SUPPORTING INFORMATION

Magainin II Modified Polydiacetylene Micelles for Cancer Therapy

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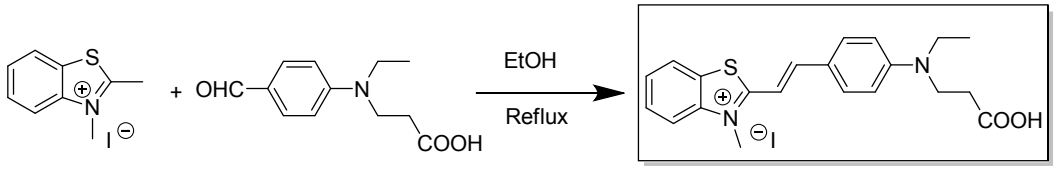
Contents

1. Synthesis of hemicyanine dye (compound 3)



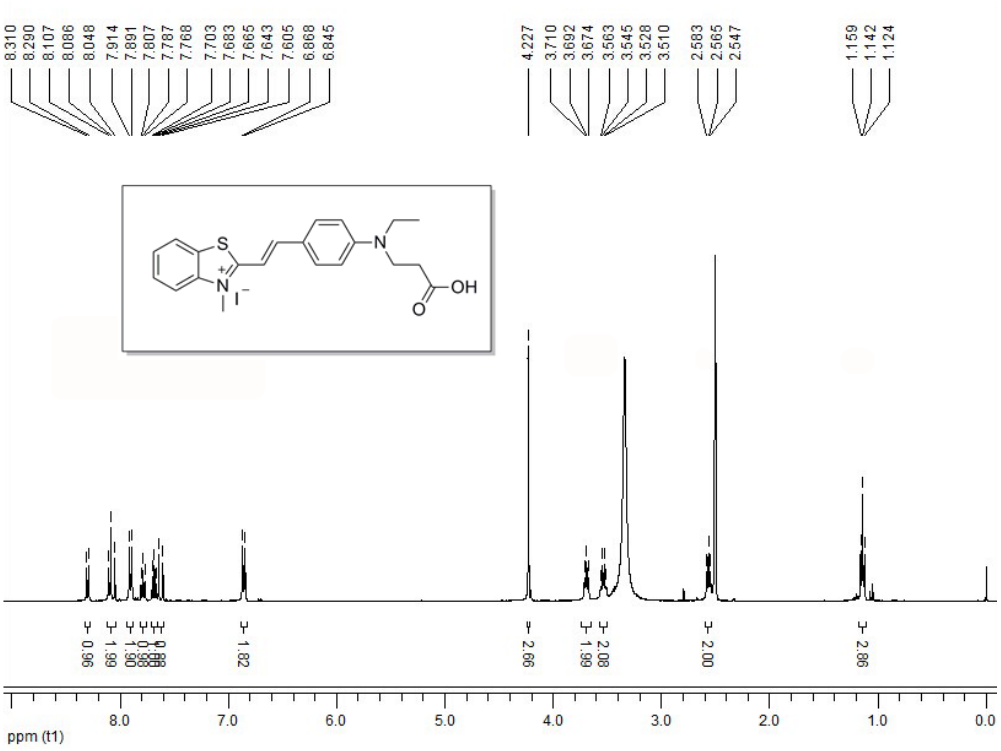
1 **2** **3**

Compound 3 was synthesized according to the literature.[1-2] A mixture of compound **1** (5.82 g, 20.0 mmol) and compound **2** (4.43 g, 20.0 mmol) in EtOH (60 mL) was refluxed for 12 h. EtOH was removed under reduced pressure to obtain a dark red residue. The residue was washed three times with Et_2O (80 mL) to remove unreacted starting materials. The crude products were purified by column chromatography on silica gel with mixed chloroform-petroleum ether as eluent. Compound **3** was obtained as a red solid powder 2.3 g, yield 23.0 %.

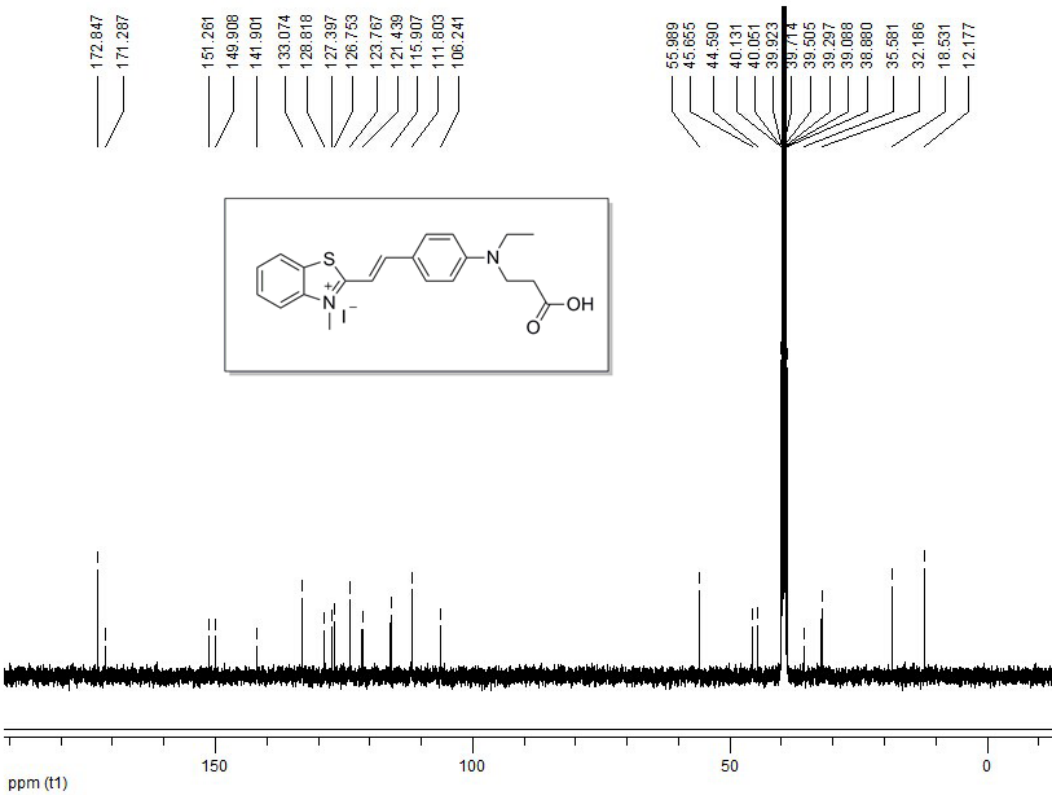
^{1}H NMR (400 MHz, DMSO-d_6): δ [ppm] 1.12-1.16 (t, J = 6.8 Hz, 3H), 2.55-2.58 (t, J = 7.2Hz, 2H), 3.51-3.56 (dd, J = 7.2 Hz, J = 14 Hz, 2H), 3.67-3.71 (t, J = 6.8 Hz, 2H), 4.23(s, 3H), 6.85-6.87 (d, J = 8.8Hz, 2H), 7.60-7.70 (m, 1H), 7.77-7.81 (m,1H), 7.90-7.91 (d, J = 9.2Hz, 2H), 8.05-8.11 (t, J = 8.8 Hz, 2H), 8.29-8.31 (d, J = 8Hz, 1H).

^{13}C NMR (100 MHz, DMSO-d_6): δ [ppm] 172.9, 171.3, 151.3, 150.0, 141.9, 133.1, 128.8, 127.4, 126.8, 123.8, 121.5, 115.9, 111.8, 106.2. 56.0, 45.1, 44.6, 40.1, 39.9, 39.7, 39.5, 39.3, 39.1, 38.9, 35.6, 32.2, 18.5, 12.2.

^{1}H NMR



^{13}C NMR



2. **HPLC analysis of MGN-II-DA**

Reverse-phase HPLC (AKTA purifier 100, GE Healthcare, USA) was performed with a RP-C18 HPLC column and UV detector. The mobile phase was a gradient of 35-100% of acetonitrile aqueous solution containing 0.1% TFA at a total flow rate of 1 mL/min. The UV absorption peaked at 530 nm was recorded for analysis.

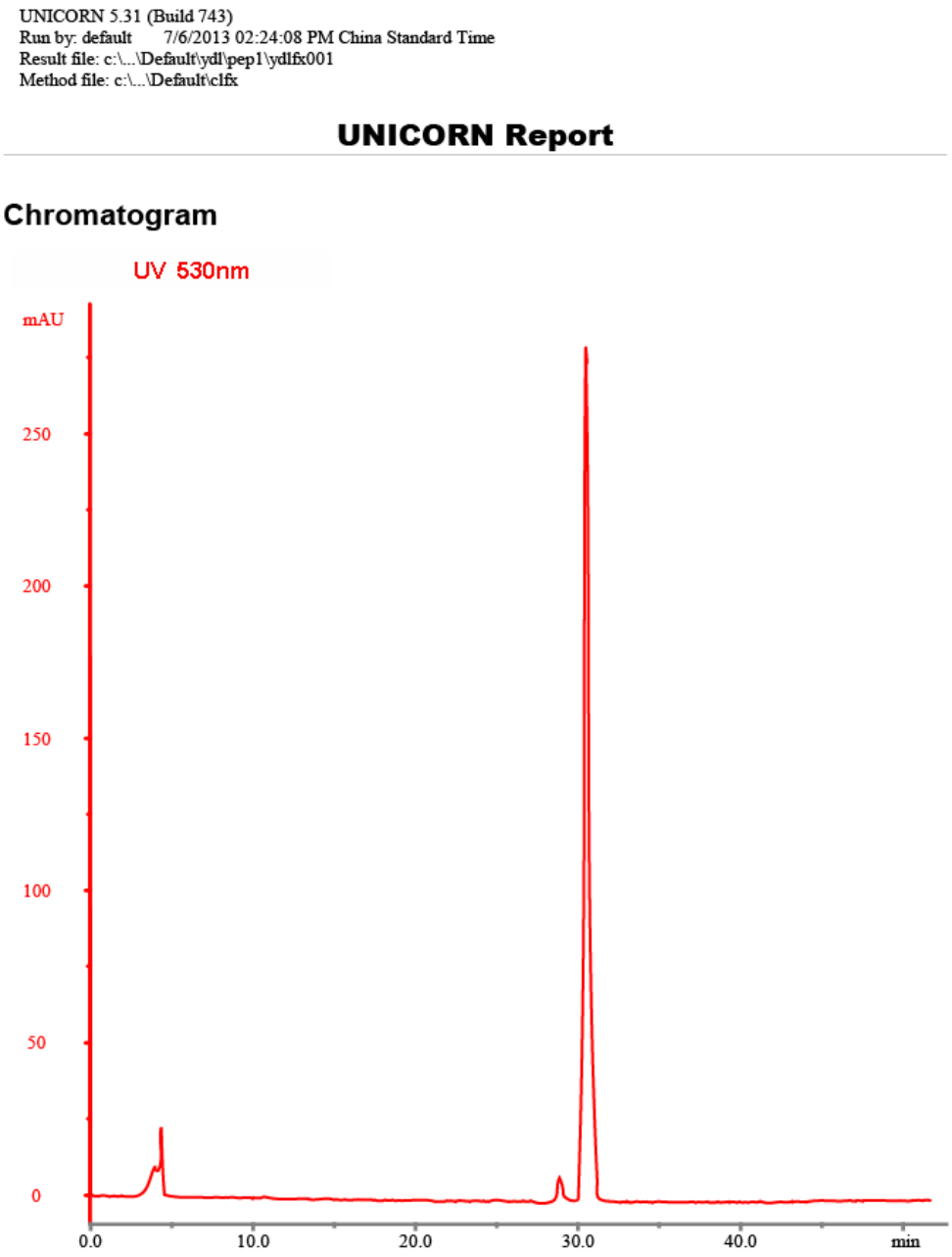
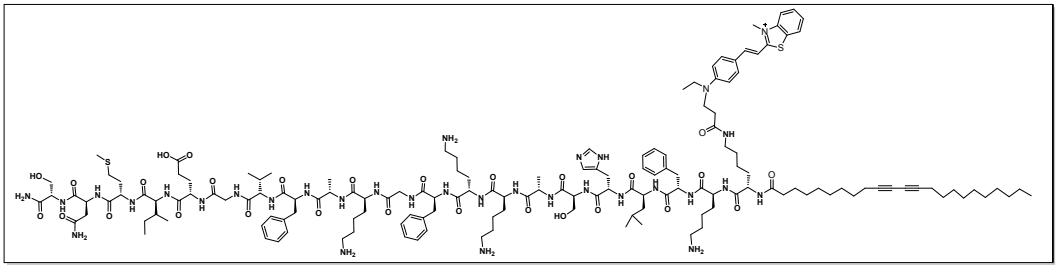


Figure S1. HPLC analysis report of MGN-II-DA

3. M-TOF analysis of MGN-II-DA

Mass spectra were acquired on a 4800 Plus MALDI TOF/TOF Analyzer (AB SCIEX, USA). Prior to MALDI-TOF-MS analysis, a volume of 0.5 μL of peptide solution was spotted on a MALDI plate, 0.5 μL of matrix solution (4 mg/mL CHCA dissolved in 50% aqueous CH_3CN containing 0.1% TFA) was deposited on the sample and allowed to air-dry at room temperature. All MALDI-TOF-MS measurement was performed in positive ion mode using a 4800 Plus M-TOF/TOF Analyzer (AB SCIEX, USA).



Scheme S1. Chemical structure of MGN-II-DA

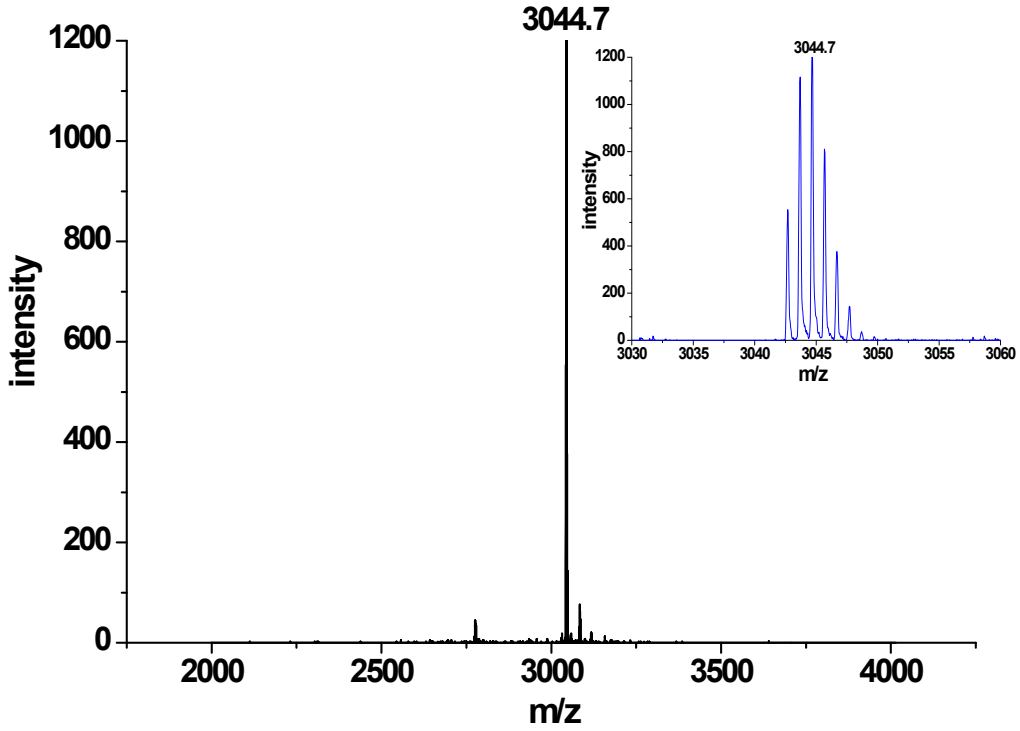


Figure S2. M-TOF analysis of MGN-II-DA

4. Zeta potential measurement.

The zeta potential of MGN-II-PDA was measured in PBS (pH = 7.4, 25°C) using Nano-ZS (Zeta sizer, Malvern) instrument. All data were corrected by subtracting a blank scan of the buffer system. Results were presented as mean ± SD (n=3).

5. The time-lapse images of A549 cells.

A549 cells were seeded in a glass-bottomed dish. After overnight culture, the cells were incubated with MGN-II-PDA in RPMI-1640 medium at a final concentration of 16 μg/mL at 37 °C.

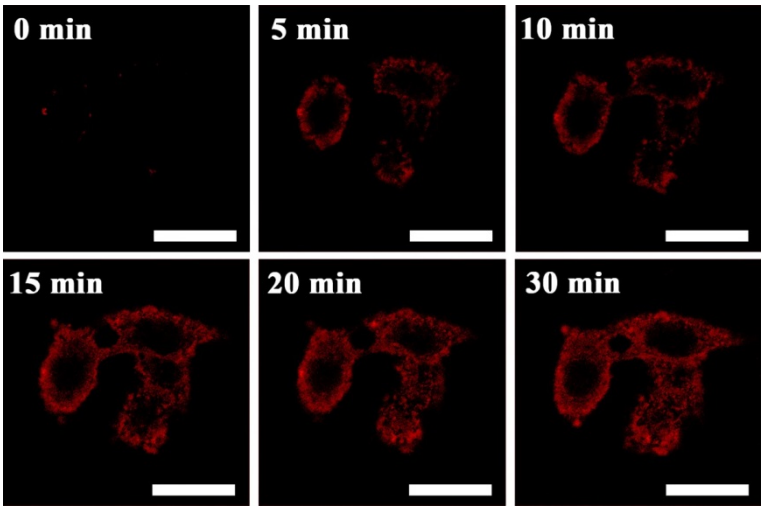


Figure S3. Time-lapse confocal laser scanning microscopy images of A549 cells were treated at the concentration of 16 μg/mL MGN-II-PDA for 0, 5, 10, 15, 20, 30 min (excitation: 488 nm, emission collected: 600-650 nm). Scale bar is 25 μm.

6. Cytotoxicity assay.

The cytotoxicity of MGN-II-PDA against the normal cell line was studied using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Briefly, the L929 murine fibrosarcoma cells in complete medium were seeded into 96-well plates at a density of 1.0×10^4 cells/well and cultured overnight at 37 °C under 5% CO_2 before treatment. After the complete medium was removed, the MGN-II-PDA solution (100 μL/well) at concentrations

of 8, 16, 32 and 64 μg/mL in RPMI-1640 was added to the wells, and the cells were incubated for 24 h at 37 °C under 5% CO_2. Subsequently, 10 μL of MTT solution (5 mg/mL in PBS) was added to the medium and the cells were incubated at 37 °C for 4 h. The resulting formazan crystals were solubilized using 100 μL of DMSO after the growth medium was removed and the quantity determined colorimetrically using a Synergy H4 Hybrid Microplate reader (Biotek, USA) at 490 nm. Cell viability (%) was calculated using the following formula: Cell viability = (mean absorbance value of the treatment group-blank /mean absorbance value of the control-blank) × 100.

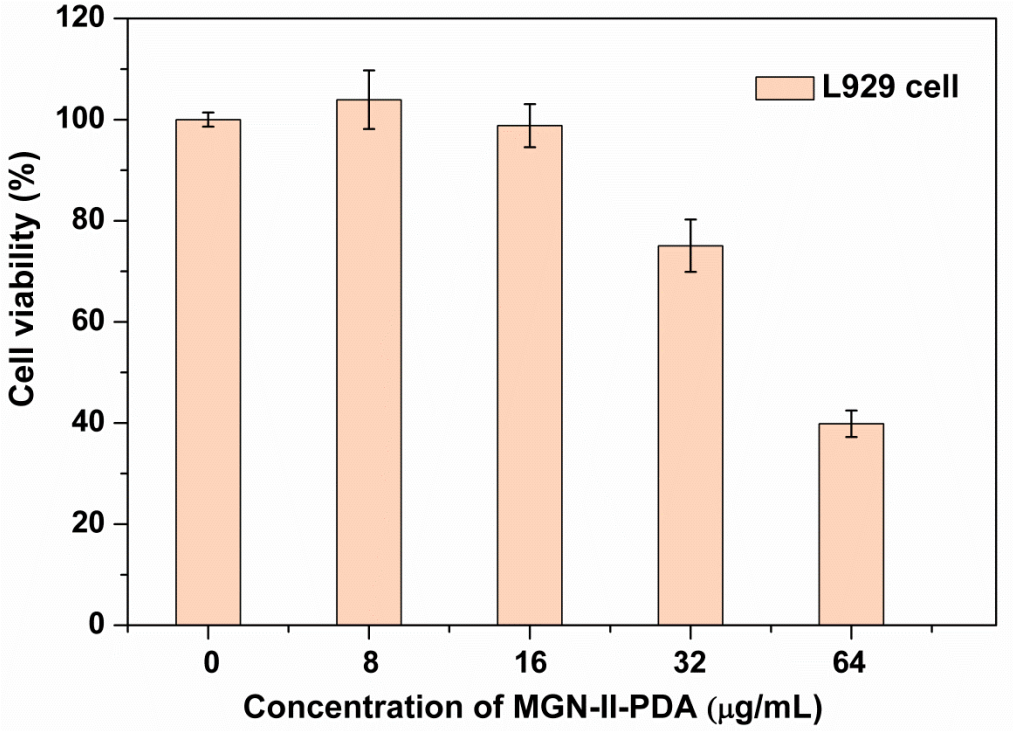


Figure S4. MTT assays for MGN-II-PDA in L929 cells; the cells were seeded at 1.0 × 10^4 cells/well on a 96-well plate, and treated with MGN-II-PDA at concentrations of 8, 16, 32 and 64 μg/mL in RPMI-1640 medium for 24 h, the data are presented as mean ± SD (n = 5).

Reference:

[1] Q.-H. Yao, F.-S. Meng, F.-Y. Li, H. Tian, and C.-H. Huang, *J. Mater. Chem.*, **2003**, *13*, 1048–1053.

[2] J. Zheng, C.-H. Huang, T.-X. Wei, Y.-Y. Huang, and L.-B. Gan, *J. Mater. Chem.*, **2000**, *10*, 921–926.