFF force field[4]. The dendrimer-drug complexes were immersed in a cubical water box using TIP3P water model[9] with a solvation shell extended up to 15 Å in all three directions.

Cytotoxicity Evaluations

The cytotoxicity of the dendrimer and SN38-loaded dendrimer were determined by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) MTT assay. Briefly, cells (4000 cells per well) were evenly seeded onto 96-well plates and incubated for 24 h. The cells were incubated with serial dilutions of free SN38, SN38-loaded dendrimers, and blank carriers for 48 h. After treatment, the cells were incubated with fresh medium containing 0.75 mg/mL of MTT for 4 h, which allowed viable cells to reduce the yellow tetrazolium salt (MTT) into dark blue formazan crystals. Finally, DMSO was added to dissolve the formazan crystals. The absorbance at 562 nm was recorded with a reference filter of 620 nm, using a microplate spectrophotometer (SpectraMax M2e, Molecular Devices, Sunnyvale, CA). Cell survival was expressed as the percentage of formazan absorbance. Results were the mean values and standard deviation (SD) from at least three different experiments in triplicate.[10]

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