Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supplementary Information

Fluorinated Aza-BODIPY Derivative for NIR fluorescence/PA/^{19}F MR Tri-modality *In Vivo* Imaging

Lianhua Liu,[a] Yaping Yuan,[a] Yuqi Yang,[a] Michael T. McMahon,[b,c] Shizhen Chen,*[a] and Xin Zhou*[a]

a. State Key Laboratory of Magnetic Resonance and Atomic and Molecular Physics, Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences; Wuhan National Laboratory for Optoelectronics, Wuhan 430071, China

E-mail: chenshizhen@wipm.ac.cn; xinzhou@wipm.ac.cn

b. Russell H. Morgan Department of Radiology and Radiological Science, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21287, United States

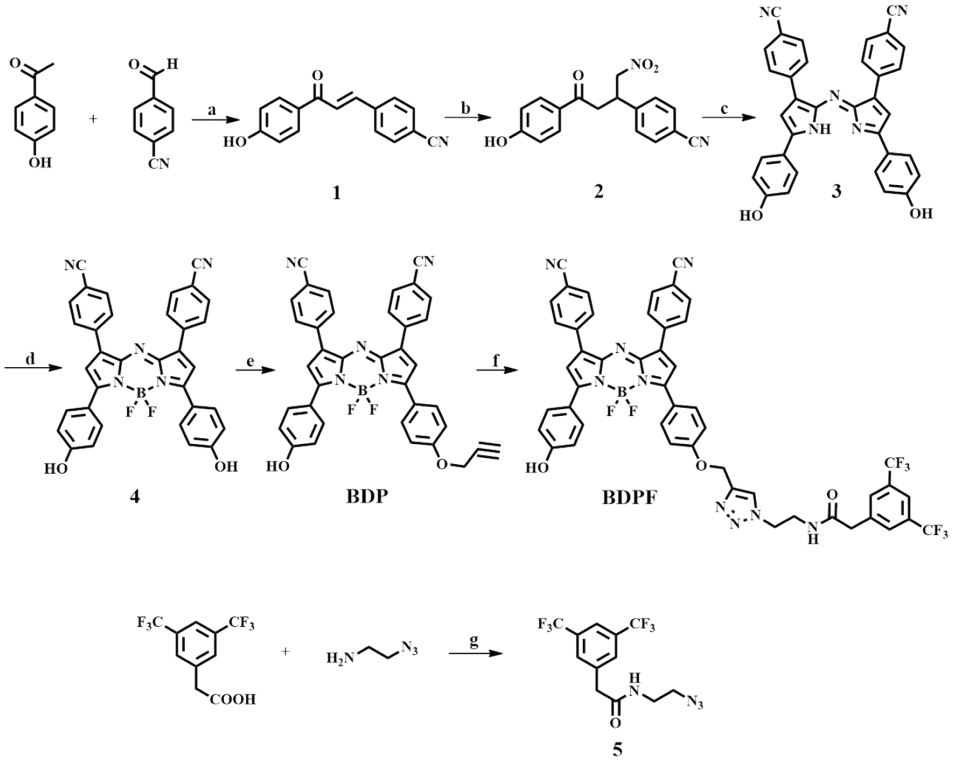
c. F.M. Kirby Research Center for Functional Brain Imaging, Kennedy Krieger Institute, Baltimore, Maryland 21287, United States

CONTENTS

1. Materials and instruments

All chemical reagents were purchased from commercial suppliers and used without further purification unless noted otherwise. Toluene was distilled over sodium and benzophenone prior to use. Zinc phthalocyanine (ZnPtc) and indocyanine green (ICG) were purchased from J&K Scientific for use as reference in the determination of the relative quantum yield and photostability study, respectively. Column chromatography was performed using 230-400 mesh silica gel. Microwave synthesis was performed on a Microwave Synthesis Reactor (Monowave 300, Anton Paar). ^{1}H, ^{13}C and ^{19}F NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Bruker 500 MHz or 600 MHz spectrometer at room temperature. High-resolution mass spectra (HRMS) were obtained at an Agilent 6530 accurate-mass Q-TOF spectrometer. UV-visible and fluorescence spectra were respectively recorded on an Evolution 220 spectrophotometer (ThermoFisher Scientific) and a FS5 spectrophotometer (EDINBURGH INSTRUMENTS).

2. Synthetic procedures



Scheme S1. The synthetic route of BDP and BDPF. (a) KOH, EtOH, rt, 12 h, 72%; (b) CH_3NO_2, Et_2NH, EtOH, reflux, 12 h, 61%; (c) ammonium acetate, EtOH, 95 °C, MW, 6 h, 35%; (d) $BF_3{\cdot}Et_2O$, DIPEA, toluene, 80 °C, 12 h, 94%; (e) 3-bromo-1-propyne, Cs_2CO_3, DMF, 80 °C, overnight, 50%; (f) 5, Na-ascorbate, $CuSO_4{\cdot}5H_2O$, CH_2Cl_2: H_2O:*t*-BuOH (1:2:2), rt, 12 h, 57%; (g) EDCI·HCl, HOBt, DIPEA, rt, overnight, 41%.

2.1 Synthesis of 1-(4-hydroxyphenyl)-3-(4-cyanophenyl)propenone (1)

4'-hydroxyacetophenone (1.36 g, 10 mmol) and 4-cyanobenzaldehyde (1.31 g, 10 mmol) were dissolved in anhydrous ethanol (50 mL). Then KOH (1.12 g, 20 mmol) was added and the reaction was continued to stir for 12 h at room temperature. The solution was acidified with 4 M HCl to pH 3. The precipitate was filtered, washed with 4 M HCl, and then dried *in vacuo* to give 1 as a light yellow solid (1.79 g, 72 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.48 (s, 1H), 8.10-8.05 (m, 5H), 7.92 (d, J = 5.0 Hz, 2H), 7.70 (d, J = 15.0 Hz, 1H), 6.90 (d, J = 5.0 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 186.89, 162.48, 140.46, 139.48, 132.65, 131.38, 129.24, 128.80, 118.65, 115.44, 112.01. HRMS (ESI) calcd for $C_{16}H_{11}NO_2$ $[M + H]^+$,

250.0863; found 250.0848.

2.2 Synthesis of 1-(4-hydroxyphenyl)-4-nitro-3-(4-cyanophenyl)-butan-1-one (2)

1-(4-hydroxyphenyl)-3-(4-cyanophenyl)propenone (120 mg, 0.48 mmol) was heated to dissolve in anhydrous ethanol (25ml). Then nitromethane (0.25 mL, 4.8 mmol) and diethylamine (0.25 mL, 2.4 mmol) were added dropwise and refluxed for 12 h. The reaction was cooled to room temperature, acidified with 4 M HCl to pH 3 and extracted with ethyl acetate. The organic layer was separated, dried over sodium sulfate. After filtration, the solvent was concentrated under reduced pressure. The residue was purified by silica gel column chromatography (eluent, hexane/EtOAc 1:1) affording 2 as a white solid (90 mg, 61 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.40 (s, 1H), 7.82-7.77 (m, 4H), 7.60 (d, J = 5.0 Hz, 2H), 6.84-6.82 (m, 2H), 5.04-4.90 (m, 2H), 4.12-4.10 (m, 1H), 3.50-3.44 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 194.98, 162.28, 146.15, 132.31, 130.50, 129.05, 127.84, 118.64, 115.22, 110.01, 79.02. HRMS (ESI) calcd for $C_{17}H_{14}N_2O_4$ $[M + H]^+$, 311.1026; found 311.1051.

2.3 Synthesis of [5-(4-Hydroxyphenyl)-3-(4-cyanophenyl)-1H-pyrrol-2-yl]-[5-(4-hydroxyphenyl)-3-(4-cyano phenyl)pyrrol-2-ylidene]amine (3)

1-(4-hydroxyphenyl)-4-nitro-3-(4-cyanophenyl)-butan-1-one (90 mg, 0.29 mmol) was dissolved in anhydrous ethanol (5 mL). Ammonium acetate (1.44 g, 18.8 mmol) was added and the reaction mixture was transferred to a microwave reaction vial (G30). Sealed with silicone septum and snap cap, the vial was microwaved for 6 h at 95 °C. Subsequently the reaction was cooled to room temperature. The generated precipitate was filtered, washed with cold ethanol and dried *in vacuo* to obtain 3 as a metallic dark blue solid (27 mg, 35 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.32 (s, 2H), 8.23 (d, J = 10.0 Hz, 4H), 7.99 (d, J = 10.0 Hz, 4H), 7.93 (d, J = 10.0 Hz, 4H), 7.72 (s, 2H), 7.01 (d, J = 10.0 Hz, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 160.46, 154.81, 148.87, 138.54, 137.76, 132.41, 128.85, 128.82, 122.06, 119.11, 116.95, 116.52, 110.06. HRMS (ESI) calcd for $C_{34}H_{21}N_5O_2$ $[M + H]^+$, 532.1768; found 532.1772.

2.4 Synthesis of BF_2 Chelate of [5-(4-Hydroxyphenyl)-3-(4-cyanophenyl)-1H-pyrrol-2-yl]-[5-(4-hydroxyphenyl) -3-(4-cyanophenyl)pyrrol-2-ylidene]amine (4)

[5-(4-Hydroxyphenyl)-3-(4-cyanophenyl)-1H-pyrrol-2-yl]-[5-(4-hydroxyphenyl)-3-(4-cyanophenyl)pyrrol-2-ylidene]amine (15 mg, 0.028 mmol) and N,N-diisopropyl-ethylamine (DIPEA) (0.1 mL, 0.28 mmol) were dissolved in dry toluene (10 mL) and stirred for 30 min. Then boron fluoride etherate (48 % BF_3, 0.2 mL, 0.48 mmol) was added dropwise and the mixture was heated at 80 °C for 12 h under N_2 atmosphere. After cool to room temperature, the reaction solution was concentrated under reduced pressure. The residue was dissolved with EtOAc, washed with water for three times. The organic layer was collected and dried over sodium sulphate. After filtration, the solvent was concentrated to afford the crude product as a dark brown solid. The solid was recrystallized from CH_2Cl_2, giving 4 as a red metallic solid (15 mg, 94 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 10.60 (s, 2H), 8.32 (d, J = 5.0 Hz, 4H), 8.12 (d, J = 10.0 Hz, 4H), 8.08 (d, J = 5.0 Hz, 4H), 7.78 (s, 2H), 6.96 (d, J = 5.0 Hz, 4H). ^{13}C NMR (126 MHz, DMSO-d_6) δ: 161.34, 157.60, 144.46, 138.94, 136.03, 132.52, 132.33, 129.23, 121.32, 121.13, 118.77, 115.99, 111.27. ^{19}F NMR (564 MHz, DMSO-d_6) δ: -131.05 (q, J = 33.8 Hz, BF_2). HRMS (ESI) calcd for $C_{34}H_{20}BF_2N_5O_2$ $[M + H + 1]^+$, 580.1787; found 580.1751. λ (DMSO)/nm = 745.

2.5 Synthesis of BF_2 Chelate of 4-{4-(4-cyanophenyl)-5-[3-(4-cyanophenyl)-5-(4-prop-2-ynyloxyphenyl)-pyrrol -2-ylideneamino]-1H-pyrrol-2-yl}phenol (BDP)

BF_2 Chelate of [5-(4-Hydroxyphenyl)-3-(4-cyanophenyl)-1H-pyrrol-2-yl]-[5-(4-hydroxyphenyl)-3-(4-cyanophenyl)pyrrol-2-ylidene]amine (50mg, 0.086mmol) and 3-bromo-1-propyne (20mg, 0.17mmo)

were dissolved in dry DMF (10mL). CS_2CO_3 (42mg, 0.13mmol) was added and heated at 80 °C for 12 h. The reaction was cooled to room temperature and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (eluent, hexane/acetone 2:1) affording BDP as a dark blue solid (30 mg, 57 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ: 10.68 (s, 1H), 8.31-8.28 (m, 4H), 8.17-8.13 (m, 4H), 8.06-8.02 (m, 4H), 7.85 (s, 1H), 7.67 (s, 1H), 7.18 (d, *J* = 6.0 Hz, 2H), 6.96 (d, *J* = 6.0 Hz, 2H), 4.95 (d, *J* = 2.4 Hz, 2H), 3.62 (t, *J* = 2.4 Hz, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ: 162.19, 162.10, 159.64, 159.45, 145.31, 143.83, 140.21, 138.29, 136.21, 135.72, 132.79, 132.51, 131.42, 129.37, 129.17, 123.85, 122.24, 120.85, 120.41, 118.70, 116.16, 115.14, 111.58, 111.13, 78.73, 78.60, 55.72. ^{19}F NMR (564 MHz, DMSO-d_6) δ: -131.98 (q, *J* = 33.8 Hz, BF_2). HRMS (ESI) calcd for $C_{37}H_{22}BF_2N_5O_2$ $[M + H + 1]^+$, 618.1944; found 618.1907. λ (DMF)/nm = 736.

2.6 Synthesis of {N-2-[4-(4-{5-[5-(4-Hydroxyphenyl)-3-(4-cyanophenyl)-1H-pyrrol-2-ylimino]-4-(4-cyanophenyl)-5H-pyrrol-2-yl}-phenoxymethyl)-[1,2,3]triazol-1-yl]ethyl}-(3,5-bis(trifluoro-methyl)phenyl acetamide (BDPF)

BF_2 Chelate of 4-{4-(4-cyanophenyl)-5-[3-(4-cyanophenyl)-5-(4-prop-2-ynyloxyphenyl)-pyrrol-2-ylideneamino]-1H-pyrrol-2-yl}phenol (8.6mg, 0.014mmol) and N-(2-azidoethyl)-3,5-bis(trifluoro-methyl) phenylacetamide (9.5mg, 0.028mmol) were dissolved in CH_2Cl_2:H_2O:*t*-BuOH (50 mL, 1:2:2) and purged with N_2 for 30 min at room temperature. Then a solution of Na-ascorbate (2.8 mg, 0.014mmol) in H_2O: *t*-BuOH (5mL, 1:1) was added dropwise to the mixture. After 10 min, asolution of $CuSO_4{\cdot}5H_2O$ (3.5 mg, 0.014 mmol) in H_2O:*t*-BuOH (5 mL, 1:1) was added dropwise over 30 min and the reaction was stirred under N_2 atmosphere for 12 h. The mixture was extracted with CH_2Cl_2 (30mL×3) and the combined organic layer was dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (eluent, CH_2Cl_2/MeOH 20:1) affording BDPF as a dark blue solid (5.5 mg, 41 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ: 8.41-8.38 (m, 4H), 8.27-8.24 (m, 4H), 8.16 (s, 1H), 8.02-7.99 (m, 4H), 7.94 (s, 1H), 7.33 (s, 1H), 7.26 (d, *J* = 6.0 Hz, 2H), 7.07-7.06 (m, 2H), 5.34 (s, 2H), 4.60 (t, *J* = 6.0 Hz, 2H), 3.81 (s, 1H), 3.76 (q, *J* = 6.0 Hz, 2H). ^{19}F NMR (564 MHz, DMSO-d_6) δ: -63.19 (s, 6F), -132.28 (q, *J* = 33.8 Hz, BF_2). HRMS (ESI) calcd for $C_{49}H_{32}BF_8N_9O_3$ $[M + Na + 1]^+$, 980.2522; found 980.2501. λ (DMF)/nm = 736.

2.7 Synthesis of N-(2-azidoethyl)-3,5-bis(trifluoromethyl)phenylacetamide (5)

The compound was synthesized according to the previously reports.[1] 3,5-bis(trifluoro methyl)phenylacetic acid (1.57 g, 5.8 mmol) was dissolved in CH_2Cl_2 (30 mL) and cooled to 0 °C. EDCI·HCl (1.66g, 8.7 mmol) and HOBT (1.17g, 8.7 mmol) were added and the mixture was allowed to stir for 30 min. 2-azidoethylamine (499 mg, 5.8 mmol) and DIPEA (4.49 g, 34.8 mmol) were added dropwise over 10 min. The ice-water bath was removed and the reaction was left to stir overnight at room temperature. Additional CH_2Cl_2 (50 mL) was added and the mixture was subsequently washed with saturated $NaHCO_3$ solution, brine and water. The organic layer was dried over sodium sulfate and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (eluent, hexane/EtOAc 20:10) affording 5 as a white solid (986 mg, 50%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.81 (s, 1H), 7.76 (s, 2H), 5.92 (s, 1H), 3.67 (s, 2H), 3.46 (d, *J* = 5.0 Hz, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ: 169.24, 136.90, 131.92 (q, $J_{C\text{-}F}$ = 32.8 Hz), 129.51 (quint, $J_{C\text{-}F}$ = 1.2 Hz), 123.14 (q, $J_{C\text{-}F}$ = 273.4 Hz), 121.32 (quint, $J_{C\text{-}F}$ = 3.8 Hz), 50.61, 42.56, 39.24. ^{19}F NMR (471 MHz, $CDCl_3$) δ: -62.90 (s, 6F). HRMS (ESI) calcd for $C_{12}H_{10}F_6N_4O$ $[M + H]^+$, 341.0832; found 341.0831.

3. Photophysical and ^{19}F NMR properties of BDP and BDPF

3.1 pK_a measurements

The pK_a values of BDP and BDPF were determined from the changes in fluorescence intensity at different pH values (4.80, 5.04, 5.24, 5.44, 5.84, 6.02, 6.22, 6.63, 6.44, 6.81, 7.00, 7.19, 7.44, 7.60, 7.80, 8.00, 8.50). All absorption spectra were obtained in 20 mM PBS buffer solution containing 1% DMSO and 0.1% CrEL. All fluorescence spectra were obtained in 20 mM PBS buffer solution containing 0.1% DMSO and 0.01% CrEL. The pK_a values were determined by using the Henderson-Hasselbalch type equation: log $[(I_{max}-I)/ (I-I_{min})]$ = pH-pK_a, where I_{max}, I_{min}, and I are the maximum, minimum, and measured fluorescence intensities at different pH, respectively. pK_a values were an average of three independent trials.

3.2 Photostability

Comparing a FDA-approved NIR dye indocyanine green (ICG), the photostability of BDP and BDPF were investigated in distilled water containing 1% DMSO and 0.1% CrEL under dark condition or continuous illumination by solar light, ultraviolet light at room temperature.[2] The process of photodegradation was monitored simultaneously by absorption spectra at different times.

3.3 ^{19}F NMR relaxation time

BDP and BDPF stock solutions were diluted with distilled water to 0.5 mM. 500 μL solution was placed in 5 mm NMR sample tube (J&K Scientific) and 10 μL D_2O was added for magnetic field locking control unit. After well mixed, T_1 and T_2 relaxation times were measured by inversion recovery method and spin echo method on a 600 MHz spectrometer equipped with Topspin 3.0 software (Bruker, Germany) at room temperature, respectively.

4. *In vitro* study of BDPF

4.1 Cell culture

Human non-small cell lung cancer A549 cells and human lung fibroblast wi-38 cells were purchased from the cell bank of Chinese academy of sciences (Shanghai, China) and cultured in MEM and RPMI-1640 media respectively. The culture media was supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 U/mL streptomycin. Cells were grown in a humidified incubator operating at 37 °C and 5% CO_2. All agents were obtained from Boster Company (China) and filtered with 0.2 μm sterile filter prior to incubation with cells.

4.2 Cellular uptake and confocal microscope imaging

A549 cells were seeded in 6-well plates at a density of 1.0×10^5 cells per well (Cellometer Auto T4 Plus, Dakewe Biotech Company) in 2 mL RPMI-1640 media supplemented with 10% FBS, 100 U/mL penicillin and 100 U/mL streptomycin and incubated for 48 h. Then culture media in each well was removed and 1 mL BDPF solution (5 μM in RPMI-1640 media) was added. After incubated for 4 h at 37 °C, the excess BDPF was removed and cells were washed with PBS for three times, fixed with 4% paraformaldehyde for 10 min and washed three times with distilled water. Subsequently, cells were treated with 4',6-diamidino-2-phenylindole (DAPI) for 5 min and the cover slip rinsed at least three times with PBS. Finally, the resulting slips were mounted and observed under the confocal laser scanning microscope (A1R/A1, Nikon, Japan).

4.3 Cytotoxicity assay

The cytotoxicity of BDPF on A549 cells and wi-38 cells were respectively determined by the standard MTT assay.[3] A549 cells and wi-38 cells were respectively seeded in 96-well culture plates at a density of

approximately 1 × 10^5 cells per well and incubated for 24 h in the incubator. BDPF stock solutions were diluted with RPMI-1640 media (1% DMSO and 0.1% CrEL as cosolvent) to 0.005 to 50 mM and added to the wells. Cells cultured with RPMI-1640 media were used as control. After incubated for 4 h at 37 °C, the media in each well was replaced with fresh culture media and cultured for additional 48 h. During the last 4 h of the culture, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) solution was added to give a final concentration of 0.5 mg/mL. Then the media was carefully removed and 200 μL of DMSO was added to each well. After thoroughly dissolving of formazan crystals, optical density at 490 nm of each well was recorded on the ELISA plate reader (Molecular Devices, USA). Taking control group as 100%, the cell viabilities of test groups were assessed as the percentages of viable cells. All data are presented as mean ± SEM, n>3.

4.4 ^{19}F MR imaging of phantoms and cells

All ^{1}H/^{19}F MR imaging of phantoms and cells were performed on a 9.4 T microimaging system (Bruker Biospec, Germany) with 10 mm inner diameter ^{1}H (400 MHz) and ^{19}F coil (376.4 MHz) for radiofrequency transmission and reception. A MSME (multi slice multi echo) pulse sequence was employed as acquisition pulse sequence in ^{19}F MR imaging and ^{1}H MR imaging.[4]

^{1}H/^{19}F MR imaging of phantoms: Different concentration of BDPF stock solutions were diluted with RPMI-1640 medium to 5 to 80 mM (5, 10, 20, 40, 80 mM). RPMI-1640 media containing 5% DMSO and 0.5% CrEL was used as comparison group. A 100 μL solution of each sample was removed and added into 1.5 mL tapered plastic centrifuge tube for MR imaging. At first, for proton imaging, the centrifuge tubes were inserted in to ^{1}H coil with the following parameters: FOV (field of view) = 4.00 x 4.00 cm^2, MTX (matrix size) = 64, SI (slice thickness) = 40.0 mm, TR (repetition time) = 1000.0 ms and TE (echo time) = 7.7 ms. Then the centrifuge tubes were inserted in to ^{19}F coil for ^{19}F MR imaging with the following parameters: FOV = 4.00 x 4.00 cm^2, MTX = 32, SI = 40.0 mm, TR = 2000.0 ms and TE = 7.7 ms.

In vitro ^{1}H/^{19}F MR imaging: A549 cells were incubated in 250 mL cell culture flasks until > 90% confluence were reached. Different concentration BDPF solutions (5, 10, 20, 40 mM) were added into the flasks and incubated with cells for 4 h. Cells without treatment was used as control. Then media was removed and the cells were carefully washed with PBS for three times. Subsequently the cells were gently scraped from culture flask surfaces with sterile cell scrapers to obtain cell suspensions with a density of approximately 2.17 × 10^6 cells/mL (8 mL). In order to get cell pellets, the suspensions were transferred to 1.5 mL tapered plastic centrifuge tubes and centrifuged at 3000 rpm for 3 min. The supernatants were cautiously replaced with PBS buffer to remove the excess BDPF and prevent destruction of cell pellets. For ^{1}H MR imaging, the centrifuge tube was inserted in to ^{1}H coil with the following parameters: FOV = 4.00 x 4.00 cm^2, MTX = 64, SI = 40.0 mm, TR = 1000.0 ms and TE = 7.7 ms. The data collection time was 32 ms. And then the centrifuge tube was inserted in to ^{19}F coil for ^{19}F MRI with the following parameters: FOV = 4.00 x 4.00 cm^2, MTX = 32, SI = 40.0 mm, TR = 2000.0 ms and TE = 7.7 ms. The total scan time was 17.06 min.

All raw data were processed using the Matlab (Mathworks, Natick, MA).

5. *In vivo* imaging with BDPF

5.1 Animal and cancer model

All experimental protocols involving animals were approved by the Animal Care and Use Committee of Wuhan Institute of Physics and Mathematics, the Chinese Academy of Sciences. BALB/c male nude mice (5-6 weeks of age, approximately 20 g) were purchased from Human SJA Laboratory Animal Co., Ltd. The nude mice were inoculated subcutaneously on the legs with 1×10^6 A549 cells in 0.1 mL PBS.[5] After 5-6

weeks breeding, the tumor-bearing mice could be used for *in vivo* experiments.

5.2 *In vivo* NIR fluorescence imaging

Prior to imaging, the A549 tumor-bearing mouse was anesthetized by intraperitoneal injection with 160 μL of 1% pentobarbital sodium in saline. And then 50 μL of BDPF (5 μM) in PBS buffer containing 1 % DMSO and 0.1 % CrEL was in situ injected. After 5 min, the NIR fluorescence imaging was measured with excitation at 730 nm using an *in-vivo* imaging system (PerkinElmer).

5.3 *In vivo* PAI

PA imaging experiments were performed using a real-time multispectral optoacoustic tomographic (MSOT) imaging system (inVision 128, iThera Medical GmbH, Neuherberg, Germany). During *in vivo* PAI, A549-bearing mouse was held under 2% isoflurane anesthesia. MOST imaging was performed before and after intravenous injection of BDPF (25 μM, 100μL). The imaged mouse skin was covered with ultrasound gel and then the mouse was placed horizontally in a holder covered with a thin polyethylene membrane. A volume ROI consisting of transverse slices with a step size of 0.3 mm spanning through the tumor region was selected by manual inspection of live MOST imaging.

5.4 *In vivo* ^{19}F MRI

In vivo ^{1}H/^{19}F MR imaging were performed on a 9.4 T microimaging system, equipped with 30 mm inner diameter ^{1}H (400 MHz) and ^{19}F coil (376.4 MHz) for radiofrequency transmission and reception. All MR imaging was obtained for the behind leg of the xenograft A549 tumor mouse. Before MR imaging, the mouse was anesthetized by 1% pentobarbital sodium and in situ injected with 100 μL of BDPF solution (100 mM). Fixed on ^{1}H coil by hooking teeth, the mouse was used for proton imaging with a RARE (Rapid Acq. With Relaxation Enhancement) pulse sequence (FOV = 4.00 x 4.00 cm^2, MTX = 256, SI = 1.50/1.50 mm, TR = 3000.0 ms, TE = 33.0 ms, and total scan time = 3.2 min). For ^{19}F MR imaging the same region with ^{1}H MRI, the mouse was fixed on ^{19}F coil and the parameters were FOV = 4.94 x 4.94 cm^2, MTX = 64, SI = 25.00 mm, TR = 3000.0 ms, TE = 7.0 ms, and total scan time = 12.8 min. All raw data were processed using the Matlab.

5.5 Histological Analysis.

Various organs including heart, liver, spleen, lung and kidney were extracted after 24 h of *in vivo* imaging. Sample preparation was carried out as follows: fixed in 4% paraformaldehyde for 24 h, dehydrated in graded alcohol (five steps: 75% (4 h), 85% (2 h), 90% (2 h), 95% (1 h), 100% alcohol (1 h)), cleared with xylene, embedded in paraffin blocks and froze under -20 °C, sectioned with a thickness of 4 μm and mounted on the glass slides. Hematoxylin and eosin (H&E) staining was performed according to the protocol[6]: deparaffination of tissue sections in xylene, rehydration using decreasing concentrations of alcohol (five steps: 100% (10 min), 95% (5 min), 90% (5 min), 80% (5 min), 70% (5 min)), rinsing in distilled water, hematoxylin (ASPEN) staining, eosin (ASPEN) staining, water removal using increasing concentrations of alcohol (four steps: 95% (5 min), 95% (5 min), 100% (5 min), 100% (5 min)), clearing with xylene, cover slide mounting. Hematoxylin will stain the nuclei in blue and the mucins in light blue. Eosin will stain the cytoplasm in pink, collagen in pale pink, red blood cells in bright red, and colloid in red. Slides were observed under Nikon Eclipse Ti-SR microscope (Nikon, Japan).

6. Supplementary figures

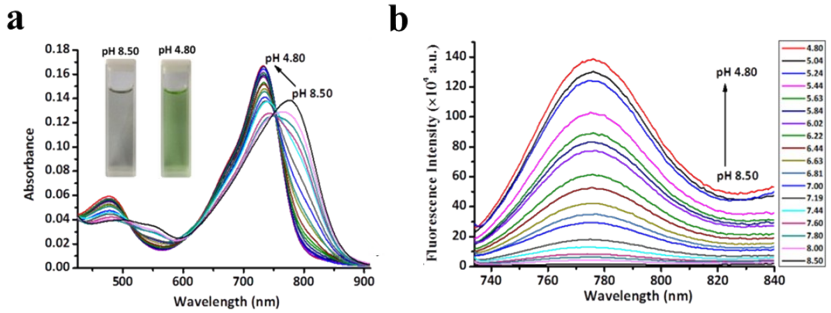


Fig. S1 Absorption (a) and fluorescence (b) spectra of BDP (5 μM and 0.5 μM, respectively) at different pH values (4.80, 5.04, 5.24, 5.44, 5.84, 6.02, 6.22, 6.63, 6.44, 6.81, 7.00, 7.19, 7.44, 7.60, 7.80, 8.00, 8.50). Inset photo presents color changes of solution upon basic (pH = 8.50) and acidic (pH = 4.80) condition.

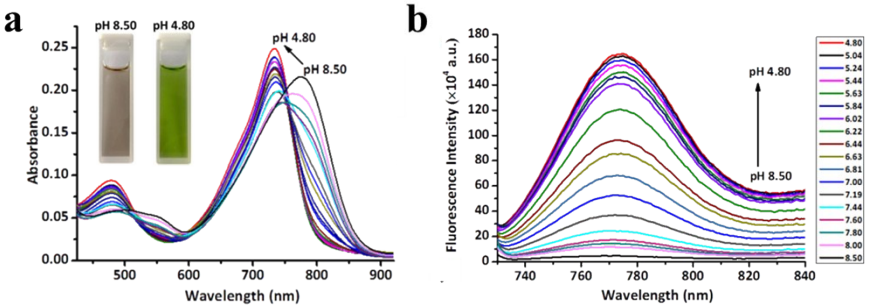


Fig. S2 Absorption (a) and fluorescence (b) spectra of BDPF (5 μM and 0.5 μM, respectively) at different pH values (4.80, 5.04, 5.24, 5.44, 5.84, 6.02, 6.22, 6.63, 6.44, 6.81, 7.00, 7.19, 7.44, 7.60, 7.80, 8.00, 8.50). Inset photo presents color changes of solution upon basic (pH = 8.50) and acidic (pH = 4.80) condition.

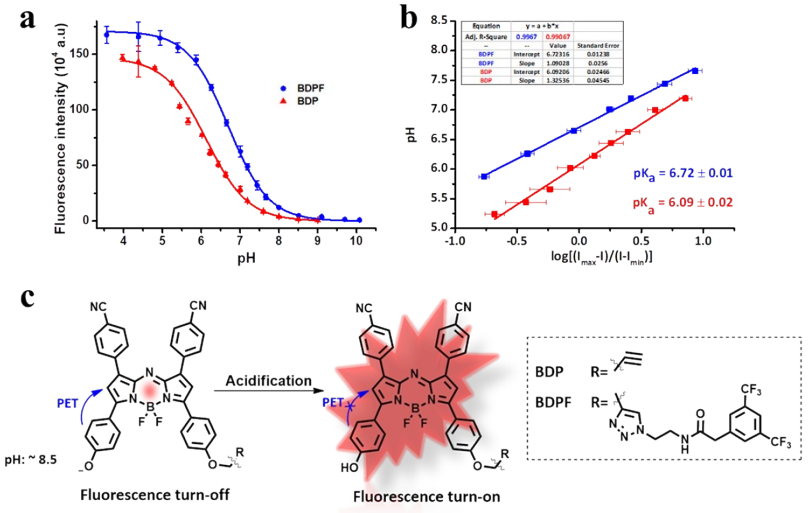


Fig. S3 (a) Plot of fluorescence intensity of BDP (red) and BDPF (blue) at 775 nm versus pH value. (b) The dependence of pH value on log [(Imax-I)/(I-Imin)]. Every point is an average of three independent measurements and error bars show the corresponding standard deviations. (e) Proposed PET-based pH-

responsive mechanism for BDP and BDPF. Upon acidic condition, protonation inhibited the process of PET from phenoxide group to aza-BODIPY unit and led to the fluorescence intensity enhancement.

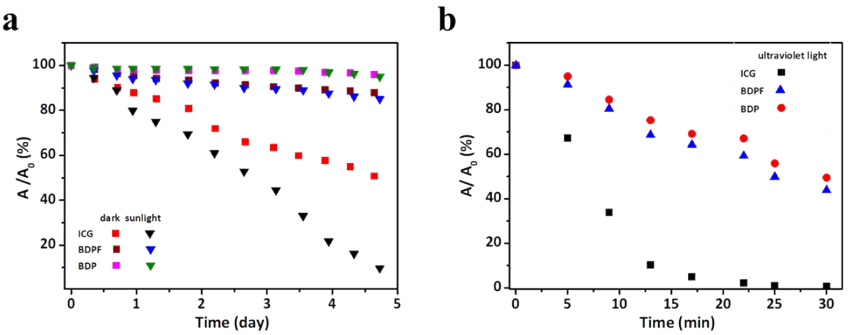


Fig. S4 The photostability experiments of BDPF (5 μM, monitored at 734 nm), BDP (5 μM, monitored at 733 nm) and ICG (5 μM, monitored at 798 nm) in aqueous solution containing 1% DMSO and 0.1% CrEL. The absorption spectra were measured after the sample was exposure to dark, sunlight or ultraviolet light in a certain time.

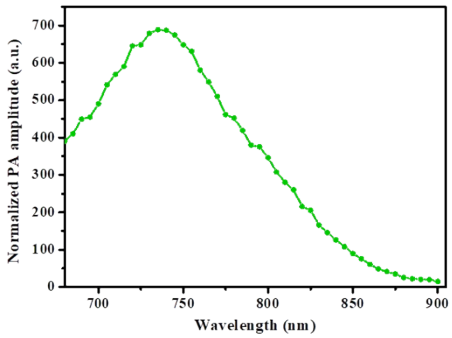


Fig. S5 PA amplitude spectrum of BDPF (5 μM) in 20 mM PBS buffer solution containing 1% DMSO and 0.1% CrEL (pH=7.44).

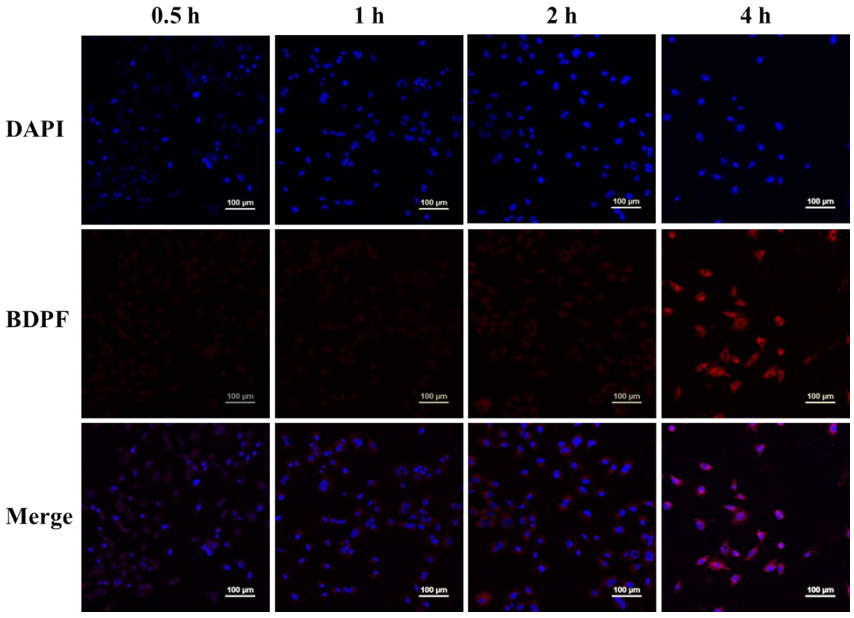


Fig. S6 Time-dependent uptake of BDPF incubated at 5 μM with A549 cells. Cell images were obtained

with the excitation wavelength of 647 nm. Cell nuclei were stained by DAPI (blue). Scale bar = 100 μm. Images showed a time-dependent increase in cell uptake.

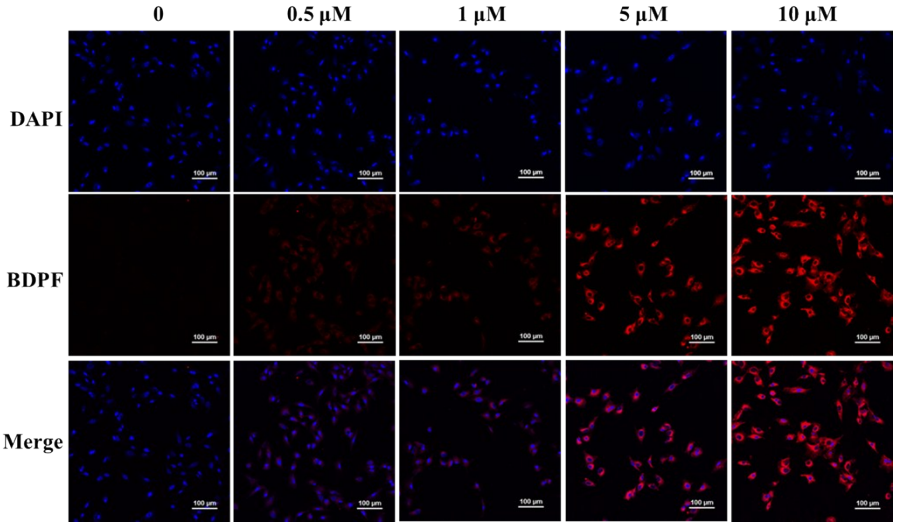


Fig. S7 Concentration-dependent uptake of BDPF incubated with A549 cells for 4 h. Cell images were obtained with the excitation wavelength of 647 nm. Cell nuclei were stained by DAPI (blue). Scale bar = 100 μm. Images showed an obvious concentration-dependent increase in cell uptake.

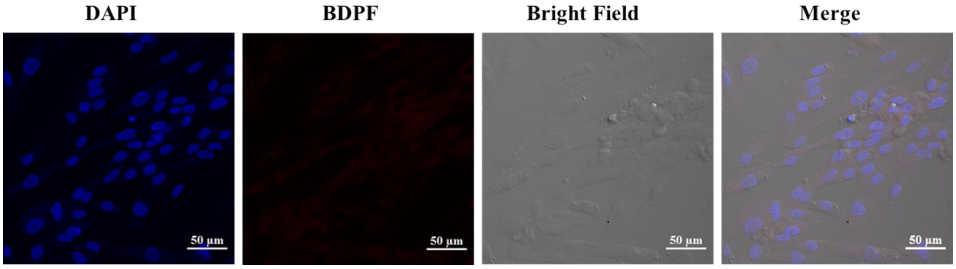


Fig. S8 (a) Confocal laser scanning microscopy images of wi-38 cells incubated with BDPF (5 μM) for 4 h at 37 °C. Cell images were obtained with the excitation wavelength of 647 nm. Cell nuclei were stained by DAPI (blue). Scale bar = 50 μm.

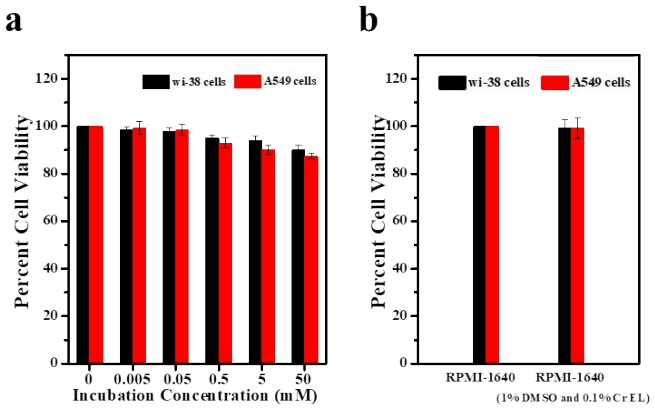


Fig. S9 The viability of wi-38 cells (black) and A549 cells (red) after co-incubated with (a) BDPF at varied concentrations (0.005 mM, 0.05 mM, 0.5 mM, 5 mM, 50 mM) and (b) RPMI-1640 (1% DMSO and 0.1%

CrEL as cosolvent). Cells cultured with RPMI-1640 served as control. Taking control group as 100%, the cell viabilities of treatment groups were assessed as the percentages of viable cells. All data are presented as mean ± SEM, n>3. Cells cultured with RPMI-1640 media were used as control.

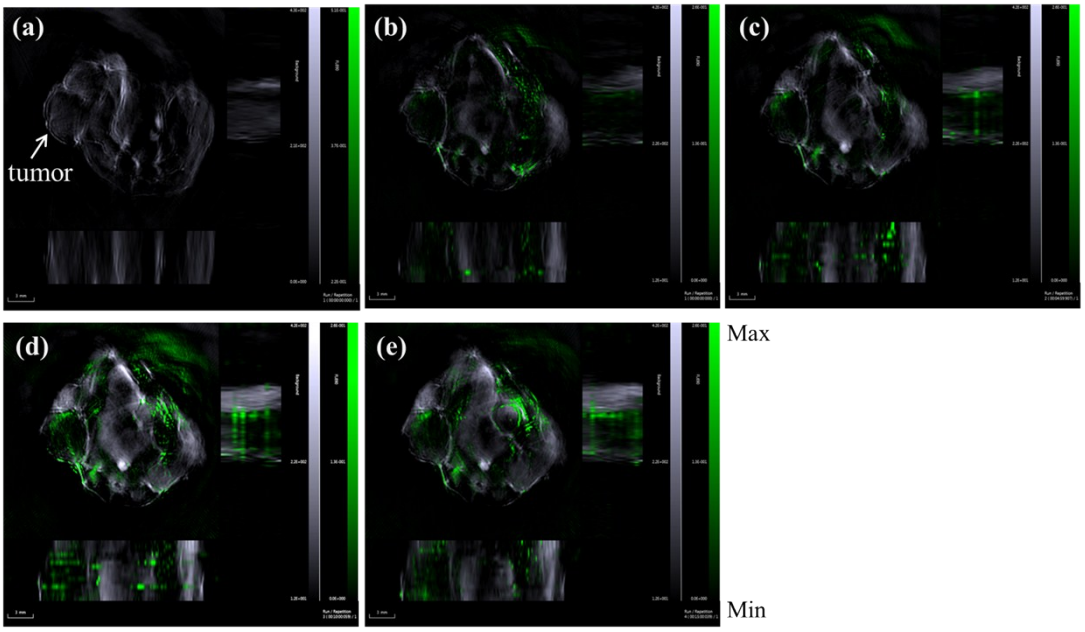


Fig. S10 *In vivo* PA images obtained at (a) 0 min, (b) 5 min, (c) 10 min, (d) 15 min, and (e) 20 min after intravenously injection of BDPF solution.

7. Characterization data

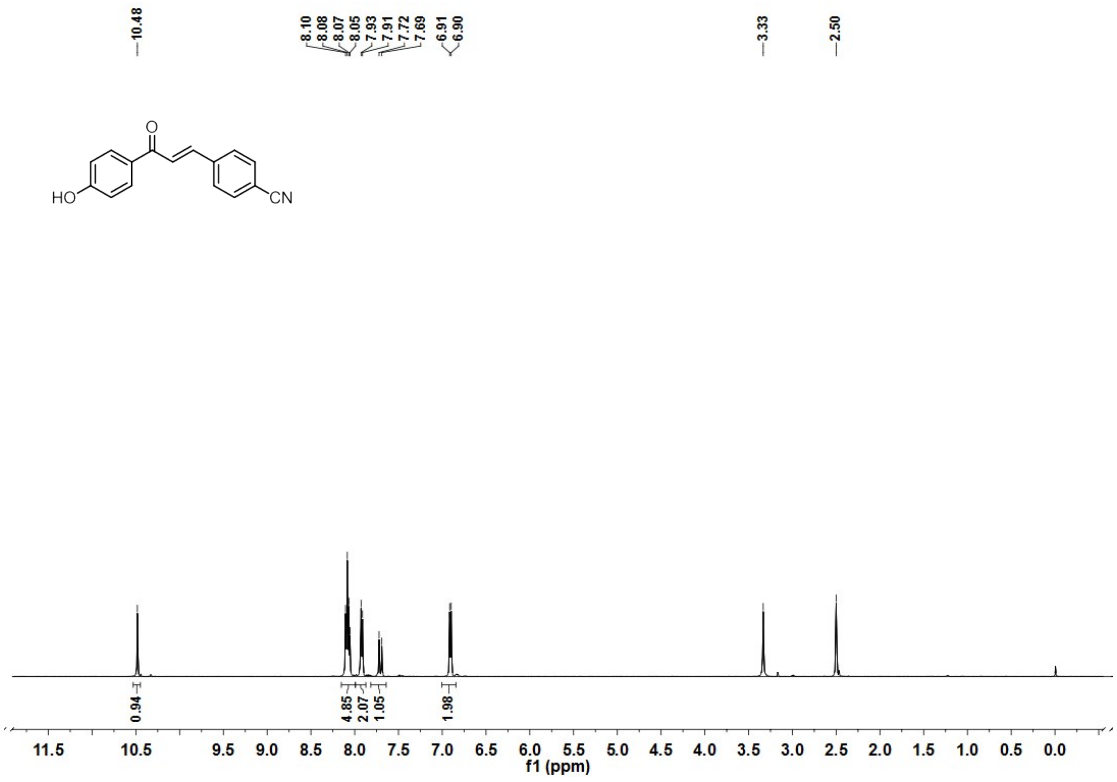


Fig. S11 ^{1}H NMR of 1

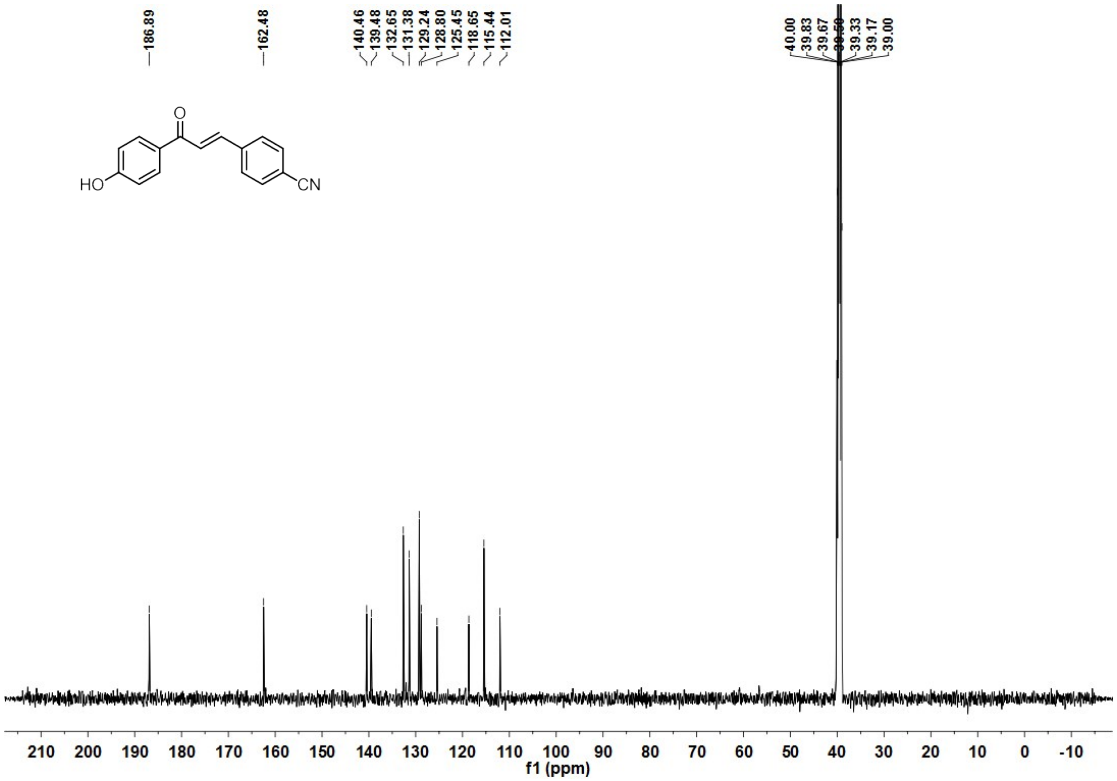


Fig. S12 ^{13}C NMR of 1

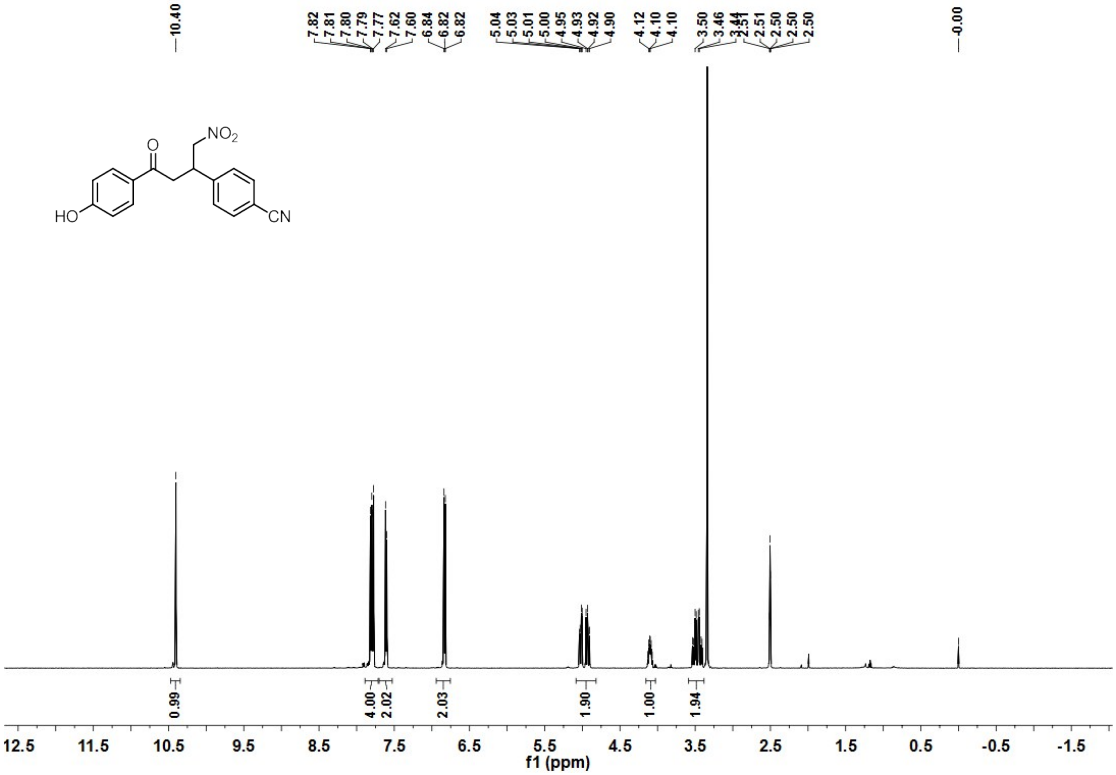


Fig. S13 ^{1}H NMR of 2

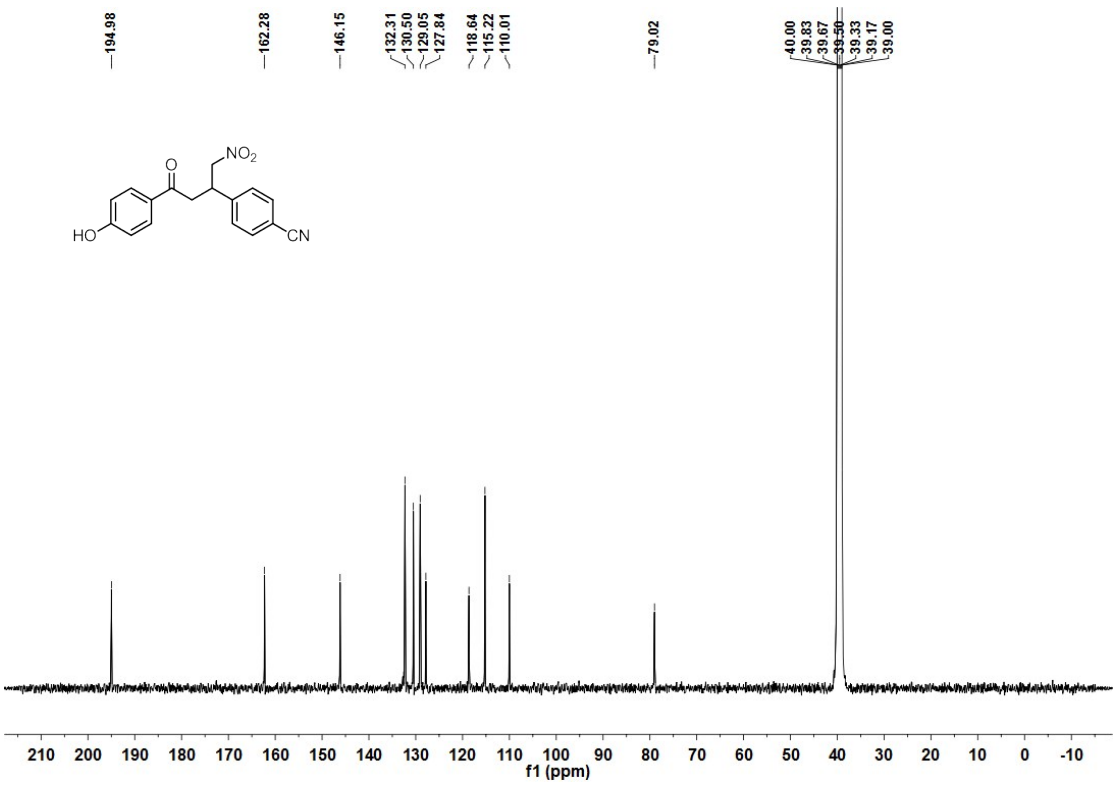


Fig. S14 ^{13}C NMR of 2

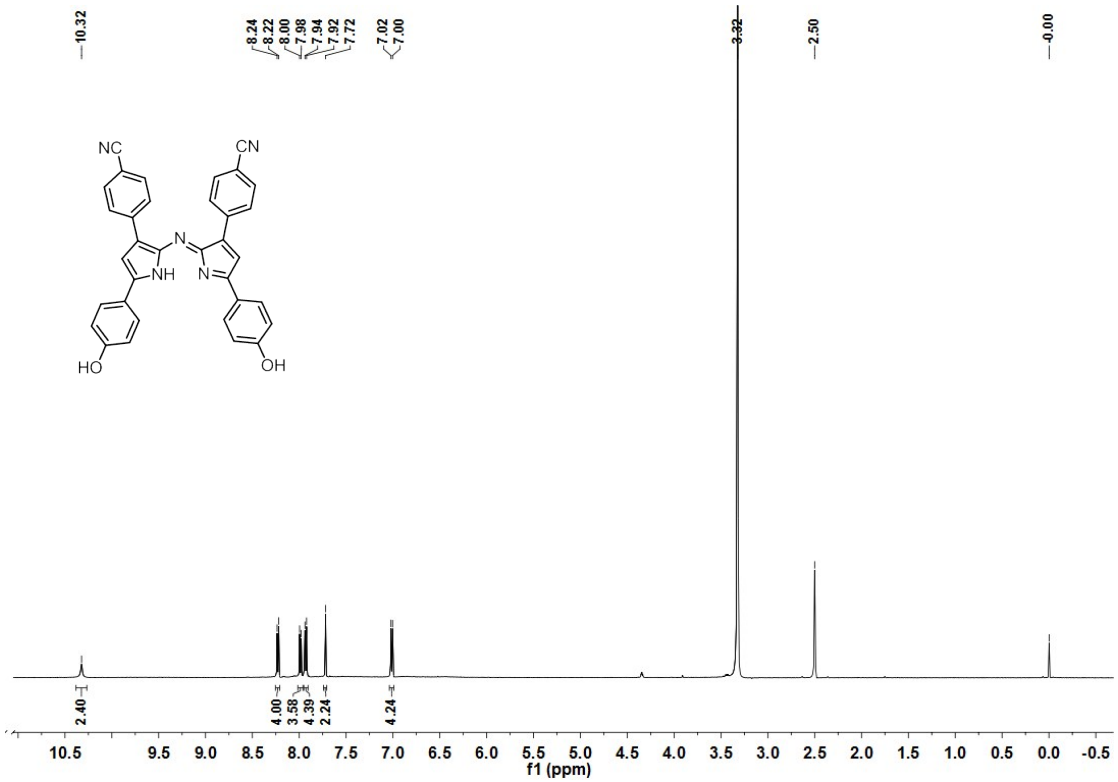


Fig. S15 ^{1}H NMR of 3

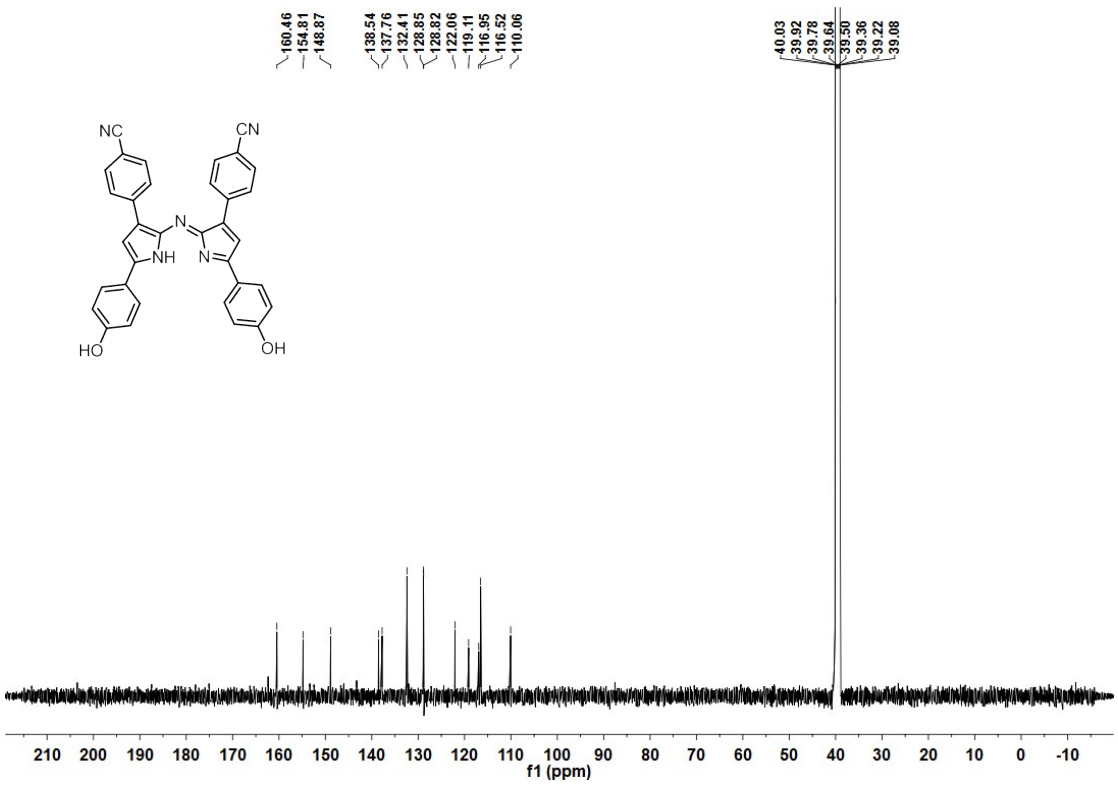


Fig. S16 ^{13}C NMR of 3

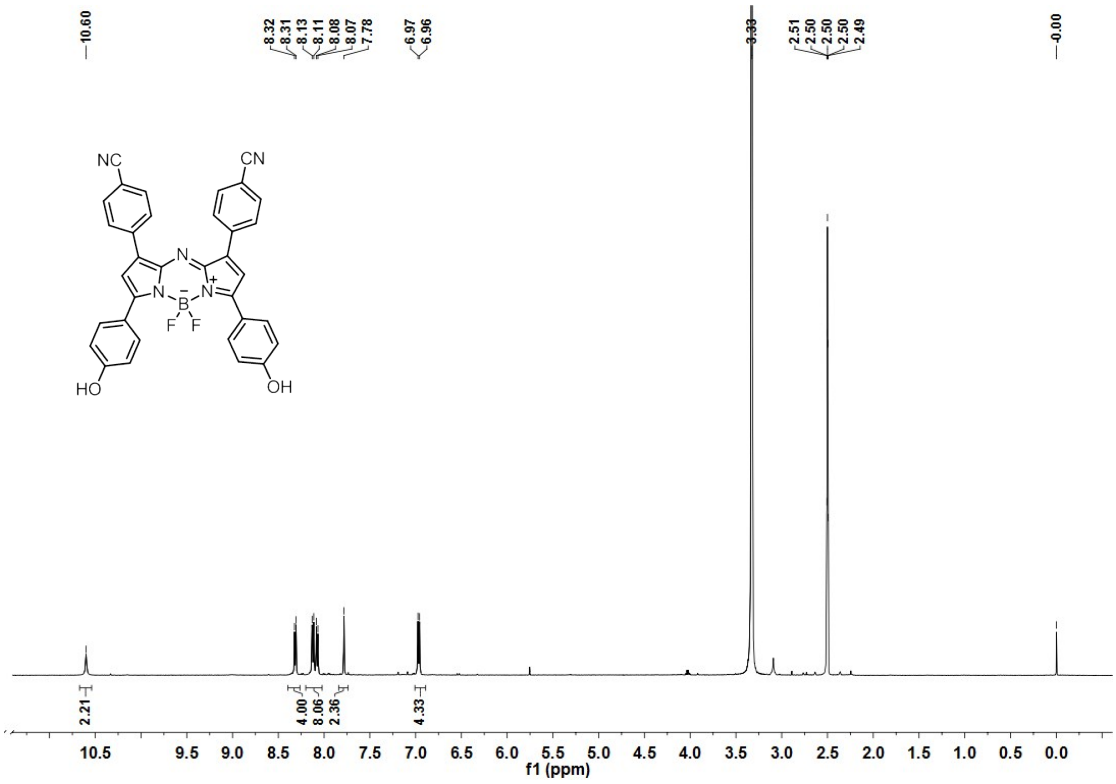


Fig. S17 ^{1}H NMR of 4

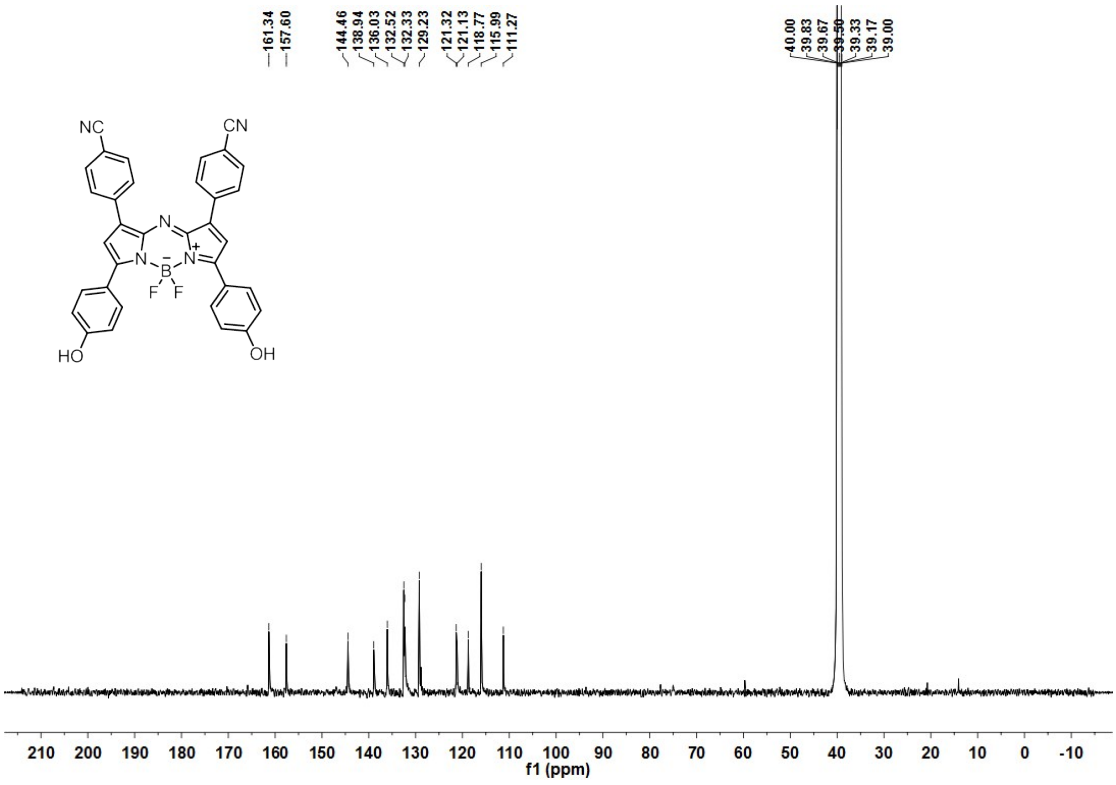


Fig. S18 ^{13}C NMR of 4

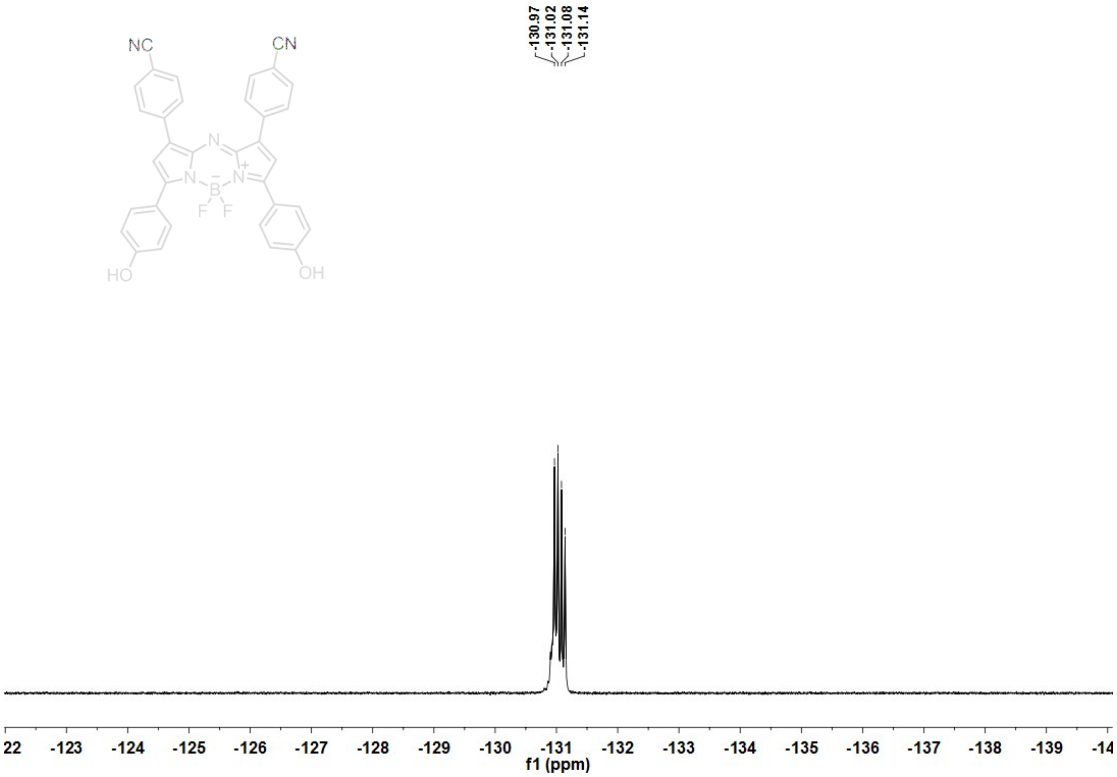


Fig. S19 ^{19}F NMR of 4

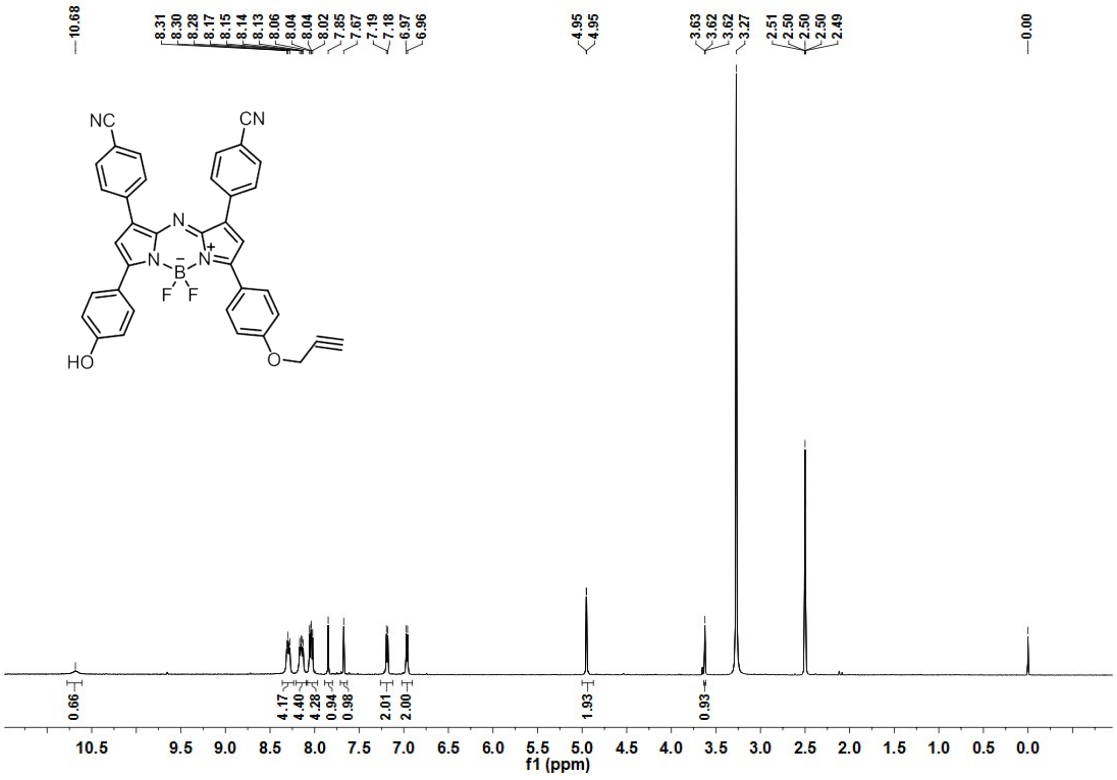


Fig. S20 ^{1}H NMR of BDP

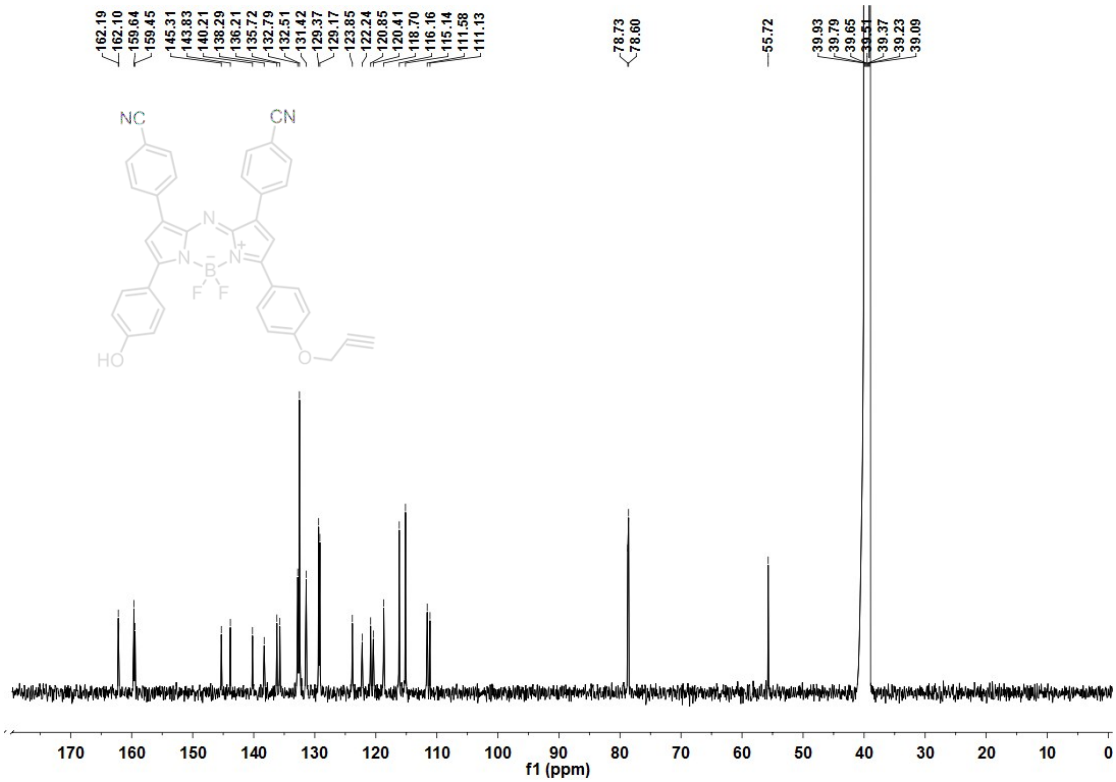


Fig. S21 ^{13}C NMR of BDP

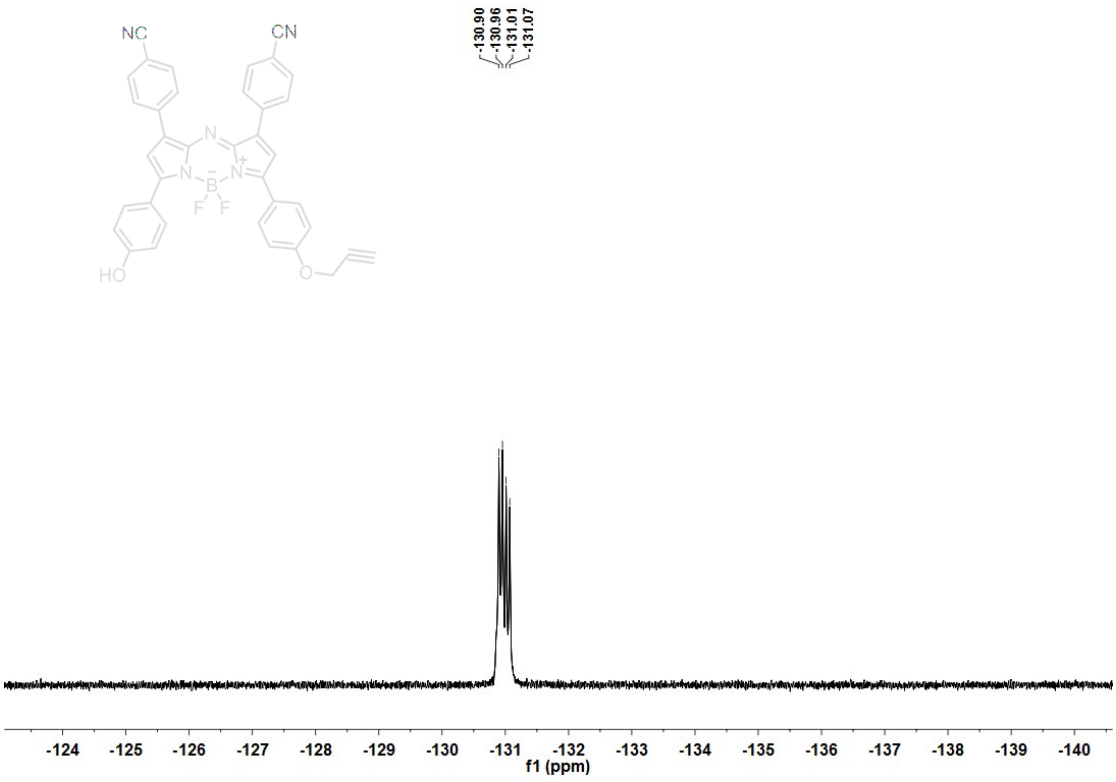


Fig. S22 ^{19}F NMR of BDP

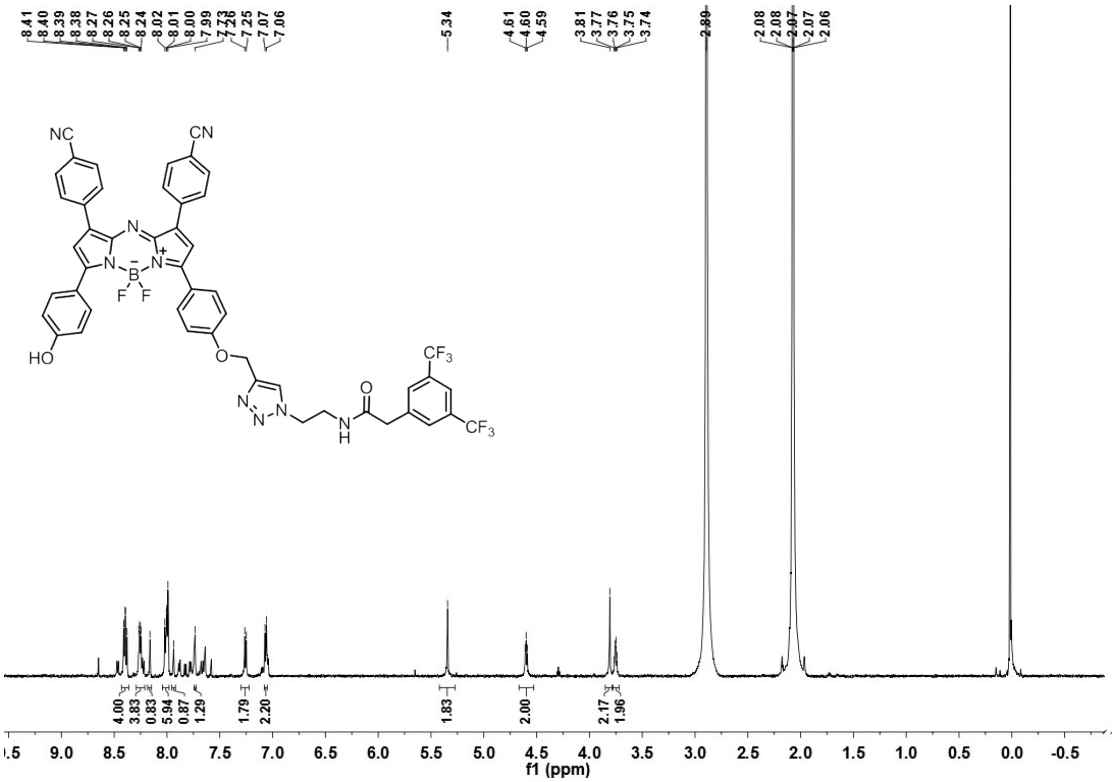


Fig. S23 ^{1}H NMR of BDPF

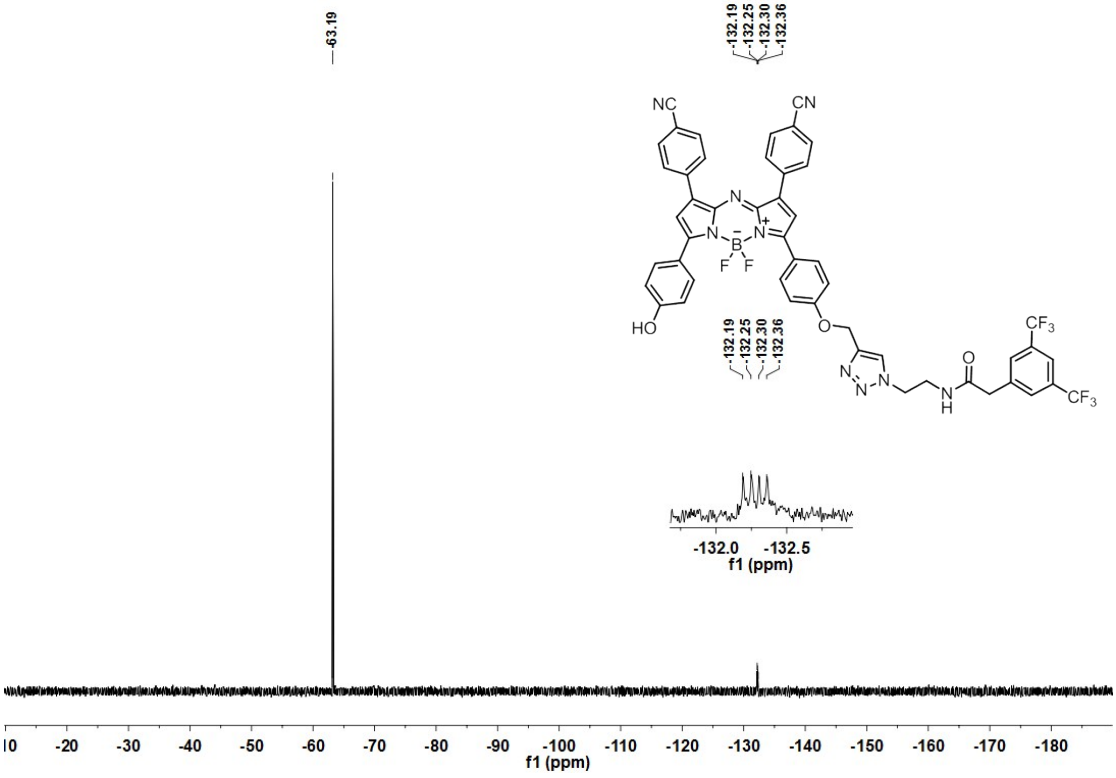


Fig. S24 ^{19}F NMR of BDPF

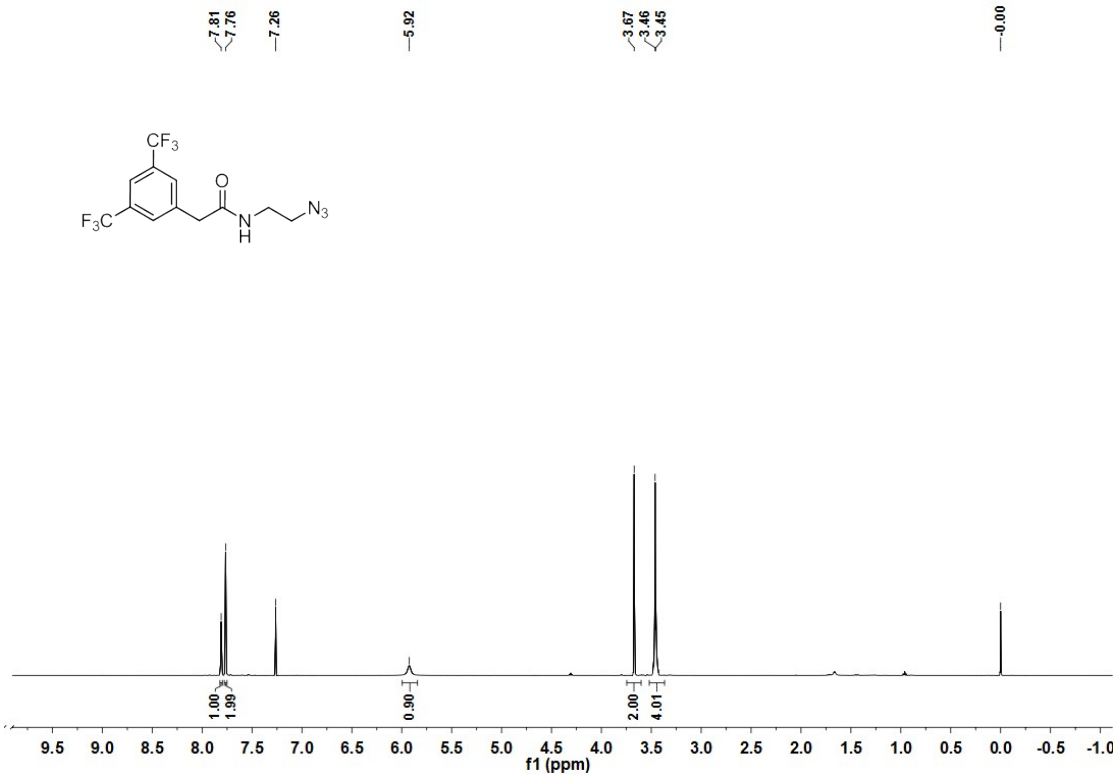


Fig. S25 ^{1}H NMR of 5

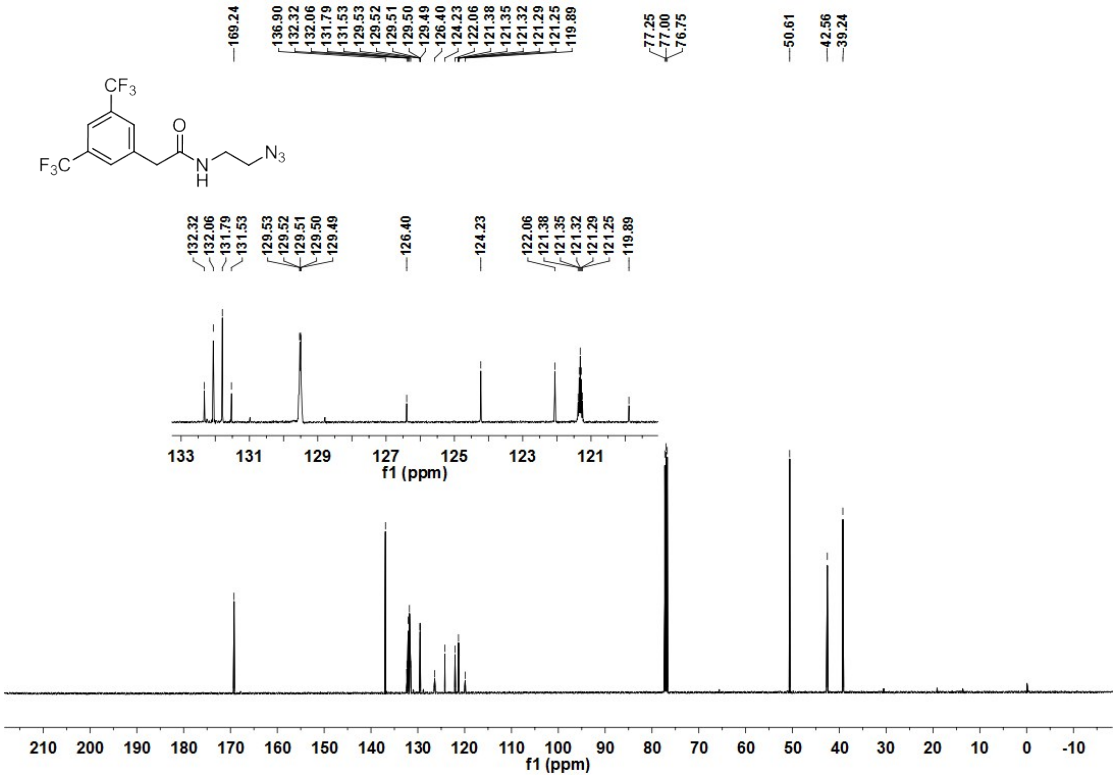


Fig. S26 ^{13}C NMR of 5

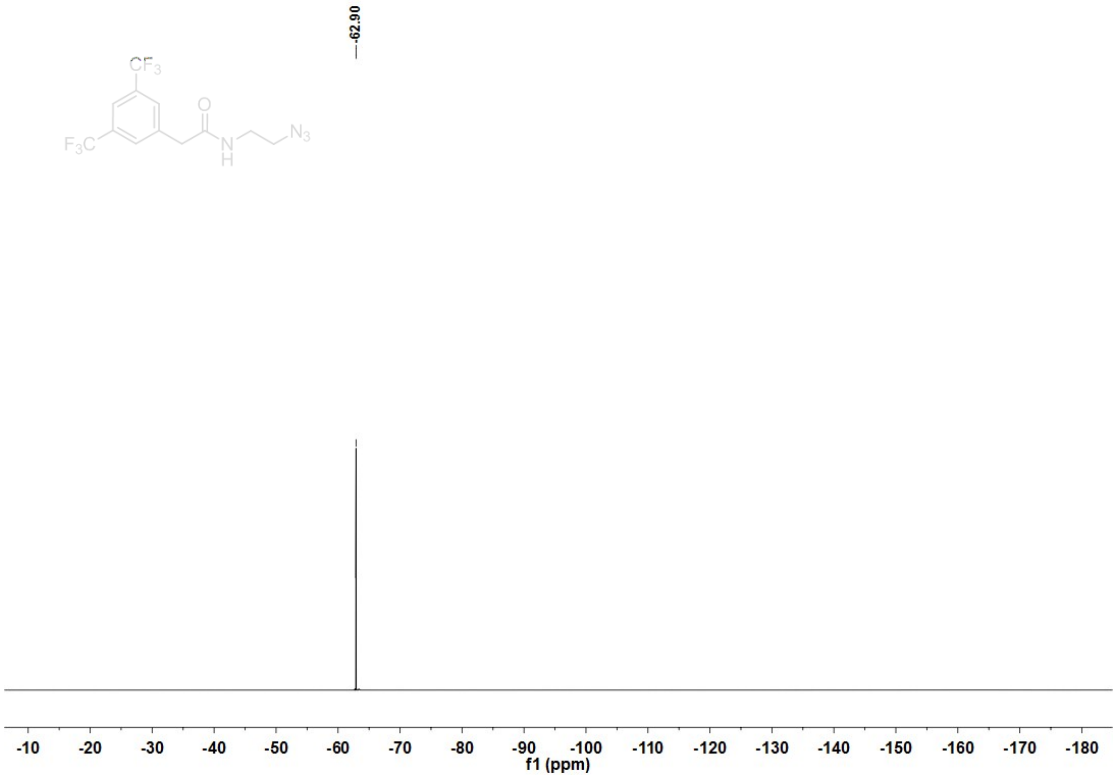


Fig. S27 ^{19}F NMR of 5

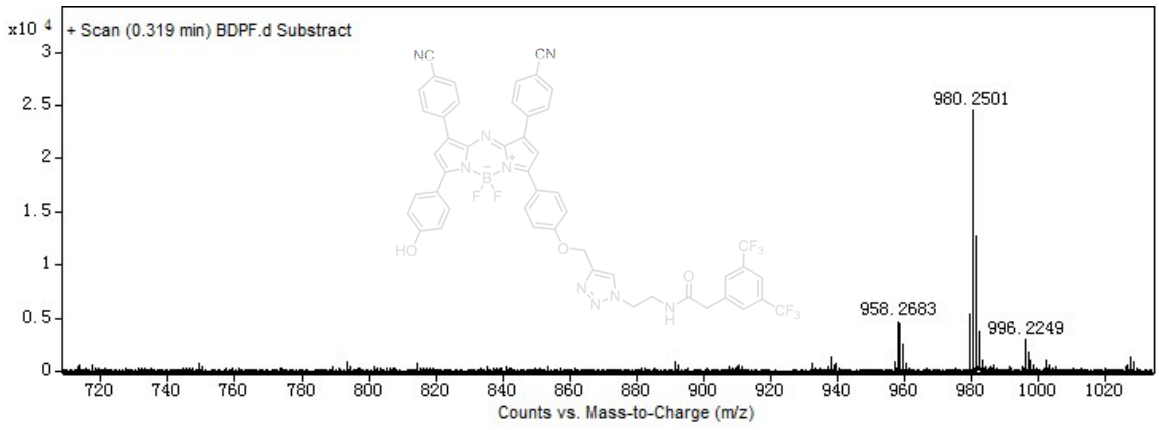


Fig. S28 HRMS (ESI) of BDPF

8. Supplementary references

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