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A−DA'D−A fused-ring small molecule-based nanoparticles for combined photothermal and photodynamic therapy of cancer

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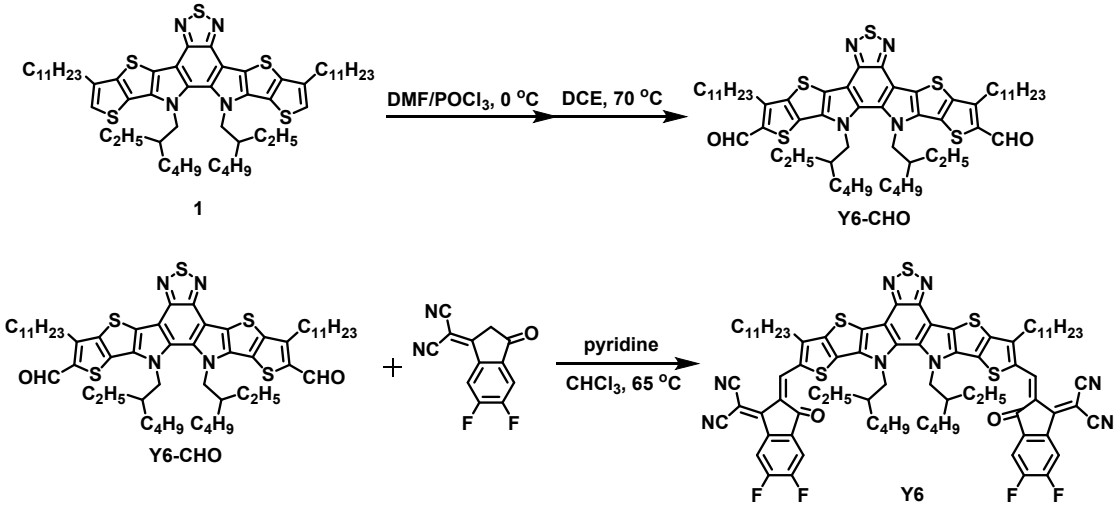
Materials

Unless stated otherwise, all the chemical reagents and solvents were obtained commercially and used without further purification. Compound 1 was purchased from SunaTech Inc. DSPE-PEG2000 was purchased from Shanghai yuanye Bio-Tech Inc. 2-(5,6-difluoro-3-oxo-2,3-dihydro-1H-inden-1-ylidene)malononitrile (2FIC) was synthesized via the reported method.[S1] Human cervical cancer cells (HeLa cells) were ordered from Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China). Fetal bovine serum (FBS) were purchased from PAA Laboratories (Austria). Other organic reagents were purchased from Meryer Chemical Inc and TCI Chemical Inc.

Synthesis



Scheme S1. The synthesis route for Y6

Y6-CHO[S2] A Vilsmeier reagent, which was prepared with phosphorus oxychloride (0.40 mL) in DMF (2.0 mL), was added to a solution of compound 1 (48.6 mg, 0.05 mmol) in 1,2-dichloroethane (20 mL) under the protection of argon. The mixture was stirred at 85 °C for 20 h. After cooling down to room temperature, the mixture was poured into ice water (50 mL) and then extracted with chloroform (2

× 50 mL). After removal of the solvent under reduced pressure, the residue was purified by column chromatography on silica gel using using petroleum ether/dichloromethane (1:1) as eluent to give an orange solid (47 mg, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.07 (s, 2H), 4.63 – 4.51 (m, 4H), 3.13 (t, *J* = 7.6 Hz, 4H), 1.97 – 1.81 (m, 6H), 1.42 – 1.37 (m, 4H), 1.30 (s, 4H), 1.21 – 1.18 (m, 28H), 0.89 – 0.77 (m, 18H), 0.62 – 0.50 (m, 12H).

Y6 [S2] To a three-necked round bottom flask were added **Y6-CHO** (51 mg, 0.05 mmol), 2FIC (47 mg, 0.20 mmol), pyridine (0.2 mL) and chloroform (20 mL). The mixture was stirred at reflux for 20 h. The mixture was washed by methanol (100 mL) and filtered. After removal of the solvent under reduced pressure, the residue was purified by column chromatography on silica gel using dichloromethane as eluent to give a dark blue solid (46 mg, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.13 – 9.08 (m, 2H), 8.56 – 8.48 (m, 2H), 7.71 (t, *J* = 7.5 Hz, 2H), 4.91 – 4.71 (m, 4H), 3.26 – 3.13 (m, 4H), 2.23 – 2.15 (m, 2H), 1.89 – 1.81 (m, 4H), 1.56 – 1.45 (m, 4H), 1.29 – 1.25 (d, 32H), 1.14 – 0.97 (m, 12H), 0.86 (t, *J* = 6.8 Hz, 6H), 0.82 – 0.76 (m, 6H), 0.72 – 0.67 (m, 6H).

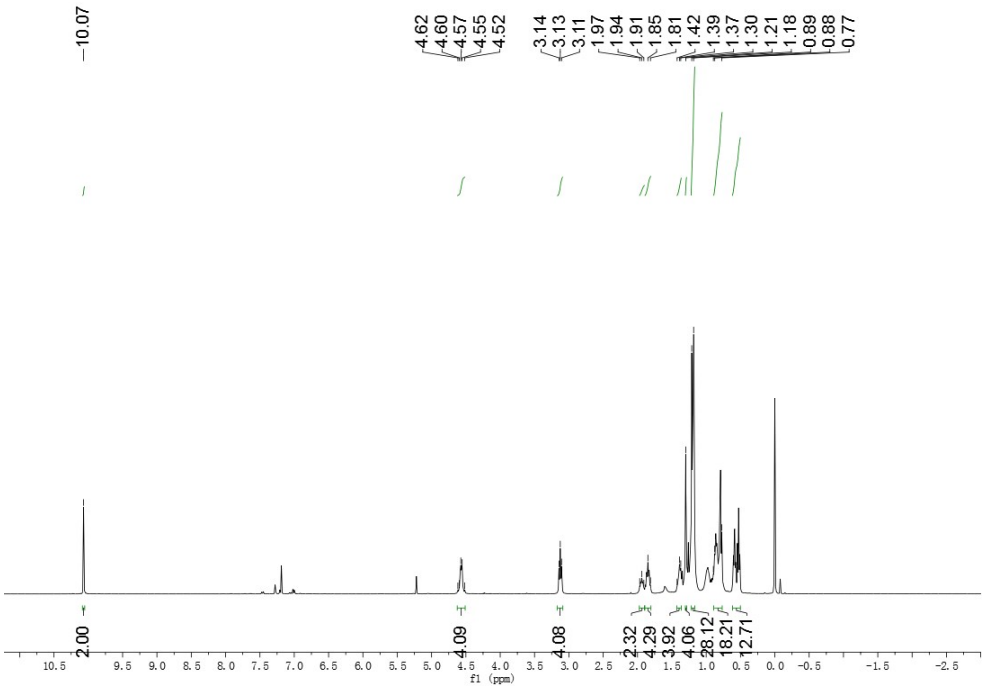


Fig. S1 the ^{1}H NMR spectrum of Y6-CHO

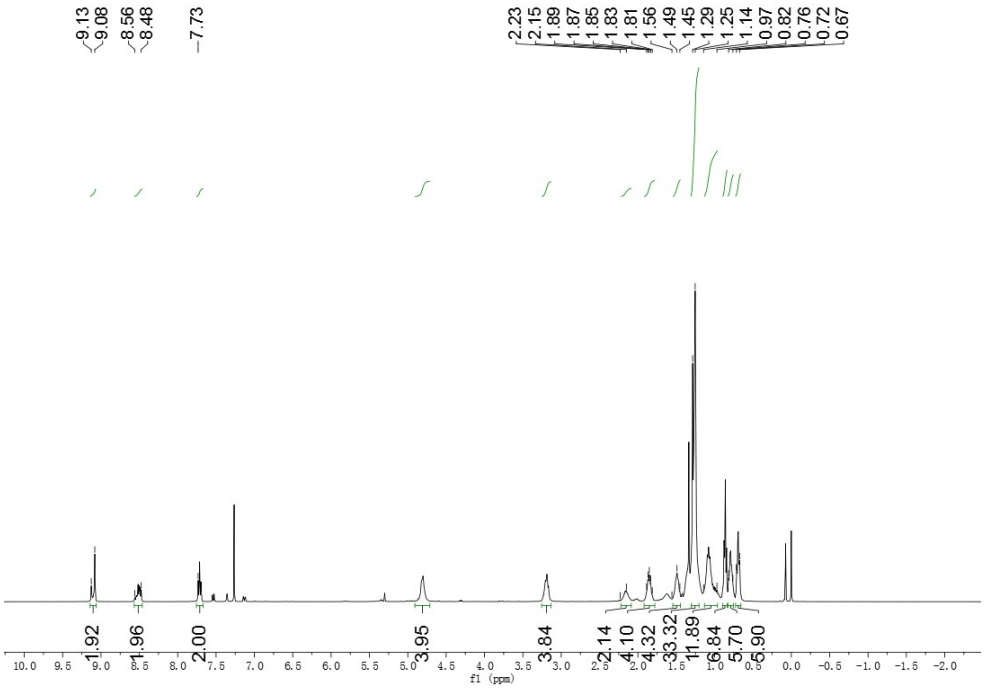


Fig. S2 the ^{1}H NMR spectrum of Y6

Y6 NPs Y6 (1 mg) and DSPE-PEG2000 (5 mg) were dissolved into THF (1 mL). Then the mixed solution was dropwise injected into 10 mL of deionized water under vigorous stirring. After the mixture was stirred for 2 h, the remaining THF was

removed through the nitrogen gas flow. The Y6 NPs aqueous solution was obtained after centrifugation.

Characterization

The ^{1}H NMR spectra were recorded using a Bruker AVANCE 400 MHz spectrometer. Scanning electron microscopy (SEM) investigations were carried out on a HitachiS-3400 SEM instrument. Dynamic light scattering measurements were performed on a goniometer ALV/CGS-3 using a UNIPHASE He-Ne laser operating at 632.8 nm. UV-Vis spectra were recorded in a quartz cell (light path 10 mm) on a Shimadzu UV-3600 spectrophotometer equipped with a PTC-348WI temperature controller. The output power of the laser was controlled by a fiber coupled laser system (LR-MFJ-808/2000mW, Changchun Lei Rui Optoelectronics Technology)

Photothermal experiment

Y6 NPs aqueous solution was continuously exposed to an 808 nm NIR laser (0.8 W/cm^2) for 12.5 min. The temperature was measured every 10 s using a digital thermometer with a thermocouple probe.

Photodynamic experiment

Y6 NPs aqueous solution (25 μM, 2.97 mL) in quartz cuvettes were mixed with DPBF in ethanol (30.0 μL, 10.0 mM), which was then irradiated by lasers at different power densities for a period of 240 s. The absorbance at 415 nm of the solution was recorded at the pre-set time points during the process. The absorbance at 415 nm of Y6 NPs aqueous solution without DPBF was also recorded, which was subtracted from the absorbance of the mixture to give the absorbance at 415 nm of DPBF. The

photodynamic experiments of ICG at same molarity were carried out following the same method.

Then the singlet oxygen quantum yield was calculated according to *eq* (1) [S3]

$$\Phi_{Y6} = \Phi_{ICG} \text{ x } (S_{Y6} / S_{ICG}) \text{ x } (F_{ICG} / F_{Y6}) \quad (1)$$

Where S is the slope of a plot of the absorbance of DPBF (at 415 nm) versus irradiation time, and F is calculated by $F = 1\text{-}10^{-OD}$, where OD represents the absorbance of Y6 and ICG at 808 nm.

Cell experiments

All the cell-based studied was carried out via the literature methods.[S4,S5]

Cytotoxicity experiments

HeLa cells were incubated in Dulbecco's modified Eagle's medium (DMEM). The medium was supplemented with 10% fetal bovine serum and 1% Penicillin-Streptomycin. HeLa cells were seeded in 96-well plates (5×10^4 cell mL^{-1}, 0.1 mL per well) for 24 h at 37 °C in 5% CO_2. Then DMEM containing different concentrations of Y6 NPs was introduced to replace the original medium. Four hours later, the cells were treated with or without an 808 nm laser (1 W cm^{-2}). After 10 min irradiation, HeLa cells were cultured for the next 24 h. The relative cellular viability was determined by the MTT assay.

Intracellular ROS detection

HeLa cells were incubated with 5 μM of Y6 NPs for 4 h followed by incubation with 10 μM DCFH-DA for 30 min. After being washed by PBS buffer for three times, cells were irradiated with 808 nm laser at a power density of 1.0 W cm^{-2} for 10 min.

Then, the fluorescence was immediately observed using confocal laser scanning microscopy.

Live-Dead Cell Staining

The same density of HeLa cells (3×10^5 cell mL^{-1}) were distributed into three confocal dishes (35 mm) for 12 h. Then the 2-plate cells were cultured with new DMEM containing Y6 NPs (25 μM). After 5 h, the cells were subjected to dark or laser irradiation (808 nm, 0.5 W cm^{-2}, 10 min). After 48 h, the cells were stained with a calcein AM/propidium iodide mixture for 30 min and washed twice using PBS. The fluorescence images eventually acquired via a confocal laser scanning microscope.

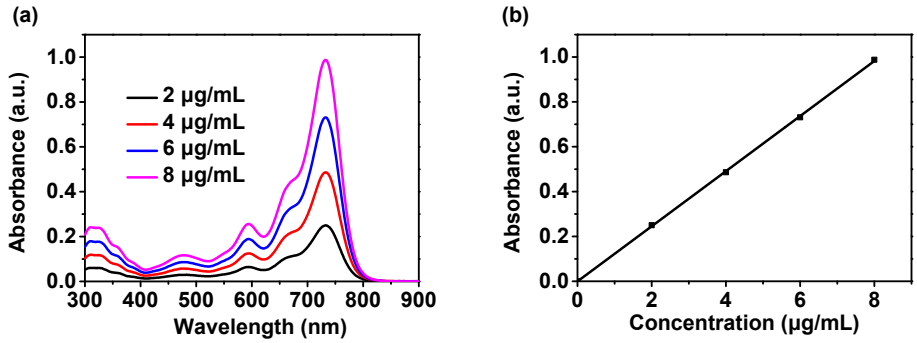


Fig. S3 (a) UV absorbance spectra of Y6 in $CHCl_3$ at different concentrations; (b) the standard curves of Y6 versus concentrations

The calculation method for the molar concentration of Y6

Y6 NPs were frozen dehydrated and then dissolved into $CHCl_3$. Then the UV absorbance at 732 nm for the mixture of Y6 and DSPE-PEG2000 can be obtained. The molar concentration of Y6 can be calculated through this absorbance at 732 nm and the standard curves of Y6 versus concentrations.

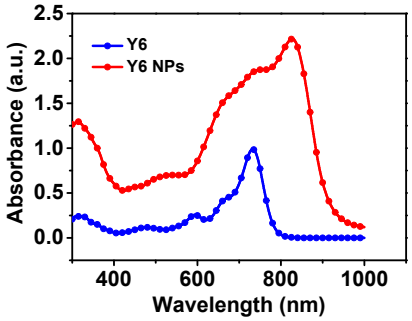


Fig. S4 The absorption spectra of Y6 in $CHCl_3$ and Y6 NPs aqueous solution

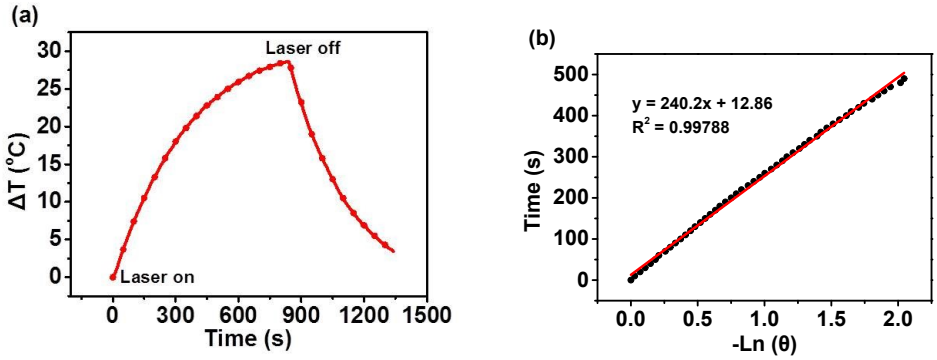


Fig. S5 (a) The temperature change curve of Y6 NPs aqueous solution with and without laser irradiation; (b) the fitting line of the cooling period versus the negative natural logarithm of the temperature decrease.

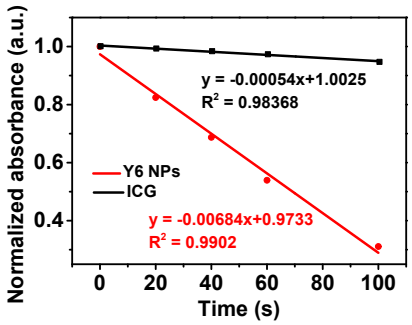


Fig. S6 The absorbance changes of DPBF in Y6 NPs and ICG aqueous solution under laser irradiation (808 nm, 1.0 W/cm^2)

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S5. Y. Ding, Y. Ma, C. Du, C. Wang, T. Chen, Y. Wang, J. Wang, Y. Yao and C.-M. Dong, *Acta Biomater.*, 2021, **123**, 335−345.