Supporting information for

Improved Targeting Aspirin Prodrug Albumin-Based Nanosystem for Visualizing and Inhibiting Lung Metastasis of Breast Cancer

Wancun Zhang[1#], Lili Xia[2#], Xiangyu Ren[2], Mengyuan Cui[2], Tianguang Liu[2], Chen Ling[2], Yanqi Xu[2], Dawei Deng[2], Xianwei Zhang[1], Yueqing Gu[2]*, Peng Wang[1,2]*

[1]*Henan Key Laboratory of Children's Genetics and Metabolic Diseases, Henan Neurodevelopment Engineering Research Center for Children, Children's Hospital Affiliated to Zhengzhou University, Henan Children's Hospital, Zhengzhou Children's Hospital, Zhengzhou 450018, China*

[2]*Department of Biomedical Engineering, School of Engineering, China Pharmaceutical University, Nanjing, 211198, China.*

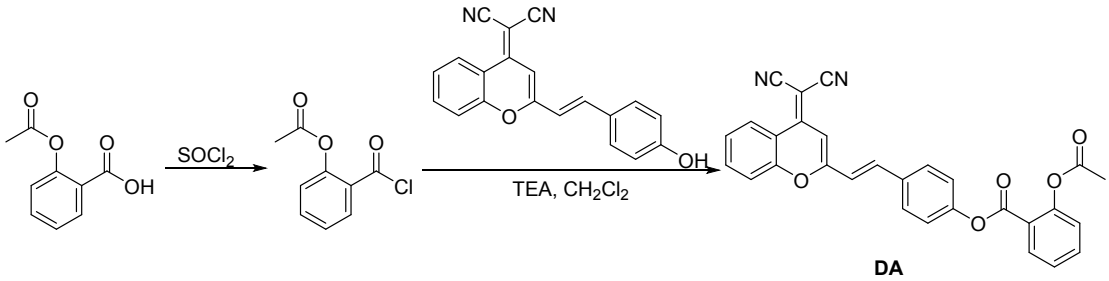
[3]*Center for Vascular and Inflammatory Diseases, University of Maryland School of Medicine, Baltimore, MD, 21201, USA*

Corresponding email: wangpeng159seu@hotmail.com

1. Experimental section

1.1 Synthesis of compound DA. Firstly, Aspirin (5.0 g, 27 mmol) was dissolved in dry dichloromethane (100 mL) in a Schlenk flask and then pyridine (2.30 mL) was added. Thionyl chloride (3.2 mL) was slowly dropped into the solution under the ice bath. Then, the mixture was vigorously stirred at room temperature for 6 h until the color of the mixture was turned to yellow. After removing the solvent under vacuum, the yellow solid was achieved. Secondly, the obtained solid (75 mg, 0.38 mmol) was added to the solution of DCM-OH (100 mg, 0.32 mmol) in dry dichloromethane (7.5 mL) at 0 °C, followed by the addition of triethylamine (TEA). The mixture was violently stirred at room temperature for overnight. Eventually, utilizing a rotary evaporator to remove the solvents, the residue was purified by silica column chromatography (CH_2Cl_2 as the eluent) to afford the compound DA (48 mg, yield 32%). ^{1}H-NMR (DMSO-*d6*, 300

MHz) δ 8.73 (d, J = 8.37 Hz, 1H), 8.17 (d, J = 7.77 Hz, 1H), 7.85-7.93 (m, 3H), 7.55-7.81 (m, 3H), 7.59-7.64 (m, 1H), 7.47-7.55 (m, 2H), 7.32-7.36 (m, 3H), 7.05 (s, 1H), 2.25 (s, 3H). ^{13}C-NMR (DMSO-*d6*, 75 MHz) δ 169.6, 162.9, 158.3, 153.4, 152.5, 152.0, 150.9, 137.9, 135.9, 135.7, 133.5, 132.2, 129.9, 129.8, 126.9, 126.6, 125.1, 124.6, 123.1, 122.9, 120.5, 119.5, 117.5, 116.2, 107.4, 21.1.(Figure S11 and S12).



Scheme S1. Synthetic route for compound DA.

2. Figures

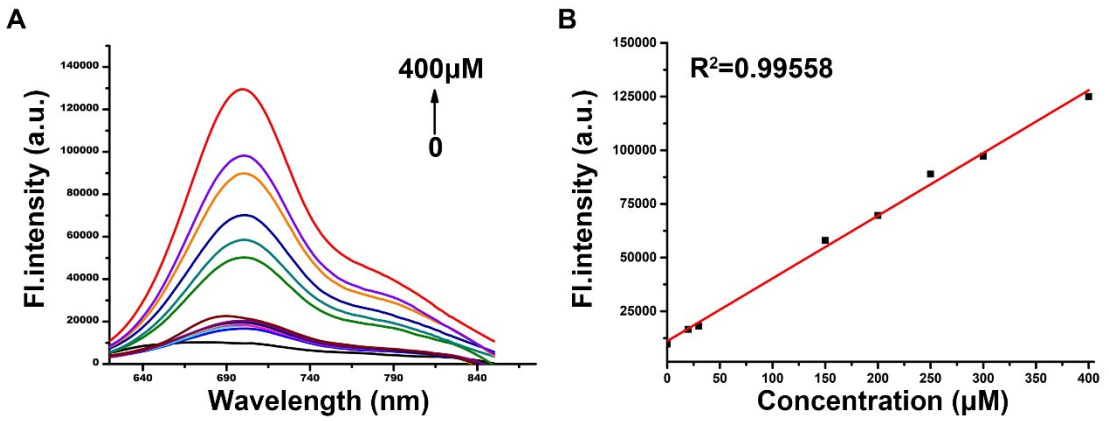


Figure S1. (A) The fluorescence spectra of prodrug DA (10 μM) to various concentrations of H_2O_2 (0–400 μM) at 37 °C for 30 min. (B) The linear correlation between the fluorescence increment at 705 nm and the concentration of H_2O_2 (0–400 μM). λex/λem = 560 nm/705 nm.

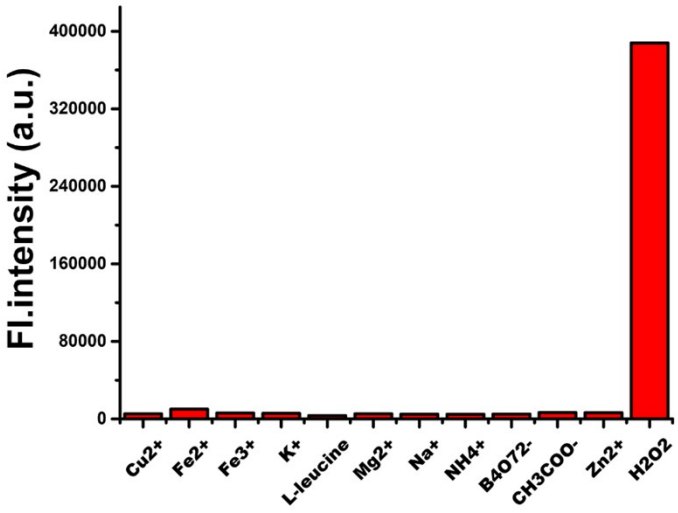


Figure S2. The fluorescence intensity of prodrug DA (10 μM) upon the addition of H_2O_2 and various of compounds, such as Cu^{2+}、Fe^{2+} 、Fe^{3+}、K^+、L-leucine、Mg^{2+}、Na^+、NH_4^+、$B_4O_7^{2-}$、CH_3COO^-、Zn^{2+}.

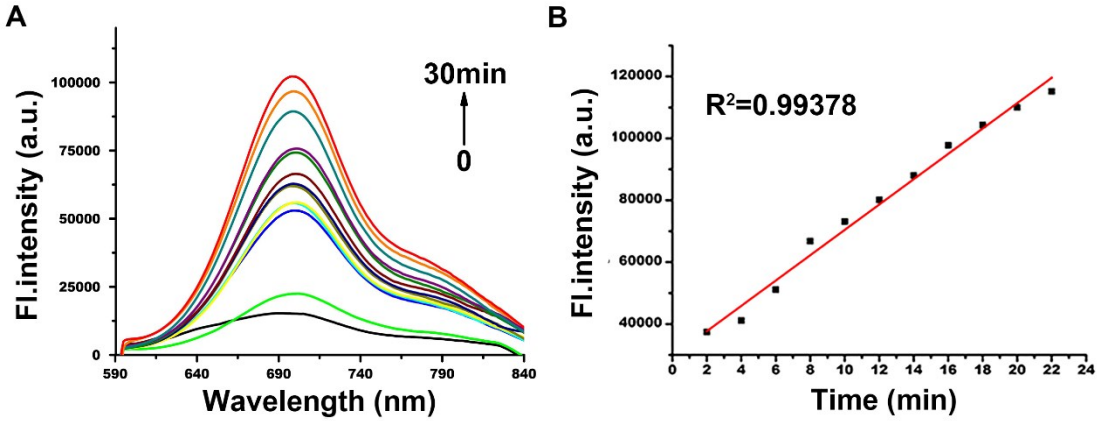


Figure S3. (A) The change of prodrug DA (10 μM) fluorescence intensity with time after adding H_2O_2 (200 μM). (B) The linear relation between fluorescence intensity at 705 nm with time (2-24 min).

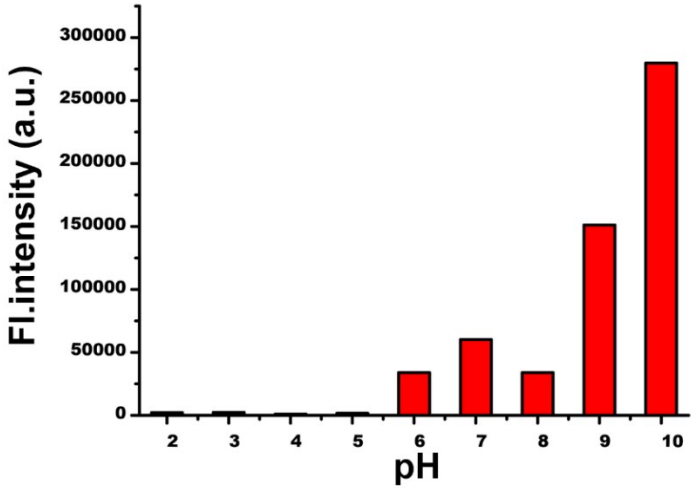


Figure S4. The fluorescence intensity of prodrug DA with H_2O_2 under the different pH.

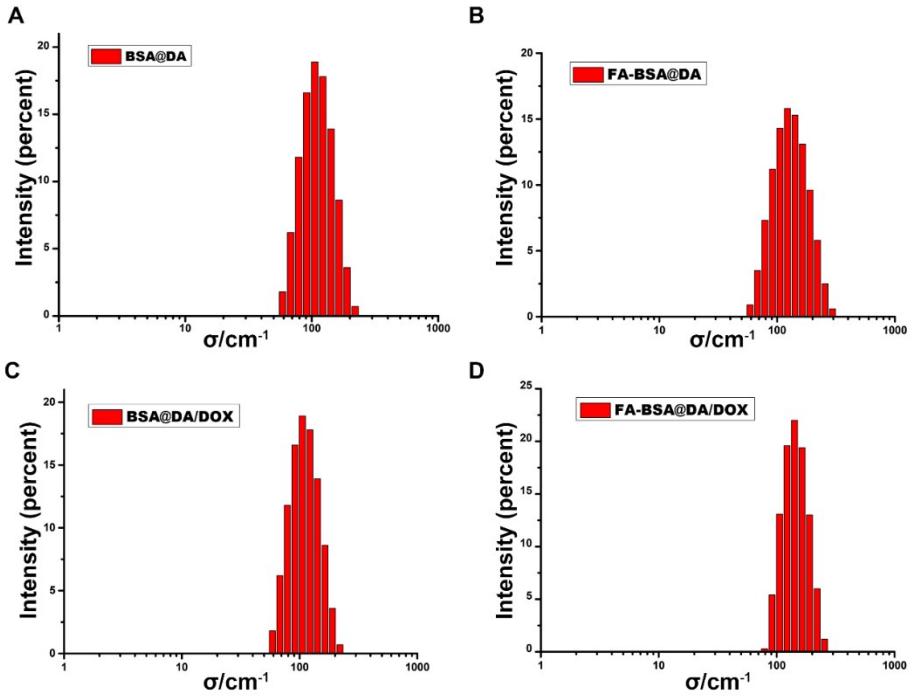


Figure S5. (A-D) The particles distribution of BSA@DA, FA-BSA@DA, BSA@DA/DOX, and FA-BSA@DA/DOX, respectively.

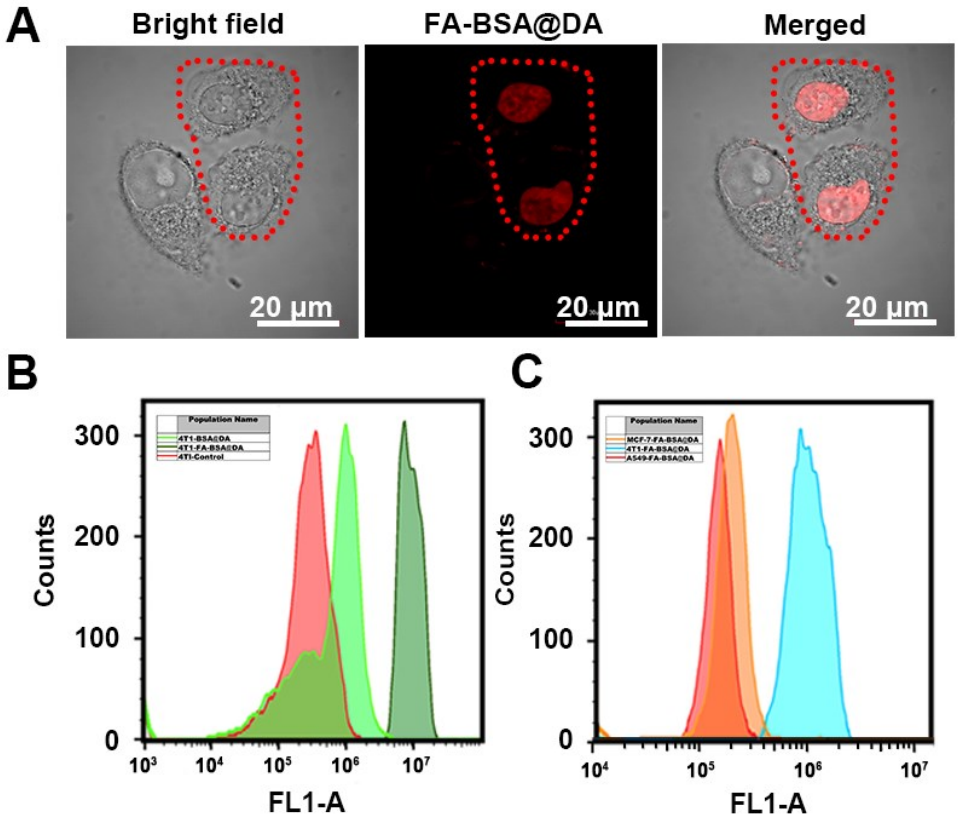


Figure S6. (A) Confocal microscopy imaging of the selectively of FA-BSA@DA in mixed cells (4T1 and MCF-7 cells, 4T1 cells was circled in red); (B) The affinity profile of 4T1 cells to BSA@DA and FA-BSA@DA, respectively. (C) The affinity profile of FA-BSA@DA to various cell lines (A549, MCF-7, and 4T1cell).

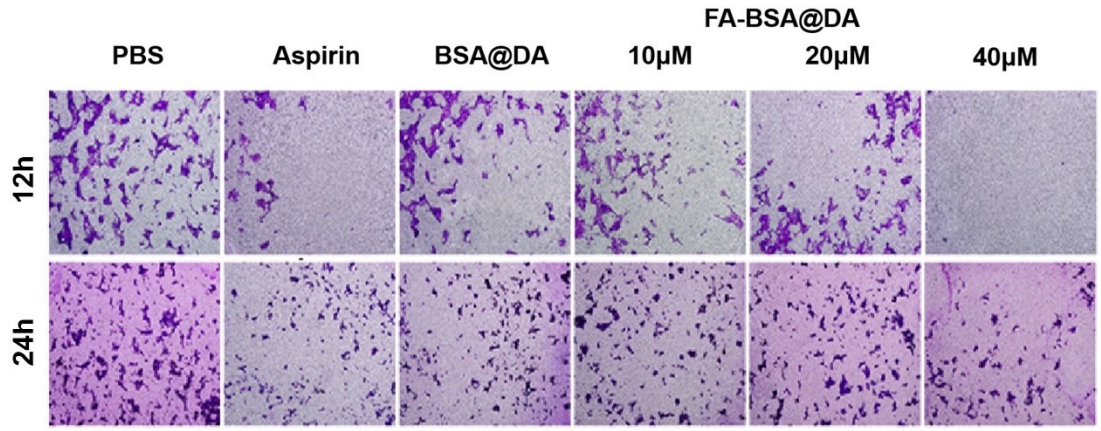


Figure S7. The images of cell migration after treatment with PBS, aspirin, BSA@DA, and different concentration of FA-BSA@DA at 12h and 24h respectively.

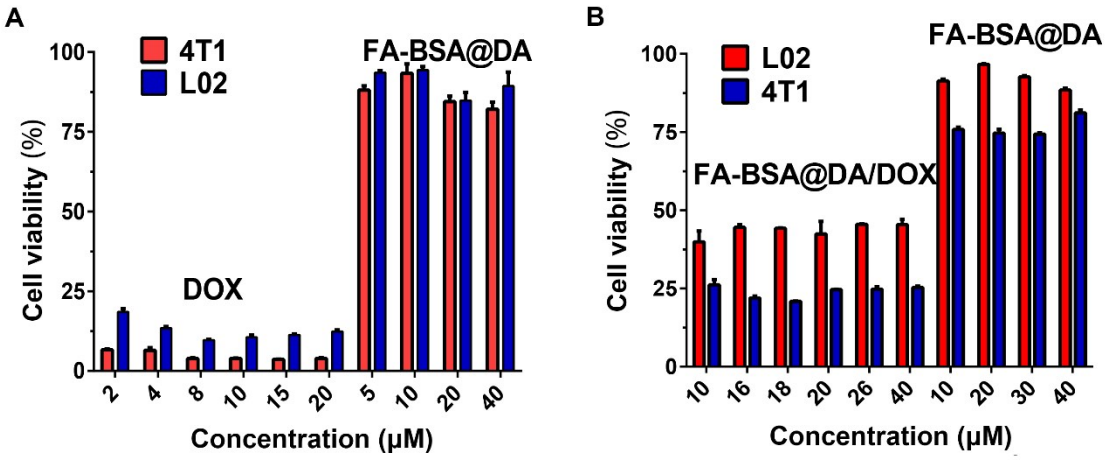


Figure S8. (A) The cytotoxicity of different concentration DOX and FA-BSA@DA nanoparticles to 4T1 cells and L02 cells. (B) The cytotoxicity in 4T1 cells and L02 cells by various concentration FA-BSA@DA and FA-BSA@DA/DOX nanoparticles.

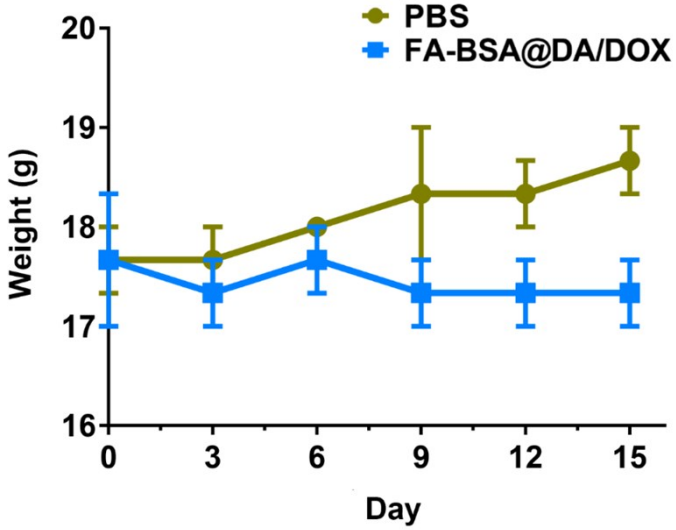


Figure S9. The weight of mice that were treated with PBS and FA-BSA@DA/DOX

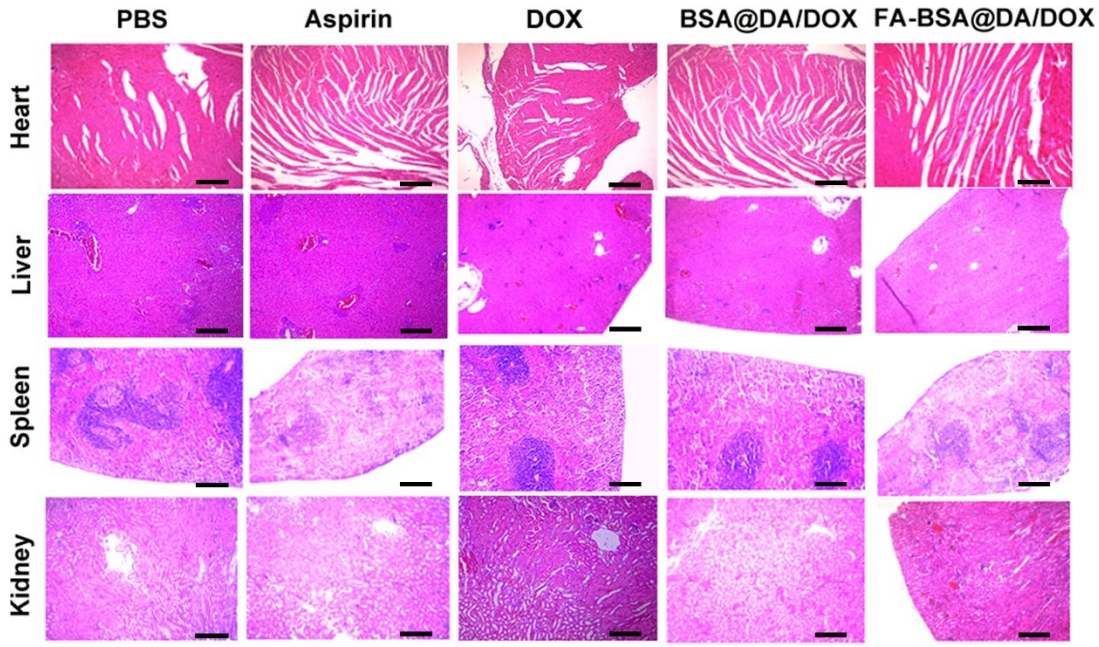


Figure S10. The H&E stained images of heart, liver, spleen and kidney on day 13 after treatment (scale bar: 100 μm).

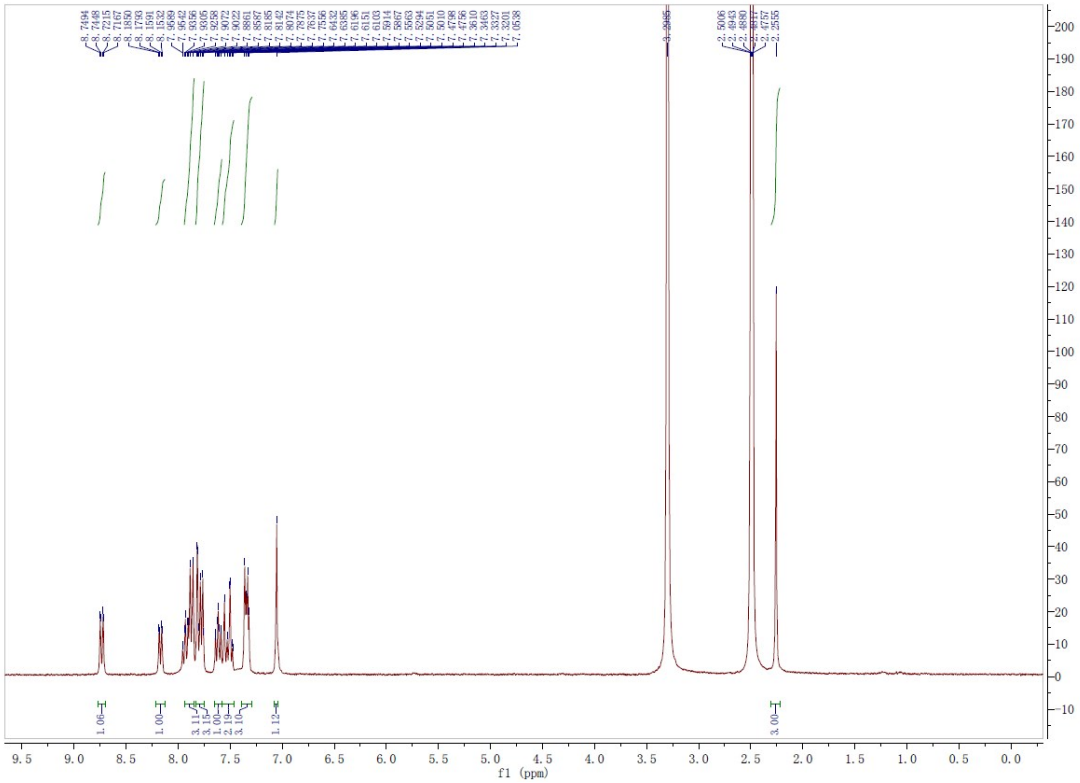


Figure S11. ^{1}H NMR spectrum of DA

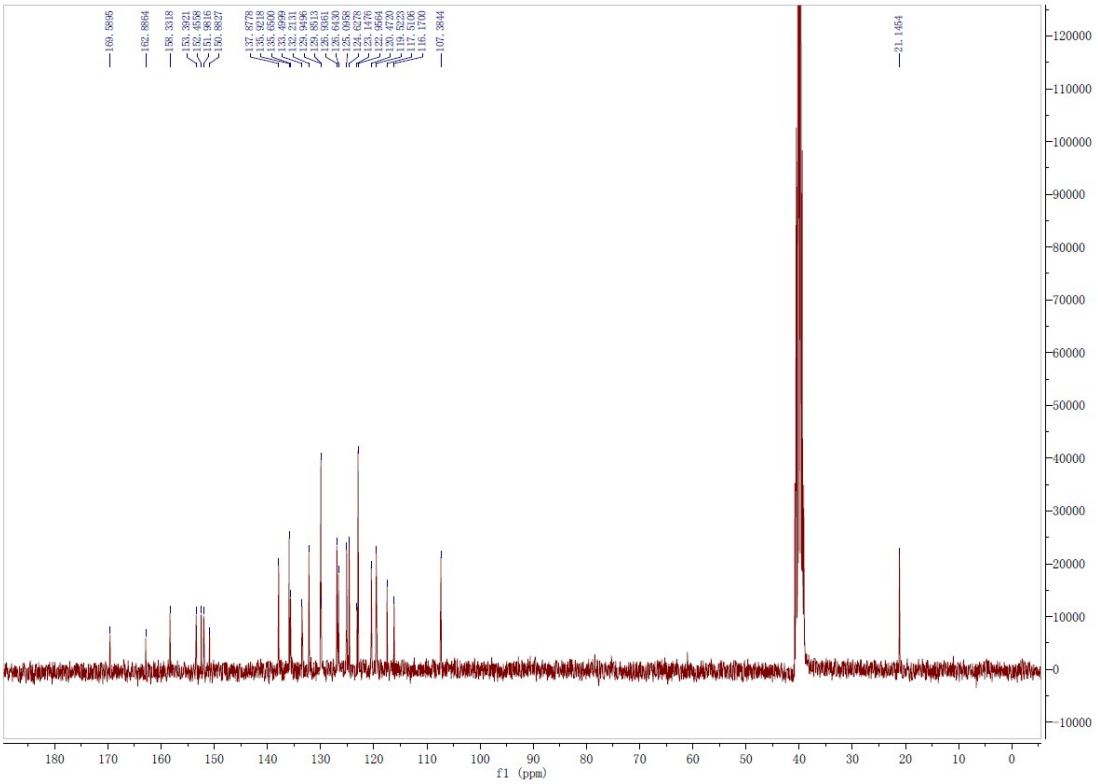


Figure S12. ^{13}C NMR spectrum of DA