

Supporting Information

Experimental:

Materials

Palladium (10 wt. % on activated carbon) was obtained from Aldrich and used as received. Dowex®50WX2-100 H⁺ resin was purchased from Lancaster Synthesis Inc. 4-(Dimethylamino) pyridinium p-toluenesulfonate (DPTS) and N-Carboxyanhydride of ω -n-dodecyl-L-glutamate were synthesized according to the literature procedure.^[1-3] The bis-MPA based fourth generation dendron (**2**) was prepared based on the double-stage convergent approach introduced by Ihre et al.^[4] All other chemicals used in this work were purchased from Alfa Aesar and used without further purification.

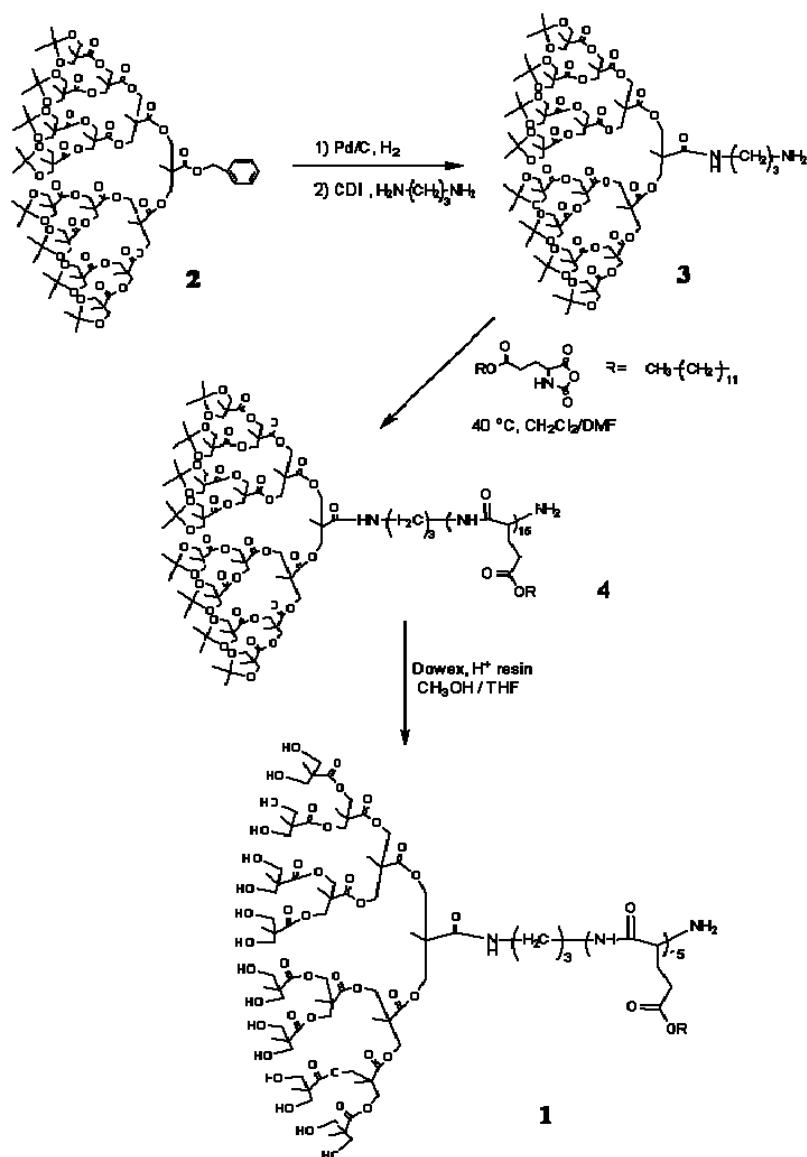
Chemical characterization

¹H NMR spectra were recorded on Varian VXR 300 MHz spectrometers. Samples (~5-10 mg/ml) were dissolved in CDCl₃, with TMS as an internal reference. Fourier transform infrared (FTIR) spectra were recorded on a Thermo Nicolet NEXUS™ 870 Series spectrophotometer by solvent casting samples onto a KBr pellet.

Gel permeation chromatography (GPC) measurements were carried out to monitor trends in the variation of molecular weight and polydispersity as measured against polystyrene standards. These measurements were performed in THF solution using a Water Breeze 1525 HPLC system equipped with four Styragel HT columns operated at 35 °C, Series 2414 Refractive Index detector, Series 1525 Binary HPLC pump and 717Plus Autosampler. Waters Breeze Chromatography Software Version 3.30 was used for data collection as well as data processing. The average molecular weight of the sample was calibrated against narrow molecular weight polystyrene standards (Polysciences, Warrington, PA).

Synthesis

The synthesis of macromolecule **1** consists of the following three steps: 1) preparation of **2**, 2-bis(hydroxymethyl)propionic acid (bis-MPA) based polyester dendron **3** with a primary amino group at the root through an amidation reaction catalyzed by 1, 1'-carbonyldiimidazole (CDI), 2) ring-opening polymerization of N-Carboxyanhydride (NCA) of γ -n-dodecyl-L-glutamate initiated with dendritic molecule **3**, and 3) Deprotection of the acetonide groups from the dendritic block to form hydroxyl end groups. (**Scheme 1**)



Scheme 1. The synthetic route toward the tree-like amphiphilic linear-dendritic copolymer.

The bis-MPA based fourth generation dendron (**2**) was prepared based on the double-stage convergent approach introduced by Ihre et al.^[4], and the structure was confirmed by TLC, IR, and NMR. After deprotection of the benzyl ester group by catalytic hydrogenolysis at the root of dendron **2**, the resulting free carboxylic acid was reacted with an excess amount of 1, 3-diaminopropane in the presence of CDI as the dehydration reagent to provide a primary amino group. The structure of dendron **3** was confirmed with NMR data compared to the dendron **2**, specifically by observing three methylene proton resonance peaks at 3.40, 1.96, and 1.82 ppm from diaminopropane. This amidation reaction also

can be verified by observing the absorptions at 1653 cm^{-1} (C=O) and 1539 cm^{-1} (N-H) at IR spectra.

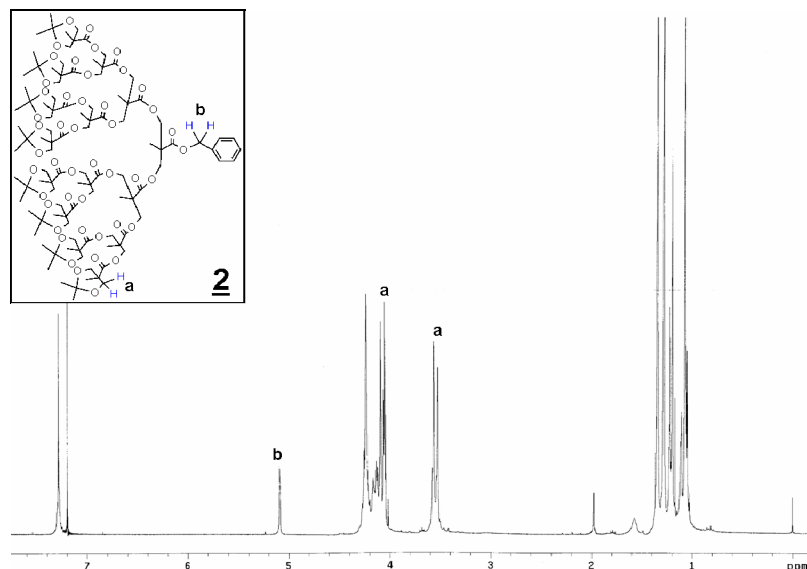


Figure S1. ^1H NMR spectra of Polyester Dendron **2**.

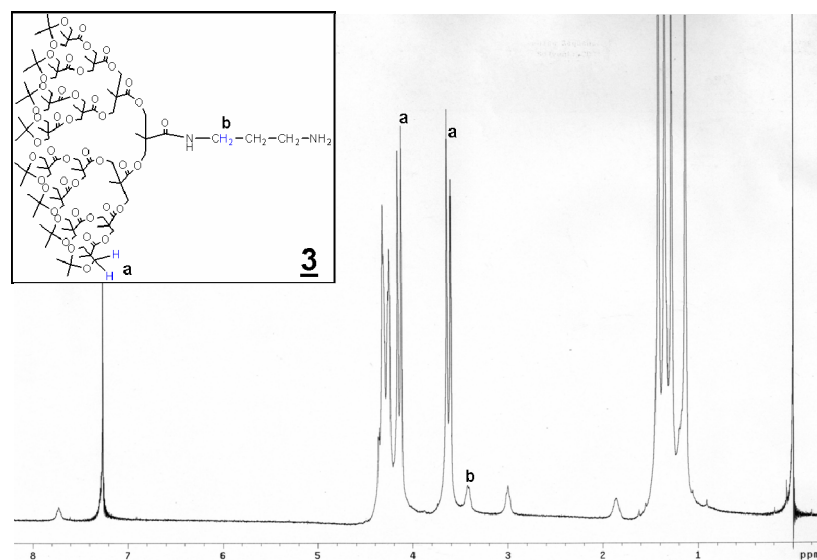


Figure S2. ^1H NMR spectra of Polyester Dendritic Initiator **3**.

By using dendron **3** as a primary aliphatic amine initiator, the NCA of γ -n-dodecyl-L-glutamate was polymerized to make a linear-dendritic copolymer (**4**). Because the primary aliphatic amines are more nucleophilic than the active chain

ends, the initiation is faster than the propagation of monomer; ^[5] all initiating dendron molecules remain attached to the growing polypeptide chain and the process has a living character, which can give nearly monodisperse polypeptides. This synthetic strategy provides a new approach to the construction of linear-dendritic block copolymers with well defined linear polypeptide structures. The polymerization was monitored by ¹H NMR by measuring the integration ratio between a typical proton peak at 3.62 ppm (16H per dendron) from the dendritic initiator and one proton peak at 1.59 ppm (2H per glutamate) from the poly (γ -n-dodecyl-L-glutamate). Consequently, the polymerization degree was calculated as 15.2, which is matched with the theoretical value of monomer feed ratio to initiator (15:1). GPC also gives a reasonable molecular weight of around 6.7 kDa with PDI at 1.08. The polypeptide backbone assumes a rigid α -helical conformation, with the long hydrocarbon side chains propagating from each repeating unit. The α -helical conformation can be identified by the strong C=O amide I absorption at 1656 cm⁻¹ and N-H amide II absorption at 1549 cm⁻¹.

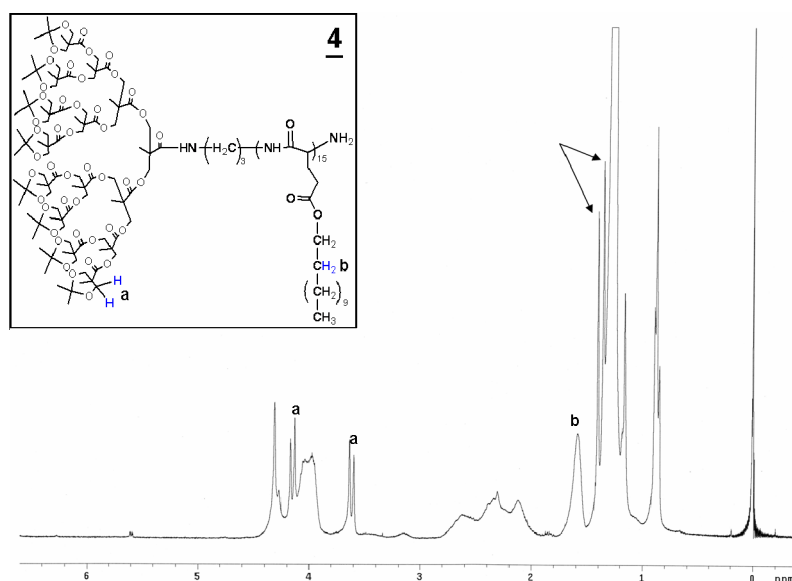


Figure S3. ¹H NMR spectra of Comb-dendritic Copolymer **4**.

The final step of the synthesis of macromolecule **1** is the deprotection of the acetonide groups from the dendritic block through trans-etherification with methanol under acidic conditions. The reaction progress was monitored by NMR for the disappearance of proton signals of acetonide groups around 1.4 ppm (as indicated by black arrows in Figure S2). Upon the reaction completion, clear shifts from the characteristic peak of the starting materials (**4**) to the products (**1**) were noted in the GPC trace with no significant polydispersity changes.

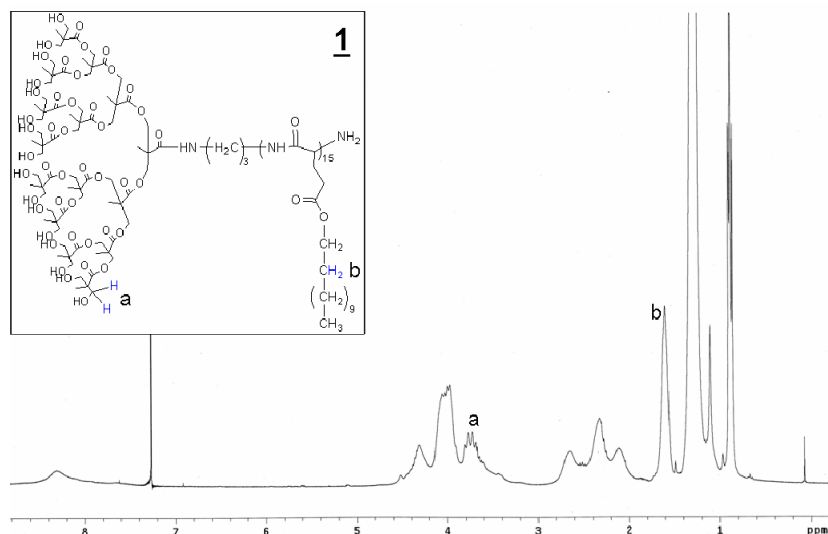


Figure S4. ^1H NMR spectra of Comb-dendritic Copolymer **1**.

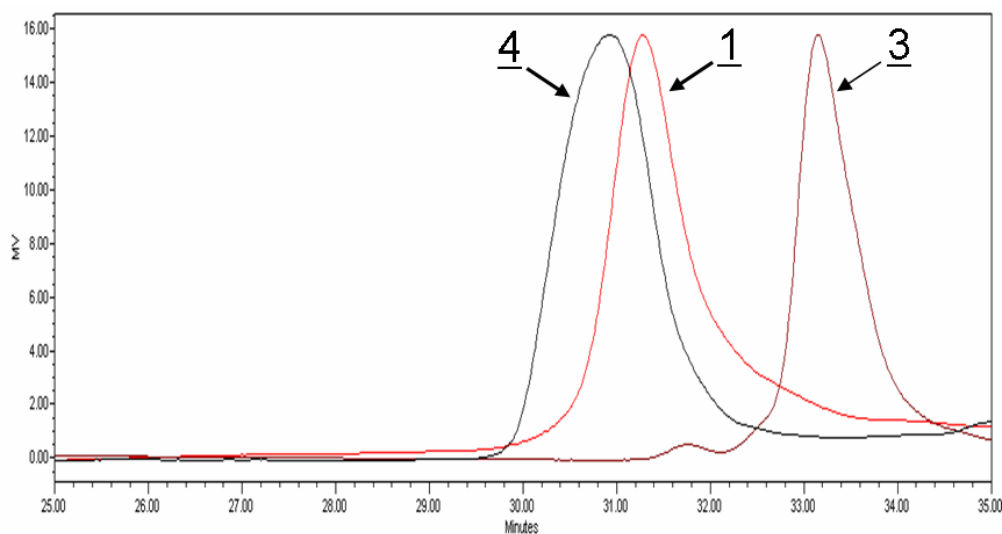


Figure S5. GPC chromatograms illustrating the differences in retention times of macromolecules **1**, **3**, and **4**.

Synthesis of Polyester Dendron (**2**):

The bis-MPA based fourth generation dendron (**2**) was prepared based on the double-stage convergent approach introduced by Ihre et al.^[4] ^1H -NMR (CDCl_3) (δ): 7.28 (m, 5H, ArH), 5.10 (s, 2H, CH_2), 4.25 (s, 16H, CH_2), 4.16 (s, 4H, CH_2), 4.12 (s, 8H, CH_2), 4.08 (d, 16H, CH_2), 3.56 (d, 16H, CH_2), 1.35 (s, 24H, CH_3),

1.29 (s, 24H, CH₃), 1.22 (s, 6H, CH₃), 1.20 (s, 12H, CH₃), 1.12 (s, 3H, CH₃), 1.08 (s, 24H, CH₃).

Synthesis of Polyester Dendritic Initiator (**3**):

The fourth generation polyester dendron (**2**) (1.0 g, 0.48 mmol) and 1, 1'-carbonyldiimidazole (CDI) (0.16 g, 0.97 mmol) were dissolved into anhydrous THF (30 ml). After stirred for 3 h under argon at room temperature, 1, 3-diaminopropane (0.29 g, 3.9 mmol) was added dropwise. The reaction mixture was stirred for one day under argon at room temperature. After the solvent was evaporated at the room temperature, the residue was then dissolved in dichloromethane (150 ml) and washed with sodium bicarbonate saturated aqueous solutions (30 ml) and with 50 ml portions of brine (3×), dried over anhydrous sodium sulfate and evaporated to dryness. The crude product was purified by chromatography using dichloromethane/methanol (9/1, v/v) as eluent. Product **3** was obtained as colorless oil (0.78 g, 73% yield). ¹H-NMR (CDCl₃) (δ): 4.31 (s, 24H, CH₂), 4.24 (s, 4H, CH₂), 4.14 (d, 16H, CH₂), 3.61 (d, 16H, CH₂), 3.40 (m, 2H, CH₂), 2.96 (m, 2H, CH₂), 1.82 (m, 2H, CH₂), 1.41 (s, 24H, CH₃), 1.34 (s, 24H, CH₃), 1.27 (s, 21H, CH₃), 1.17 (s, 24H, CH₃). IR (KBr, cm⁻¹): 3363, 1539 (N-H), 2990, 2877 (C-H), 1738, 1653 (C=O), 1240, 1155, 1123, 1082 (C-O). GPC: Mw = 2.1 kDa, PDI = 1.02.

Synthesis of Comb-dendritic Copolymer (**4**):

The polyester dendritic initiator (**3**) (0.9 g, 0.42 mmol) and N-Carboxyanhydride (NCA) of γ -n-dodecyl-L-glutamate (2.2 g, 6.4 mmol) were dissolved in a mixture of dichloromethane/DMF (5/1, v/v). The ring-opening polymerization proceeded homogeneously at 40 °C for 24 h under argon. The solvents were then removed under vacuum. The crude product was purified by precipitation into methanol (250 ml) from dichloromethane (15 ml). The Product **4** was obtained as white solid (2.3 g, 84% yield). ¹H-NMR (CDCl₃) (δ): 4.31 (s, 24H, CH₂), 4.27 (s, 4H, CH₂), 4.15 (d, 16H, CH₂), 4.03 (m, 15H, CH), 3.97 (t, 30H, CH₂), 3.62 (d, 16H, CH₂), 3.18 (m, 4H, CH₂), 2.62 (m, 30H, CH₂), 2.33 (m, 30H, CH₂), 2.21 (m, 30H, CH₂), 1.85 (m, 2H, CH₂), 1.59 (m, 30H, CH₂), 1.41 (s, 24H, CH₃), 1.36 (s, 24H, CH₃), 1.25 (m, 21H, CH₃; 270H, CH₂), 1.16 (s, 24H, CH₃), 0.87 (t, 45H, CH₃). IR (KBr, cm⁻¹): 3292, 1549 (N-H), 2925, 2855 (C-H), 1737, 1656 (C=O), 1246, 1173, 1123, 1083 (C-O). GPC: Mw = 6.7 kDa, PDI = 1.08

Synthesis of Amphiphilic Comb-dendritic Copolymer (**1**):

The comb-dendritic copolymer (**4**) (2.0 g, 0.30 mmol) was dissolved into 80 ml of methanol/THF mixture (1:3, v/v). Acidic Dowex 50W-X2 resin (2.0 g) was added into the reaction flask. After stirring under argon for 24 h, the reaction mixture was filtered. The filtrate was evaporated to dryness and then diluted by dichloromethane (400 ml) and washed with 50 ml portions of brine (3×), dried

over anhydrous sodium sulfate and evaporated to dryness. The final product was obtained as colorless glassy solid (1.6 g, 84% yield). $^1\text{H-NMR}$ (CDCl_3) (δ): 4.31 (m, 28H, CH_2), 4.06 (m, 15H, CH), 3.99 (t, 30H, CH_2), 3.79 (d, 16H, CH_2), 3.71 (d, 16H, CH_2), 3.20 (m, 4H, CH_2), 2.64 (m, 30H, CH_2), 2.32 (m, 30H, CH_2), 2.11 (m, 30H, CH_2), 1.85 (m, 2H, CH_2), 1.60 (m, 30H, CH_2), 1.26 (m, 21H, CH_3 ; 270H, CH_2), 1.10 (s, 24H, CH_3), 0.88 (t, 45H, CH_3). IR (KBr, cm^{-1}): 3294, 1550 (N-H), 2924, 2854 (C-H), 1737, 1656 (C=O), 1247, 1174, 1123, 1040 (C-O). GPC: $M_w = 6.0$ kDa, PDI = 1.05.

Bilayer and Vesicles Characterizations

Differential Scanning Calorimetry (DSC) was carried out by using a TA Instruments Q1000, the heating and cooling rate was 5°C min^{-1} . Small angle X-ray scattering (SAXS) data were collected with a Siemens 2-D small angle X-ray scattering detector. The X-rays were Cu $K\alpha$ radiation with a wavelength 0.1542 nm, set at 40 kV and 0.66 mA. Silver behenate was used to calibrate the sample to detector distance with a first order scattering vector of q of 1.076 nm^{-1} (with $q = (4\pi\sin\theta)/\lambda$ where 2θ is the scattering angle and λ is the wavelength).

Reflection	Expected ratio to first order peak	q expected (nm^{-1})	q observed (nm^{-1})
1st order	-	-	0.931
2nd order	2	1.86	1.82
3rd order	3	2.79	2.71

Transmission electron microscopy (TEM) was performed on a JEOL JEM 200CX electron microscope (JEOL Ltd., Japan). The samples were prepared for TEM analysis by dip-casting of the polymer solutions onto the plasma treated carbon coated copper grids and allowing it to dry freely in air. Before analysis, the samples were stained with 1.0 wt% phosphotungstic acid.

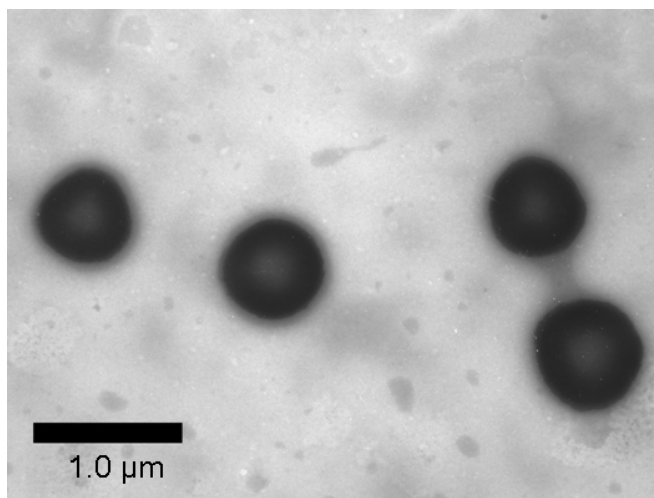


Figure S6. A TEM image of polymer vesicles in THF (1.0 mg/mL).

Dynamic light scattering (DLS) study was performed by using a Brookhaven Instruments, Co. (Holtville, NY) system consisting of a model BI-200SM goniometer, a model EMI-9865 photomultiplier, and a model BI-9000AT digital correlator, and a Coherent Innova® 90C Series ion laser (Santa Clara, CA) operated at 514 nm. The DLS measurements were taken at angle of 90° at 25 ± 0.1 °C. All determinations were made in triplicate.

Circular dichroism (CD) spectroscopy of vesicle dispersion in water was carried out by using an Aviv Model 202 CD Spectrometer. Measurements were performed at 25 ± 0.1 °C, sampling every 0.2 nm with a 10 s averaging time over the range of 200 – 250 nm (bandwidth = 1.0 nm). Polymer concentration was 50 μg/ml. The secondary structure (i.e., α -helical conformation) of comb block poly(γ -n-dodecyl-L-glutamate) within vesicular bilayer membrane was verified by observing characteristic negative ellipticity at 209 and 222 nm.

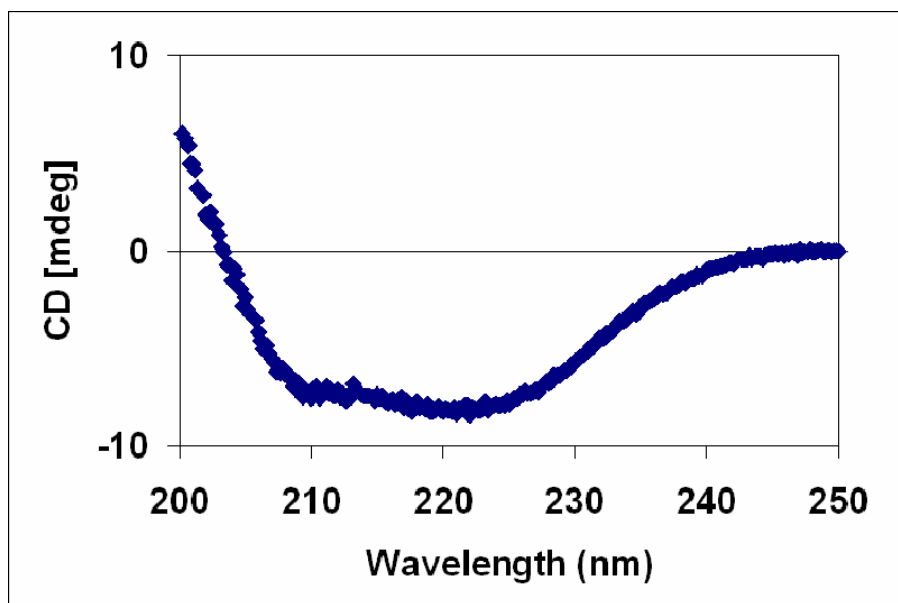


Figure S7. Circular dichroism of vesicle aqueous dispersion of macromolecule **1**.

Cytotoxicity Study

The cytotoxicity properties of macromolecule **1** were evaluated *in vitro* with Hep G2 (American Type Culture Collection, Manassas, VA) human hepatocellular carcinoma cells. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS), 25 mM HEPES buffer, 100 units/ml penicillin, and 100 $\mu\text{g/ml}$ streptomycin (Invitrogen, MA). The cytotoxicity of the polymer was determined with an MTT assay. Cells were seeded into 96-well plates at a density of 1×10^4 cells per well. After incubation for 24 hours, the medium was aspirated and 100 μL of varying concentrations of polymer were added to the wells in quadruplicate. The solutions of varying polymer concentration were prepared from a 1 mg/ml solution of polymer suspended in DMSO. The polymer/DMSO solution was diluted to concentrations from 1 μM to 0.001 μM with DMEM containing 10% FBS and filtered through a 0.2 μm syringe filter. The cells were incubated for 24 or 48 hours. After incubation, 10 μL of MTT in PBS (5 mg/ml) was added to each well and incubated for 3 hours. 50 μL of 50/50 DMF/Water with SDS was added, and the 96-well plate was incubated overnight. The optical densities at 565 nm were obtained using a SpectraMax Plus384 (Molecular Devices).

An MTT assay was used to determine the biocompatibility of the polymer to Hep G2 cells. The cells were treated to varying concentrations of polymer for 24 hours or 48 hours. The results were compared to control cells that were only treated with media. The control cells were considered to have a 100% growth, and the optical density results of all other treated cells were normalized to the optical density of the control cells to obtain a relative percentage of cell viability.

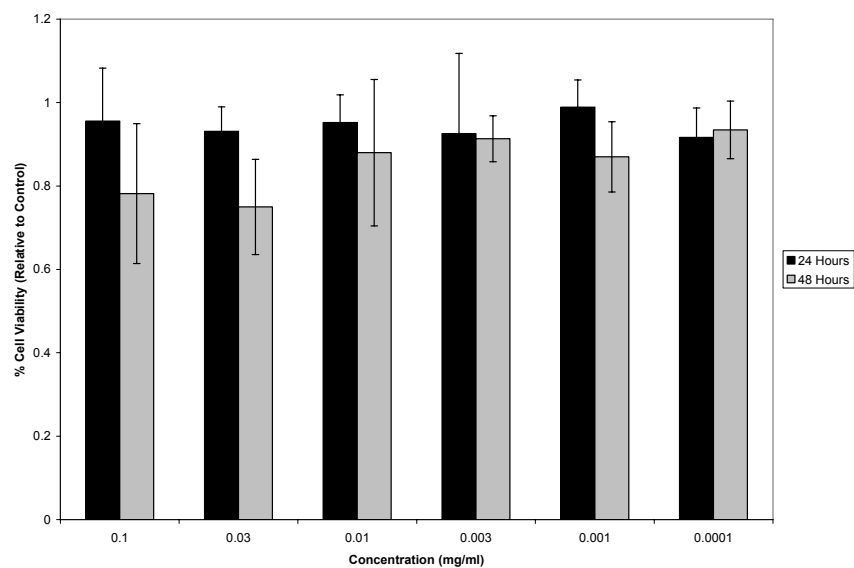


Figure S8. Viability of Hep G2 cells after 48 h exposure with varying concentrations of macromolecule 1.

Reference

- [1] J. S. Moore, S. I. Stupp, *Macromolecules* **1990**, *23*, 65.
- [2] D. Wasserman, J. D. Garber, F. M. Meigs, US, **1966**, p. 12.
- [3] W. H. Daly, D. Poche, *Tetrahedron Letter* **1988**, *29*, 5859.
- [4] H. Ihre, A. Hult, J. M. J. Frechet, I. Gitsov, *Macromolecules* **1998**, *31*, 4061.
- [5] W. H. Daly, D. Poche, I. I. Negulescu, *Progress in Polymer Science* **1994**, *19*, 79.