Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Hydrogen-bond Super-amphiphile based Drug Delivery System: Design, Synthesis and Biological Evaluation

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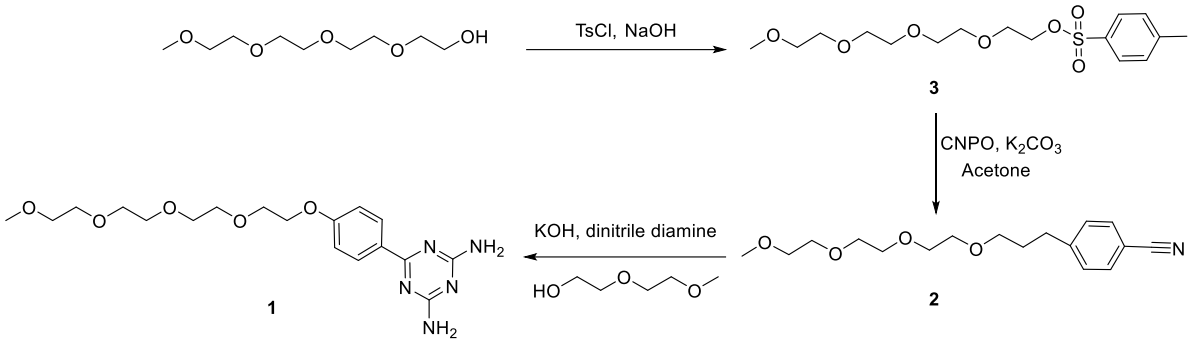
Scheme S1. Synthesis of compound **1.**



Synthesis of compound 3. Tetraethylene glycol monomethyl ether (2.961 g, 0.014 mmol) was slowly added to the mixture of p-toluenesulfonyl chloride (TsCl) (5.330 g, 0.028 mmol) and sodium hydroxide (2.240 g, 0.056 mmol) in mortar. The reaction mixture was grinded for 30 min and extracted with dichloromethane (3 × 20 mL). The combined organic phase was washed with brine (3 × 20 mL), dried over anhydrous Na_2SO_4, filtrated, and evaporated under reduced pressure. The crude product was dried in vacuum to give **3** (4.006 g, 77.8%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, J = 8.3 Hz, 2H), 7.34 (d, J = 8.2 Hz, 2H), 4.16 (dd, J = 9.9, 5.2 Hz, 2H), 3.71 – 3.52 (m, 14H), 3.37 (s, 3H), 2.44 (s, 3H).

Synthesis of compound 2. Potassium carbonate (4.582 g, 33.15 mmol) and 4-hydroxybenzonitrile (CNPO) (1.315 g, 11.05 mmol) was added to the stirred solution of compound **3** (4.006 g, 11.05 mmol) in dry acetone, and the reaction mixture was refluxed for 12 h. Then, the solvent was removed and the mixture was extracted with EtOAc (3 × 20 mL). The combined organic phase was washed with brine (3 × 20 mL), dried over anhydrous Na_2SO_4, filtrated, and evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (EA: PE = 2: 1) to give **2** (2.267 g, 63.0%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (d, J = 8.3 Hz, 2H), 7.34 (d, J = 8.2 Hz, 2H), 4.16 (dd, J = 9.9, 5.2 Hz, 2H), 3.71 – 3.52 (m, 14H), 3.37 (s, 3H), 2.44 (s, 3H).

Synthesis of compound 1. A mixture of potassium hydroxide (0.193 g, 4.02 mmol), diethylene glycol methyl ether, dinitrile diamine (0.339 g, 4.02 mmol) and compound **2** (1.244 g, 4.02 mmol) were refluxed for 12 h. The mixture was slowly added with 1 N HCl under stirring until pH reached 7. The solvent was removed and the mixture was extracted with water (3 × 20 mL). The combined water phase

was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (EA: PE = 2: 1) to give **1** (0.79 g, 50.0%) as a light-yellow solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.79 (d, J = 8.3 Hz, 2H), 7.34 (d, J = 8.2 Hz, 2H), 4.16 (dd, J = 9.9, 5.2 Hz, 2H), 3.71 – 3.52 (m, 14H), 3.37 (s, 3H), 2.44 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.75, 161.40, 129.85, 128.54, 125.97, 114.39, 72.74, 71.73, 70.41, 70.27, 70.23, 70.03, 69.33, 67.72, 58.51. HR-MS (ESI): calculated for $C_{18}H_{27}N_5O_5$ $[M + Na]^+$ 416.1898, found: 416.1896.

Preparation of HBS-DDS with Nile Red. Firstly, Nile red solution (1 mg/mL) was prepared by dissolving Nile red in chloroform solution. Then, the Nile red solution (20 μL) was added to the glass bottle and dried. Finally, the HBS-DDS (1.6 mL, 500 μM) was added to the glass bottle with Nile red and sonicated for 30 min to obtain the Nile red loaded HBS-DDS.

Determination of critical micelle concentration (CMC). Firstly, the Nile red was dissolved in chloroform. Then, the chloroform with Nile red was added to a series of vials and the chloroform was evaporated. Next, a measured amount of compound **1** and HCFU was added to each vial and the deionized water was added to the vials to make the concentrations of compound **1** and HCFU ranging from 2 to 1024 μM. The vials were vibrated at room temperature overnight, and then the fluorescence emission intensity at the wavelengths of 640 nm (excited at 485 nm) was measured. The critical micelle concentration was obtained as the intersection of the tangents to the two linear portions of the graph of the fluorescence intensity. From **Figure S**7, the CMC of amphiphile 1 was ~41.6 μM, which was higher than the minimum concentration for dilution stability. Above results might be caused by the hydrogen bond between HCFU and compound **1**, which could act as a cross-linking point to enhanced the stability of HBS-DDS.

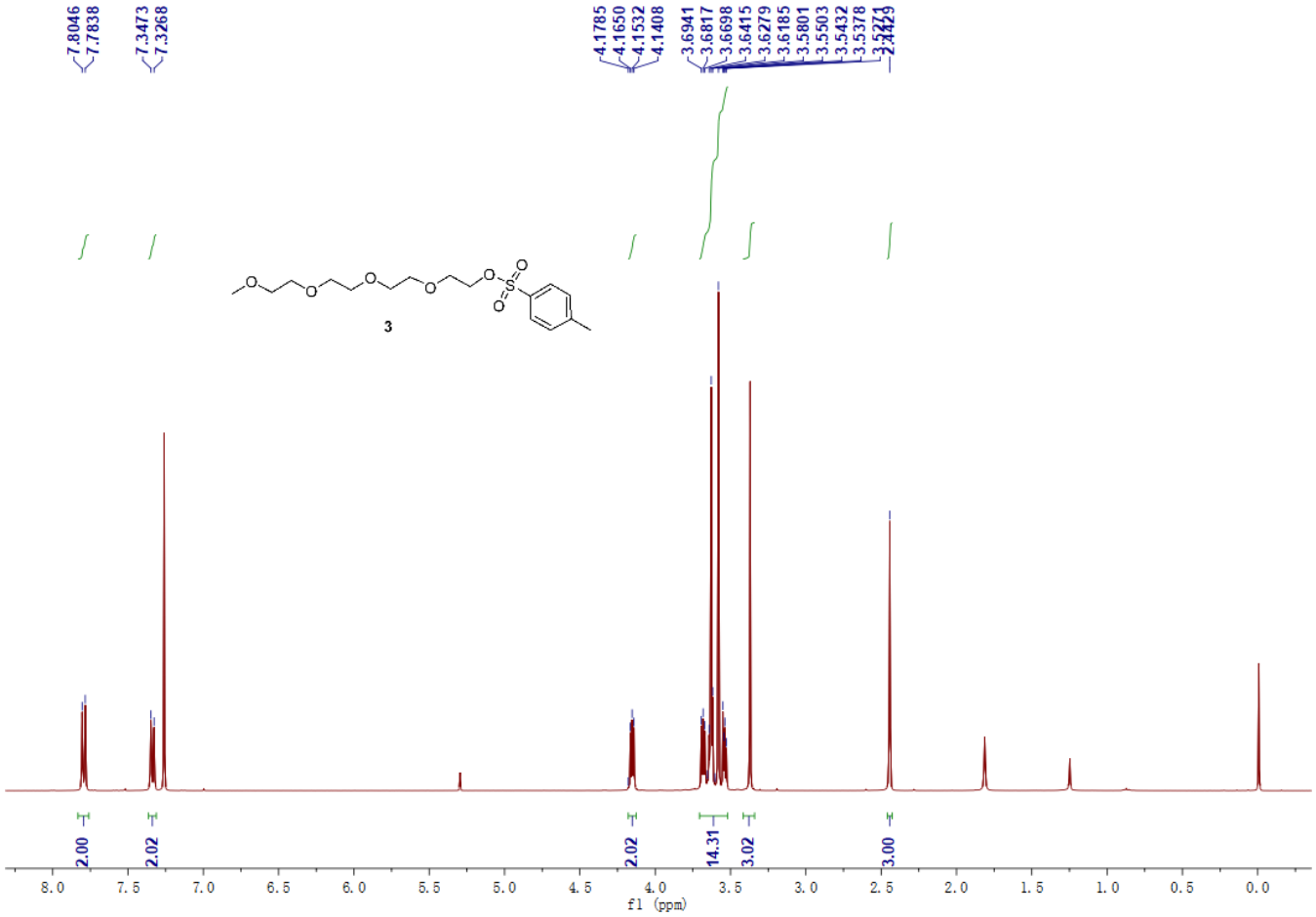


Figure S1. ^{1}H NMR of compound **3**.

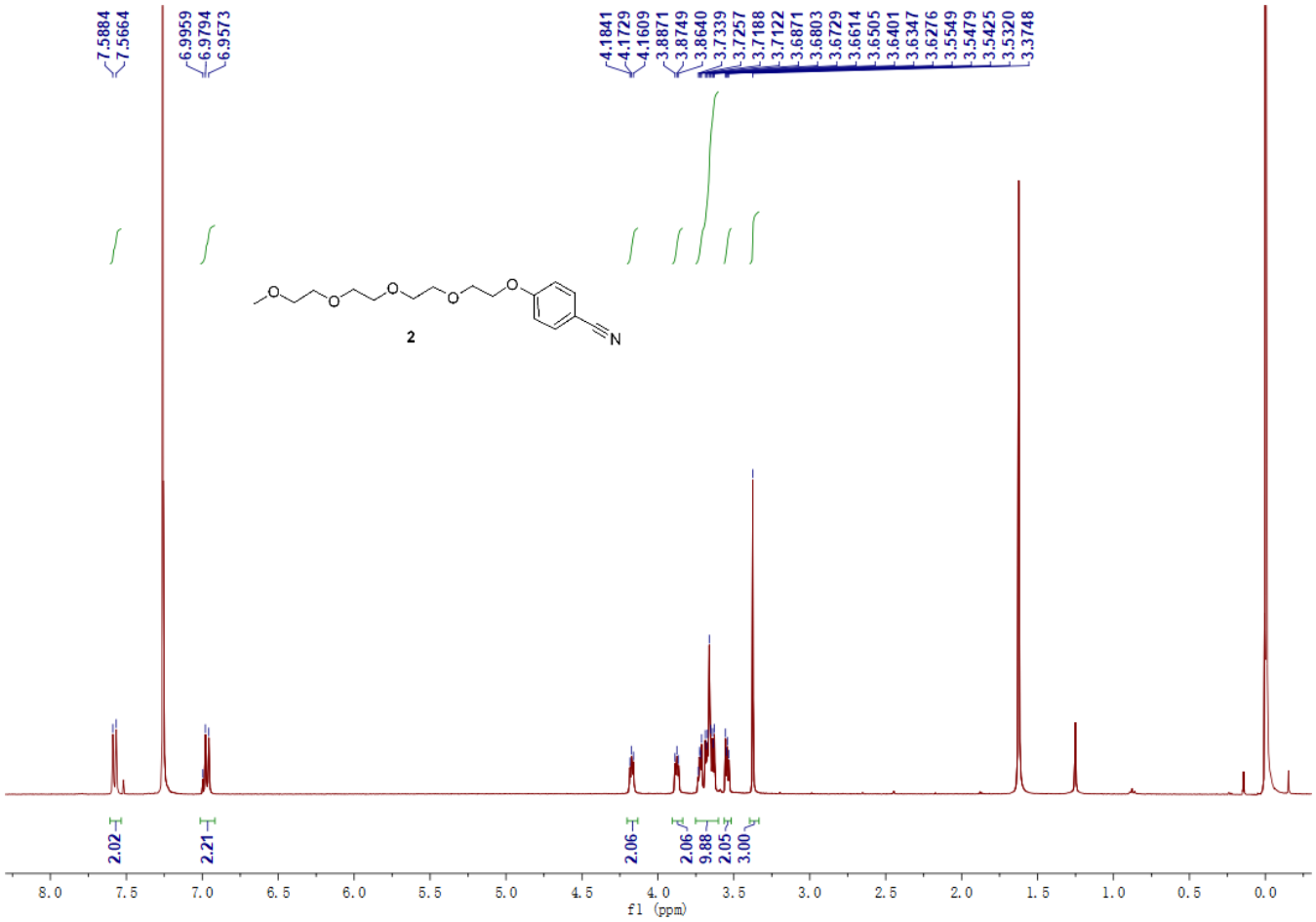


Figure S2. ^{1}H NMR of compound **2**.

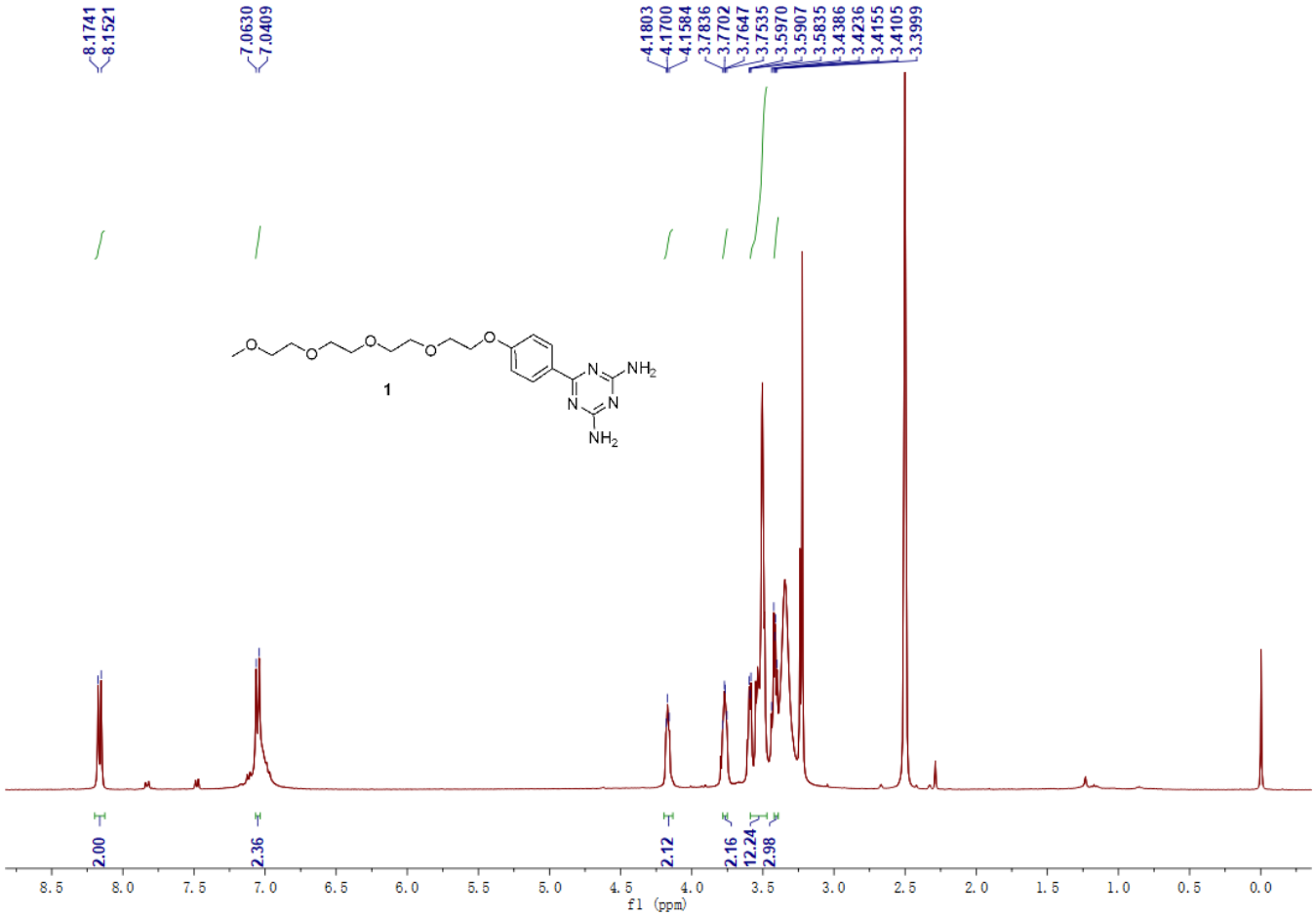


Figure S3. ^{1}H NMR of compound **1**.

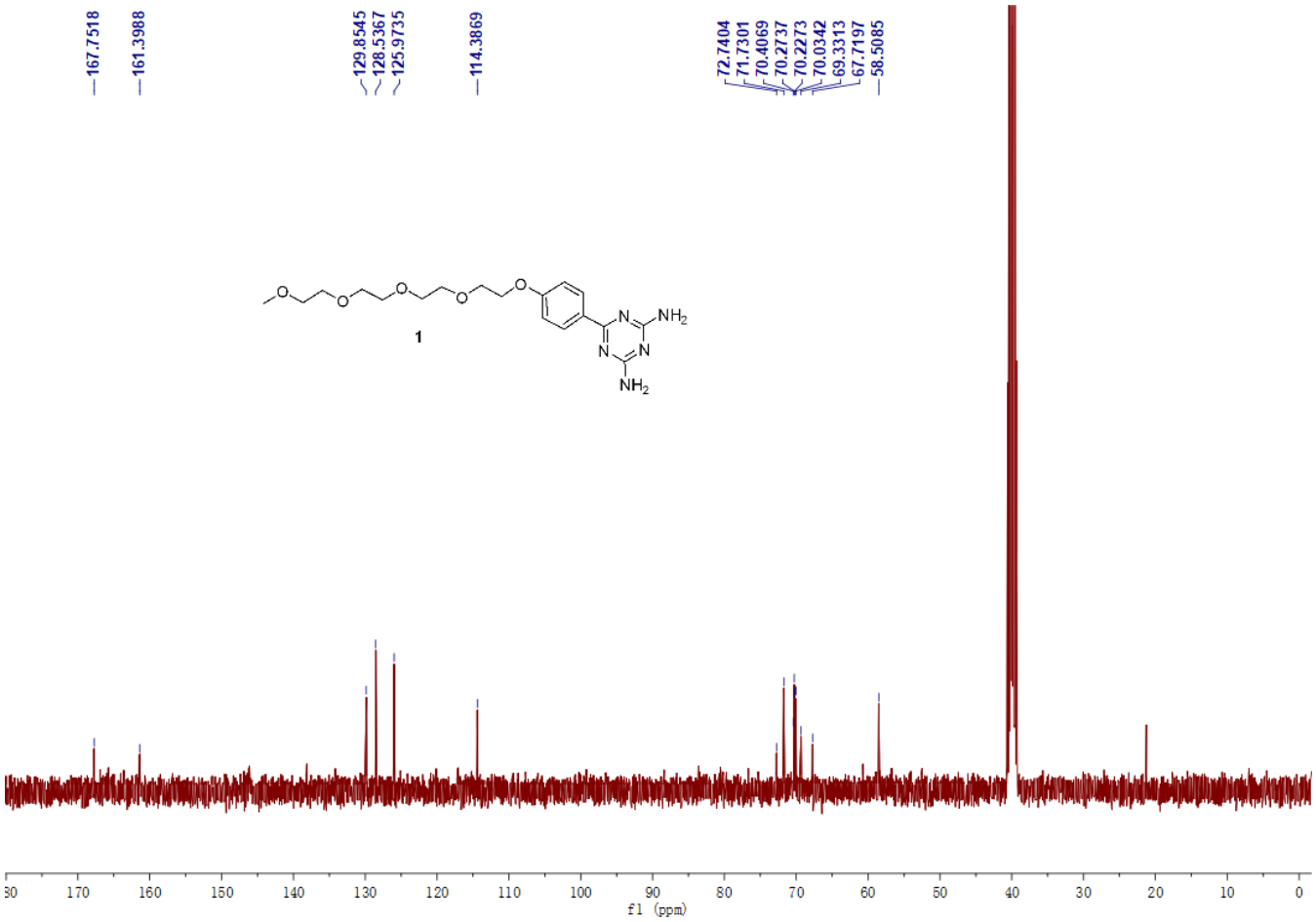


Figure S4. ^{13}C NMR of compound **1**.

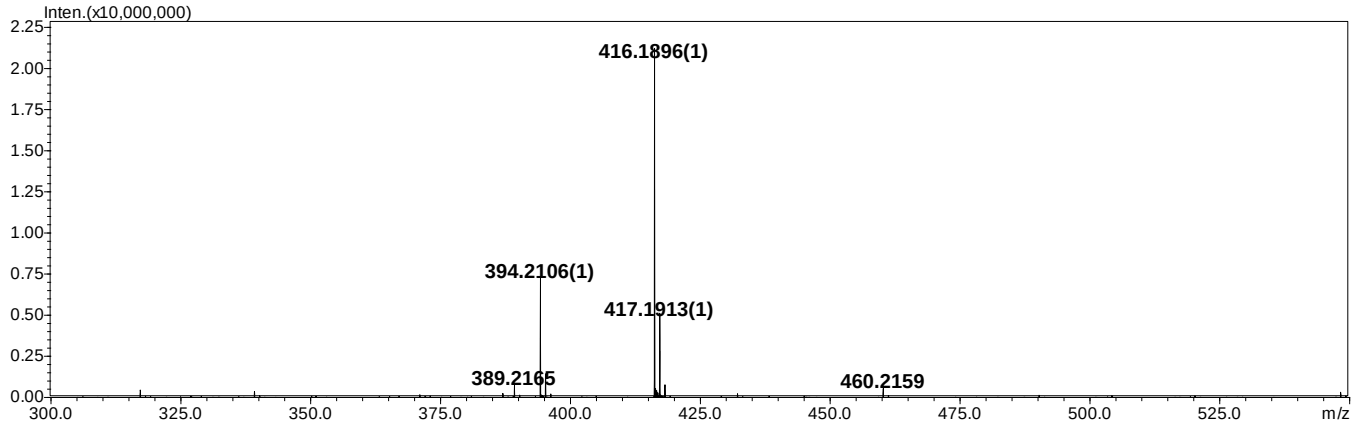


Figure S5. High resolution mass spectrum of compound **1**.

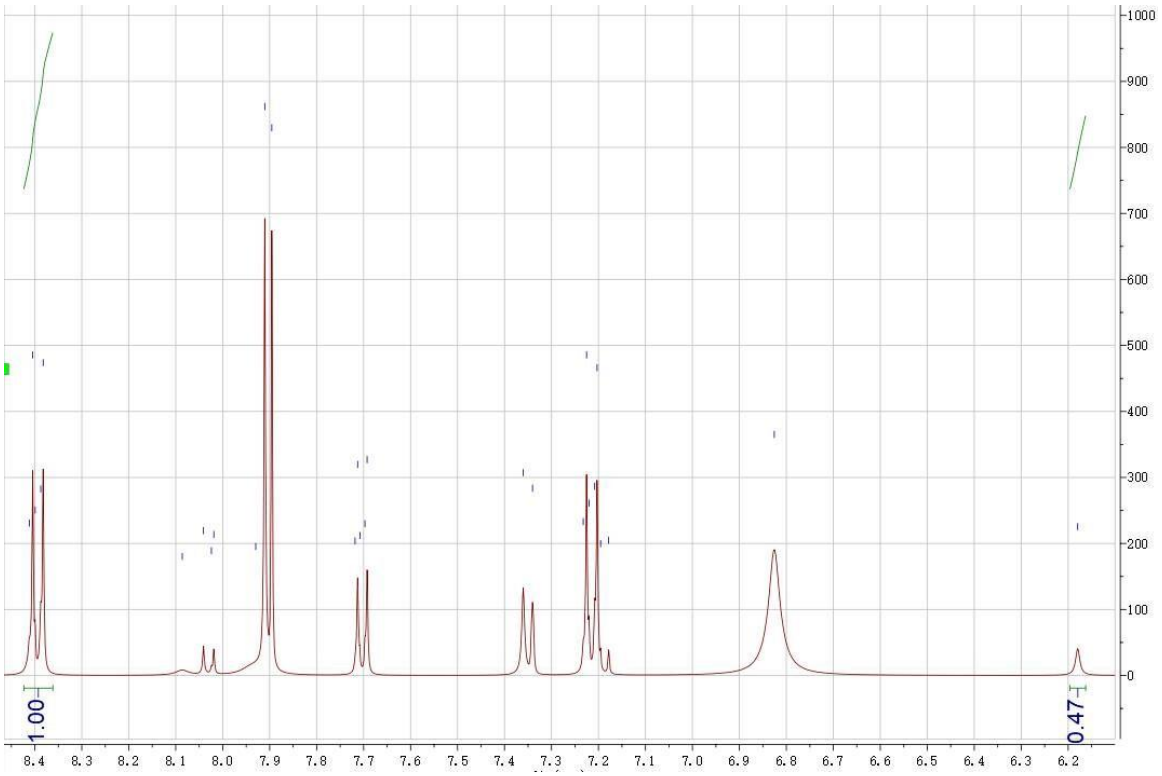


Figure S6. ^{1}H NMR of HSB-DDS.

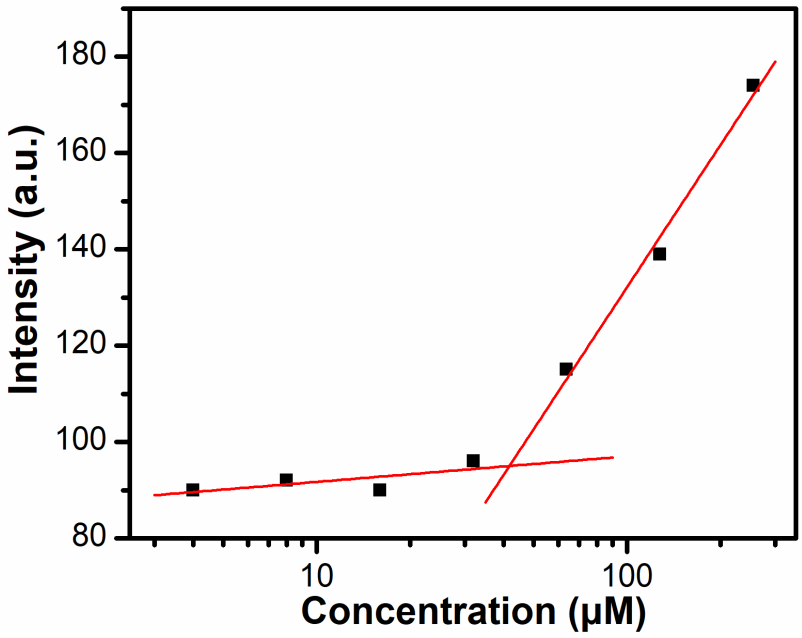


Figure S7. Emission intensity at 640 nm of Nile red as a function of concentrations of compound **1** and HCFU in water.

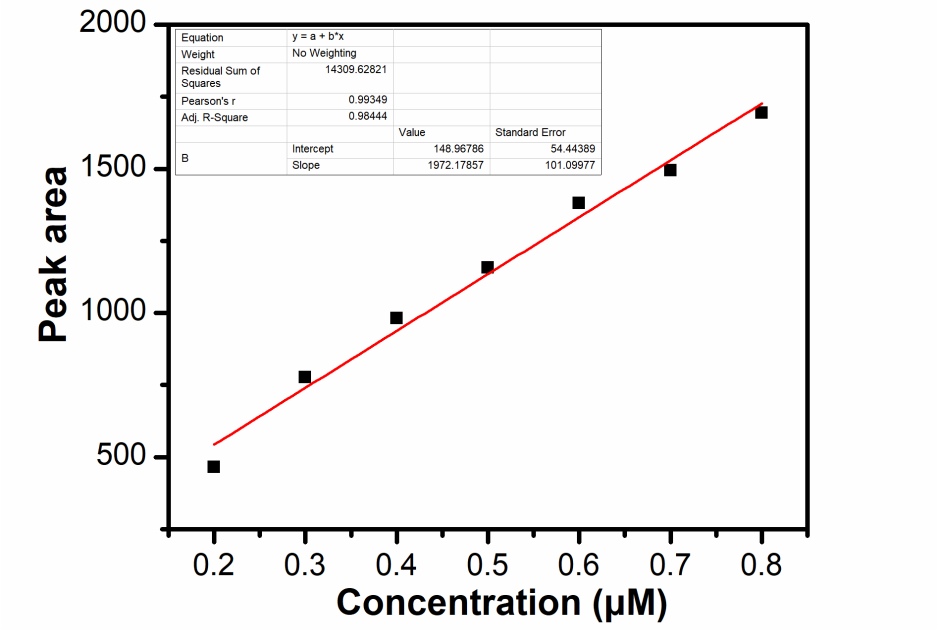


Figure S8. The calibration curve of HCFU by a HPLC.

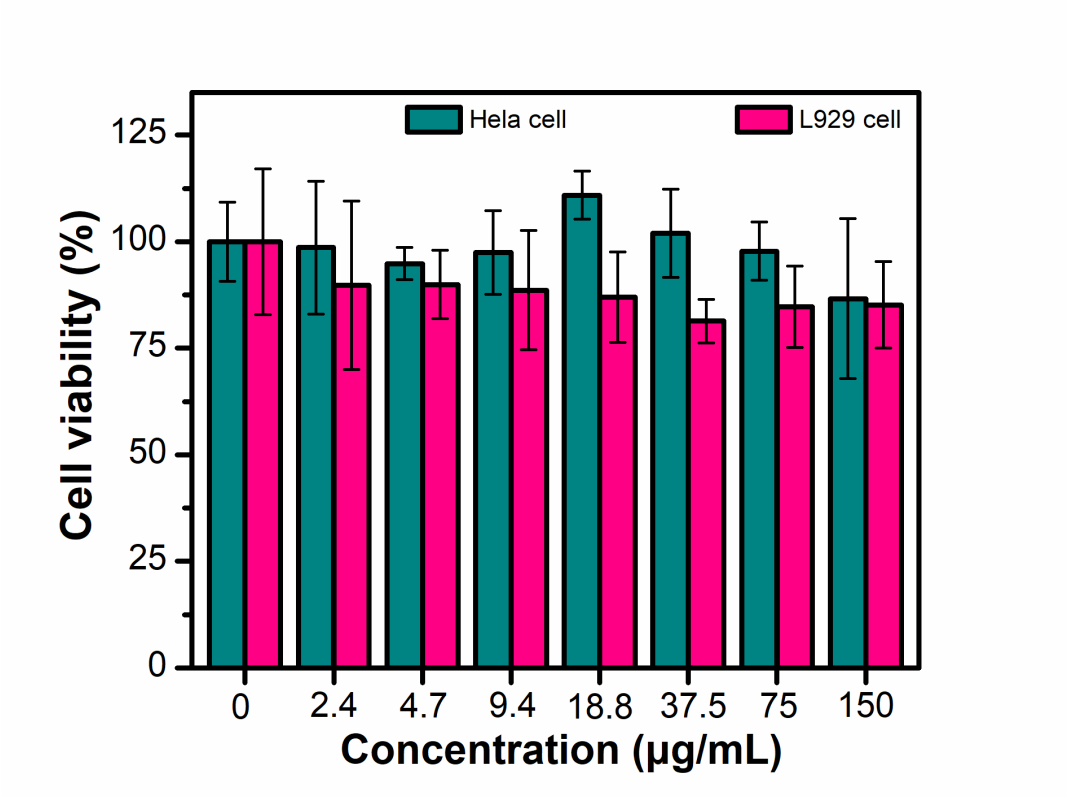


Figure S9. Cell viability of compound **1** against (a) Hela cells and (b) L929 cell after incubation for 48 h at 37 °C with a series of concentrations.

Table S1. Composition of compound **1** and HCFU in HSB-DDS.

Component	compound **1**	HCFU
Molar ratio	1	0.94