Supporting Information

Facile construction of boranil complexes with aggregation-induced emission characteristics and their specific lipid droplets imaging applications

Na Zhao,* Chengcheng Ma, Weiyao Yang, Wei Yin, Jiahui Wei and Nan Li*

Key Laboratory of Macromolecular Science of Shaanxi Province

Key Laboratory of Applied Surface and Colloid Chemistry of Ministry of Education

Key Laboratory of the Ministry of Education for Medicinal Resources and Natural Pharmaceutical Chemistry

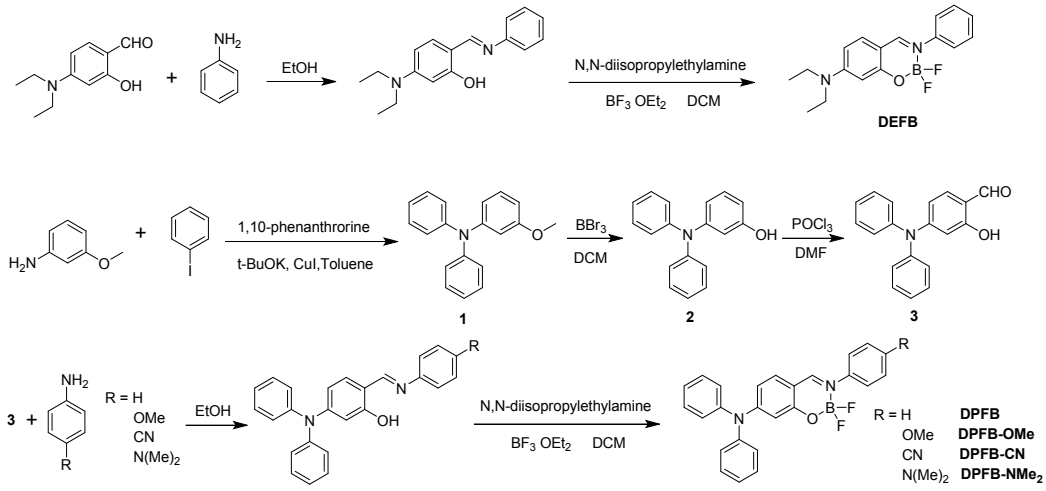
Chemistry and School of Chemistry & Chemical Engineering, Shaanxi Normal University, Xi'an, 710119, China.

E-mail: *nzhao@snnu.edu.cn*; *nli@snnu.edu.cn*.

1. General Information

Toluene was distilled over sodium and benzophenone. DMF was distilled over CaH_2. Petroleum ether and ethyl acetate for chromatography were distilled before used. All other reagents and solvents were used directly from the corresponding supplier without further purification. All starting materials were purchased from Alfa Aesar, Aladdin, Energy, Accela and use directly. HCS LipidTOX™ Deep Red Neutral Lipid was purchased from Thermo Fisher. Stain Analytical thin-layer chromatography (TLC) was carried out using commercial silica gel plated (GF254). Nuclear magnetic resonance spectra (^1H, ^{13}C NMR) were recorded on a Bruker Ascend 600 (^1H at 600 MHz, ^{13}C at 151 MHz). The chemical shifts are reported as ppm and solvent residual peaks were shown as following: $CDCl_3$ δ H (7.26 ppm) and δ C (77.16 ppm). UV-visible absorption spectra were measured on Purkinje TU-1950 spectrometer. Fluorescence spectra were recorded on a Hitachi F-7000 spectrometer. Fluorescence quantum yields were measured using Hamamatsu C9920-02G. Fluorescence lifetime was measured using Edinburgh FLS980 spectrometer. Single crystal was collected on Oxford diffraction Eos CCD detector or Bruker CMOS PHOTON 100 detector, respectively. The fluorescence imaging was collected on Olympus FV1200. Dynamic Light Scattering (DLS) was carried out on Malvern Zetasizer Nano ZS90. High-resolution Mass spectra (HRMS) were obtained on a Bruker Maxis and Microflex and reported as m/z (relative intensity).

2. Synthetic Procedure



Scheme S1. Synthetic routes to **DEPB** and **DPFB** derivatives.

Synthesis of DEFB. 4-(Diethylamino)-2-hydroxybenzaldehyde (38.65 mg, 0.2 mmol) and aniline (18 uL, 0.2 mmol) was dissolved in ethanol (5.00 mL). The reaction was refluxed at 95 °C for 24 hours, and then the ethanol was removed under reduced pressure to the intermediate imine compound. The obtained imine was dissolved in dichloromethane (5.00 mL), $BF_3{\cdot}OEt_2$ (148 μL, 1.2 mmol) and N,N-diisopropylethylamine (198 μL, 1.2 mmol) were added sequentially into the reaction mixture. The reaction was refluxed until the imine was completely consumed based on TLC. Then the reaction solution was concentrated under reduced pressure and purified by column chromatography on silica gel (petroleum ether/ethyl acetate = 40/1) to afford DFEB as a light yellow solid (39 mg, 61% yield). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.04 (d, *J* = 3.8 Hz, 1H, CH), 7.50 (d, *J* = 7.9 Hz, 2H, Ar H), 7.43 (t, *J* = 7.9 Hz, 2H, Ar H), 7.34 (t, *J* = 7.4 Hz, 1H, Ar H), 7.22 (d, *J* = 9.0 Hz, 1H, Ar H), 6.37-6.35 (m, 1H, Ar H), 6.25 (d, *J* = 2.3 Hz, 1H), 3.47-3.44 (q, 4H, CH_2), 1.24 (t, *J* = 7.1 Hz, 6H, CH_3); ^{13}C NMR (151 MHz, $CDCl_3$) δ 162.06, 158.53, 156.39, 143.41, 134.00, 129.50, 127.79, 123.42, 106.75, 98.30, 45.31, 12.79. HRMS (ESI-TOF): m/z calcd for $C_{17}H_{19}BF_2N_2O$ $[M+H]^+$ 339.1500, found 339.1448.

Compound 1-3. Compound **1-3** were synthesized according to the literature.[1-3]

Synthesis of DPFB derivatives. The DPFB derivatives were synthesized similar to the synthetic route of **DEFB**. Yield: 51%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.14 (s, 1H, CH), 7.51 (d, *J* = 7.9 Hz, 2H, Ar H), 7.44 (t, *J* = 7.8 Hz, 2H, Ar H), 7.42 – 7.35 (m, 5H, Ar H), 7.26 – 7.18 (m, 7H, Ar H), 6.51 (dd, *J* = 8.8, 2.2 Hz, 1H, Ar H), 6.47 (d, *J* = 2.1 Hz, 1H, Ar H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 161.64, 159.69, 157.70, 145.06, 143.04, 133.24, 130.04, 129.60, 128.39, 127.38, 126.53, 123.50, 112.08, 109.64, 105.68. HRMS (ESI-TOF): m/z calcd for $C_{25}H_{19}BF_2N_2O$ $[M+Na]^+$ 435.1500, found 435.1450.

DFPB-OMe. Yield: 35%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.10 (s, 1H, CH), 7.44 (d, *J* = 8.9 Hz, 2H, Ar H), 7.38 (t, *J* = 7.9 Hz, 4H, Ar H), 7.28 – 7.16 (m, 7H, Ar H), 6.95 (d, *J* = 9.0 Hz, 2H, Ar H), 6.51 (dd, *J* = 8.8, 2.2 Hz, 1H, Ar H), 6.47 (d, *J* = 2.0 Hz, 1H, Ar H), 3.83 (s, 3H, CH_3); ^{13}C NMR (151 MHz, $CDCl_3$) δ 161.34, 159.70, 158.92, 157.40, 145.19, 136.18, 132.96, 130.01, 127.34, 126.41, 124.57, 114.79, 112.06,

109.67, 105.88, 55.73. HRMS (ESI-TOF): m/z calcd for $C_{26}H_{21}BF_2N_2O_2$ $[M+Na]^+$ 465.1600, found 465.1560.

DPFB-CN. Yield: 32%. ^1H NMR (600 MHz, $CDCl_3$) δ 8.13 (s, 1H, CH), 7.74 (d, *J* = 8.6 Hz, 2H, Ar H), 7.64 (d, *J* = 8.5 Hz, 2H, Ar H), 7.41 (dd, *J* = 10.8, 5.0 Hz, 4H, Ar H), 7.28 (t, *J* = 7.5 Hz, 2H, Ar H), 7.25 – 7.19 (m, 5H, Ar H), 6.51 (dd, *J* = 8.9, 2.2 Hz, 1H, Ar H), 6.42 (d, *J* = 1.9 Hz, 1H, Ar H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.26, 159.15, 158.66, 146.41, 144.52, 133.86, 133.57, 130.16, 127.49, 127.06, 124.10, 118.19, 112.34, 111.73, 109.61, 104.78. HRMS (ESI-TOF): m/z calcd for $C_{26}H_{18}BF_2N_3O$ $[M+Na]^+$ 460.1400, found 460.1430.

DPFB-NMe$_2$. Yield: 47%. ^1H NMR (600 MHz, $CDCl_3$) δ 8.09 (s, 1H, CH), 7.42 – 7.33 (m, 6H, Ar H), 7.21 (m, 7H, Ar H), 6.72 (d, *J* = 9.1 Hz, 2H, Ar H), 6.53 – 6.47 (m, 2H, Ar H), 2.99 (s, 6H, CH_3); ^{13}C NMR (151 MHz, $CDCl_3$) δ 160.93, 157.39, 156.86, 150.50, 145.44, 132.56, 132.28, 129.95, 127.26, 126.16, 123.98, 112.62, 112.09, 110.05, 106.34, 40.59. HRMS (ESI-TOF): m/z calcd for $C_{27}H_{24}BF_2N_3O$ $[M+Na]^+$ 478.1900, found 478.1871.

Reference:

1. A. G. Jadhav, S. Kothavale, N. Sekar, *Dyes and Pigments*, 2017, **138**, 56–67.
2. S. W. Kwak, H. Jin, J. H. Lee, H. Hwang, M. Kim, Y. Kim, Y. Chung, K. M. Lee, M. H. Park, *Inorg. Chem.*, 2019, **58**, 2454–2462.
3. L. Han, J. He, J. Zhao, S. Jiang, *Res. Chem. Intermed.*, 2017, **43**, 5779–5794.

3. Cell Culture and Imaging

Cell Viability Assay. HeLa cells were cultured in a 96 well-plate at the density of 10000 cells per well. After 48 hours incubation, the culture medium was replaced by 100 μL of fresh medium containing various concentrations (0, 0.5, 1, 5, 10, 20 and 50 μM) of **DPFB** derivatives. After that, the cells were incubated for 12 hours, and then 10 μL MTT solutions (5 mg/mL in phosphate buffer solution) were added into each well. The cells were incubated with MTT for another 4 hours and 100 μL of the lysate buffer was added into each well and incubated for further 4 hours to completely dissolve the formazan crystal, The absorbance of each well at 570 nm was measured

via a Spectra Max M384 (Molecular Devices) and the data was recorded by using Softmax Pro 6.4 software. Each of the experiments was performed 6 times as a parallel test.

Cell Culture. HeLa cells grown on a 35-mm Petri dish with a cover slip were cultured in DMEM medium containing 10% fetal bovine serum (FBS) and 5% CO_2 at 37 °C. For fluorescence imaging, cultured HeLa cells were pretreated with 40 μM oleic acid for 4 h to induce the formation of lipid droplets and then stained with **DPFB** derivatives.

Co-localization Experiments. The oleic acid-treated HeLa cells were firstly incubated with 5 μM of **DPFB** derivatives (by adding 5 μL of 1 mM DMSO stock solution of **DPFB** derivatives into 1 mL DMEM medium without phenol red) and HCS LipidTOX™ Deep Red Neutral Lipid (1: 1000) at 37 °C for 30 min. Then the medium was removed and resulted cells were washed with phosphate buffered saline (PBS) twice before imaging. For **DPFB** derivatives, the emission filter was 535–605 nm (**DPFB**) and 530–630 nm (**DPFB-NMe_2**), for HCS LipidTOX™ Deep Red Neutral Lipid, the emission filter was 655–755 nm. The excitation was 405 nm for **DPFB** derivatives and 635 nm for HCS LipidTOX™ Deep Red Neutral Lipid.

Wash-Free Imaging. The oleic acid-treated HeLa cells were incubated with **DPFB** derivatives (5 μM), BODIPY493/503 (500 nM) and Nile Red (500 nM) for 30 min, respectively. After that, the cells were imaged directly using confocal microscopy without washing by PBS. The emission filter was 500–550 nm (BODIPY493/503) and 570–660 nm (Nile Red). The excitation was 488 nm for BODIPY493/503 and 559 nm for Nile Red.

Fast-Staining Experiments. The oleic acid-treated HeLa cells were washed with PBS twice. After that, the **DPFB** derivatives in DMEM medium (phenol red free) were added to give the final concentration of 5 μM. Then the fluorescent images were collected over time.

Zebrafish Imaging. Zebrafish embryos were incubated at 27 °C in EM culture medium containing 150 mM NaCl, 0.5 mM KCl, 1.0 mM $CaCl_2$, 0.37 mM KH_2PO4, 0.05 mM Na_2HPO_4, 2.0 mM $MgSO_4$ and 0.71 mM $NaHCO_3$. After 24~48h incubation,

PTU (75 μM) was added into EM culture medium to prevent the formation of melanin, which could keep the fish body optically transparency. For confocal imaging experiments, zebrafish larvae were soaked in 1 mL EM containing **DPFB** derivatives (5 μM) for 30 min. Then the dye-stained zebrafish larvae were washed with fresh PBS solution for three times. The zebrafish larvae were anesthetized by 0.003% tricaine methane sulfonate (MS222) before confocal imaging.

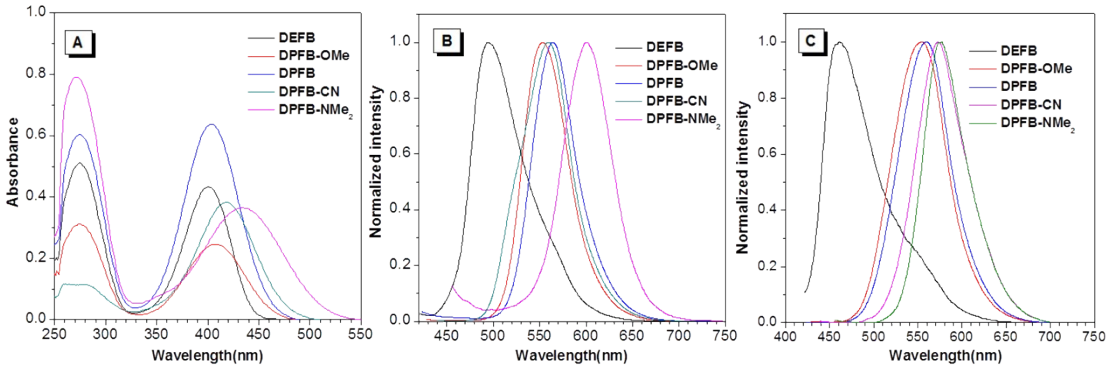


Fig. S1 (A) Absorption spectra of all complexes in DMSO solution. Normalized emission spectra of all complexes (B) in solid state and (C) in PBS solution.

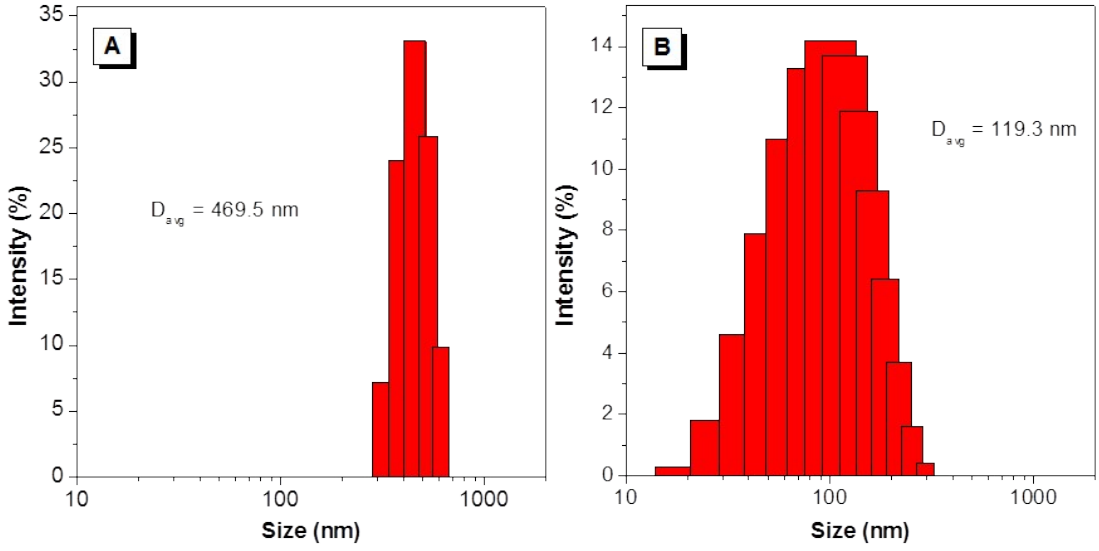


Fig. S2 Size distribution of (A) **DEFB** and (B) **DPFB** in DMSO/water mixture with f_w 99%.

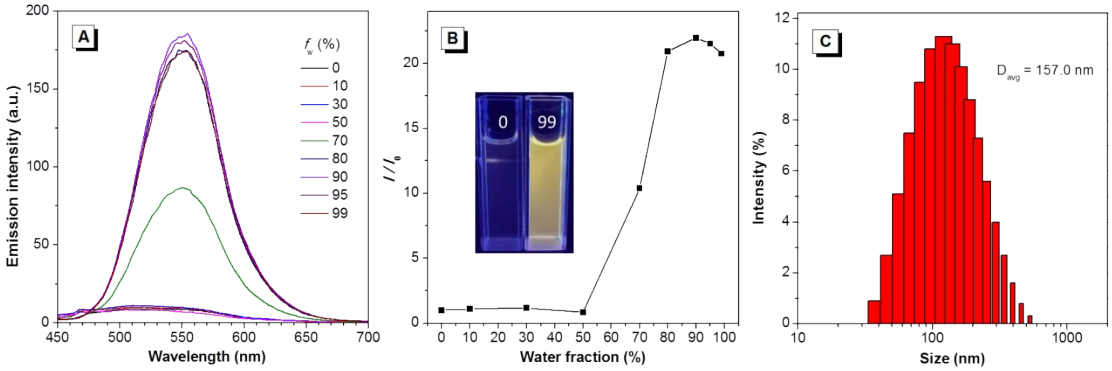


Fig. S3 (A) The Emission spectra of **DPFB-OMe** in DMSO/water mixtures with varied water fractions (f_w). (B) Plot of I/I_0 of **DPFB-OMe** versus f_w of the solvent mixture (I_0 represents the emission intensity in DMSO). (C) Size distribution of **DPFB-OMe** in in DMSO/water mixture with 99% f_w. Inset: photograph of (B) **DPFB-OMe** in DMSO/water mixtures with 0 and 99% f_w under irradiation of 365 nm UV light. λ_{ex}: 408 nm.

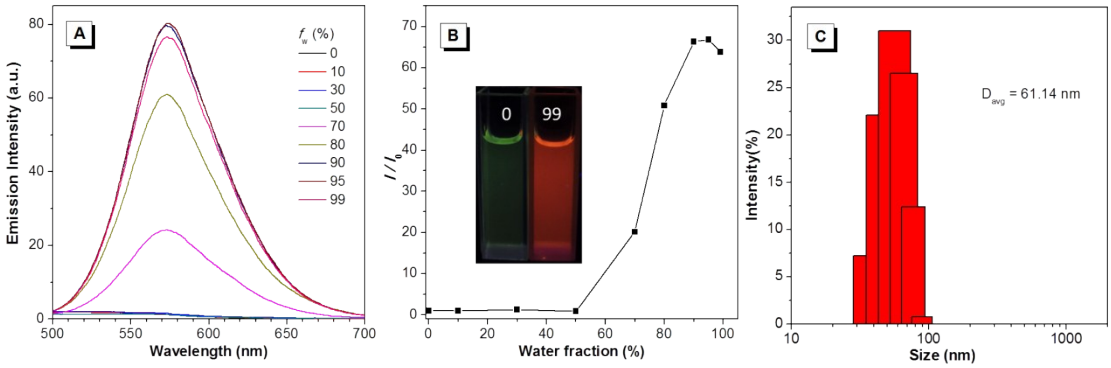


Fig. S4 (A) The Emission spectra of **DPFB-CN** in DMSO/water mixtures with varied water fractions (f_w). (B) Plot of I/I_0 of **DPFB-CN** versus f_w of the solvent mixture (I_0 represents the emission intensity in DMSO). (C) Size distribution of **DPFB-CN** in DMSO/water mixture with 99% f_w. Inset: photograph of (B) **DPFB-CN** in DMSO/water mixtures with 0 and 99% f_w under irradiation of 365 nm UV light. λ_{ex}: 419 nm.

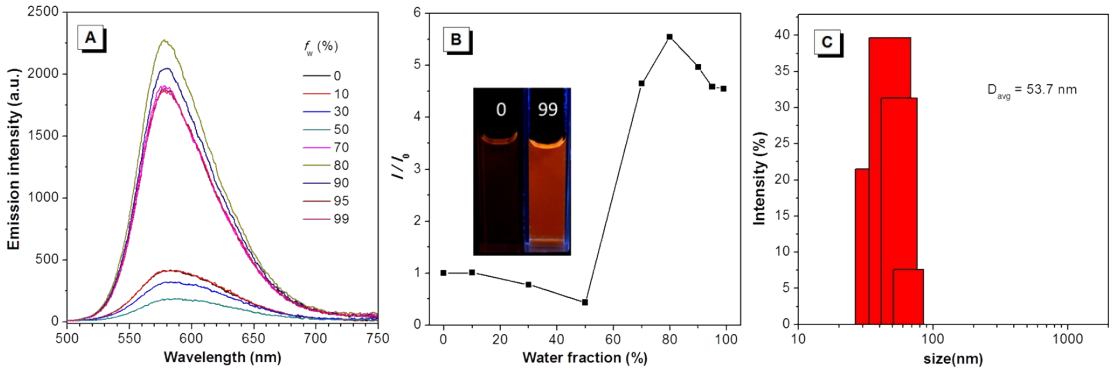


Fig. S5 (A) The Emission spectra of **DPFB-NMe$_2$** in DMSO/water mixtures with varied water fractions (f_w). (B) Plot of I/I_0 of **DPFB-NMe$_2$** versus f_w of the solvent mixture (I_0 represents the emission intensity in DMSO). (C) Size distribution of **DPFB-NMe$_2$** in in DMSO/water mixture with 99% f_w. Inset: photograph of (B) **DPFB-NMe$_2$** in DMSO/water mixtures with 0 and 99% f_w under irradiation of 365 nm UV light. λ_{ex}: 435 nm.

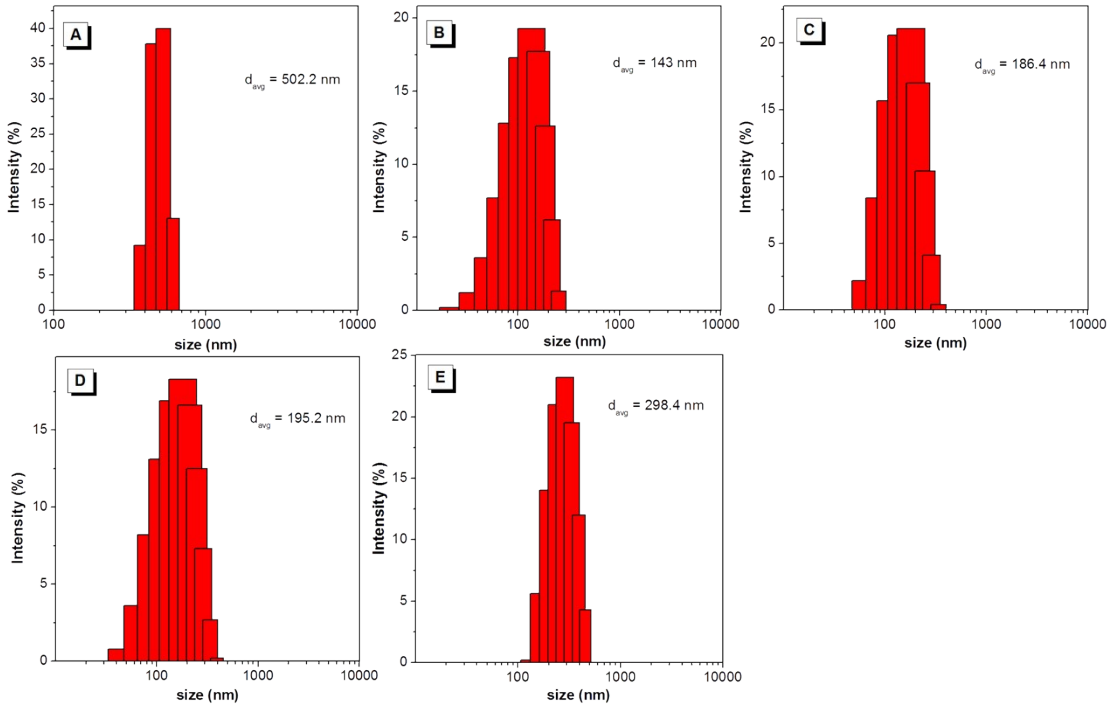


Fig. S6 Size distribution of (A) **DEFB**, (B) **DPFB**, (C) **DPFB-OMe**, (D) **DPFB-CN** and (E) **DPFB-NMe$_2$** in DMSO/PBS mixtures with PBS content of 99%.

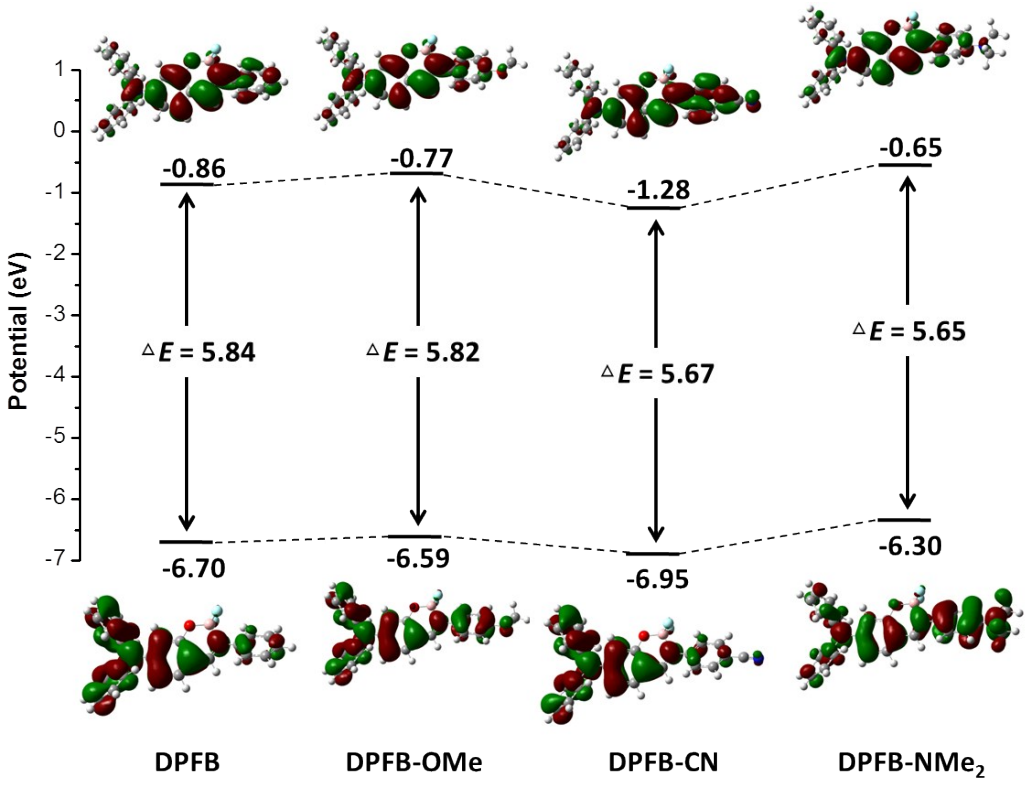


Fig. S7 The energy gaps between HOMO and LUMO for **DPFB** derivatives calculated from DFT based on 6-31G basis set.

Table S1. Crystallographic data of **DEFB**, **DPFB-CN** and **DPFB-NMe$_2$**.

Crystal	**DEFB**	**DPFB-CN**	**DPFB-NMe$_2$**
formula	$C_{17}H_{19}BF_2N_2O$	$C_{26}H_{18}BF_2N_3O$	$C_{27}H_{24}BF_2N_3O$
crystal system	monoclinic	triclinic	triclinic
space group	P 1 21/c 1	C 1 2/c 1	P –1
a [Å]	6.3794 (2)	16.1393 (7)	6.3782 (3)
b[Å]	17.4457 (5)	7.0248 (4)	11.9598 (5)
c[Å]	13.9241 (4)	37.8519 (18)	18.7878 (8)
α [deg]	90	90	93.233 (3)
β [deg]	92.880 (3)	93.108 (5)	95.900 (4)
γ [deg]	90	90	105.309 (4)
V [Å^3]	1547.70 (8)	4285.1 (4)	1369.77 (11)
Z	1	8	1
μ [mm^{-1}]	0.834	0.786	2.464
T [K]	293	293	293
θ_{min}–θ_{max} [deg]	5.988–72.013	4.860–71.962	3.847–71.941
R	0.0734	0.0726	0.0715
wR_2	0.2052	0.2108	0.2128
GOOF	1.086	1.042	1.071
CCDC number	1915931	1915930	1915932

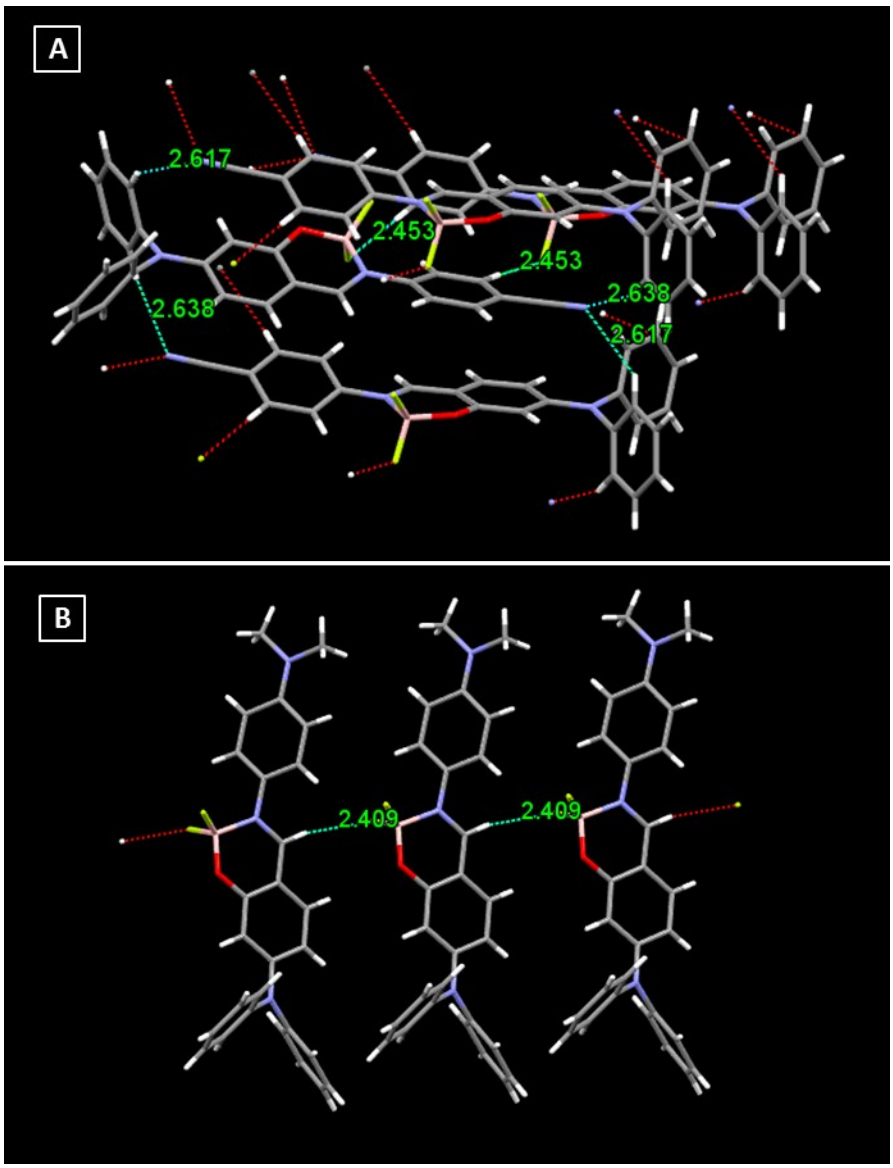


Fig. S8 The view of short contacts of (A) **DPFB-CN** and (B) **DPFB-NMe$_2$**.

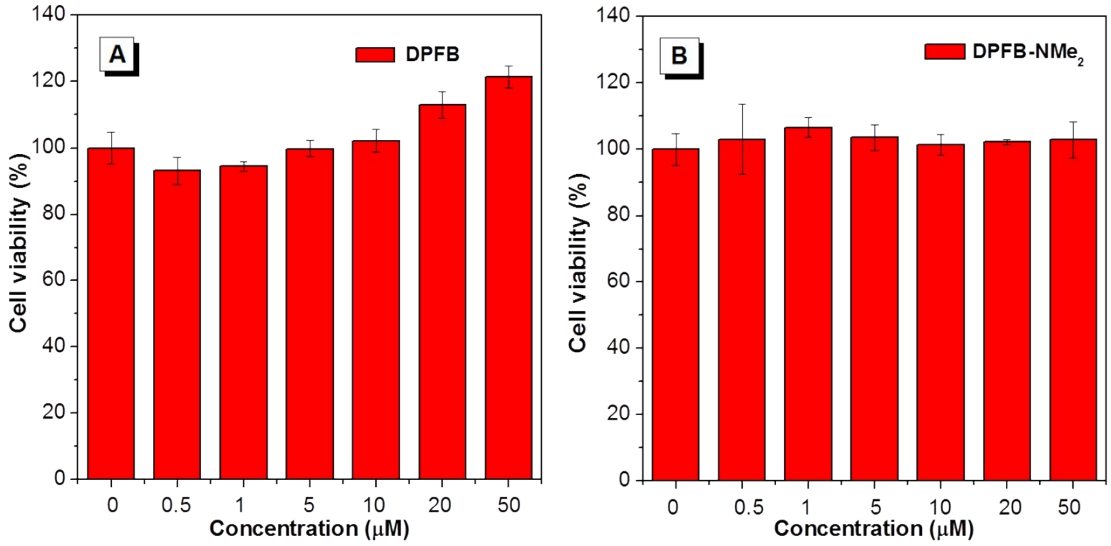


Fig. S9 Cell viabilities of Hela cells after incubated with different concentration of **DPFB** and **DPFB-NMe$_2$**.

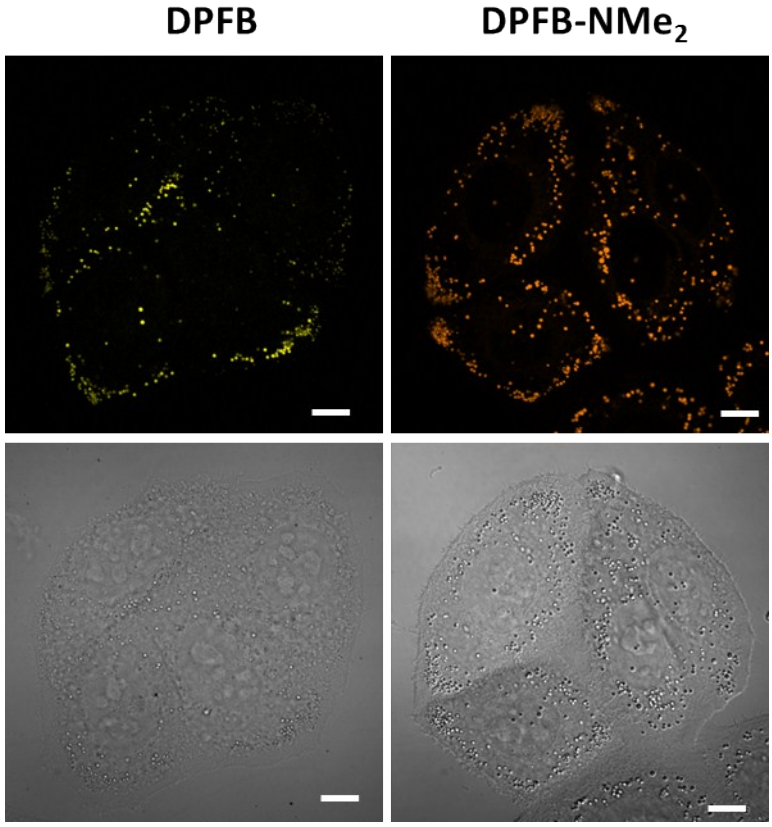


Fig. S10 CLSM images of HeLa cells stained with **DPFB** (5 μM) and **DPFB-NMe$_2$** (5 μM). Scale bars: 10 μm.

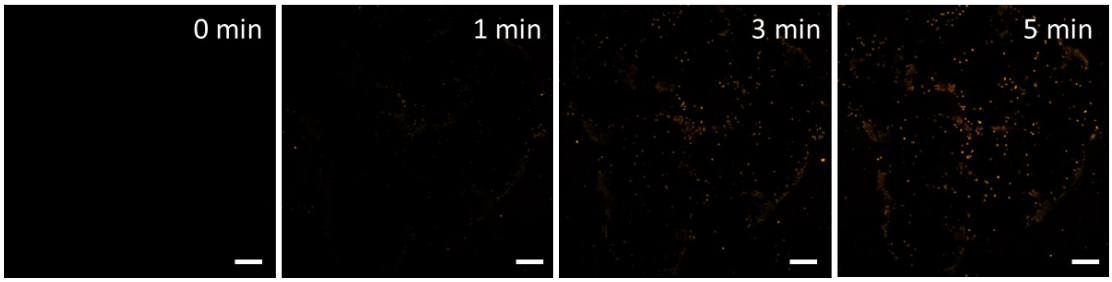


Fig. S11 Time-dependent images of **DPFB-NMe$_2$** (5 μM). Scale bar: 10 μm.

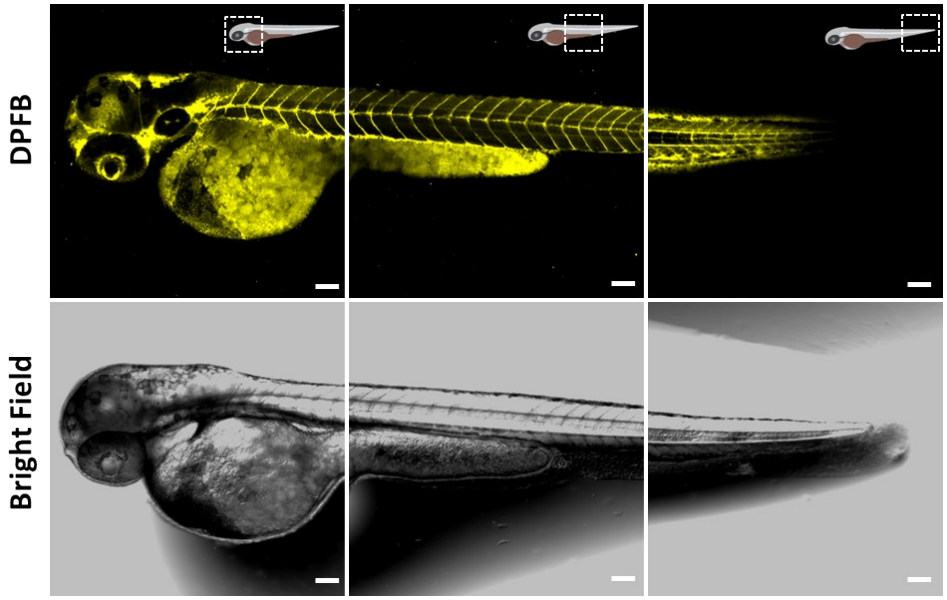


Fig. S12 CLSM images of zebrafish stained with **DPFB** (5 μM). Scale bar: 100 μm.

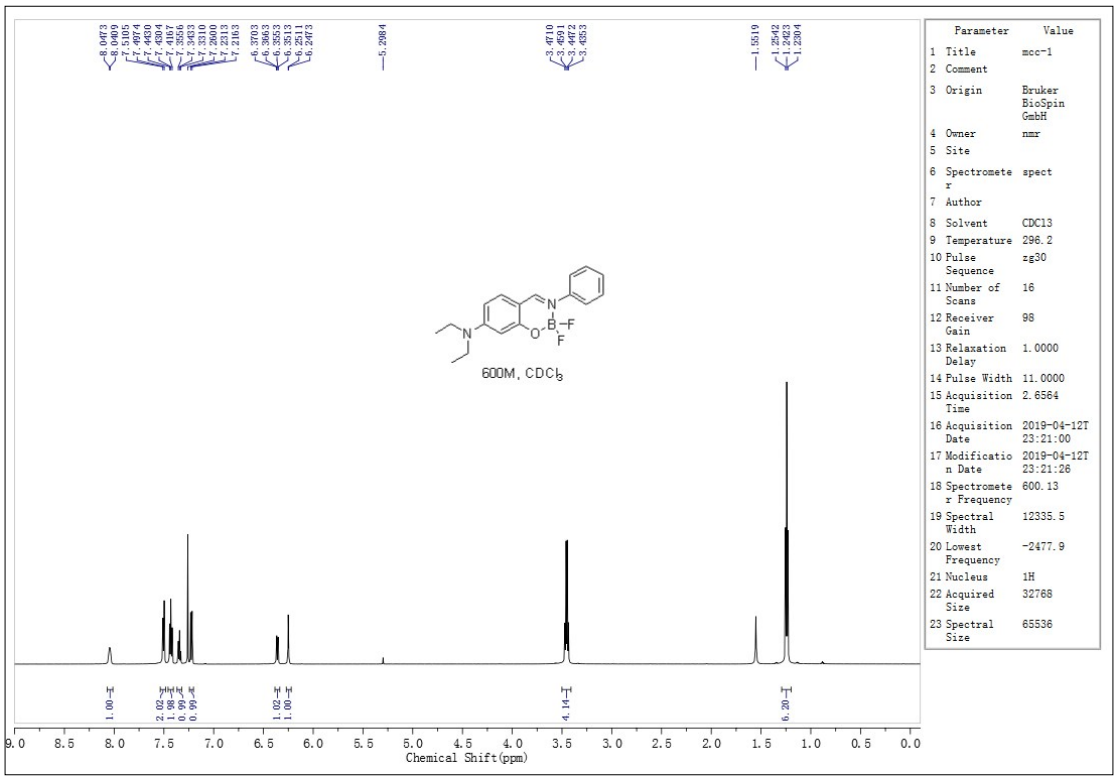


Fig. S11 ^{1}H NMR spectrum of **DEFB** in $CDCl_3$ (600 MHz).

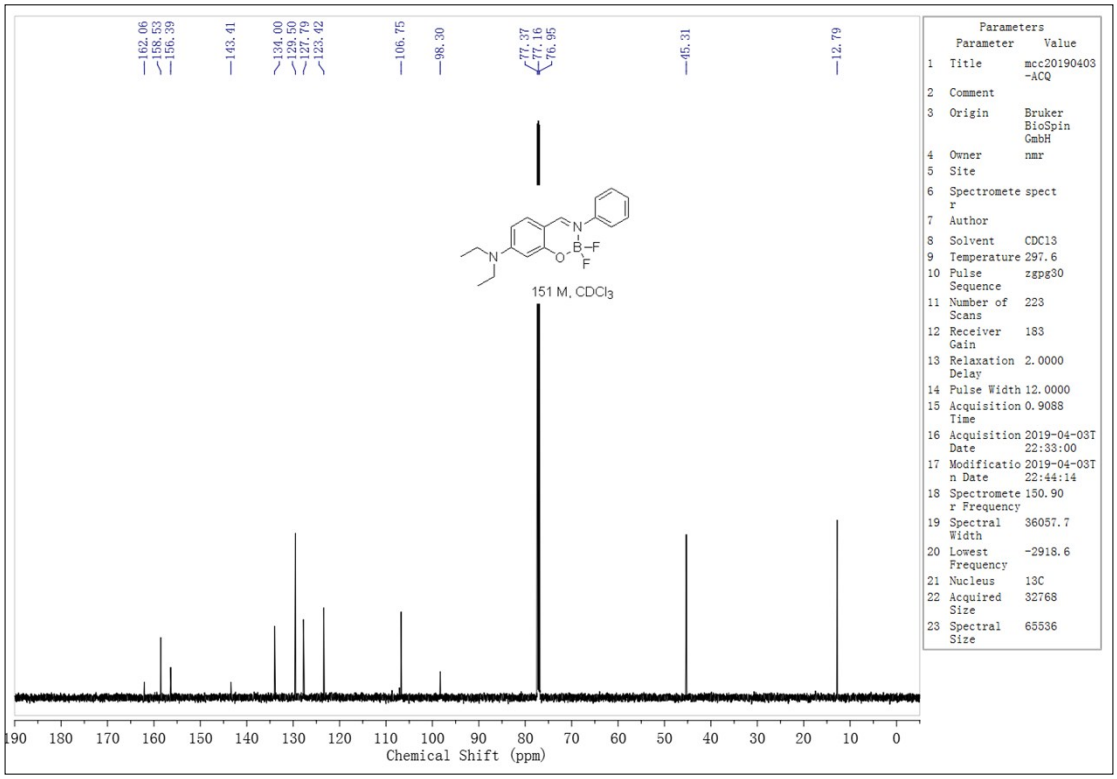
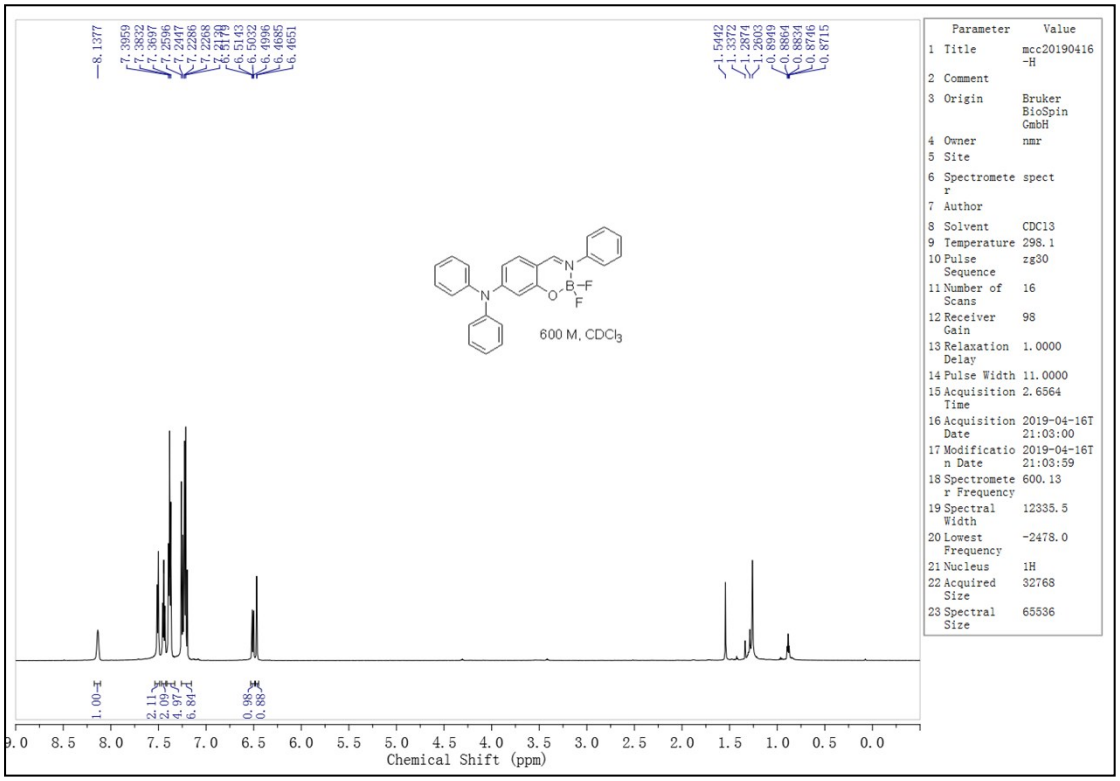
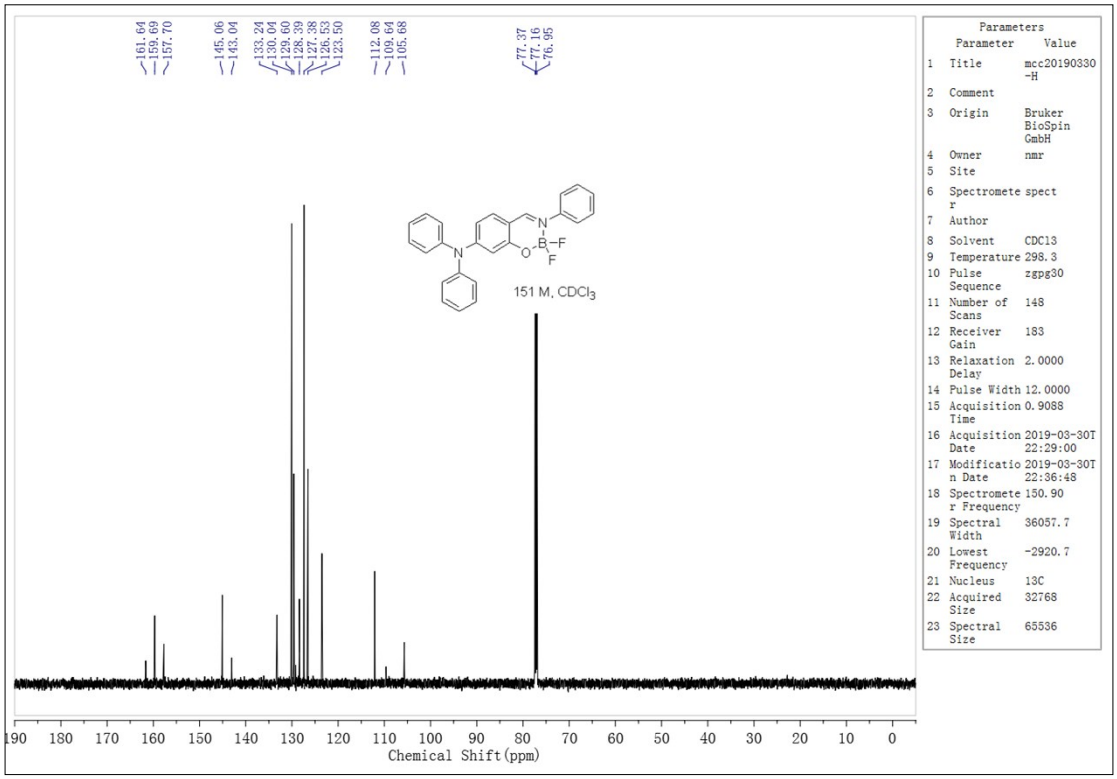


Fig. S12 ^{13}C NMR spectrum of **DEFB** in $CDCl_3$ (151 MHz).



	Parameter	Value
1	Title	mcc20190416-H
2	Comment	
3	Origin	Bruker BioSpin GmbH
4	Owner	nmr
5	Site	
6	Spectrometer	spect r
7	Author	
8	Solvent	CDC13
9	Temperature	298.1
10	Pulse Sequence	zg30
11	Number of Scans	16
12	Receiver Gain	98
13	Relaxation Delay	1.0000
14	Pulse Width	11.0000
15	Acquisition Time	2.6564
16	Acquisition Date	2019-04-16T 21:03:00
17	Modification Date	2019-04-16T 21:03:59
18	Spectrometer Frequency	600.13
19	Spectral Width	12335.5
20	Lowest Frequency	-2478.0
21	Nucleus	1H
22	Acquired Size	32768
23	Spectral Size	65536

Fig. S13 ^{1}H NMR spectrum of **DPFB** in $CDCl_3$ (600 MHz).



	Parameter	Value
1	Title	mcc20190330-H
2	Comment	
3	Origin	Bruker BioSpin GmbH
4	Owner	nmr
5	Site	
6	Spectrometer	spect r
7	Author	
8	Solvent	CDC13
9	Temperature	298.3
10	Pulse Sequence	zgpg30
11	Number of Scans	148
12	Receiver Gain	183
13	Relaxation Delay	2.0000
14	Pulse Width	12.0000
15	Acquisition Time	0.9088
16	Acquisition Date	2019-03-30T 22:29:00
17	Modification Date	2019-03-30T 22:36:48
18	Spectrometer Frequency	150.90
19	Spectral Width	36057.7
20	Lowest Frequency	-2920.7
21	Nucleus	13C
22	Acquired Size	32768
23	Spectral Size	65536

Fig. S14 ^{13}C NMR spectrum of **DPFB** in $CDCl_3$ (151 MHz).

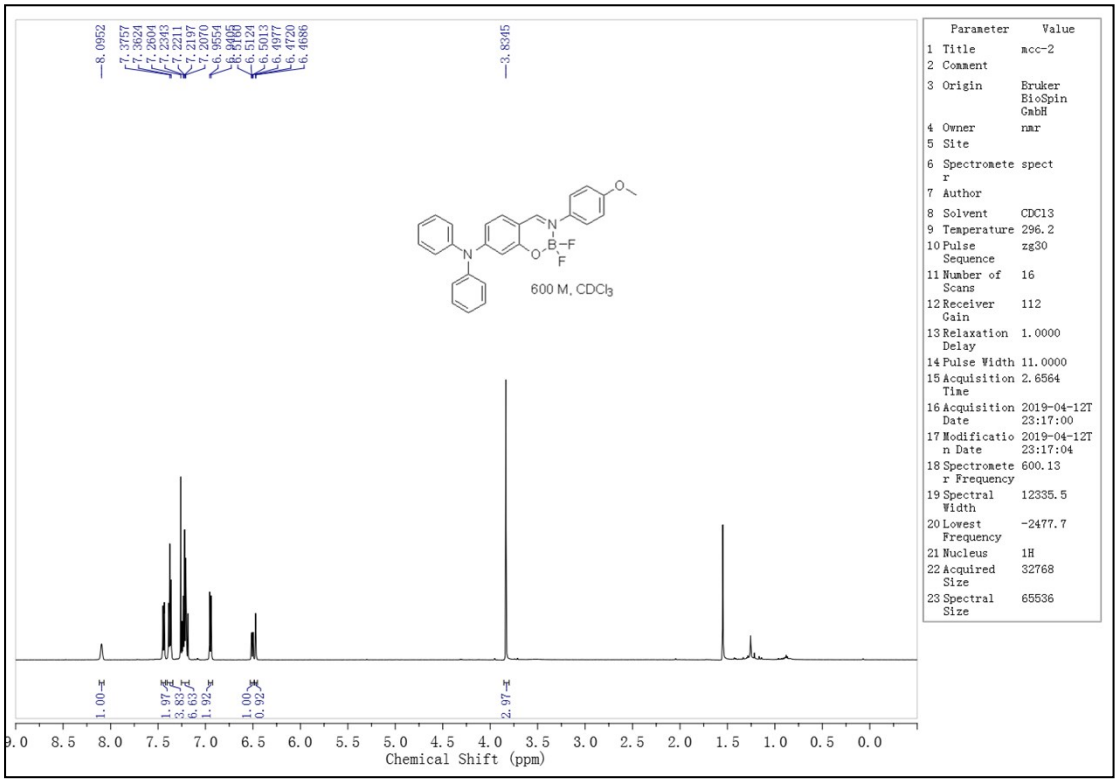


Fig. S15 ^{1}H NMR spectrum of **DPFB-OMe** in $CDCl_3$ (600 MHz).

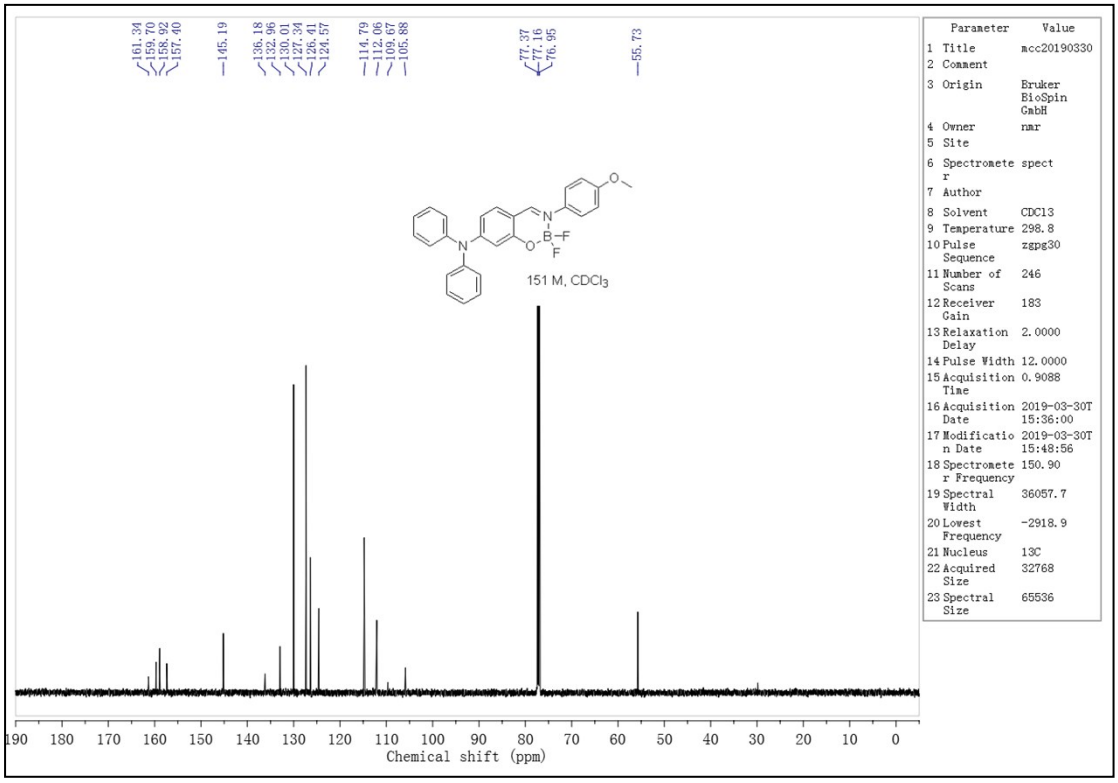


Fig. S16 ^{13}C NMR spectrum of **DPFB-OMe** in $CDCl_3$ (151 MHz).

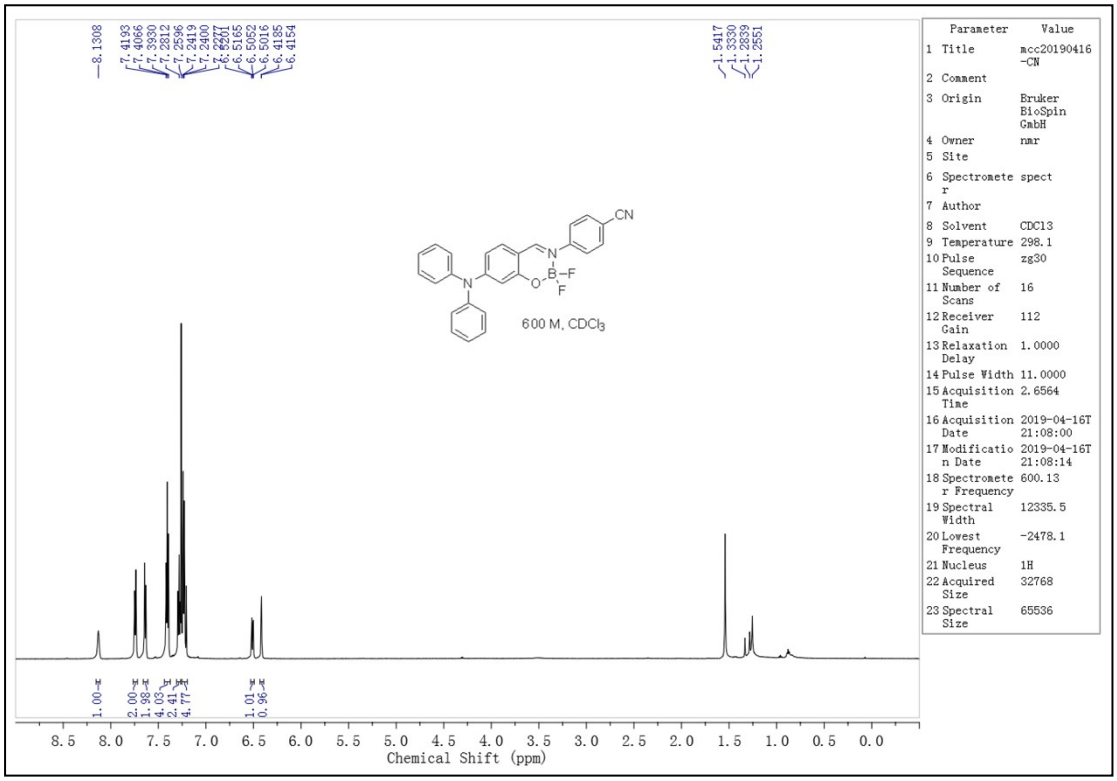


Fig. S17 ^{1}H NMR spectrum of **DPFB-CN** in $CDCl_3$ (600 MHz).

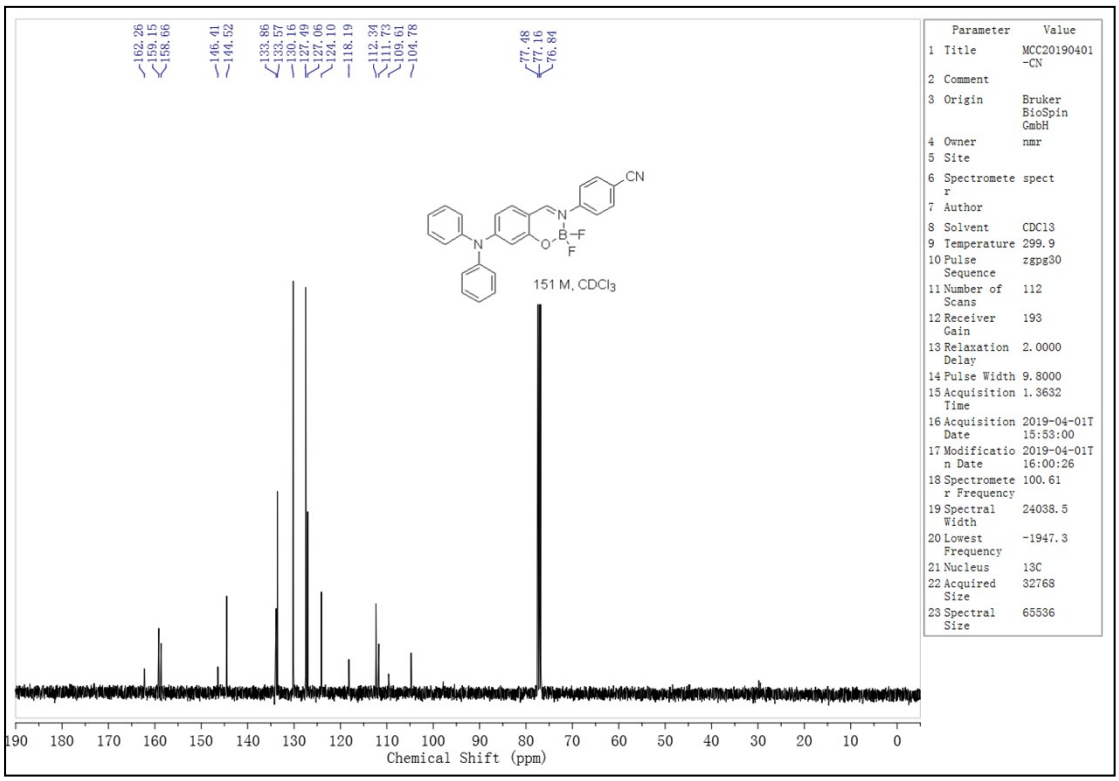


Fig. S18 ^{13}C NMR spectrum of **DPFB-CN** in $CDCl_3$ (151 MHz).

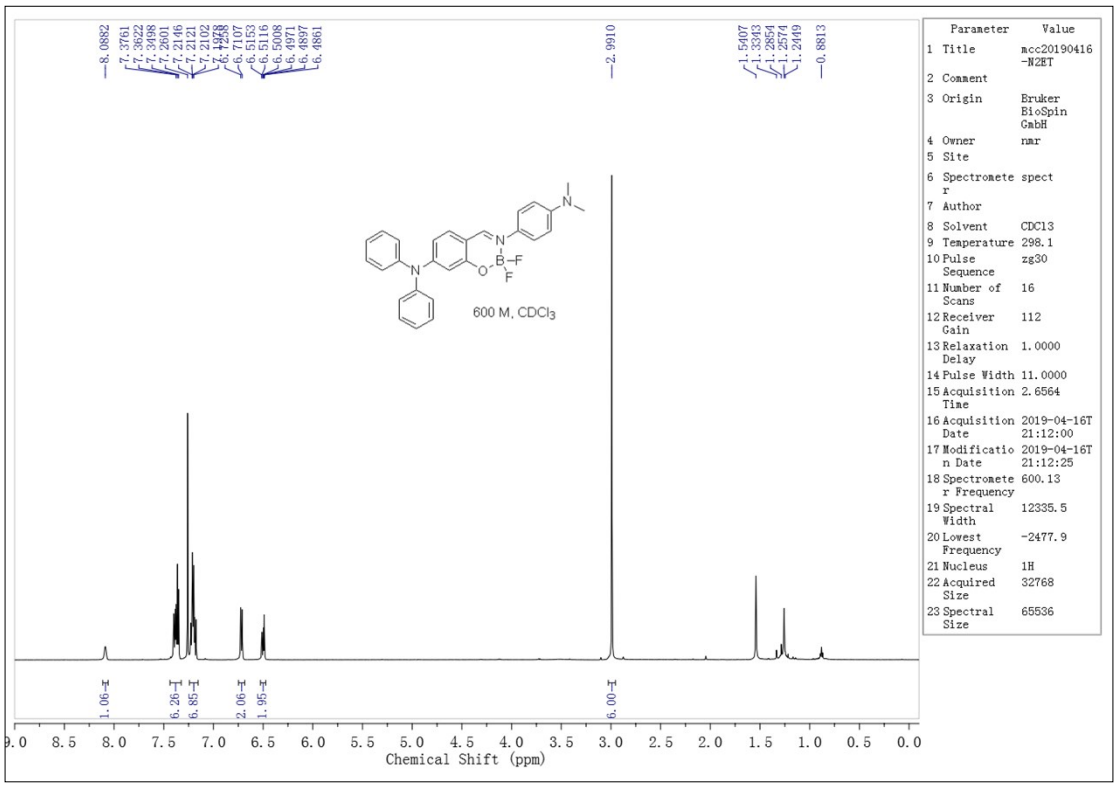


Fig. S19 ^{1}H NMR spectrum of **DPFB-NMe$_2$** in $CDCl_3$ (600 MHz).

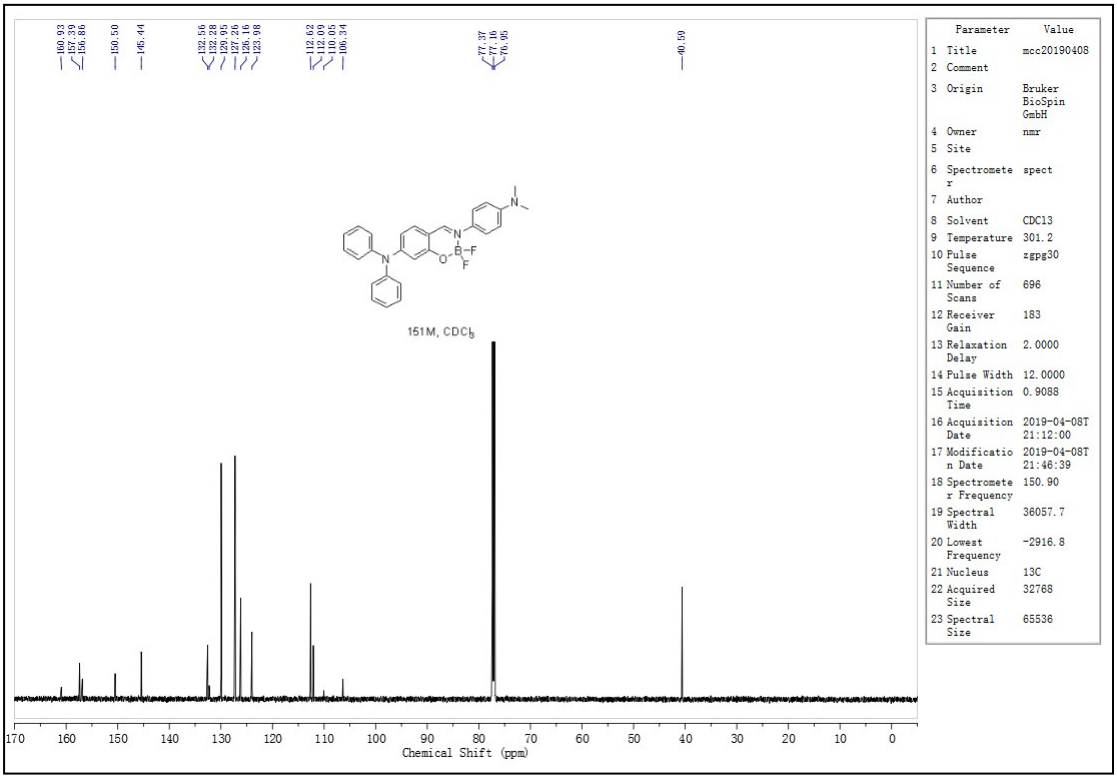


Fig. S20 ^{13}C NMR spectrum of **DPFB-NMe$_2$** in $CDCl_3$ (151 MHz).