Electronic Supplementary Material (ESI) for Organic Chemistry Frontiers.

Supporting Information

BODIPY-based Probe for Amyloid-*β* Imaging *In Vivo*†

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1. General information

Reagents, Materials, and Animals

All reagents were commercial products and were used without further purification unless otherwise stated. All the experimental water was deionized (DI). 2,4-dimethylpyrrole, Boron trifluoride etherate ($BF_3 \cdot Et_2O$), and triethylamine (Et_3N) were purchased from Beijing Innochem Technology Co., Ltd. 3,5-Dimethylpyrrole-2-carbaldehyde was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. 4-(Boc-amino) benzaldehyde was purchased from Shanghai Haohong Scientific Co., Ltd. Phosphoryl trichloride （$POCl_3$）was purchased from Shanghai Adamas Reagent Co., Ltd. Amyloid-$\beta_{(1–42)}$human ($A\beta_{1–42}$) were purchased from Shanghai Macklin Biochemical Technology Co., Ltd.

All the cell experimental reagents were sterile and used in a super-clean bench to guarantee a sterile environment. High-glucose Dulbecco's modified Eagle's medium (DMEM), heat-inactivated fetal bovine serum (FBS), penicillin and streptomycin, and PBS (pH = 7.2-7.4, 0.01 M) were purchased from Hyclone. The human blood sample was provided by Beijing China-Japan Friendship Hospital. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) kit was purchased from KeyGEN BioTECH (Jiangsu, China).

C57BL/6J mice (18 months, female), and APP/PS1 (C57BL/6J, 18 months, female) were purchased from Beijing Huafukang Biotechnology Co., Ltd. All protocols requiring the use of animals were approved by the animal care committee of China-Japan Friendly Hospital. The approval number is zryhyy12-20-10-2.

Instruments

^1H and ^{13}C spectra were obtained by a Bruker Avance III (400 MHz, Germany) spectrometer at room temperature (r.t.) in $CDCl_3$. Absorption spectra were recorded on a UV-vis spectrophotometer (HITACHI, U-3900H, Japan). Fluorescence spectra were collected on a fluorescence spectrophotometer (HITACHI, F-4600, Japan). Mass spectra results were obtained from the Beijing Mass Spectrometry Center, Institute of Chemistry, Chinese Academy of Sciences. Cell viability values were recorded on a microplate reader (PerkinElmer Enspire, USA). The fluorescence signals of mouse brains were captured by an IVIS Lumina IV system (PerkinElmer Enspire, USA). The Gaussian and Multiwfn calculation were supported by the high-performance computing platform of the Beijing University of Chemical Technology (BUCT).

Cytotoxicity Experiment

The cell used for the cytotoxicity test was PC12, purchased from the Chinese National Infrastructure of Cell Line Resource. The experiment was divided into two parts, cell culture and cytotoxicity test. The PC12 cells were first cultured in cell culture fluid under 5% CO_2 at 37 °C. After a period of incubation, Hela was transferred into a sterile 96-well plate (1×10^4 cells per well) and cultured for 24 h under 5% CO_2 at 37 °C. After that, the cell culture fluid was removed, and gradient concentrations of BocBDP in the cell culture fluid (0, 2, 5, 10, 15, and 20 μM) were added into the 96-well plate and incubated for another 24 h. After that, the gradient concentration of BocBDP was removed and MTT (5× MTT was diluted into 1× MTT using dilution buffer, 50 μL per well) was added; the mixture was incubated at 37 °C for 4 h. The supernatant was removed, and DMSO (150 μL per well) was added; the mixture was placed on a shaker to be mixed well. The cell viability was determined by a microplate reader at 490 or 540 nm.

Hemolysis Experiment

The blood sample was first diluted with 2 mL of PBS solution (1 mL, provided by China-Japan Friendship Hospital in Beijing, China). Samples were centrifuged for 10 min (8000 rpm) and washed 5 times with PBS to separate red blood cells from serum. Finally, the red blood cells were dispersed in 10 mL of PBS solution, from which 0.2 mL was removed, and 0.8 mL of different reagents (PBS, DI water, and 10, 20, 30, 50, and 100 μM BocBDP) were added. After mixing, the samples were allowed to stand at room temperature for 3 h and then centrifuged (12,000 rpm, 5 min) to determine hemolysis. The supernatant was removed and tested for UV absorption. The hemolysis rate (HR) of erythrocytes was calculated using the following formula.

$$\mathrm{HR} = \frac{\mathrm{Abs_{sample}} - \mathrm{Abs_{pbs}}}{\mathrm{Abs_{H_2O}} - \mathrm{Abs_{pbs}}}$$

Theoretical Calculation and Docking studies

The ground-state geometries were optimized using the density functional theory (DFT) method with B3LYP hybrid functional at the basis set level of 6-311+(d,p). The calculations were performed using Gaussian 09 package and Multiwfn software. The crystal structures of A$\beta_{1\text{-}42}$ (PDB ID: 5KK3) were obtained from the Protein Database (www.rcsb.org) and optimized by the Discovery Studio 4.5 software. The molecular docking simulation was performed with Autodock Vina. Figures were generated using the PyMOL 2.4.0 software package.

Preparation of A$\beta_{1\text{-}42}$ Aggregates

A$\beta_{1\text{-}42}$ aggregates were prepared as described previously.[1] Briefly, lyophilized A$\beta_{1\text{-}42}$ peptides were dissolved in 1,1,1,3,3,3-hexafluoro-2-isopropanol (HFIP) at a concentration of 2 mg/mL and incubated at 25 °C for 2 h. Then HFIP was removed under a gentle flow of nitrogen. A$\beta_{1\text{-}42}$ (1 mg) was dissolved in aqueous NaOH (0.5 mL, 2 mM), and the pH was adjusted to 10.5 using 0.1 M NaOH (~15 μL). The solution was sonicated in an ultrasonic (KQ5200DE) water bath for 20 min at 0 °C and then centrifuged at 1200 r/min in a bench-top centrifuge (3H16RI) for 25 min at 4 °C. The supernatant was filtered through a 0.22 μm filter. The solution was diluted to 40 μM with PBS buffer and shaken on a shaker (300 rpm) at 37 ℃ for 3 d to form the A$\beta_{1\text{-}42}$ aggregates.

Saturated Binding Assay

A solution containing a gradient concentration of BocBDP and A$\beta_{1\text{-}42}$ aggregates (1 μM) in PBS were freshly prepared. The mixture was immediately transferred to a fluorescence spectrophotometer (U-3900H, HITACHI, Japan) to record their spectrum (Ex slit = 10 nm, Em slit = 10 nm). All the samples were prepared in triplicate. The obtained data was analyzed by Origin 2022 software, and the K_d value was calculated using nonlinear regression. The K_d was calculated according to the formula as follows.

$$Y = \frac{B_{max} \cdot X}{k_d + X}$$

where X is the concentration of probes, Y is change in fluorescence intensity, B_{max} is the maximum specific binding has the same units as Y, K_d is the equilibrium binding constant.

***In Vitro* A$\beta_{1\text{-}42}$ staining in solution**

A$\beta_{1\text{-}42}$ aggregates solution (20 μL) was added dropwise, spread, and stand still to a slide (CITOTEST) until it was dry. ThT (10 μM, 20 μL) was added dropwise and spread until it was dry. BocBDP was added dropwise and spread until it was dry. Anti-fluorescence quenching reagent (10-20 μL) was

added dropwise and covered with a cover slide. The fluorescence signal was captured by CLSM. λ_{ex} (ThT) = 488 nm, λ_{em} (ThT) = 508-550 nm; λ_{ex} (BocBDP) = 543 nm, λ_{em} (BocBDP) = 563-700 nm.

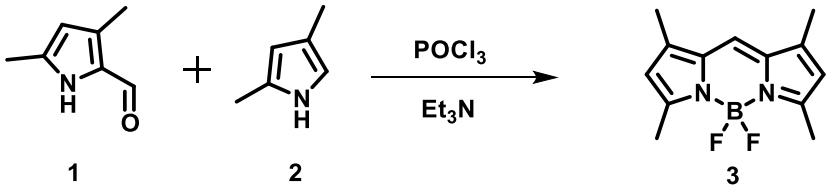
***In Vitro* Slice Fluorescence Staining**

The paraffin-embedded blank sections were immersed in xylene for 5 min for deparaffinization and then washed with ethanol for 2 min and water for 5 min. The brain slices were incubated with 40 μL of 10 mg/mL ThT for 5 min and washed with 50% ethanol solution for 3 min. Next, the brain slices were incubated with 10 μL of 100 μM probes for 20 min. After absorbing the residual liquid with dust-free paper, the antifade mounting medium was added dropwise, and neutral gum was used for mounting. The slices were then placed under a laser confocal microscope (Lecia SP8) for imaging.

***In Vivo* Fluorescence Imaging**

The mice used for *in vivo* fluorescence imaging were APP/PS1 (C57BL/6J, 18 months, female) and age-matched wild-type mice (WT, 18 months, C57BL/6J, female). For *in vivo* fluorescence imaging, the APP/PS1 mice and WT mice were first head-shaved to reduce the effect of hair on fluorescence imaging. Before *in vivo* fluorescence imaging, the APP/PS1 and WT mice were first placed into an imaging box to obtain background signals. BocBDP was injected intravenously, and the mice were transferred into an imaging box. The mice were anesthetized under 5% isoflurane gas and 1.0 L/min oxygen flow during the imaging process. The fluorescence signals from the brain at various time points before and after dosing were collected on an IVIS Lumina III system (PerkinElmer). The acquired images were analyzed by drawing ROI with the area around the brain region using the Living Image 4.5.5 software.

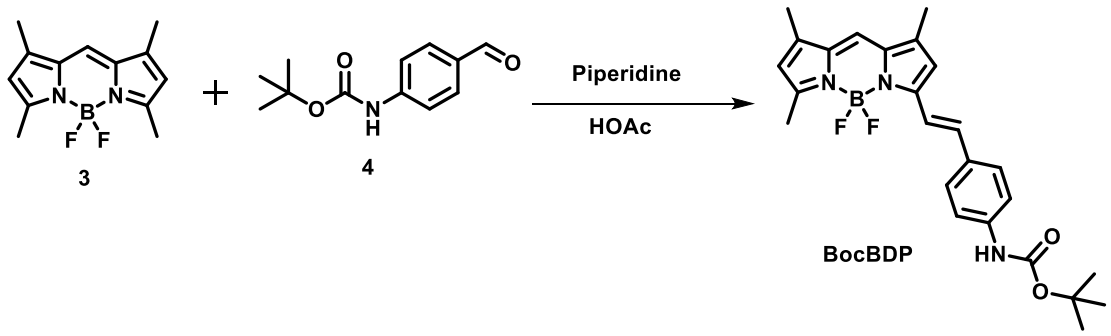
2. Synthesis



Scheme S1. Synthetic route of compound 3.

Synthesis of 3

The synthesis method of compound 3 refers to the known literature.[2] 3,5-Dimethylpyrrole-2-carbaldehyde (100 mg, 0.81 mmol) and 2,4-dimethylpyrrole (70 mg, 0.74 mmol) were dissolved in dry DCM (15 mL) and the reaction mixture was cooled to 0 °C and stirred under nitrogen atmosphere for 10 min, then $POCl_3$ (124 mg, 0.81 mmol) was added slowly over 5 min. The reaction mixture was stirred at 0 °C for 1 hour and then at 25 °C for an additional 4 h. Anhydrous TEA (750 mg, 7.4 mmol) was added, followed by $BF_3{\cdot}Et_2O$ (0.93 ml, 7.4 mmol) after 15 min. After 2 h, the reaction mixture was evaporated in a vacuum, washed with ethyl acetate (200 mL), then H_2O (3 × 50 mL), and dried over Na_2SO_4. The crude product was purified by silica gel column chromatography (petroleum ether/ethyl acetate = 5:1, v/v) to give 109 mg (60%) **3** as red crystal. The ^1H NMR spectrum of compound 3 was shown in Fig. S5. ^1H NMR (400MHz, $CDCl_3$, δ): 2.17 (s, 6H), 2.46 (s, 6H), 5.97 (s, 2H), 6.96 (s, 1H). MS m/z calcld for $C_{13}H_{15}N_2BF_2$, 248.12; found: 248.

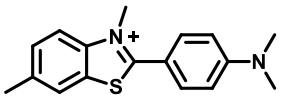
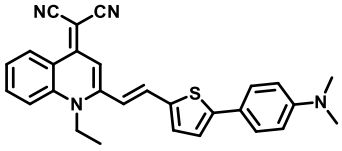
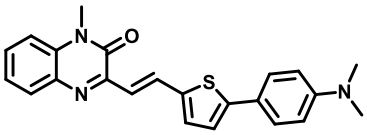
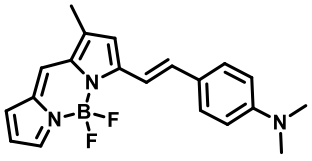


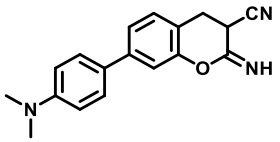
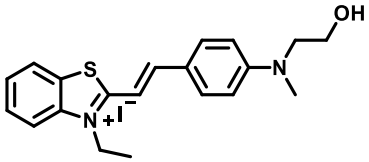
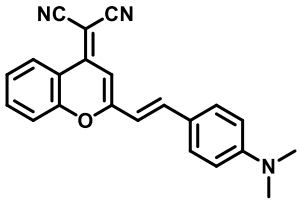
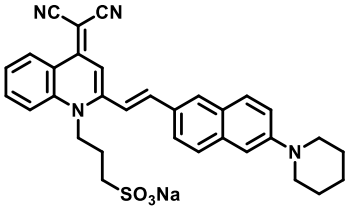
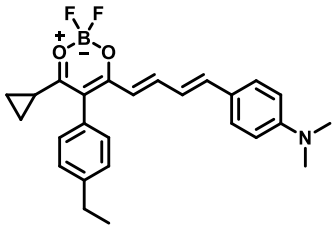
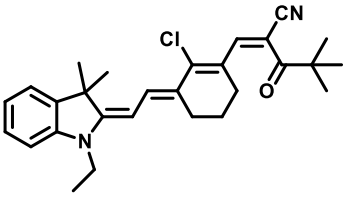
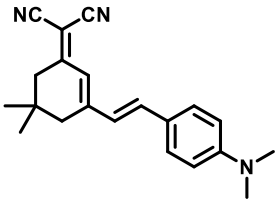
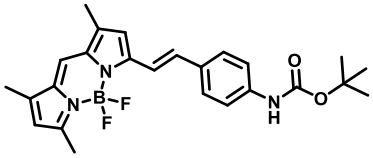
Scheme S2. Synthetic route of compound BocBDP.

Synthesis of BocBDP

Compound 3 (440 mg, 2 mmol) and 4-(Boc-amino) benzaldehyde (120 mg, 0.5 mmol) were dissolved in 5 ml of toluene. Under a nitrogen atmosphere, 0.35 ml of acetic acid and 0.43 ml of piperidine were added, and the mixture was heated to reflux for 48 h. The reaction mixture was evaporated in vacuo, washed with ethyl acetate (200 mL), then H_2O (3 x 50 mL) and dried over Na_2SO_4, the crude product was purified by silica gel chromatography (petroleum ether/dichloromethane = 7/3, v/v) to give 34 mg (15%) BocBDP as green crystal. The ^1H NMR spectrum of BocBDP was shown in Fig. S6. ^1H NMR (400 MHz, $CDCl_3$, δ): 7.52 (dd, J = 9.3, 7.4 Hz, 3H), 7.39 (d, J = 8.3 Hz, 2H), 7.22 (d, J = 16.3 Hz, 1H), 7.02 (s, 1H), 6.64 (d, J = 21.2 Hz, 2H), 6.09 (s, 1H), 2.59 (s, 3H), 2.29 (d, J = 10.5 Hz, 6H), 1.55 (s, 9H). The ^{13}C NMR spectrum of BocBDP was shown in Fig. S7. ^{13}C NMR (100 MHz, $CDCl_3$, δ): 156.20, 154.32, 152.40, 140.58, 140.48, 139.17, 136.03, 134.98, 133.78, 131.25, 128.43, 118.99, 118.42, 118.36, 117.56, 115.42, 80.85, 28.33, 14.80, 11.35, 11.32. HRMS m/z calcd for $C_{25}H_{28}BF_2N_3O_2$, 451.2242; found 451.2237.

Table S1. Comparison of our probe with reported probes

	Probes	Molecule structure	K_d	clog *P* [a]	Target Analyte	Reference
1	ThT		890 nM	-0.14	Aβ aggregates	3
2	QM-FN		-	5.92	Aβ aggregates	4
3	QNO-AD-3		23.84 nM	4.51	Aβ aggregates	5
4	BAP-1		44.1 nM	3.52	Aβ aggregates	6

5	IRI-1		374 nM	3.31	Aβ aggregates	7
6	RM-28		175.69 ± 4.87 nM	0.86	Aβ aggregates	8
7	PAD-3		94.0 ± 20.6 nM	4.56	Aβ aggregates	9
8	TM-2		92 nM	-	Aβ aggregates	10
9	PTO-29		oligo: 248 ± 48 nM	7.75	Aβ oligomers	11
10	ZT-1		445 nM	6.93	Aβ aggregates	12
11	DCIP-1		674.3 nM	4.85	Aβ aggregates	13
12	BocBDP		67.8 ± 3.18 nM	3.74	Aβ aggregates	-

[a] All clog *P* values were calculated using the online ALOGPS 2.1 program.

Table S2. The spectra data of BocBDP in different solvent polarity

Probe	Solvents	Ex [nm]	Em [nm]	Stokes shift [nm]
BocBDP	Toluene	585	591	6
	THF	579	590	11
	DCM	578	588	10
	MeCN	571	583	12
	DMSO	581	595	14

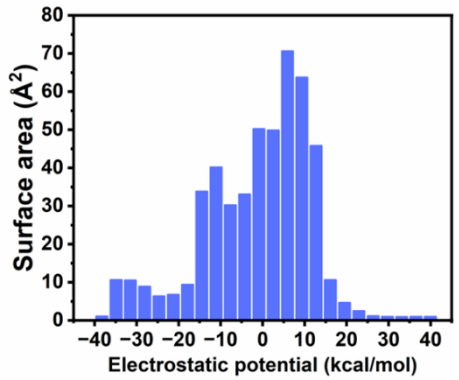


Fig. S1 The electrostatic potential distribution area in BocBDP surface.

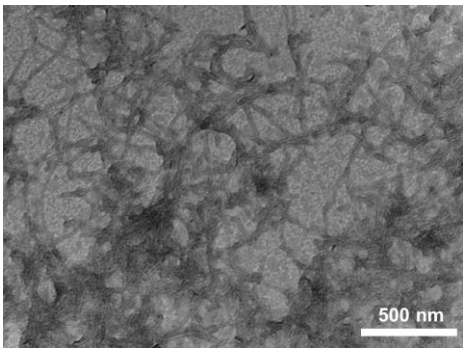


Fig. S2 TEM of A$\beta_{1\text{-}42}$ aggregates

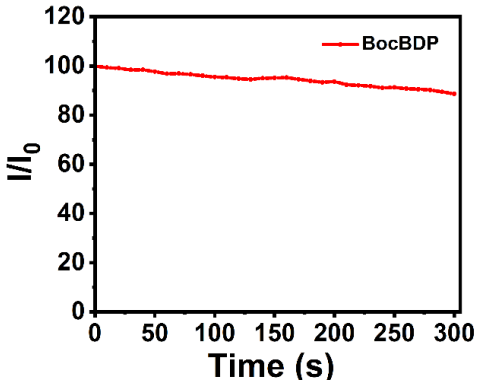


Fig. S3 The photostability of BocBDP within 300 s (λ_{ex} = 543 nm)

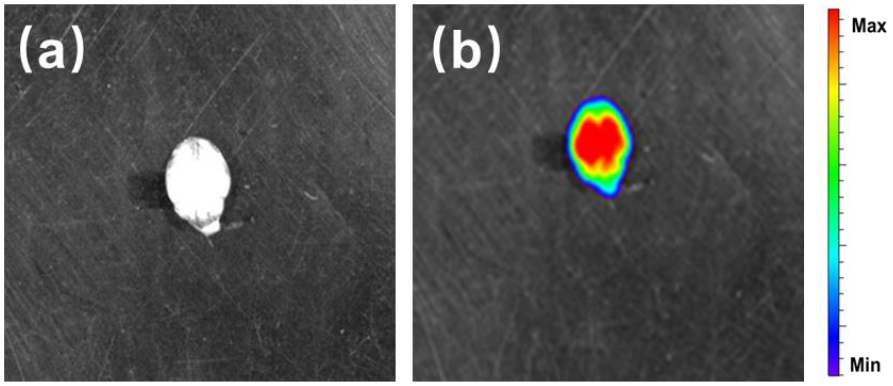


Fig. S4 (a) The picture of WT mouse brain. (b) The fluorescence signal of WT mouse brain after intravenous injection of BocBDP (2 mg/kg)

3. NMR and HRMS spectra

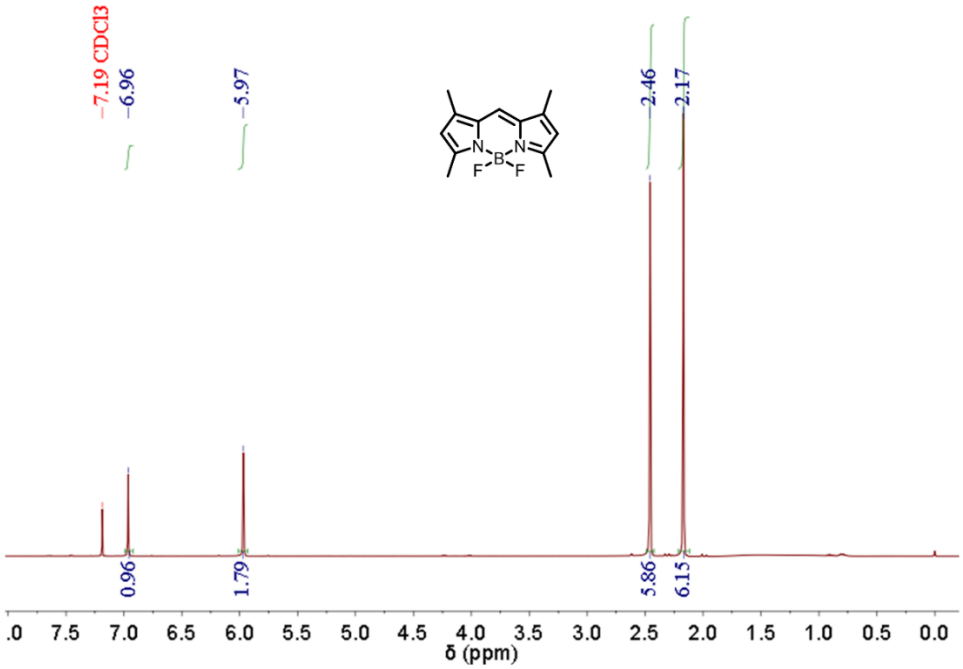


Fig. S5 ^{1}H NMR spectrum of compound 3 in $CDCl_3$

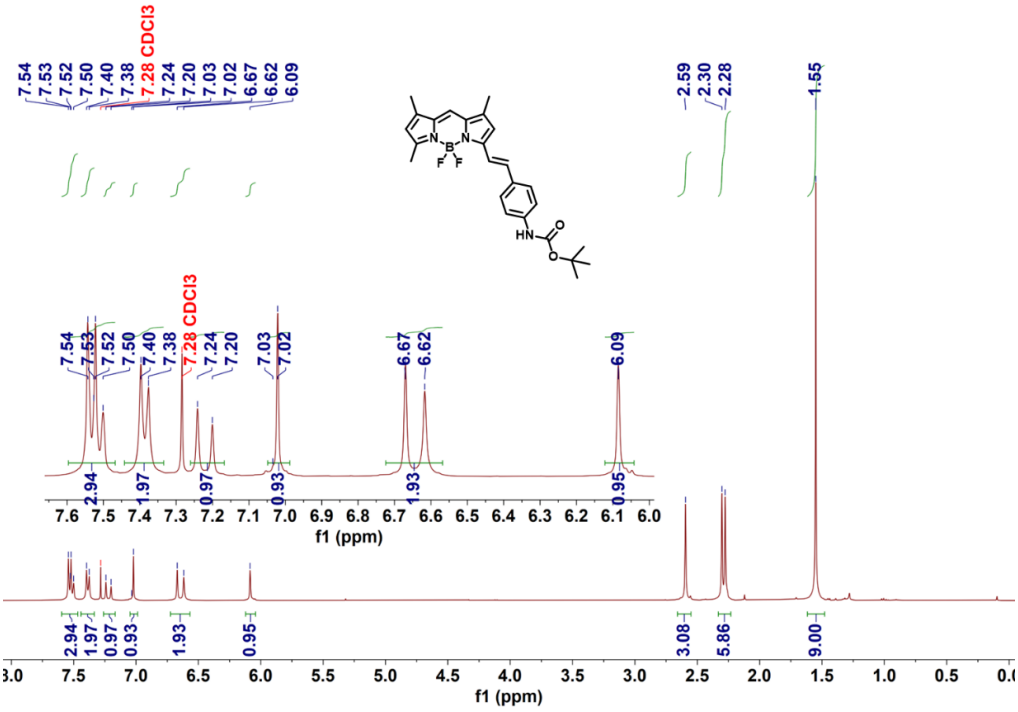


Fig. S6 ^{1}H NMR spectrum of BocBDP in $CDCl_3$

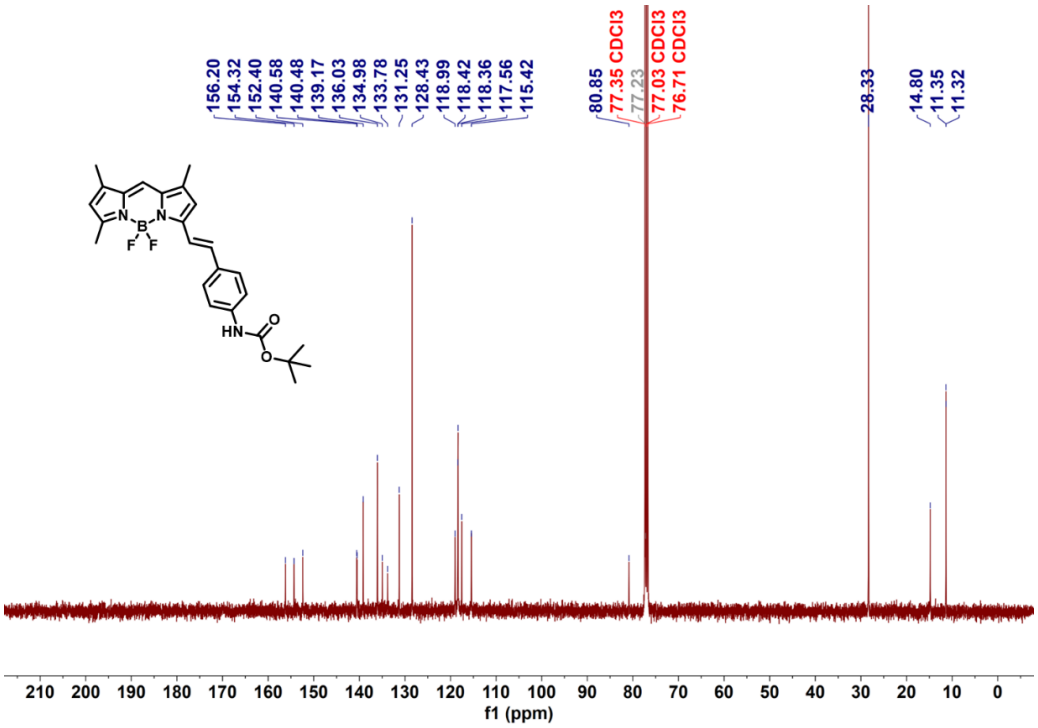


Fig. S7 ^{13}C NMR spectrum of BocBDP in $CDCl_3$

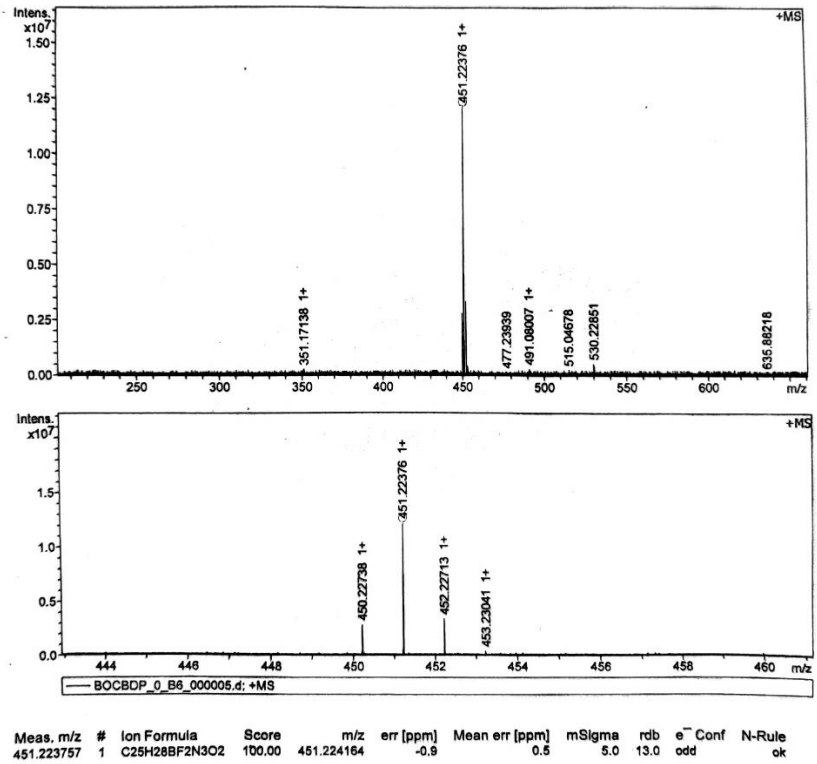


Fig. S8 HRMS spectrum of BocBDP

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