Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

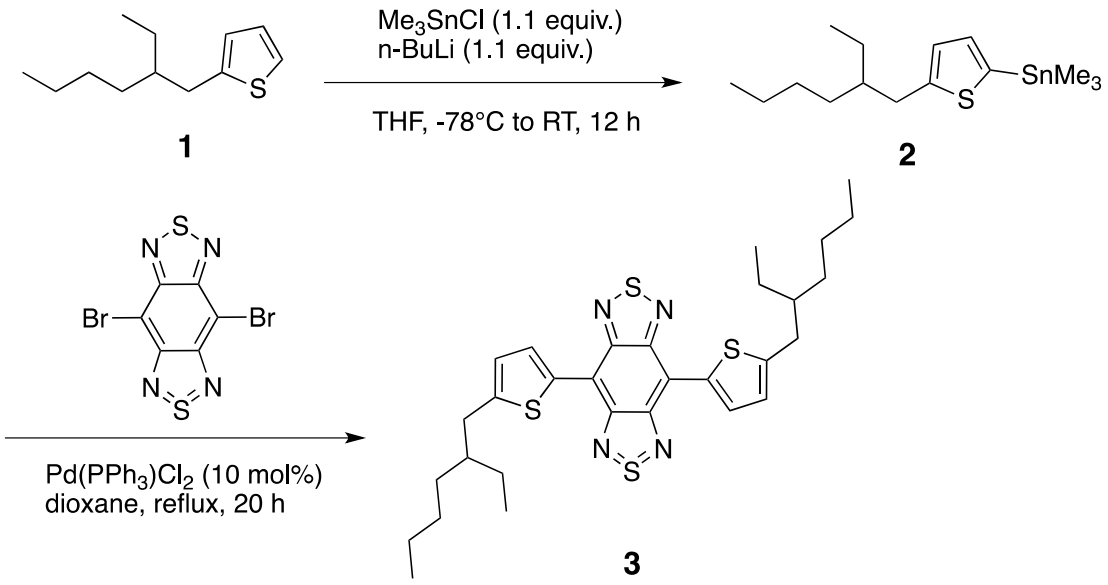
A narrow-bandgap benzobisthiadiazole derivative with high near-infrared photothermal conversion efficiency and robust photostability for cancer therapy

Shuo Huang,[a] *Ravi Kumar Kannadorai,*[a] *Yuan Chen,*[a] *Quan Liu,*[a,]* *Mingfeng Wang*[a,]*

[a]School of Chemical and Biomedical Engineering, Nanyang Technological University, 62 Nanyang Drive, Singapore 637459

Correspondence: E-mail: Q. Liu (quanliu@ntu.edu.sg); M. Wang (mfwang@ntu.edu.sg)

Supplementary Methods



(5-(2-ethylhexyl)thiophen-2-yl)trimethylstannane(compound 2). In a two neck 100 mL round bottom flask, to the solution of 2-ethylhexyl)thiophen (**1**) (2.11 g, 10.75 mmol) in 50 mL of THF was dropwise added *n*-BuLi (11.8 mmol, 4.72 mL of 2.5 M in hexane) at -78 ^{o}C under Ar atmosphere. The resulting mixture was stirred for 1 h at the same temperature and then allowed to warm to 0 ^{o}C and stirred at this temperature for 30 min, and again cooled to -78 ^{o}C, followed by slow addition of Me_3SnCl (11.8 mmol, 11.8 mL of 1.0 M in hexane). The resulting solution was slowly warmed to room temperature and stirred overnight. The reaction was quenched with water and extracted with dichloromethane (30 mL x 2), the combined organic layers was dried over $MgSO_4$, filtered, concentrated to give a yellow oil (3.55 g, 92%) compound (**2**) which was used directly for the next step without further purification.

4,8-di(2-ethylhexyl)thiophen-2-yl)-benzo[1,2-c;4,5-c′]bis[1,2,5]thiadiazole (compound 3). 4,8-dibromo--benzo[1,2-c;4,5-c′]bis[1,2,5]thiadiazole (1.03 g, 2.9 mmol),

$Pd[PPh_3]_2Cl_2$ (0.21 g, 0.29 mmol), **Compound 2** (2.48 g, 6.9 mmol) and anhydrous 1,4-dioxane (43 mL) was added into a round bottom 100 mL Schlenck flask. The mixture was bubbled with Ar for 10 min and then refluxed under N_2 overnight. The mixture was cooled down to room temperature under N_2. Then 2-trimethylstanny-3-hexylthiophene (1.2 mL) and $Pd[PPh_3]_2Cl_2$ (0.2 g) was added to the reaction mixture. The mixture was bubbled with Ar for 10 min and refluxed again under N_2 for 24 h. The mixture was cooled down to room temperature and poured into methanol (150 mL). The crude product was purified further by column chromatography (silica gel, hexane/toluene (4:1, v/v)) to give dark green solid BBTEHT (337mg, 20%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta =$ 8.71 (s, 2H), 6.90 (s, 2H), 2.82 (d, J = 6.9 Hz, 4H), 1.77 (s, 2H), 1.30 (br, m, 16H), 0.87 (m, 12H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta =$ 150.97, 150.79, 135.54, 132.25, 126.51, 113.18, 34.98, 34.36, 32.41, 28.84, 25.73, 23.13, 14.22, 10.94 ppm; MALDI-TOF-MS m/z: 582.3 (M^+); M_w calcd. for $C_{30}H_{38}N_4S_4$ = 582.9; T_m = 122 ℃; FT-IR (KBr, cm^{-1}): 2956, 2923, 2857, 1442, 1376, 1115, 932, 869.

General procedure for the synthesis of the nanoparticles:

A mixture of 5 mg of BBTEHT and 83 mg of Pluronic 127 was completely dissolved in 1 *mL of* THF overnight. Then 10 *mL* of deionized-water was quickly injected into the mixture under vigorous stirring at room temperature. After being stirred for 5 min, the dispersion was dialyzed against deionized-water by 4 KDa dialysis membranes for 48 h to remove THF. The NPs were separated by centrifugation at 10000 rpm for 5 min to remove unencapsulated

surfactant and then redispersed in deionized-water before characterization and cell study. The concentrations used for following measurements and cell study are calculated based on the amount of BBTEHT used in NP preparation.

General materials characterization:

Scanning electron microscopy (SEM) was performed on a Field Emission Scanning Electron Microscopy (FESEM, Model JEOL JSM 6701F) operated at 10 kV. Transmission electron microscope (TEM) measurements were carried out on a TEM Carl Zeiss Libra 120 Plus operating at an acceleration voltage of 120 kV. UV-vis transmission spectrum was recorded on a Varian Cary 4000 UV-Vis spectrophotometer. ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on a Varian Inova-400 Instrument (400 MHz). MADLI-TOF MS spectra were carried on MALDI TOF/TOFABI 4800.

Photothermal evaluation

The stock dispersion of BBTEHT NPs was diluted to 50, 35 and 20 μg/mL, respectively. At each concentration, a total of 1 *mL* dispersion was used in the evaluation of its photothermal effect under irradiation of a 808-nm laser with a power density of 1.77 W/cm^2. Every sample was irradiated for 20 min and allowed to cool down to the room temperature for the next 20 min, which was counted as one cycle. Water and gold nanorods at a concentration of 35 μg/mL served as the reference group and control group, respectively. The temperature was recorded using a T-type thermocouple probe (HYPO, Omega, Connecticut, USA) coupled to a thermocouple controller (NI 9211, National Instruments, Texas, USA), which was controlled by a computer.

Stability evalulation of BBTEHT NPs and gold nanorods

To test the stability of sample, and compare with gold nanorods, we irradiated 1-mL BBTEHT NPs and commercial gold nanorods under the 808-nm NIR laser with a power density of 1.77 W/cm^2 for 4 h. Temperature was recorded automatically every second. The UV-Vis spectra and TEM images of the samples were measured both before and after laser irradiation to evaluate the changes in the transmission spectrum and morphology.

Preparation of gold nanorods and cell line

CTAB-coated Au nanorods with an aspect ratio of 3.8 (10 *nm* in diameter and 38 *nm* in length) were purchased from Strem chemicals Inc. (Massachusetts, USA). The Au nanorods were delivered in water suspension with a concentration of about 35 μg/mL. To make the nanorods biocompatible, the Au nanorods were PEGylated with mPEG (Sigma-Aldrich, Missouri, United States). The 1-μM mPEG solution was incubated in GNR suspension with a ratio of 1:4 for 6 h and re-suspended in PBS for future use.

Human renal cell carcinoma cells (Caki-2 cell line) were purchased from American Type Culture Collection (Virginia, USA). Caki-2 cells were cultured in Iscove's Modified Dulbecco's Medium (IMDM) supplemented with 10% fetal bovine serum (FBS) and 500 μL of Antibiotic (Antimycotic, GE Healthcare, England), and then were incubated at 37°C with 5% CO_2. Cultures were maintained by adding or replacing the medium every 3-4 days to maintain a cell density of 10^5-10^6 cells per mL. Since Caki-2 cell lines adhered to the surface of the culture flask during each passaging, the adherent cells were removed by replacing the culture medium by 1× Trypsin (Life Technologies, CA, USA) and putting it back in the

incubator for 10 mins. Culture medium was then added to neutralise trypsin and cell counting was performed to check the cell density.

In the photothermal study, a total of 1-mL cell suspension with a cell density of ~1×10^6 cells/mL was transferred to a well plate, where it was mixed with either BBTEHT NPs or PEGylated Au nanorods and incubated for another 6 hours in the incubator. The BBTEHT NPs and PEGylated Au nanorods were washed twice with the culture medium prior to mixing with the Caki-2 cells. After 6 hours, the culture medium was removed and the petridish was replaced with PBS by washing the cells gently twice in PBS to get rid of Au nanorods in the extracellular matrix. To evaluate cell death due to necrosis, trypan blue solution (Sigma-Aldrich, Missouri, USA) was added to the cell sample and was left for 10 minutes in room temperature before the observation under a bright-field microscope. Dead cells were stained blue while live cells stayed transparent as their cytoplasm remained intact.

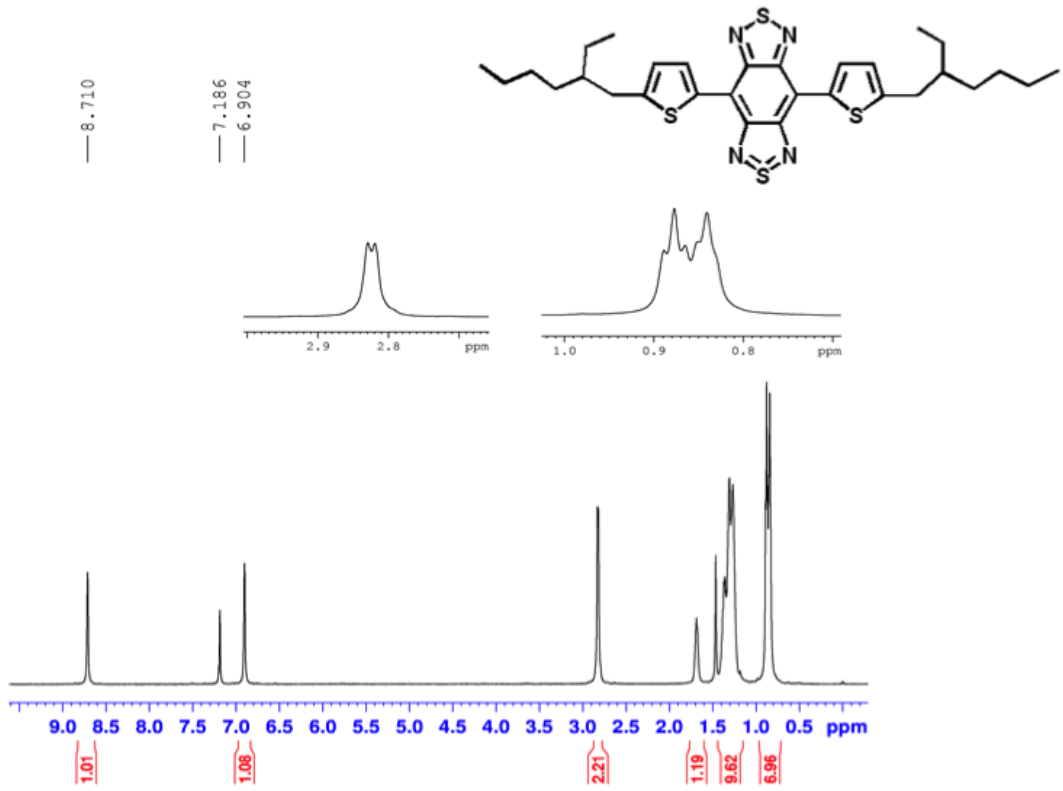


Figure S1. ^{1}H-NMR ($CDCl_3$, 400 MHz) spectrum of BBTEHT.

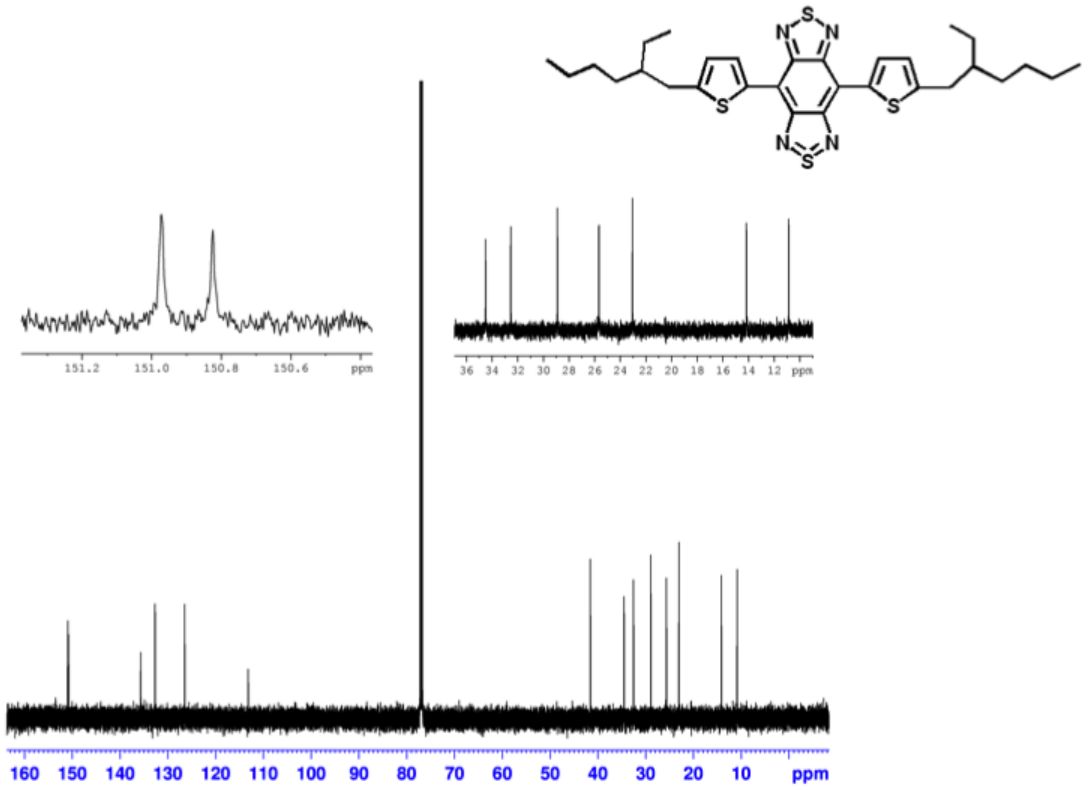


Figure S2. ^{13}C-NMR ($CDCl_3$, 100 MHz) spectrum of BBTEHT.

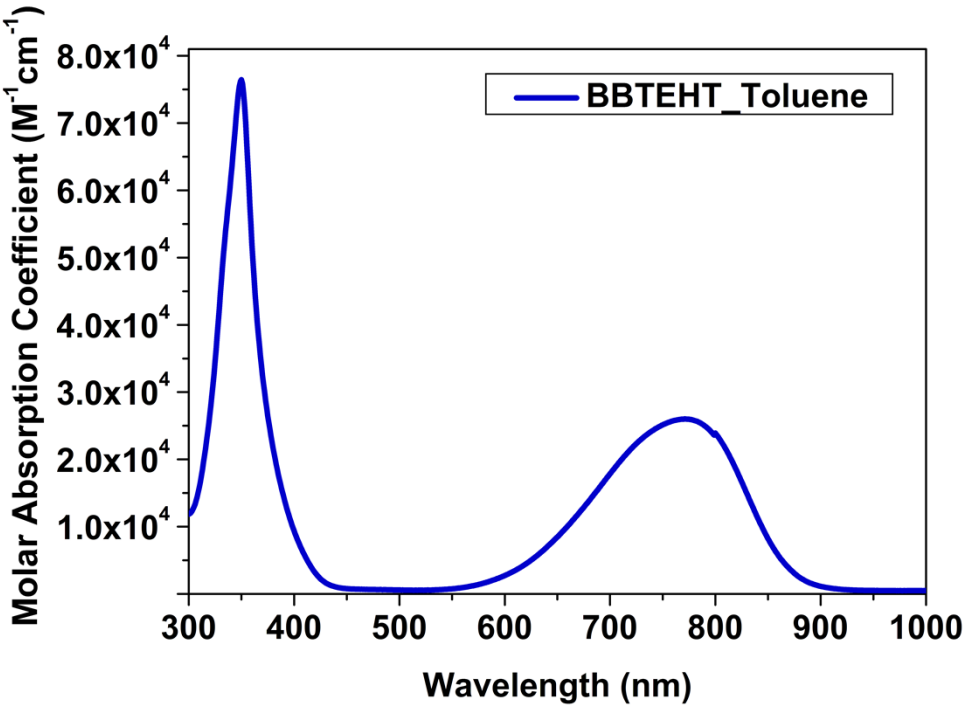


Figure S3. The molar absorption coefficient *vs.* wavelength graph of BBTEHT in toluene.

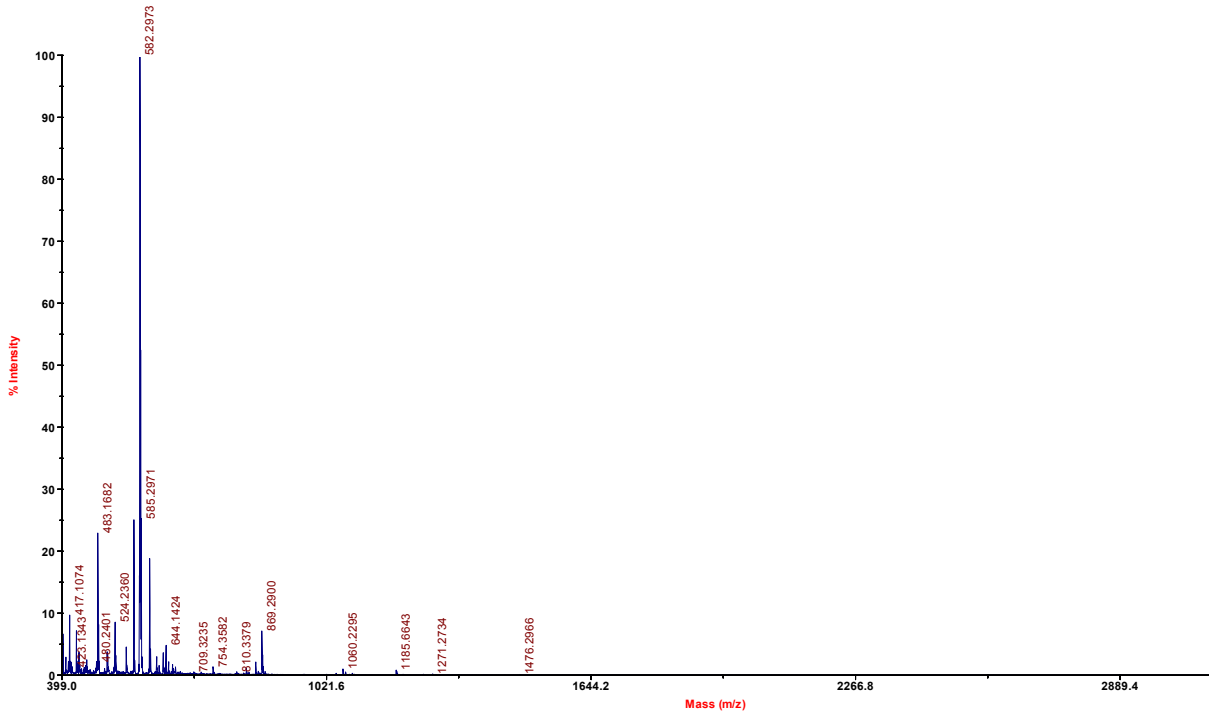


Figure S4. MADLI-TOF mass spectrum of BBTEHT.

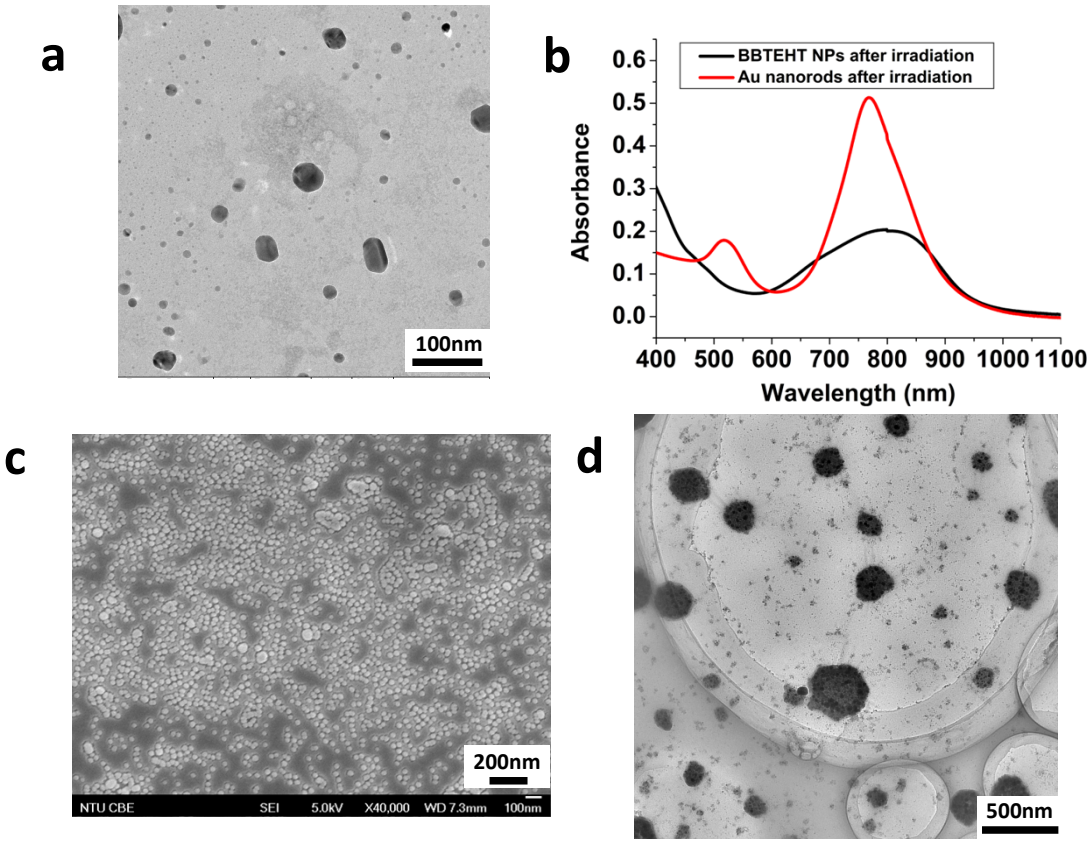


Figure S5. (a) TEM image of BBTEHT NPs from a dispersion of 35 μg/mL before laser irradiation (b) UV–VIS-NIR absorption spectra of BBTEHT NPs and Au nanorods after 4 h irradiations of a 808-nm laser with a power density of 1.77 W/cm^2. (c-d) SEM (c) and TEM (d) images of BBTEHT NPs after 4-h irradiation of a 808-nm laser with a power density of 1.77 W/cm^2.