Electronic Supplementary Information

A Novel Boron Ketoiminate-Based Conjugated Polymer with Large Stokes Shift: AIEE Feature and Cell Imaging Application

Chunhui Dai,*[a,d] Dongliang Yang,[b] Yanling Hu,[c] Yue Deng,[d] Xiaoman Yang,[d] and Zhonglin Liu[d]

[a] Jiangxi Key Laboratory for Mass Spectrometry and Instrumentation, East China University of Technology, Nanchang 330013, P. R. China

[b] School of Physical and Mathematical Sciences, Nanjing Tech University (Nanjing Tech), 30 South Puzhu Road, Nanjing 211816, P. R. China

[c] School of Electrical and Control Engineering, Nanjing Polytechnic Institute, 625 Geguan Road, Nanjing, 210048, P. R. China

[d] School of Chemistry, Biology, and Materials Science, East China University of Technology, Nanchang 330013, P. R. China

E-mail: daichunhui@163.com

Materials and Measurements

All organic solvents and chemical reagents were commercially available. PSMA was purchased from Sigma-Aldrich Chemical Co. Ltd. NMR spectra were measured using a 400-Bruker for ^{1}H NMR, 376 MHz for ^{19}F NMR and 100 MHz for ^{13}C NMR. Fluorescence spectra were obtained from a Shimadzu RF-5301PC Spectrofluorometer. UV-vis spectra were obtained using a Perkin-Elmer Lambda 35 spectrophotometer. Elemental analyses were performed on an Elementar Vario MICRO analyzer. Cyclic voltammetry (CV) measurement was performed on a BAS100W. The cyclic voltammogram was recorded in redistilled THF under the protection of nitrogen at room temperature. Molecular weight of as-prepared polymer was determined by GPC. THF was used as solvent and relative to polystyrene standards. The particle size distributions of CPNs was measured by dynamic light scattering (DLS) using a particle size analyser (BI-200SM, Brookhaven instruments Corp., Holtsville, NY). The morphology of CPNs was characterized on transmission electron microscope (TEM) (JEOL JEM-200CX, Japan). Confocal laser scanning microscope images of CPNs were taken on a Olympus Fluo-view 1000. Milli-Q water was supplied by Milli-Q Plus System (Millipore Corporation, Breford, USA).

Synthesis of monomers and conjugated polymer

Synthesis of 5. Pentane-2,4-dione (5.0 g, 50 mmol), amberlyst H-15 (5.0 g), activated 4Å molecular sieves (5.0 g) and *n*-hexane (80 mL) were mixed. The mixture was stirred for 10 minutes and then aniline (4.65 g, 50 mmol) was added dropwise. The resulting mixture was stirred at room temperature for 48 h. The mixture was then filtered off. The filtrate was concentrated under vacuum, and the residue was purified by column chromatography on neutral aluminum oxide column (petroleum ether/ethyl acetate, *v*/*v*, 30:1) to give the product **5** as a white solid (3.51 g, 40% yield). ^{1}H NMR (400 MHz, DMSO): δ 12.49 (s, 1H, OH), 7.40–7.36 (m, 2H, Ar-H), 7.22–7.19 (m, 3H, Ar-H), 5.25 (s, 1H, CH), 2.02 (s, 3H, C(OH)CH_3), 2.00 (s, 3H, C(NPh)CH_3); ^{13}C NMR (100 MHz, DMSO): δ 195.0, 159.7, 138.3, 129.2, 125.1, 123.8, 97.5, 40.1, 39.9. 39.7, 39.5, 39.3, 39.1, 38.9, 28.9, 19.4; MS (EI, m/z): 176.10 $[M+H]^+$. Anal. calcd for $C_{11}H_{13}NO$: C, 75.40; H, 7.48; N, 7.99. Found: C, 75.29; H, 7.42; N, 7.93%.

Synthesis of 6. Compound **5** (0.7 g, 4.0 mmol) was dissolved in 20 mL of CH_2Cl_2, boron trifluoride etherate (3 mL) was then added. The reaction mixture was left refluxing overnight. After cooling, the mixture was concentrated to dryness and purified by column chromatography on neutral aluminum oxide column (petroleum ether/ethyl acetate, v/v, 5:1) to give the product **6** as a white solid (0.5 g, 56% yield). ^{1}H NMR (400 MHz, DMSO): δ 7.50–7.46 (m, 2H, Ar-H), 7.43–7.39 (m, 1H, Ar-H), 7.21 (d, J = 7.5 Hz, 2H, Ar-H), 5.84 (s, 1H, CH), 2.15 (s, 3H, C(O)CH_3), 1.95 (s, 3H, C(NPh)CH_3); ^{13}C NMR (100 MHz, DMSO): δ 175.7, 172.4, 139.4, 129.1, 128.0, 126.2, 98.9, 40.1, 39.9, 39.7, 39.5, 39.3, 39.1, 38.9, 22.2, 21.0; ^{19}F NMR (376 MHz, DMSO): δ -131.1 (dd, J_{F-F} = 32.2 Hz, J_{B-F} = 15.8 Hz); MS (EI, m/z): 224.3 $[M+H]^+$; Anal. calcd for $C_{11}H_{13}BF_2NO$: C, 58.97; H, 5.85; N, 6.25. Found: C, 59.11; H, 5.66; N, 6.25%.

Synthesis of M2. Four drops of piperidine were added to a solution of **6** (0.36 g, 1.6 mmol) and 4-bromobenzaldehyde (0.74 g, 4.0 mmol) in toluene (20 mL). The resulting mixture was refluxed for 24 h. After cooling to room temperature, the precipitate was collected by filtration and further washed with toluene three times to afford **M2** as a dark red solid (0.57 g, 64% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86 (d, J = 16.2 Hz, 1H, Ar-H), 7.72–7.62 (m, 7H, Ar-H), 7.54–7.45 (m, 3H, Ar-H), 7.39 (d, J = 8.5 Hz, 2H, Ar-H), 7.29 (d, J = 7.2 Hz, 2H, BrPhCHCHC(O)), 7.13 (d, J = 15.9 Hz, 1H, BrPhCHCHC(N)), 6.68 [s, 1H, C(O)CHC(N)], 6.50 (d, J = 16.2 Hz, 1H, BrPhCHCHC(N)); ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.4, 165.3, 142.6, 139.3, 137.8, 134.2, 133.6, 132.2, 132.0, 130.0, 129.9, 129.2, 128.2, 126.6, 124.4, 123.5, 123.3, 120.4, 107.0, 97.1, 40.1, 39.9, 39.7, 39.5, 39.3, 39.1, 38.9; ^{19}F NMR (376 MHz, $CDCl_3$): δ -132.45 (d, J_{F-F} =

25.9 Hz); MS (EI, m/z): 224.00 $[M]^+$. Anal. calcd for $C_{25}H_{18}BBr_2F_2NO$: C, 53.90; H, 3.26; N, 2.51. Found: C, 53.69; H, 3.36; N, 2.51%.

Synthesis of EBKCP. In a 50-mL Schlenk tube, **M1** (321.3 mg, 0.50 mmol), **M2** (278.5 mg, 0.50 mmol), and $[Pd(PPh_3)_4]$ (28.9 mg, 0.025 mmol) was added. 10 mL of K_2CO_3 aqueous solution (2 M), and 15 mL of toluene were added after the tube was degassed with Ar. The mixed solution was heated to 80 °C stirred for 24 h under Ar atmosphere. The mixture was cooled and extracted with ethyl acetate for three times. The organic phases were combined and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure and the residue was dissolved in a small quantity of THF. The solution was added into 50 mL of methanol to precipitate the polymer, which was dried in a vacuum to give 212.6 mg of yellow solids in 54% yield. GPC results: Mw = 11410, Mn = 7540, PDI = 1.51. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.91 (d, J = 15.5 Hz, 1H), 7.71–7.33 (m, 21H), 6.88–6.76 (m, 1H), 6.60–6.49 (m, 1H), 6.23–6.16 (m, 1H), 2.02 (s, 4H), 1.40 (d, J = 2.4 Hz, 1H), 1.26 (s, 2H), 1.17-1.06 (m, 20H), 0.83–0.70 (m, 10H).

Preparation of functionalized CPNs

The PSMA functionalized CPNs were prepared via reprecipitation method. EBKCP was first dissolved in anhydrous THF to make a stock solution (40 μg/mL). PSMA was also dissolved in THF and mixed with a stock solution of EBKCP to produce solution mixtures with an EBKCP concentration of 20 μg/mL and a PSMA concentration of 5 μg/mL, respectively. In a typical preparation, 2 mL of the resulting mixture solution was injected rapidly into 8 mL MilliQ water under sonication. The mixture was further concentrated by evaporation under reduced pressure, followed by an additional filtration step with a 0.22 μm syringe filter to give the functionalized CPNs suspension.

Cell culture

HeLa cells used in this work were cultured at 37 °C in a humidied environment containing 5% CO_2 in Dulbecco's modied Eagle's medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin. The cells were pre-cultured prior to experiments until confluence was reached.

Cytotoxicity assay

The metabolic cytotoxicity of CPNs was evaluated using MTT assays. HeLa cells were firstly seeded in 96-well plates (Costar, IL, USA) at an intensity of 4×10^4 cells mL^{-1}. After 24 h incubation, the old medium was replaced by a series of doses of CPNs with different concentrations. And the cells were then incubated for 48 h. The sample wells were washed twice with 1×PBS buffer and 100 mL of freshly prepared MTT (0.5 mg mL^{-1}) solution in culture medium was added into each sample well. The MTT medium solution was carefully removed after 3 h of incubation, the control wells without addition of MTT solution were washed twice with 1×PBS buffer. Filtered DMSO (150 μL) was then added into each well and the plate was gently shaken for 10 min at room temperature to dissolve all the precipitates formed. The absorbance of individual wells at 570 nm was then monitored by the microplate Reader (BioTek, PowerWave XS2, Vermont, USA). The absorbance of MTT in the sample well was determined by the differentiation between the absorbance of the sample well and that of the corresponding control well. Cell viability was expressed by the ratio of absolute absorbance of the cells incubated with NP suspensions to that of the cells incubated with culture medium only.

Photostability of CPNs

The photostability of CPNs inside HeLa cells was studied by monitoring their respective fluorescence intensity changes in a phosphate buffer solution (PBS) at 37 °C by using confocal microscopy. The CLSM images of each sample were recorded at 2 min interval under continuous laser scanning at an excitation wavelength of 458 nm with 5 mW laser power. The fluorescence intensity of each image was analyzed by Image Pro Plus software, and was further expressed by I/I_0, where I_0 is the initial fluorescent intensity of fresh CPNs suspension and I is that of CPNs suspension after continuous laser scanning.

Cell staining and imaging

After HeLa cells 80% confluence, the medium was removed and the adherent cells were washed with 1×PBS buffer (2 mL×3). Then, the cells were coincubated with ~4 μM CPNs suspension for 2 h at 37 °C. The solution was then removed, and the cells were washed with 1×PBS (2 mL×3) before observation. The cellular images were recorded by a confocal laser scanning microscope (Olympus, FV-1000). The excitation wavelength was fixed at 458 nm, and the fluorescent signals were collected at 500-600 nm.

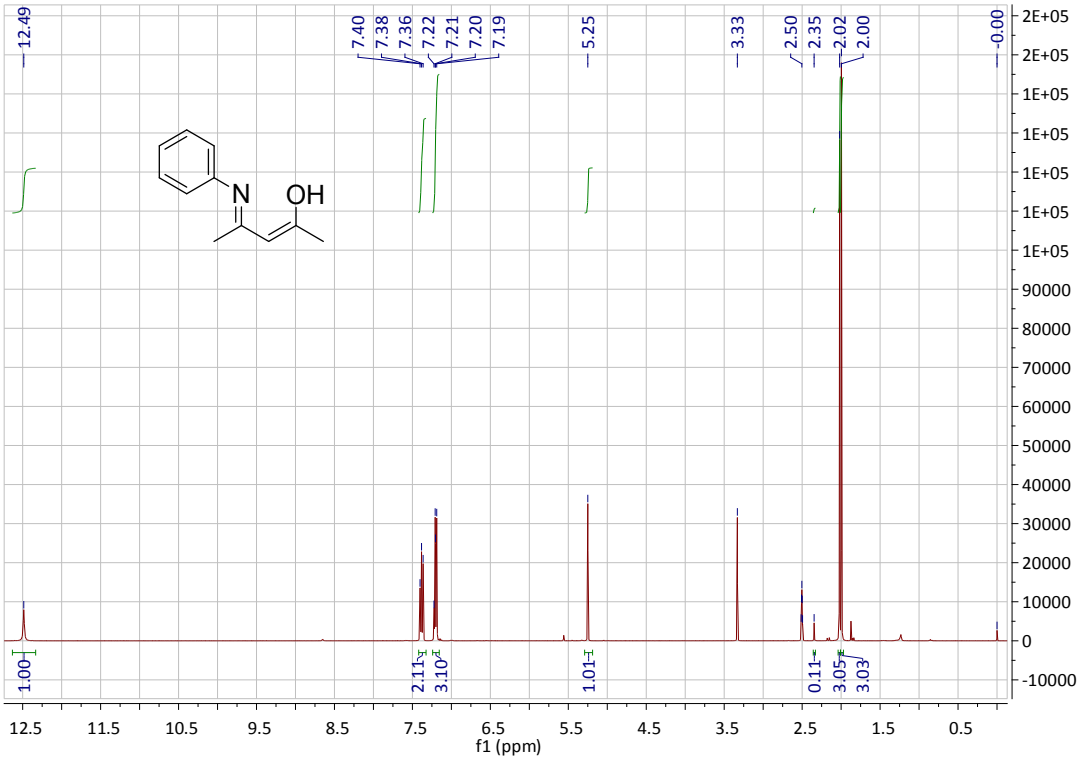

Figure S1. ^{1}H NMR of **5** in DMSO

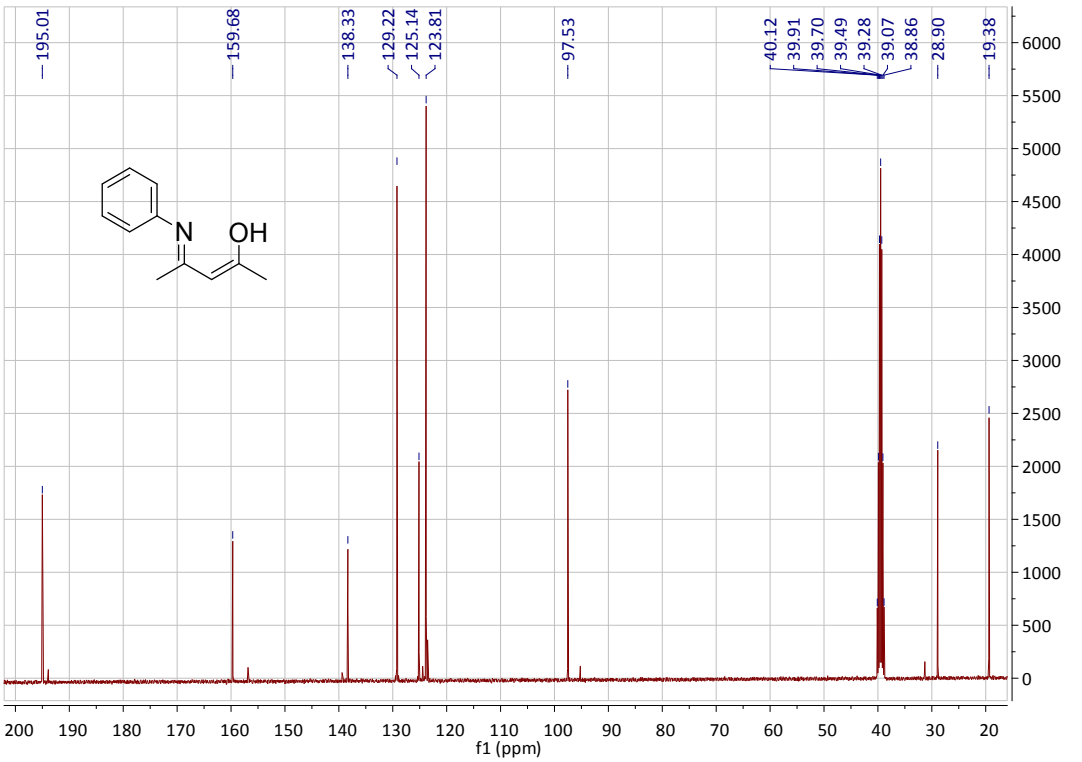

Figure S2. ^{13}C NMR of **5** in DMSO

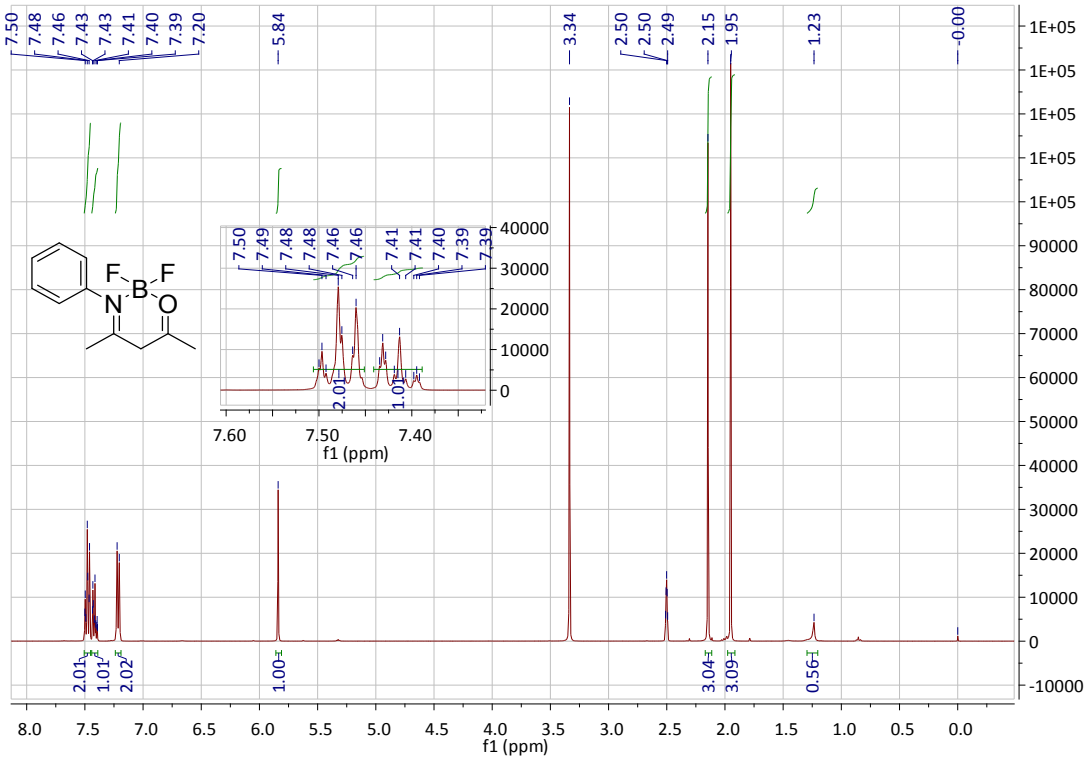

Figure S3. [1]H NMR of **6** in DMSO

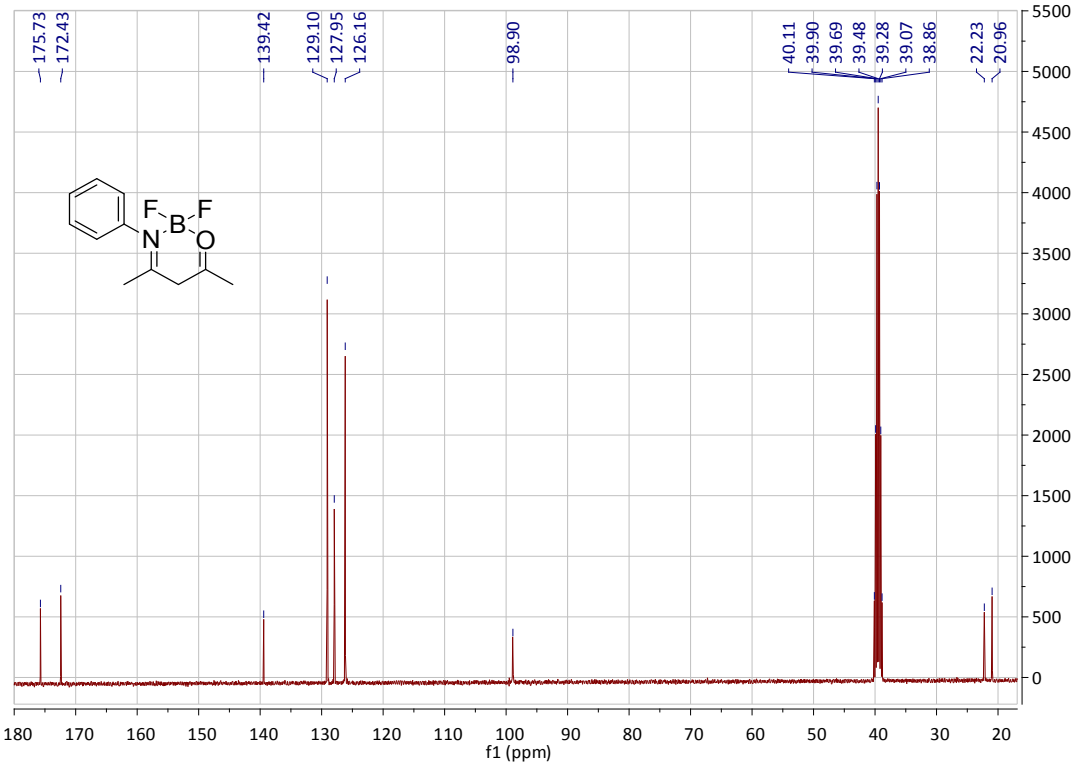

Figure S4. [13]C NMR of **6** in DMSO

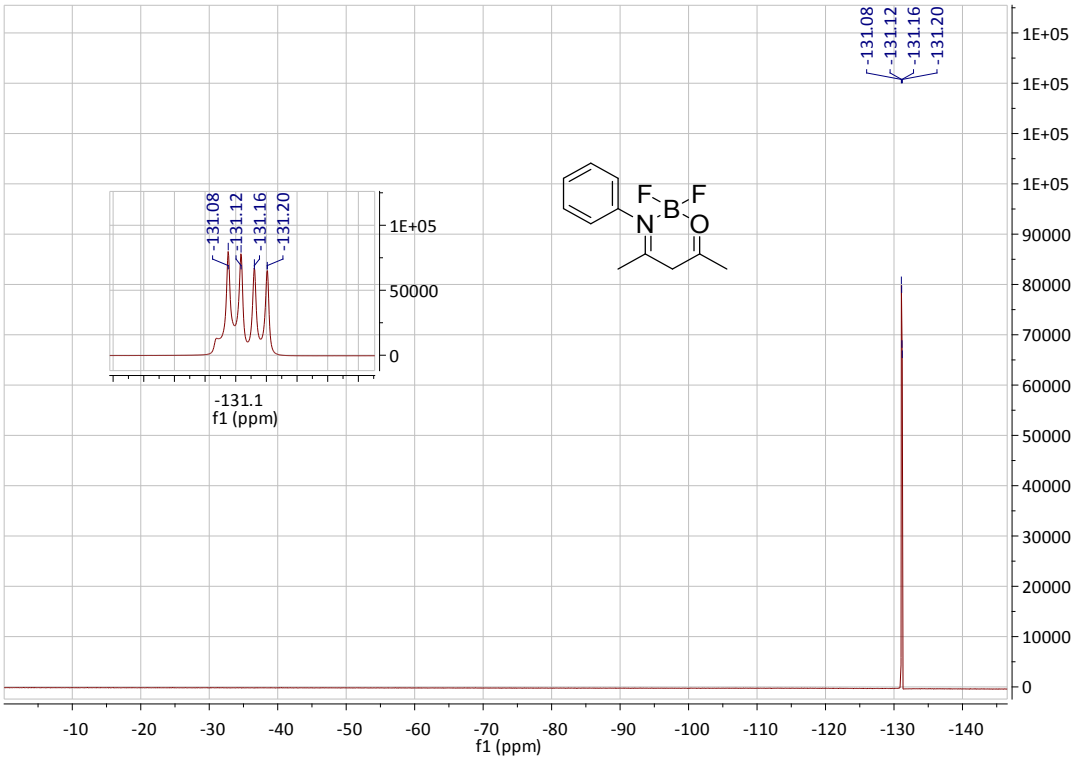

Figure S5. ^{19}F NMR of **6** in DMSO

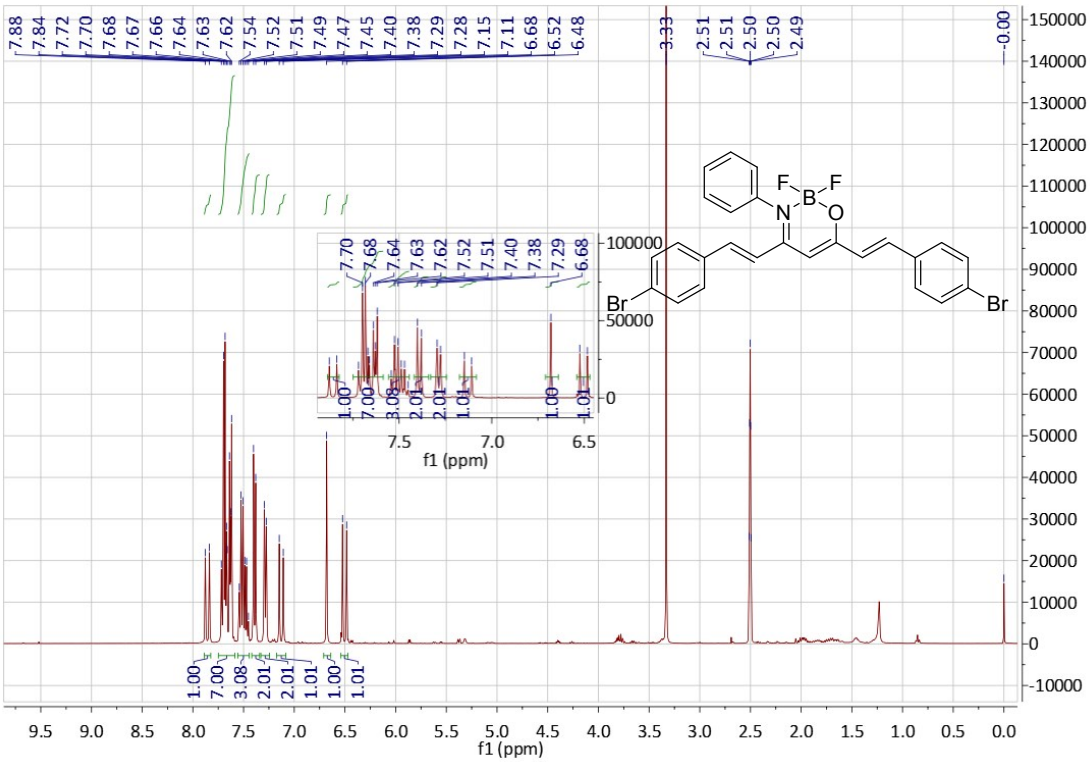

Figure S6. ^{1}H NMR of **M2** in DMSO

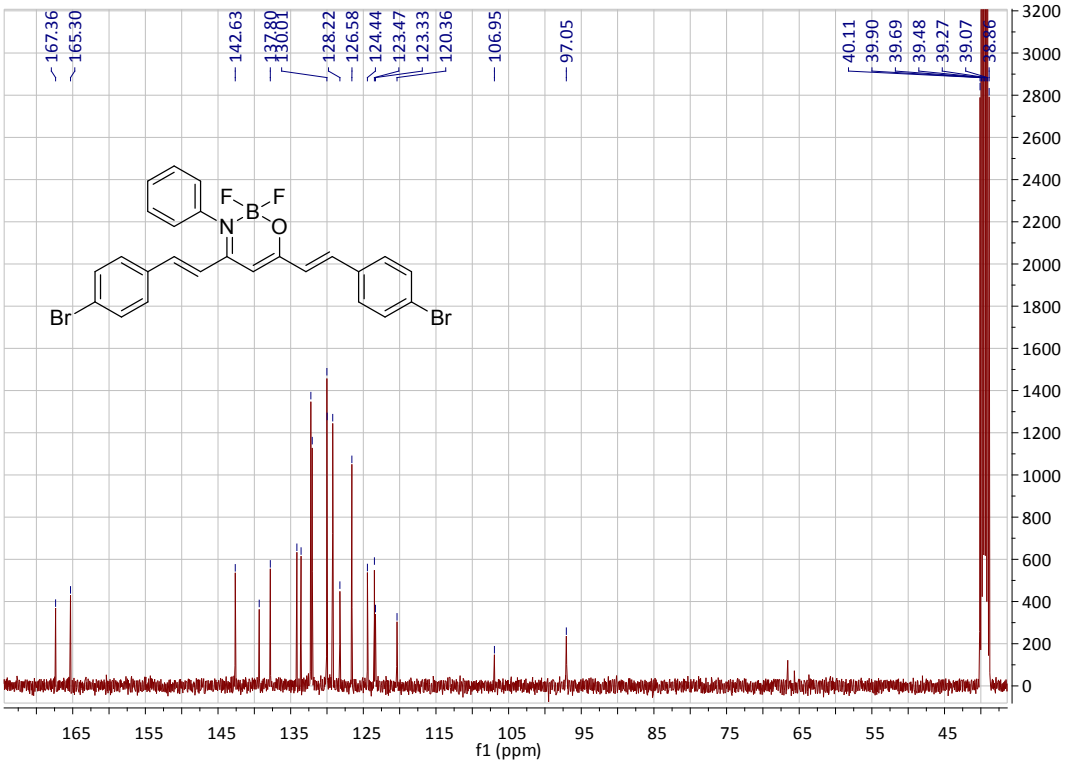

Figure S7. ^{13}C NMR of **M2** in DMSO

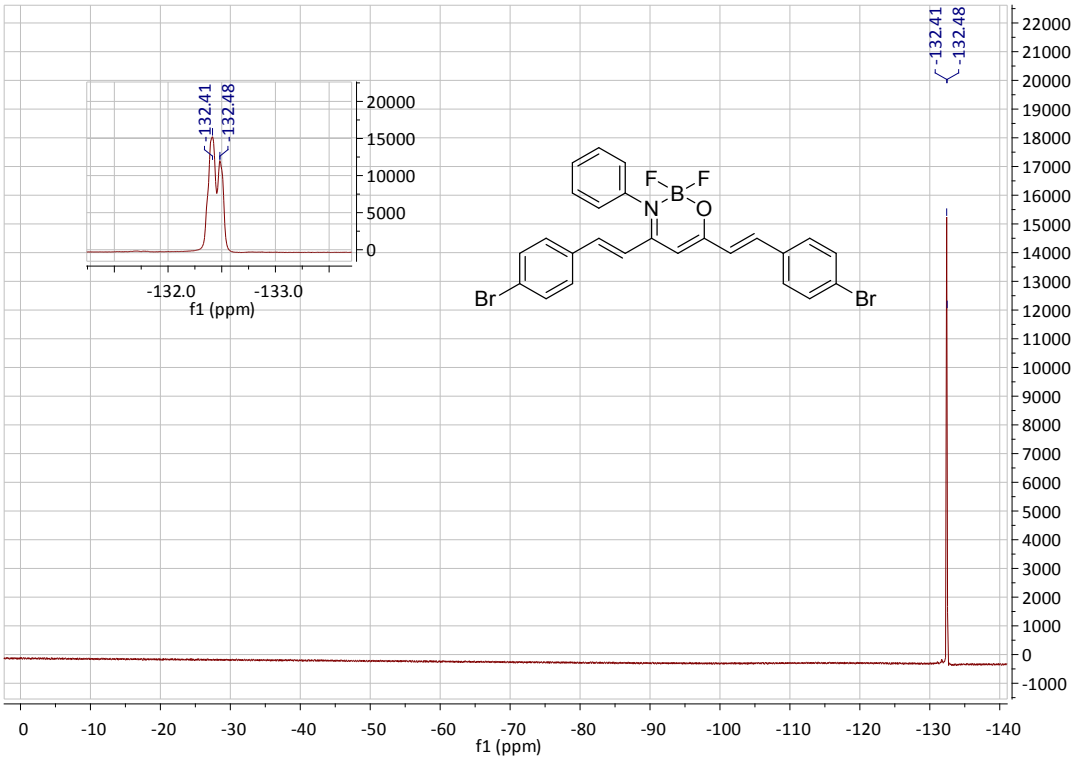

Figure S8. ^{19}F NMR of **M2** in DMSO

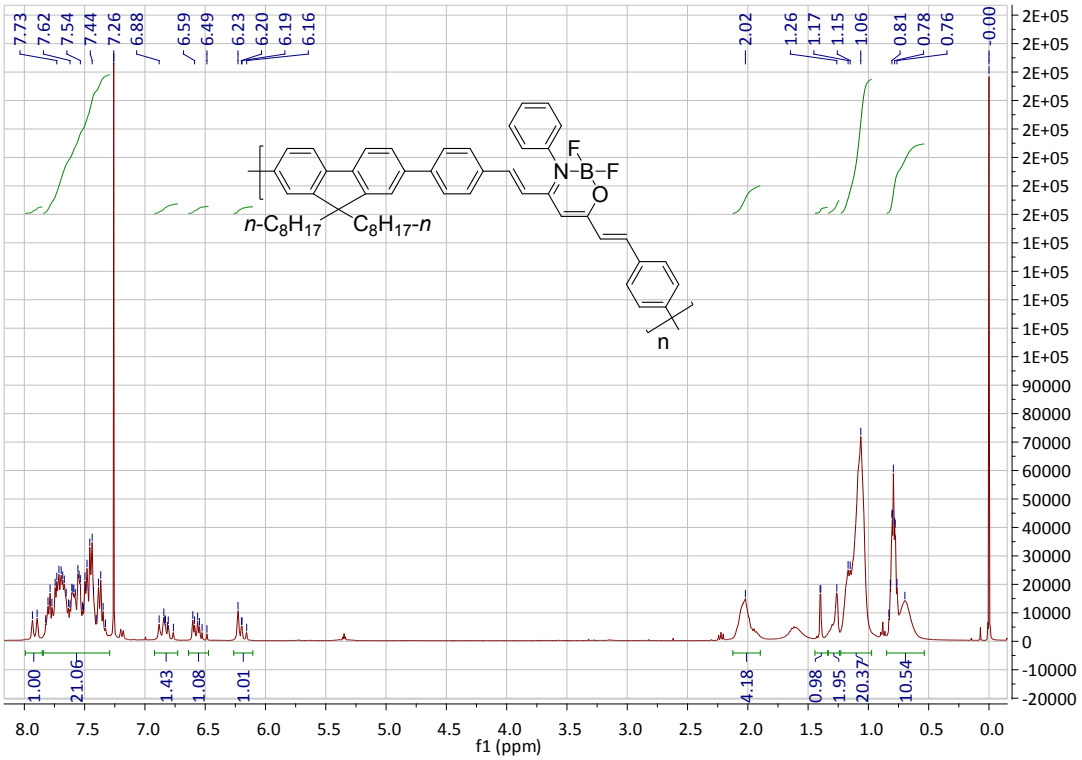

Figure S9. ^{1}H NMR of **EBKCP** in $CDCl_3$

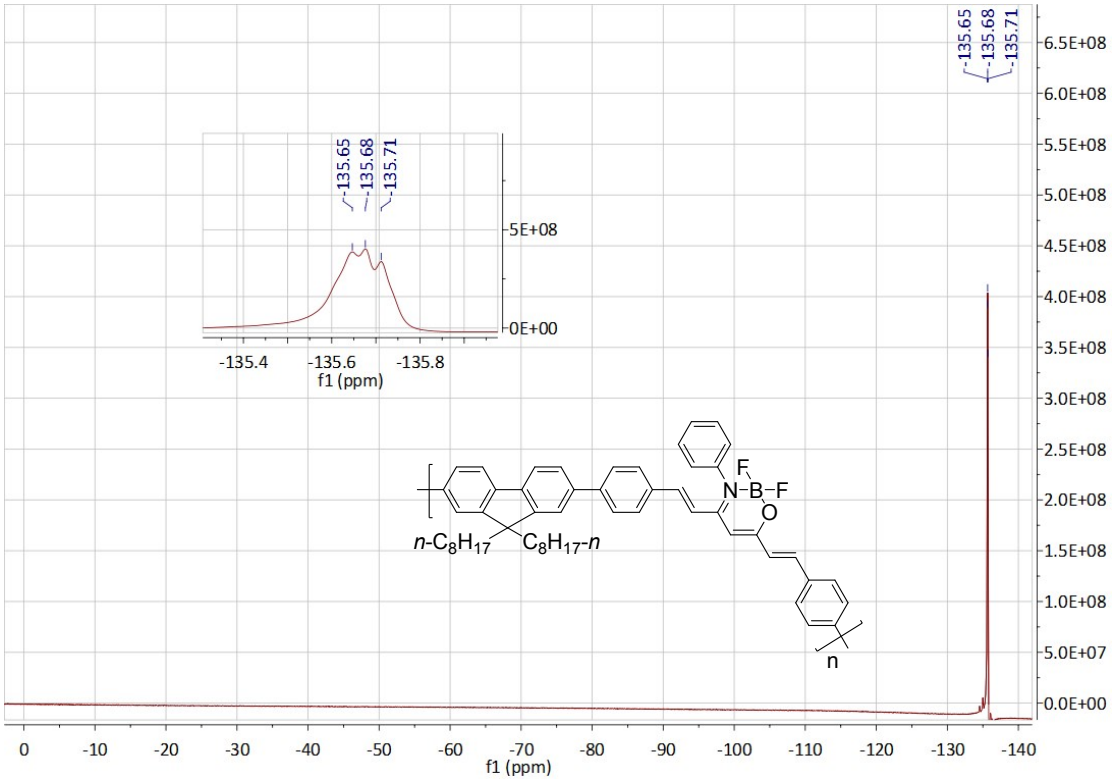

Figure S10. ^{19}F NMR of **EBKCP** in $CDCl_3$

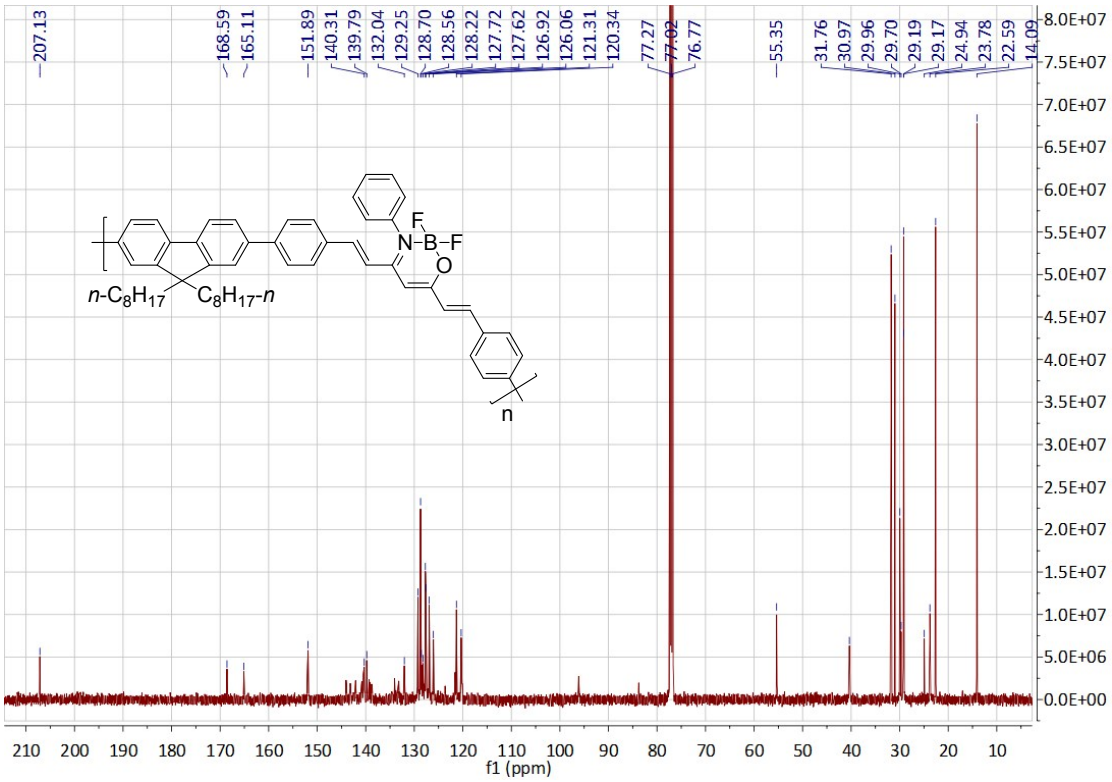

Figure S11. ^{13}C NMR of **EBKCP** in $CDCl_3$

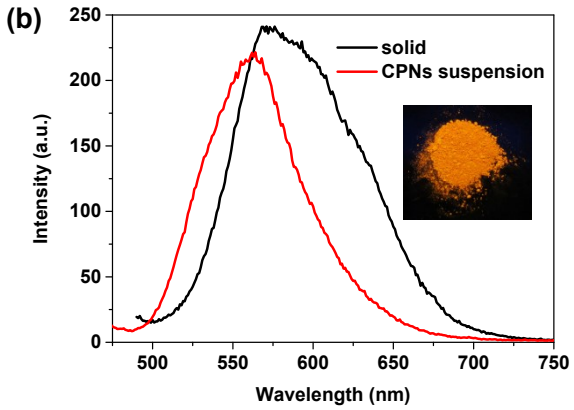

Figure S12. Fluorescence spectra of EBKCP solid and PSMA functional CPNs suspension. Inset shows photos of EBKCP solid and its nanoparticles suspension taken under UV illumination. The excitation wavelength is 460 nm in all fluorescence measurements.

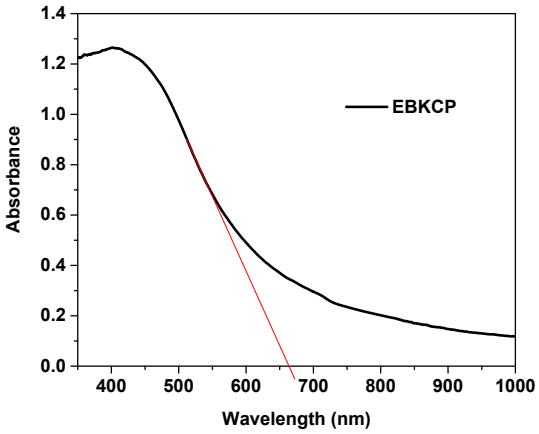

Figure S13. UV-vis diffuse reflectance spectra (DRS) of EBKCP powders.

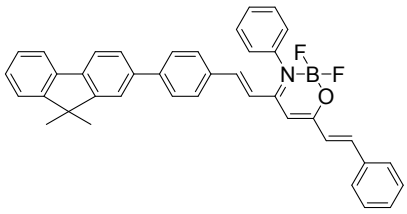

Figure S14. Optimized model compound from EBKCP for DFT calculation.