Supporting Information

A Phosphine-based fluorescent probe for fluorescent imaging of hypochlorous acid in living cells and zebrafish

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Contents

Experimental section

Reagents, materials, and apparatus:

All the solvents used in the experiment were of analytic grade. The reaction progress was monitored by thin-layer chromatography (TLC) on silica gel plates (GF_{254}) visualized by UV light. 200-300 mesh silica gel was used for column chromatography. NMR experiments were carried out on a Bruker AV-400 NMR spectrometer with chemical shifts reported in ppm (in $CDCl_3$, DMSO-d_6, or TMS as an internal standard). Mass spectra were measured on an Agilent 1290 LC-MS spectrometer. All pH measurements were made with a Sartorius basic pH-Meter PB-10. Fluorescence spectra were determined on a PerkinElmer LS55 Fluorescence spectrophotometer. Absorption spectra were collected on a Shimadzu UV 2501(PC)S UV-Visible spectrophotometer. The excitation and emission widths for **BBP** were all 1.5.

Preparation of various ROS and RNS species:[1]

HOCl: Take an appropriate amount of commercially available hypochlorous acid solution and prepare about 10^{-2} M hypochlorous acid stock solution with deionized water. Dilute the hypochlorous acid solution, and calibrate its concentration (ε = 350 $M^{-1}cm^{-1}$) through the ultraviolet spectrum absorption value at 292 nm.

ONOO-: To a vigorously stirred solution of $NaNO_2$ (0.6 M, 10 mL) and H_2O_2 (0.7 M, 10 mL) in deionized H_2O at 0 °C was added HCl (0.6 M, 10 mL), immediately followed by the rapid addition of NaOH (1.5 M, 20 mL). Excess hydrogen peroxide was removed by passing the solution through a short column of MnO_2. The concentration of $ONOO^-$ was determined by UV analysis with the extinction coefficient at 302 nm (ε = 1670 M^{-1} cm^{-1}). The solution were stored at -20 °C for use.

NO: A solution of the H_2SO_4 (3.6 M) was added dropwise into a stirred solution of $NaNO_2$ (7.3 M). The emitted gas was allowed to pass through a solution of NaOH (2 M) first and then deionized H_2O to make a saturated NO solution of 2.0 mM.

$^1O_2$: $NaMoO_4$ (10 mM) and H_2O_2 (10 mM) was prepared in PBS (10 mM, pH 7.4). Equal aliquots of these solutions were then mixed to yield $^1O_2$ of 5 mM.

H_2O_2: Take an appropriate amount of commercially available H_2O_2 solution and prepare about 10^{-2} M H_2O_2 stock solution with deionized water. And its concentration is calibrated by the ultraviolet absorption value at 240 nm ($\varepsilon = 43.6 M^{-1}cm^{-1}$).

·OH: ·OH was generated by Fenton reaction. To a solution of H_2O_2 (1.0 mM, 1.0 mL) in PBS (10 mM, pH 7.4) was added $FeSO_4$ solution (1.0 mM, 100 μL) at ambient temperature (stock solution 0.1 mM).

ROO·: ROO· was generated from 2, 2'-azobis(2-amidinopropane)dihydrochloride, which was dissolved in PBS (10 mM, pH 7.4) 1 h before use to make a stock solution of 10 mM.

Additional spectroscopic data

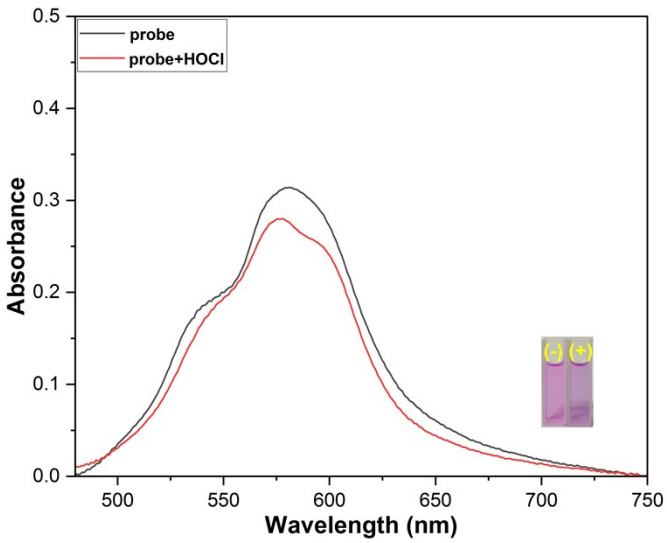


Fig. S1 The UV-vis spectrum of **BBP** (10.0 μM) with and without of HOCl (2.0 equiv.) in PBS buffer solution (10 mM, pH 7.2, containing 10% EtOH). Bottom inset: Cuvette images of the probe **BBP** before and after adding HOCl.

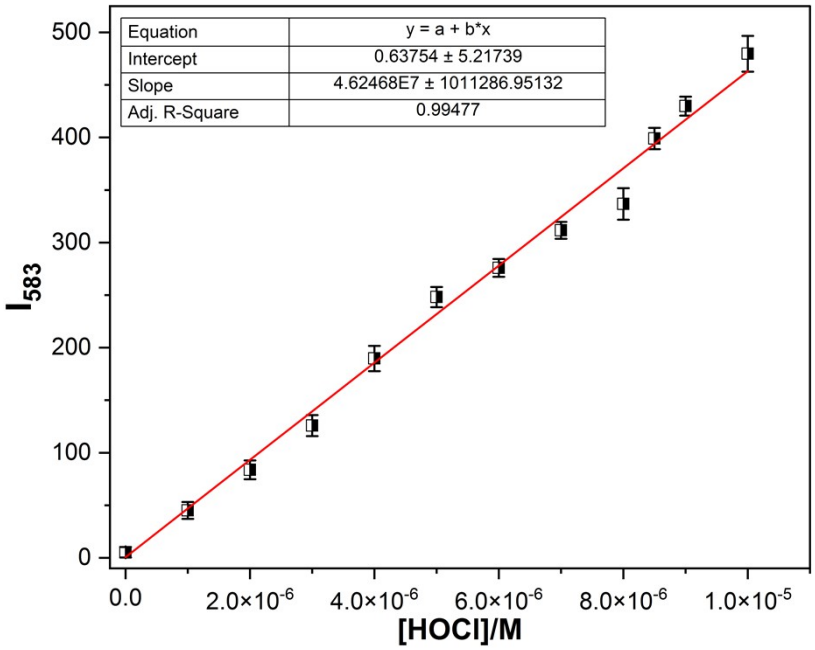


Fig. S2 The fluorescent intensity of **BBP** (10.0 μM) at 583 nm ((I_{583}) as a function of HOCl concentration (0-10.0 μM) in PBS buffer solution (10 mM, pH 7.2, containing 10% EtOH, λ_{ex} = 552 nm).

The detection limit (DL) of HOCl using **BBP** was determined from the following equation: [2]

$$DL = 3*\sigma/K$$

Where σ is the standard deviation of the blank solution; K is the slope of the calibration curve.

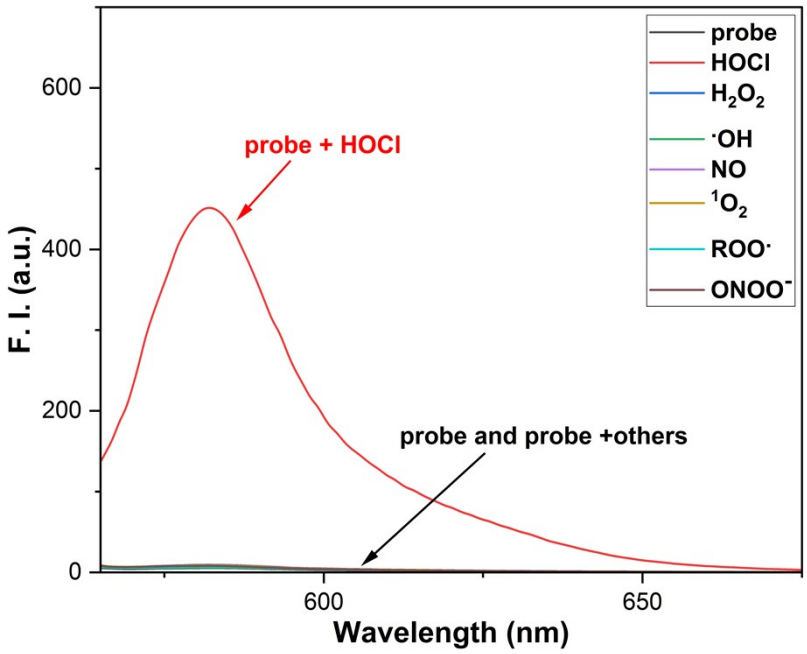


Fig. S3 The fluorescent spectra of **BBP** (10 μM) before and after the addition of various analytes (100 μM each, including $^1O_2$, H_2O_2, NO, ·OH, ROO·, and $ONOO^-$), and HOCl (10 μM), (in PBS buffer solution, 10 mM, pH 7.2, containing 10% EtOH, λ_{ex} = 552 nm).

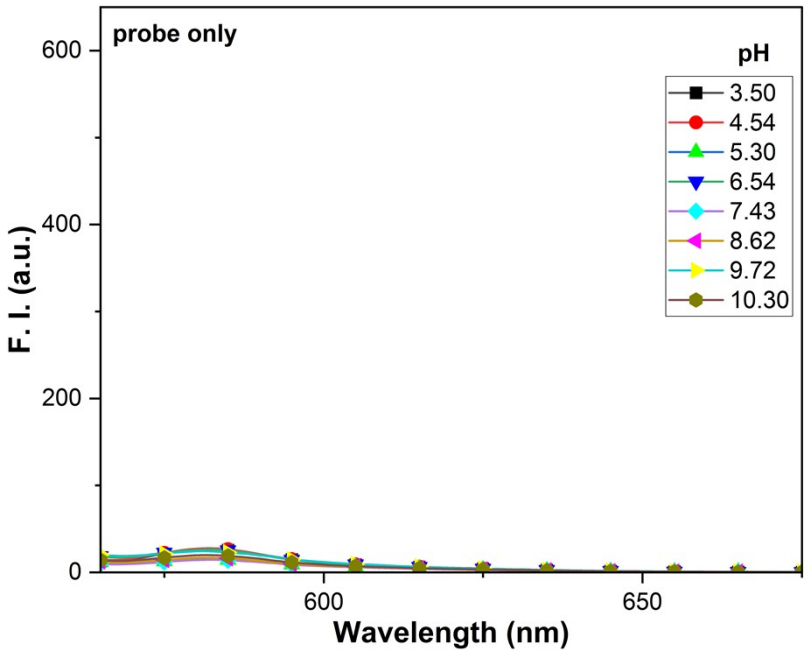


Fig. S4. The fluorescence spectra of the probe **BBP** (10 μM) alone at different pH values (λ_{ex} = 552 nm).

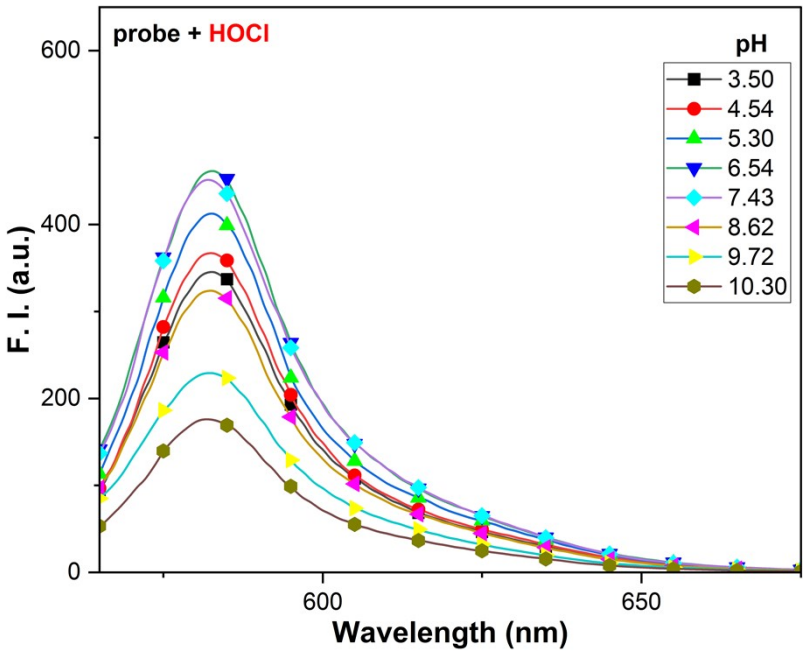


Fig. S5. The fluorescence spectra of the probe **BBP** (10 μM) in the present of HOCl (10 μM) at different pH values (λ_{ex} = 552 nm).

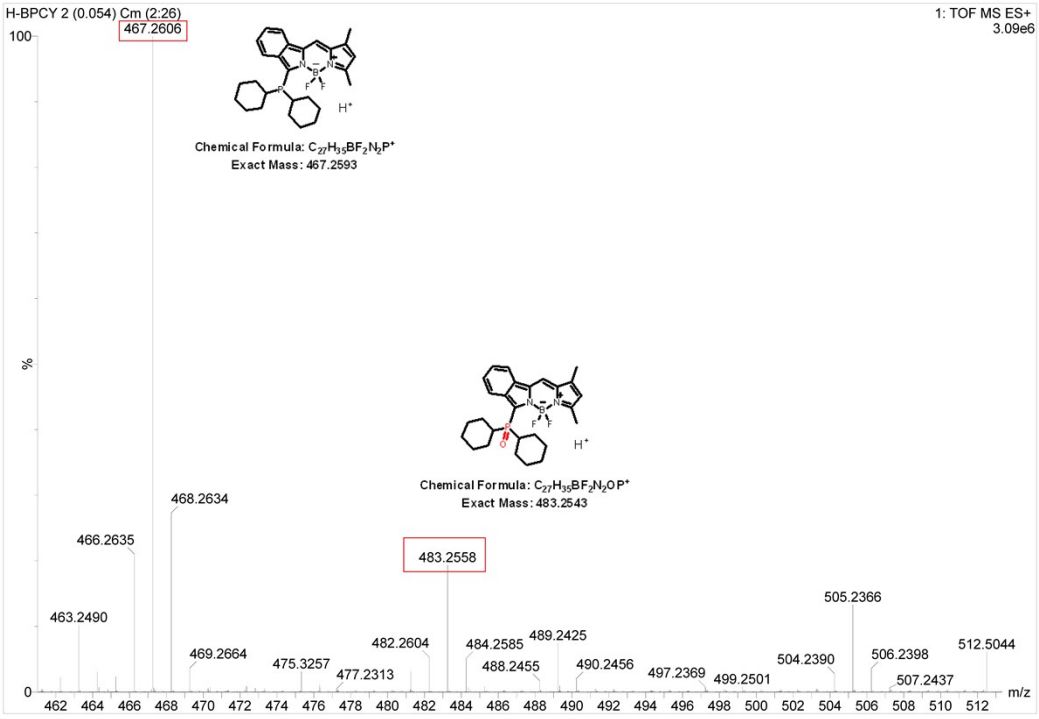


Fig. S6. The HR ESI-MS spectrum of **BBP** and HOCl mixture.

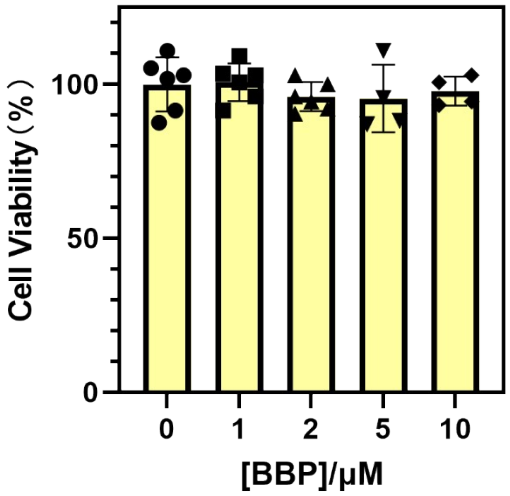
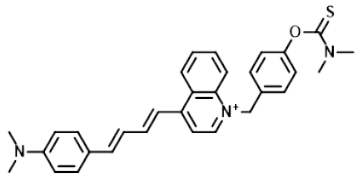
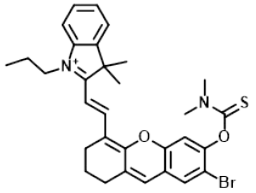
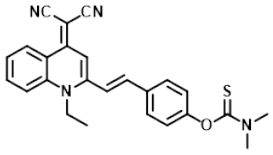
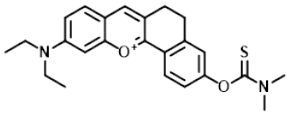
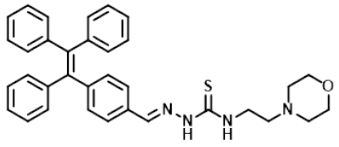
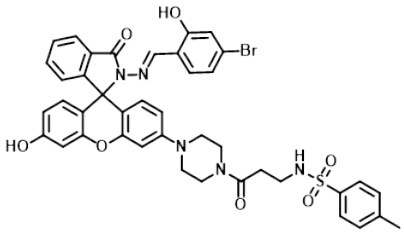
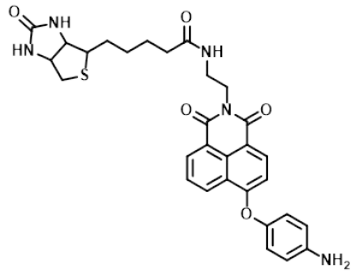
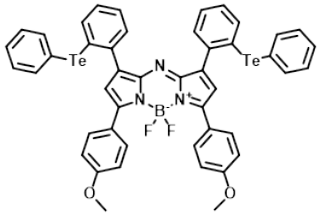
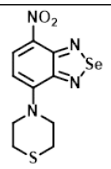
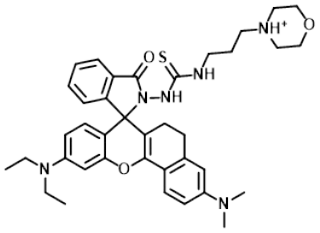
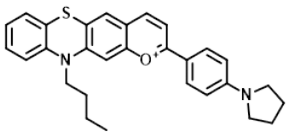
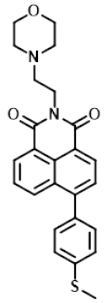
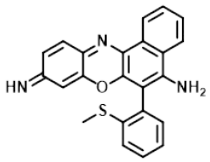
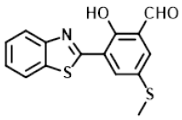
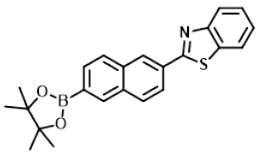
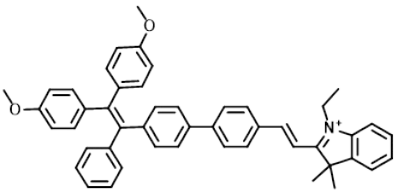
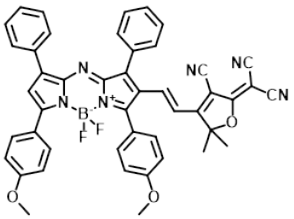
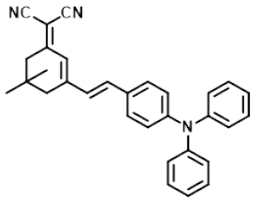
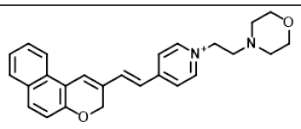
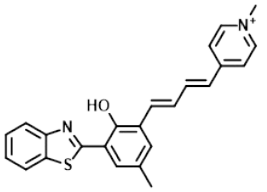
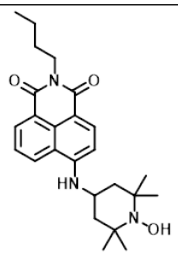
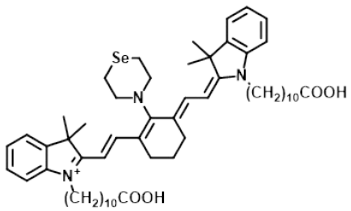
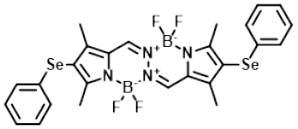
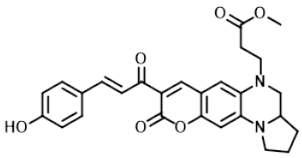
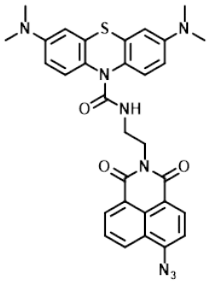
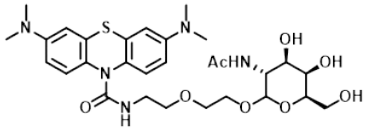


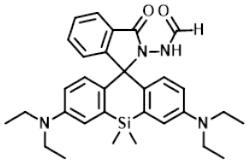
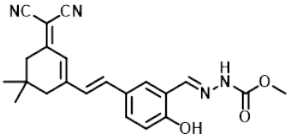
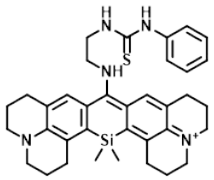
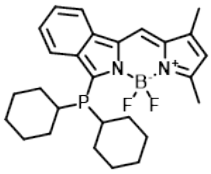
Fig. S7. Cell viability of the probe in a standard MTT assay in living RAW 264.7 cells for 24 h. The experiment was repeated three times.

Table S1. Comparison of fluorescent probes for HOCl detection.

No.	Structures	Abs/Em	Stocks Shift	LOD	Cell Imaging	Mouse/Zebra Fish Imaging	Refs
1		387/590	203	2.4 nM	√	×	[3]
2		694/712	18	89.7 nM	√	√	[4]
3		430/620	190	30.8 nM	√	×	[5]
4		567/623	56	5.8 μM	√	√	[6]
5		350/492	142	250 nM	√	×	[7]
6		513/556	43	785 nM	√	×	[8]
7		468/553	85	17 nM	√	×	[9]
8		692/738	46	90 nM	√	×	[10]

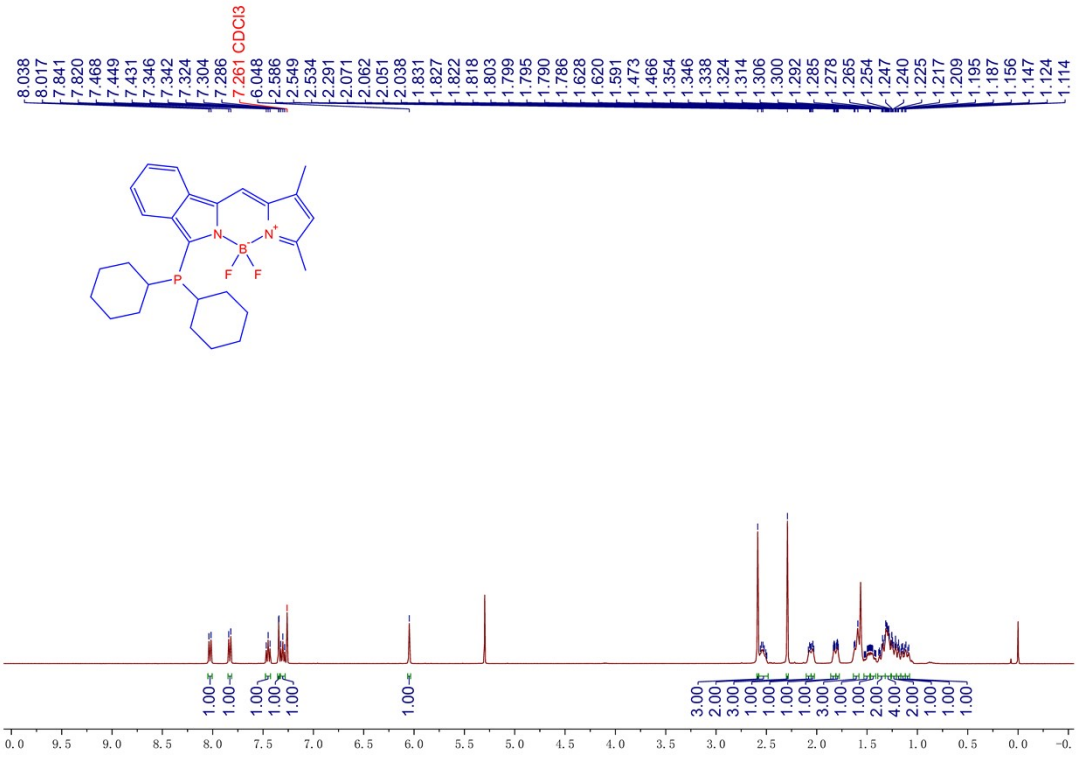
9		520/620	100	-	×	×	[11]
10		622/655	33	30 nM	√	√	[12]
11		438/503	65	25.8 nM	√	×	[13]
12		374/595	221	118 nM	√	√	[14]
13		596/638	42	94.7 nM	√	×	[15]
14		435/456	21	0.14 μM	√	×	[16]
15		328/394	66	-	√	×	[17]
16		364/530	166	32 μM	√	×	[18]
17		700/-	-	0.36 μM	√	×	[19]

18		510/650	140	3.3 nM	√	×	[20]
19		450/640	190	12 nM	√	√	[21]
20		402/680	278	24.5 nM	√	√	[22]
21		445/530	85	10.3 nM	√	×	[23]
22		710/790	80	31.5 nM	√	√	[24]
23		452/484	32	0.63 μM	√	×	[25]
24		5741/-	-	55 nM	√	√	[26]
25		378/530	152	32 nM	√	√	[27]
26		664/700	36	15 nM	√	√	[28]

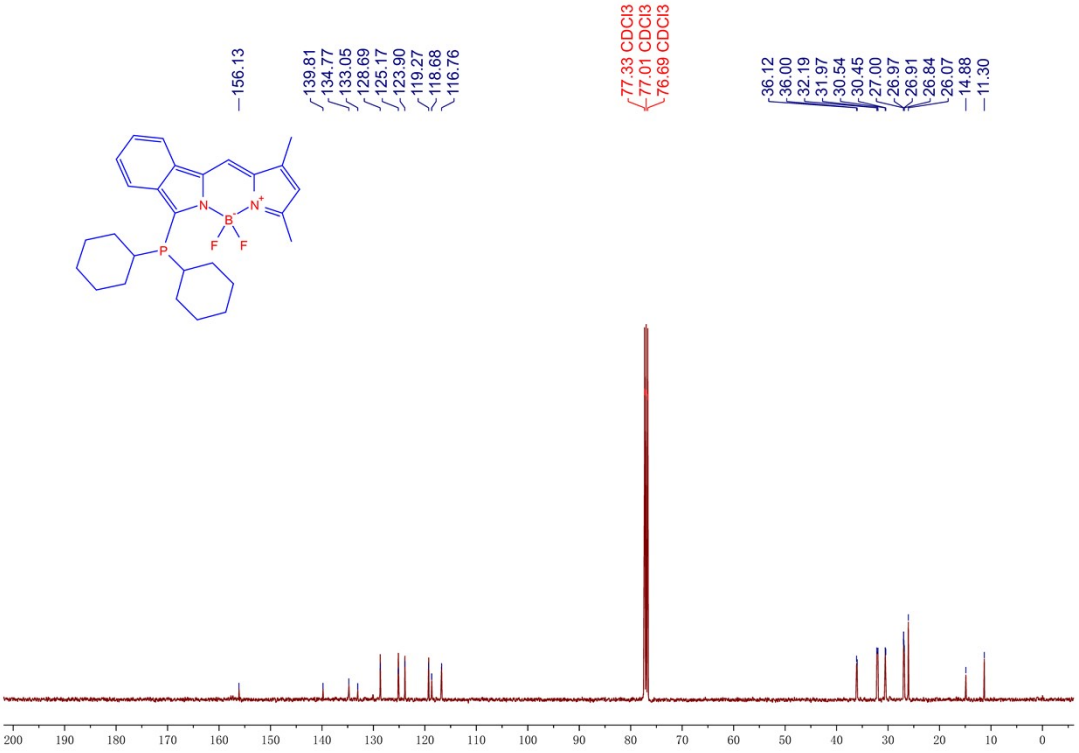
27		653/669	16	0.23 μM	√	×	[29]
28		425/653	228	15.3 nM	√	×	[30]
29		504/642	142	16.1 nM	√	√	[31]
30		575/583	8	15.3 nM	√	√	This work

The characterization data of BBP

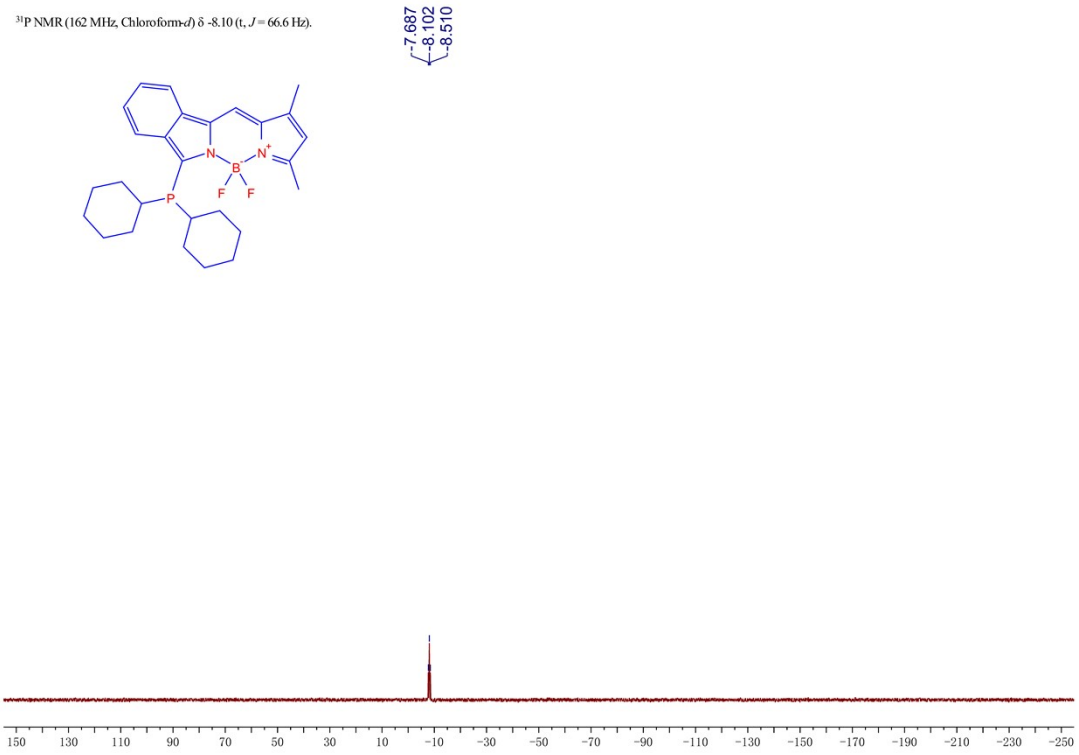
^{1}H NMR of **3 (BBP)**



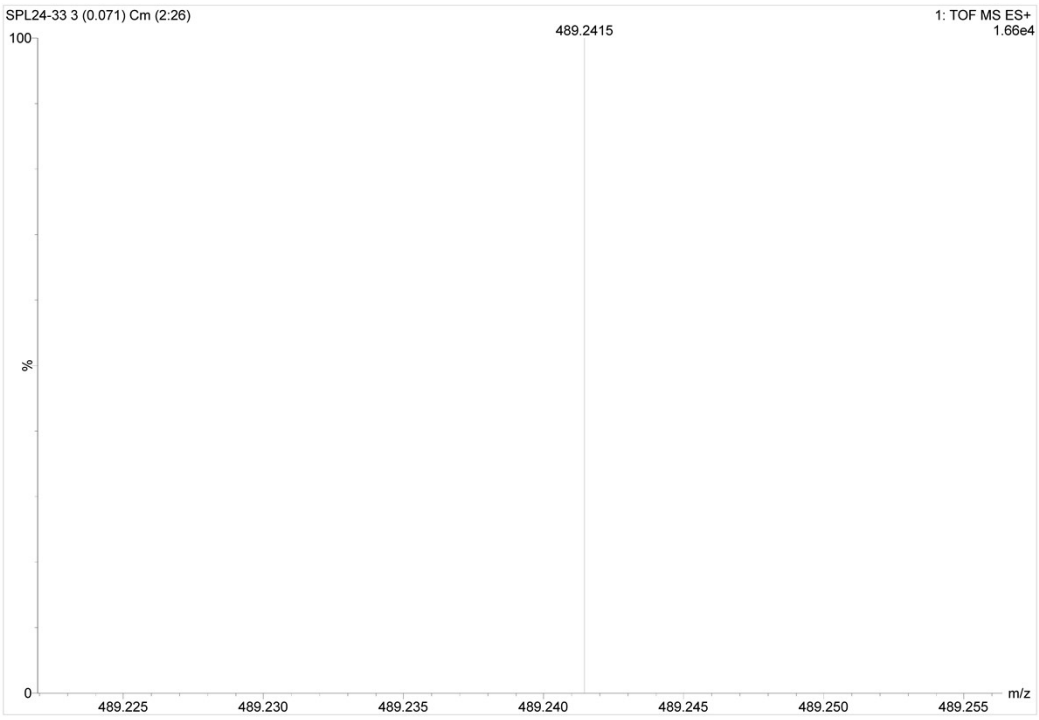
^{13}C NMR of **3 (BBP)**



^{31}P NMR of **3 (BBP)**



HR-MS of **3 (BBP)**



References

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