Electronic Supplementary Information

Chiral amino acid modified boron-dipyrromethene nanoparticles with different photodynamic activities

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Experimental section

Materials and characterizations

1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl) was purchased from Shanghai Yuanye Biological Technology Co., Ltd.. 1,3-Diphenylisobenzofuran (DPBF) was bought from TCI (Shanghai) Development Co., Ltd.. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and 2′,7′-dichlorofluorescin diacetate (DCFH-DA) probe were purchased from Shanghai Beyotime Biological Technology Co., Ltd.. SYTO was purchased from Jiangsu KeyGEN Biotechnology Co., Ltd.. Cell culture dishes were purchased from Guangzhou Jet Bio-Filtration Co., Ltd.. 96-Well plates were purchased from Wuxi NEST Biotechnology Co., Ltd.. PI Cell Viability/Cytotoxicity Assay Kit were purchased from Shanghai Beyotime Biological Technology Co., Ltd.. The other chemicals were used as obtained commercially. Analytical balance (XS105DU) and Rainin Pipettes from METTLER TOLEDO were used to quantify solid and liquid respectively. TEM and DLS results were determined by JEOL JEM-1011 electron microscope (acceleration voltage of 100 kV) and Malvern Zeta-sizer Nano. CLSM images were obtained from a Zeiss LSM 700 (Zurich, Switzerland). The energy levels were calculated using density functional theory (DFT) at the B3LYP/6-31 G* level by Gaussian 09 package.

Synthesis of BDP-Asp

BDP-COOH and BDP were synthesized via reported methods.[1,2] Then, BDP (100 mg), NHS (149 mg), and EDC·HCl (170 mg) were added to anhydrous dichloromethane (10 mL) and reacted for 12 h. BDP-NHS was obtained by column chromatography. Then, BDP-NHS (30 mg), triethylamine (150 μL), and L-ASP or D-Asp (37 mg) were added to DMSO (2 mL) and reacted for 24 h. BDP-LAsp and BDP-DAsp were obtained by column chromatography.

Preparation of NPs

BDP-LAsp (1 mg) was dissolved in DMSO (1 mL), and then added into deionized water (9 mL) under stirring. After stirring for 1 h, the solution was dialyzed for 24 h to remove DMSO and obtain BDP-LAsp NPs. BDP-DAsp NPs were obtained according to the same method.

Reactive oxygen species (ROS) generation detection

DPBF was used as a probe to determine the ability of BDP-LAsp NPs and BDP-DAsp NPs for singlet oxygen generation. Briefly, 50 μL of DPBF (1 mg mL^{-1} in DMSO) and 1 mL of BDP-LAsp NPs or BDP-DAsp NPs (10 μM in water) were added into water (1.3 mL), and DPBF alone in water was used as the control. The mixed solution was irradiated with green LED light (12 mW cm^{-2}) for 140 s, and the absorption spectra of the mixed solution were measured every 20 s.

ROS production was further detected by 2′,7′-dichlorofluorescein (DCFH). First,

10 μL of 2′,7′-dichlorofluorescein diacetate (DCFH-DA, 10 mM) was added into 90 μL of NaOH (1 mM) aqueous solution. The solution was sonicated for 15 min to cleave the ester bond and generate DCFH, then 900 μL of PBS was added. After that, 100 μL of DCFH solution was added into BDP-LAsp NPs or BDP-DAsp NPs (2 mL, 5 μM). The mixed solution was irradiated with green LED light (12 mW cm^{-2}) for 270 s, and then the fluorescence changes were measured with an excitation wavelength of 480 nm every 30 s.

Cell lines and cell culture

HeLa (human cervical carcinoma) and 4T1 (mouse breast cancer) cells were cultured in Dulbecco's modified Eagle's medium (DMEM) and Roswell Park Memorial Institute 1640 (RMPI 1640) with 10% (v/v) FBS. Cells were cultured in a humidified incubator at 37 °C with 5% CO_2, and the culture medium was replaced once every day.

Cell viability assays

The cytotoxicity of BDP NPs against different cells was examined via MTT assays. HeLa and 4T1 cells were seeded in 96-well plates. After adhering overnight, the incubation media were discarded and replaced with BDP-LAsp NPs or BDP-DAsp NPs diluted with the fresh media to desired concentrations. Then, the cells were incubated for an additional 4 h at 37 °C and irradiated with green LED light (12 mW cm^{-2}) for 10 min or not. After 24 h, 20 μL of MTT (5 mg mL^{-1} in PBS) was added and

the cells were incubated at 37 °C for another 4 h. Then, the culture medium supernatant was carefully removed and 150 μL of DMSO was added to each well to dissolve the formed violet formazan crystals. Ultimately, the plates were shaken for 4 min, and the absorbance of violet product was determined at 490 nm by a microplate reader.

Cellular uptake of BDP NPs measured by CLSM

The cellular uptake of BDP NPs was evaluated by CLSM. First, HeLa cells were seeded in six-well culture plates and allowed to adhere for 24 h. Then, the medium was removed and replaced by BDP NPs (5 μM) and incubated for an additional 4 h at 37 °C. After being stained by Hoechst 33258 for 8 min to track cellular nuclei, the cells were imaged by CLSM.

Intracellular detection of ROS

As a fluorescent ROS probe, DCFH-DA was used to detect ROS generation in cells. First, HeLa cells were treated with or without BDP NPs (5 μM) for 4 h, and then irradiated with green LED light (12 mW cm^{-2}) for 10 min or not, followed by washing with PBS for 3 times. Subsequently, DCFH-DA was added, and cells were incubated for 30 min. After the media were removed, the cells were washed with PBS and the samples were imaged by CLSM.

Live-dead cell staining

HeLa cells were pretreated with PBS or BDP-Asp NPs (3 μM), and 4 h later, the

cells were irradiated with green LED light (12 mW cm^{-2}) for 10 min or not. After 20 h of incubation at 37 °C, cells were stained with calcein-AM/propidium iodide (PI) solution for 30 min at room temperature. Finally, the samples were imaged by a fluorescence microscope.

Bacterial culture

The single colony on the solid agar was transferred to 3 mL of broth medium, and the bacteria were in exponential growth stage after shaking at 37 °C for 6 h. The bacteria were centrifuged at 3000 rpm for 4 min and washed with PBS. After discarding the supernatant, the bacteria were suspended in PBS and diluted to an optical density (OD) at 600 nm of 1.0 (OD_{600}=1.0 with about 10^9 CFU mL^{-1}).

Antibacterial performance of BDP NPs

The bacteria (OD_{600}=1.0) were diluted to 2×10^5 CFU mL^{-1} with broth medium by serial dilution. The bacteria (2×10^5 CFU mL^{-1}, 1 mL) were mixed with different concentrations of NPs (1 mL per group) and the final bacteria concentration was 10^5 CFU mL^{-1}, then the mixture was placed into 96-well plate. The bacteria were irradiated with/without green LED light (12 mW cm^{-2}) for 10 min after being co-cultured at 37 °C for 30 min.

The antibacterial ability of BDP-LAsp NPs and BDP-DAsp NPs was confirmed by bacterial colony counting. *S. aureus* (10^5 CFU mL^{-1}) were incubated with BDP-Asp NPs (1.5 μM) or PBS for 30 min, followed by irradiation with green LED light

(12 mW cm^{-2}) for 10 min or not. After that, the bacterial suspensions were diluted 100 fold, and then the diluted bacterial suspensions (100 μL) were inoculated on LB agar plates. After incubation at 37 °C for 24 h, the agar plates were photographed.

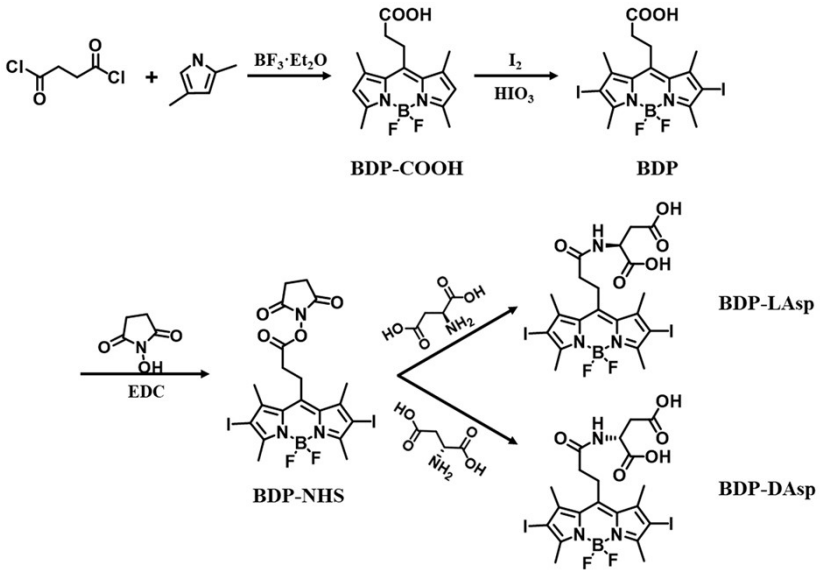

Fig. S1 The synthetic routes of BDP-LAsp and BDP-DAsp.

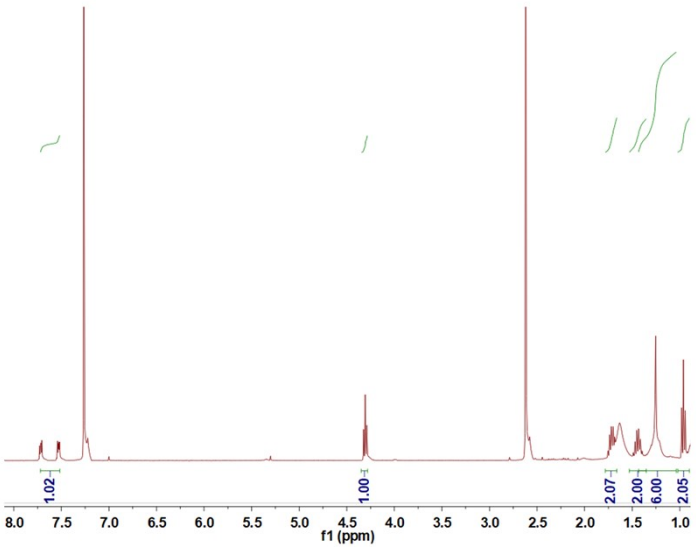

Fig. S2 ^{1}H NMR spectrum of BDP-LAsp.

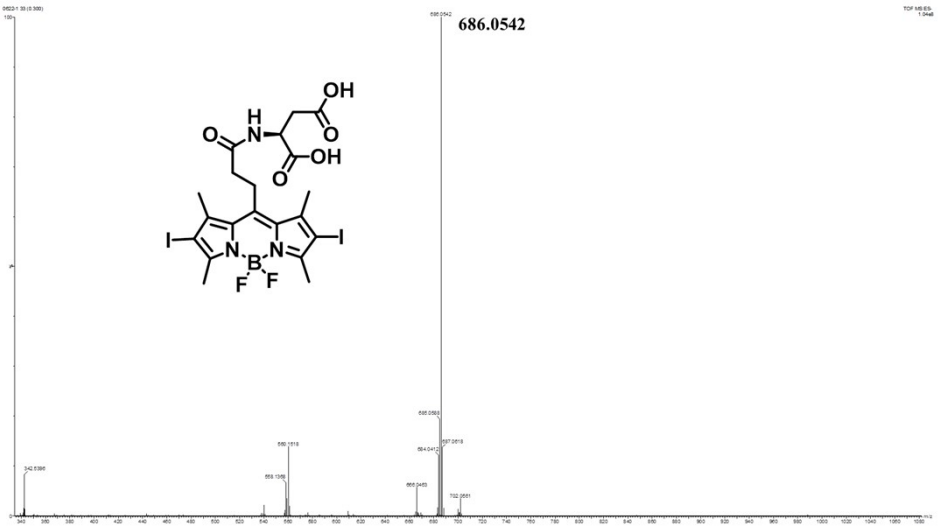

Fig. S3 Mass spectrum of BDP-LAsp.

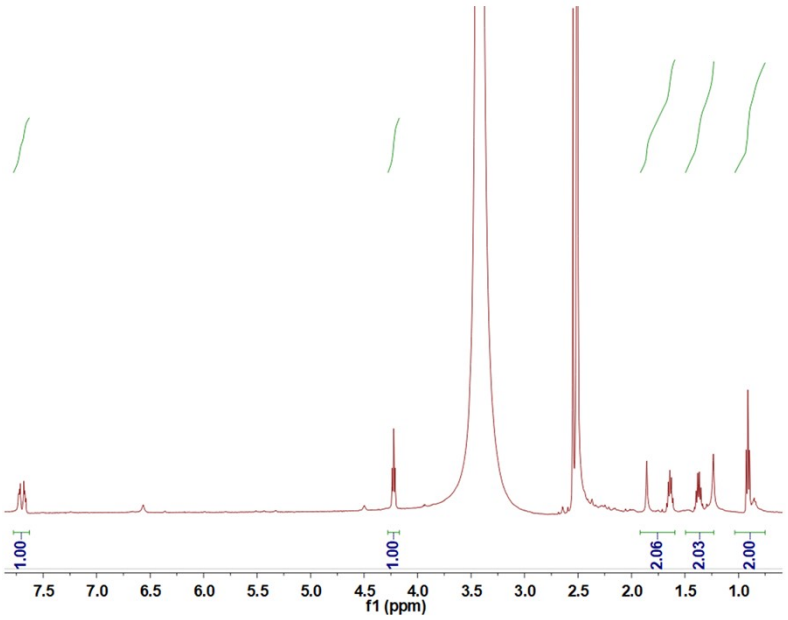

Fig. S4 ^{1}H NMR spectrum of BDP-DAsp.

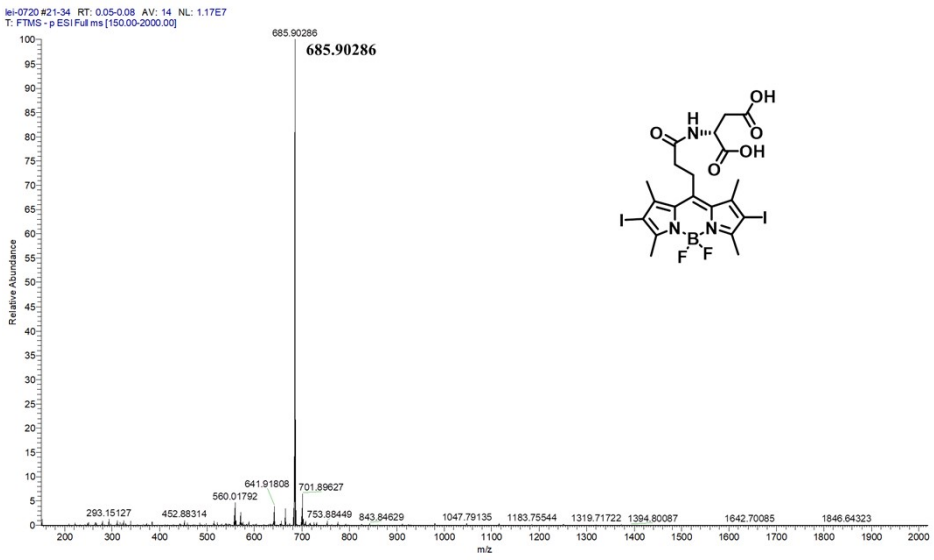

Fig. S5 Mass spectrum of BDP-DAsp.

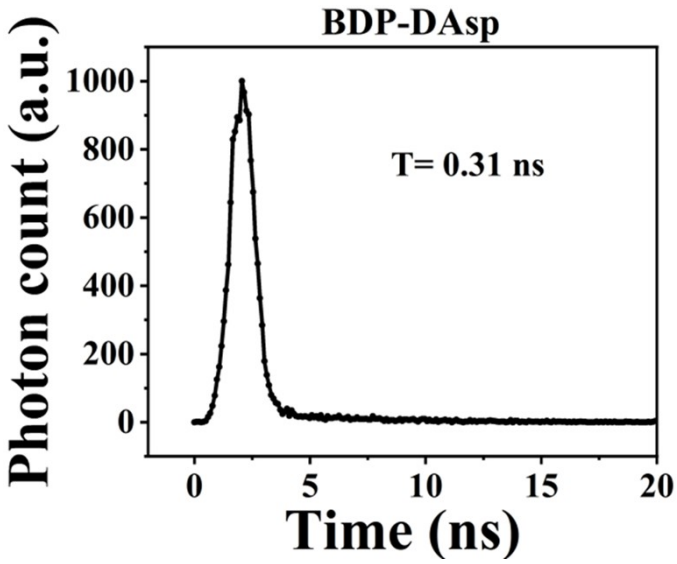

Fig. S6 The fluorescence lifetime of BDP-DAsp.

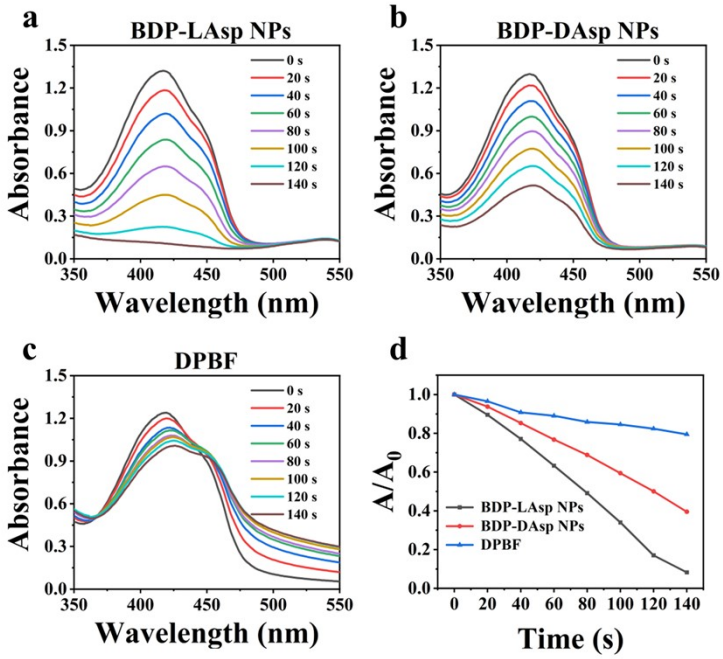

Fig. S7 The absorption spectra change of DPBF induced by ROS generation from (a) BDP-LAsp NPs, (b) BDP-DAsp NPs, and (c) water with green LED light irradiation (12 mW cm^{-2}). (d) Ratio of the absorbance of DPBF (A) to the initial absorbance (A_0) under irradiation for different times.

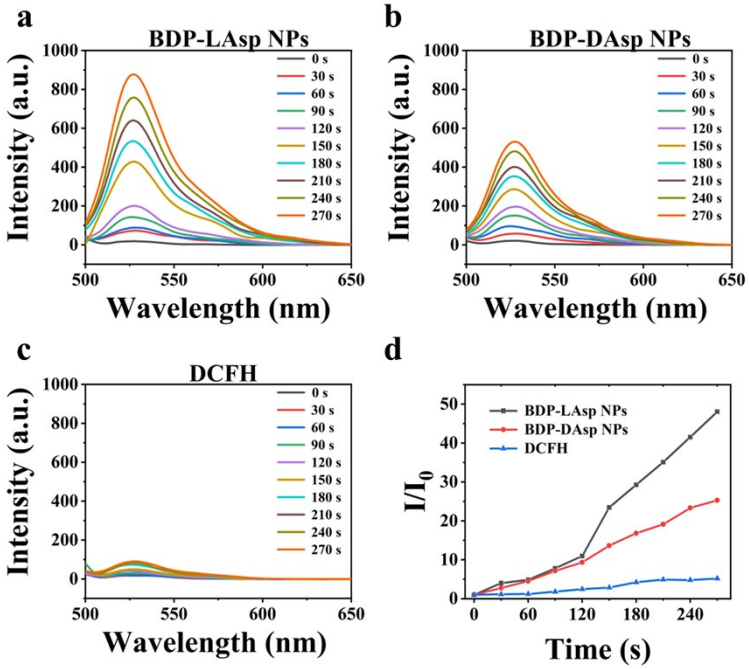

Fig. S8 The fluorescence spectra change of DCF induced by ROS generation from (a) BDP-LAsp NPs, (b) BDP-DAsp NPs, and (c) water with green LED light irradiation (12 mW cm^{-2}). (d) Ratio of the fluorescence intensity of DCF (I) to initial intensity (I_0) under irradiation for different times.

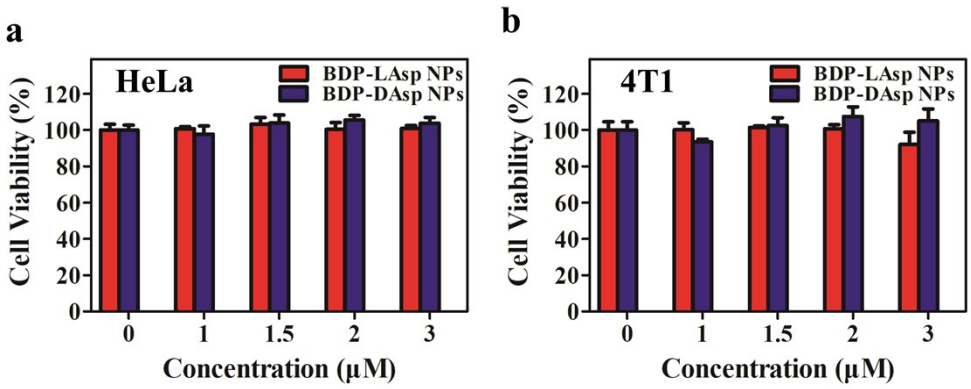

Fig. S9 Cytotoxicity of different concentrations of BDP-LAsp NPs and BDP-DAsp NPs (0–3 μM) against (a) HeLa and (b) 4T1 cells without green light irradiation.

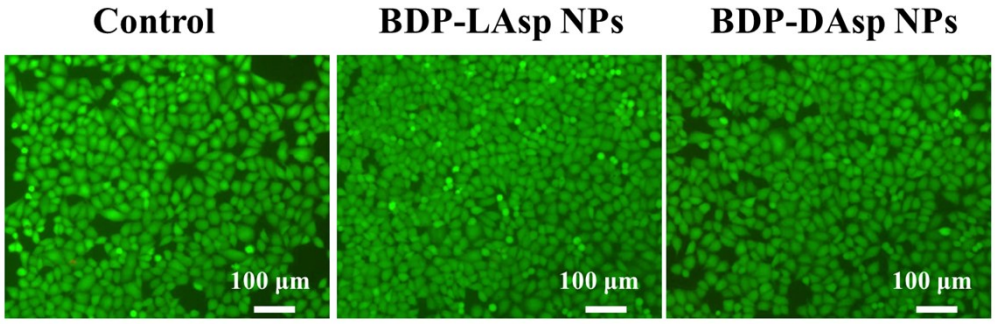

Fig. S10 Fluorescence images of calcein-AM/PI co-stained HeLa cells treated with BDP-LAsp NPs and BDP-DAsp NPs (3 μM).

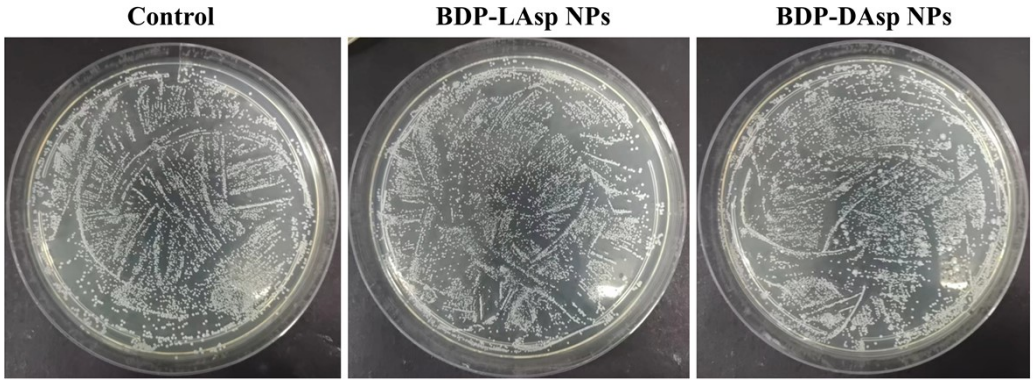

Fig. S11 Photos of bacterial colonies of *S. aureus* treated with PBS, BDP-LAsp NPs, or BDP-DAsp NPs without green light illumination.

Reference

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2 M. Gonzalez-Bejar, M. Liras, L. Frances-Soriano, V. Voliani, V. Herranz-Perez, M. Duran-Moreno, J. M. Garcia-Verdugo, E. I. Alarcon, J. C. Scaiano and J. Perez-Prieto, *J. Mater. Chem. B*, 2014, **2**, 4554-4563.