Supporting Information

A specific fluorescent probe for NO based on a new NO binding group

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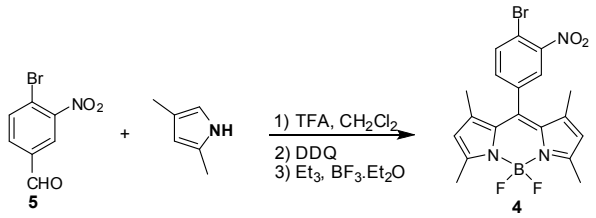
1. General information and methods. All reagents and solvents were purchased from commercial suppliers and used without further purification unless otherwise stated. Deionized water was used throughout all experiments. All reactions were magnetically stirred and monitored by thin layer chromatography (TLC). Column chromatography was conducted over silica gel (mesh 200–300). Fluorescence spectra were taken on HITACHI F-7000 fluorescence spectrometer with the excitation and emission slit widths at 5.0 and 5.0 nm respectively. The ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 MHz and 75 MHz, respectively. High resolution mass spectra were obtained on a Varian QFT-ESI mass spectrometer. The following abbreviations were used to explain the multiplicities: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; br = broad.

2. Synthesis

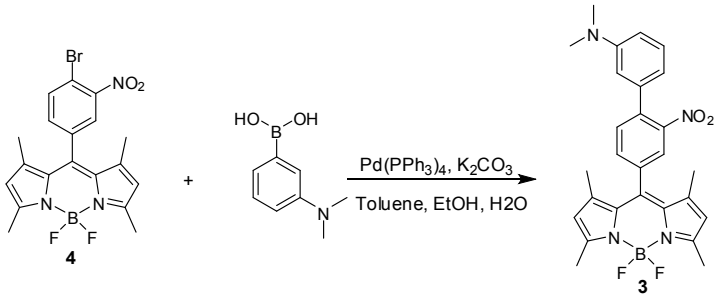
Compound 4



To a solution of 4-Bromo-3-nitrobenzaldehyde **5** (2.30 g, 10 mmol) and 2,4-dimethyl-pyrrole (1.90 g, 20 mmol) in dry CH_2Cl_2 (250 mL) under N_2 was added three drops of trifluoroacetic acid. After stirring the mixture for 3 hours at room temperature under

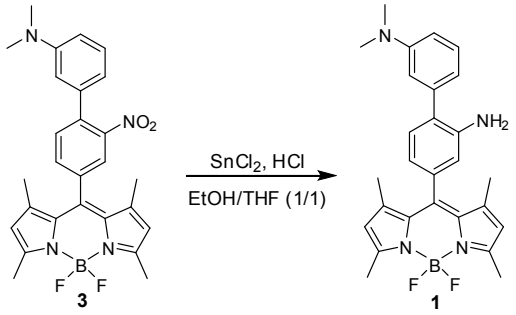
N_2 and in the dark, 2, 3-dicyano-5, 6-dichloroquinone (DDQ, 2.5 g, 11 mmol) was added. The solution immediately turned dark violet, and then was stirred for 30 min at room temperature. Freshly distilled triethylamine (25 mL) was introduced, followed by $BF_3 \cdot Et_2O$ until green appeared in the solution (~25 mL). The mixture was washed with water and dried over Na_2SO_4, filtered, and evaporated to afford a dark oil. The latter was purified by silica gel chromatography with CH_2Cl_2 : PE = 1 : 1 to yield **4** as a red solid (2.0 g, 4.4 mmol, 44%). ^{1}H-NMR ($CDCl_3$, 300MHz) δ (ppm): 7.93 (d, J = 8.4 Hz, 1H); 7.83 (s, 1H); 7.43 (d, J = 8.4 Hz, 1H); 6.04 (s, 2H); 2.56 (s, 6H); 1.45 (s, 6H). ^{13}C-NMR ($CDCl_3$, 75MHz): 157.9, 151.4, 143.3, 136.9, 134.1, 131.7, 126.6, 123.0, 116,1, 48.2, 16.0. HR MS: calculated for $[M]^+$: 447.0565; found: 447.0586.

Compound 3



To a suspension of compound **4** (0.223 g, 0.5 mmol), 3-dimethylaminophenylboronic acid (0.165 g, 1 mmol) and K_2CO_3 (0.690 g, 5 mmol) in toluene (20 mL), were added water (2 mL) and Ethanol (2 mL). The mixture was N_2 flushed, then $Pd(PPh_3)_4$ (5 mol%) was introduced. After degassing by bubbling the mixture with N_2 for another 15 min, the reaction mixture was stirred at 80°C for 12 hours under N_2. The organic phase was washed with saturated NH_4Cl and water then dried over Na_2SO_4, filtered, and evaporated to dryness to afford a dark residue which was purified by column chromatography with CH_2Cl_2 to yield **3** as red solid (0.15 g, 0.30 mmol, 60%). ^{1}H-NMR ($CDCl_3$, 300MHz) δ (ppm): 7.76 (s, 1H); 7.66 (d, J = 7.5 Hz, 1H); 7.56 (d, J = 8.7 Hz, 1H); 7.30 (s, 1H); 6.72-6.85 (m, 3H); 6.04 (s, 2H); 3.01 (s, 6H); 2.57 (s, 6H); 1.50 (s, 6H). ^{13}C-NMR ($CDCl_3$, 75MHz): 157.2, 151.2, 150.6, 143.2, 138.2, 137.5, 135.5, 133.2, 131.7, 130.2, 124.4, 122.4, 116,5, 113.3, 112.0, 41.1, 15.6. HRMS: calculated for $[M+H]^+$: 489.2273; found: 489.2272.

Probe 1



Tin(II) chloride (0.448 g, 2 mmol) and conc. HCl (2.5 ml) were added to a solution of compound **3** (97.6 mg, 0.2 mmol) in THF-EtOH (1:1, 30 ml), and the reaction mixture was stirred for 6 h. A sat. solution of K_2CO_3 was added and the reaction mixture was partitioned between EtOAc (100 ml) and water (20 ml). The organic phase was separated, washed several times with water, dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by column chromatography to yield probe **1** as orange red solid (60 mg, 65%). ^{1}H-NMR ($CDCl_3$, 300MHz) δ (ppm): 7.42 (m, 1H); 7.31(s, 1H); 6.91 (m, 3H); 6.78 (d, J = 8.1 Hz, 1H); 6.73 (s, 1H); 6.07 (s, 2H); 4.01 (b, 2H), 3.10 (s, 6H); 2.64 (s, 6H); 1.69 (s, 6H). ^{13}C-NMR ($CDCl_3$, 75MHz): 156.3, 152.1, 145.5, 144.5, 143.4, 140.7, 135.8, 132.6, 132.2, 130.8, 130.2, 122.2, 118,9, 118.4, 115.7, 114.2, 112.9, 41.9, 15.7. HR MS: calculated for $[M+H]^+$: 459.2562; found: 459.2565.

3. Preparation of the test solution

DEA•NONOate was synthesized according to the literature.[1] Dehydroascorbic acid (DHA), ascorbic acid (AA), and methylglyoxal (MGO) were purchased from Aldrich and used without further purification. Deionized water and spectroscopic grade EtOH were used for spectroscopic studies. Superoxide solution (O_2^-) was prepared by adding KO_2 (1 mg) to dry dimethyl sulfoxide (1 mL) and stirring vigorously for 10 min. Hydroxyl radical (OH•) was generated *in situ* by the Fenton reaction. Singlet oxygen ($^1O_2$) was generated from ClO^- and H_2O_2. Hypochlorite and hydrogen peroxide solution was prepared by dilution of commercial NaClO solution and H_2O_2

solution in deionized water. Peroxynitrite was prepared following literature procedure[2] and assayed using a spectrophotometer using ε 302 nm = 1670 $cm^{-1}M$. The aqueous solutions of $NaNO_2$ and $NaNO_3$ were freshly prepared and used as nitrite (NO_2^-) and nitrate (NO_3^-) sources, respectively. A stock solution of DEA·NONOate was prepared in 0.01M NaOH solution. Various analytes (10 equiv. for NO, $ONOO^-$ and $^1O_2$; 100 equiv. for others analytes), represented by H_2O_2, NO_3^-, NO_2^-, HClO, O_2^-, OH•, AA, DHA, and MGO, were added to the solution of probe **1** (5 μM) in EtOH-PBS buffer (20 mM, pH 7.4, 1:1, v/v), respectively. The resulting solution was kept at 37 °C for 30 min, and then the fluorescence spectra were recorded with excitation at 480 nm.

4. Cell culture and fluorescence imaging:

The HL-7702 cell line was provided by Institute of Biotechnology of Shanxi University. Cells were grown in Dulbecco's Modified Eagle' medium (DMEM) supplemented with 10% FBS (Fetal Bovine Serum) and 1% antibiotics at 37 ºC in humidified environment of 5% CO_2. Cells were plated on 6-well plate and allowed to adhere for 12 hours. Before the experiments, cells were washed with PBS and then incubated with **1** (5 μM) in DMEM medium for 1 h at 37 ºC and then washed three times with PBS. After incubating with 50 μM DEA·NONOate for another 1 h at 37 ºC, the HL-7702 cells were rinsed with PBS three times, and the fluorescence images were acquired through a fluorescence microscopy OLMPUS-IX51 equipped with camera.

Reference

1. R. S. Drago, F. E. Paulik, *J. Am. Chem. Soc.*, 1959, **82**, 96.

2. R. M. Uppu, W. A. Pryor, *Anal. Biochem.*, 1996, **236**, 242.

5. ^{1}H NMR, ^{13}C NMR, and HRMS charts

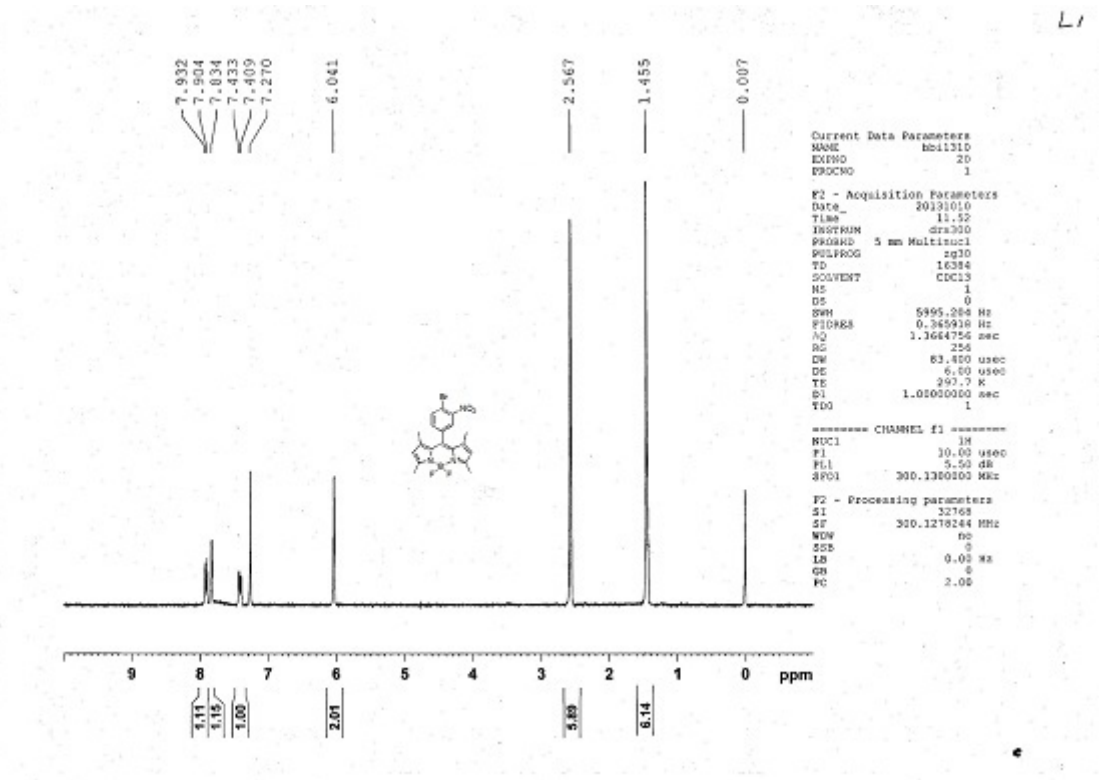


Figure S1 ^{1}H NMR chart of compound **4** ($CDCl_3$, 300 MHz).

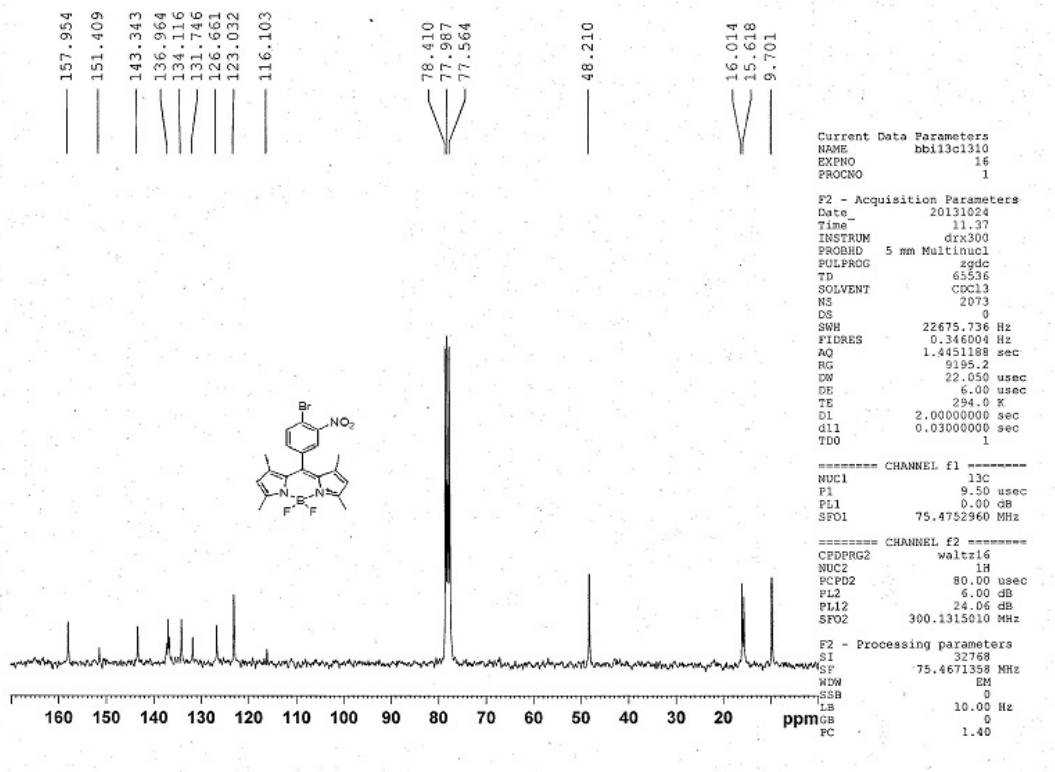


Figure S2 ^{13}C NMR chart of compound **4** ($CDCl_3$, 75 MHz).

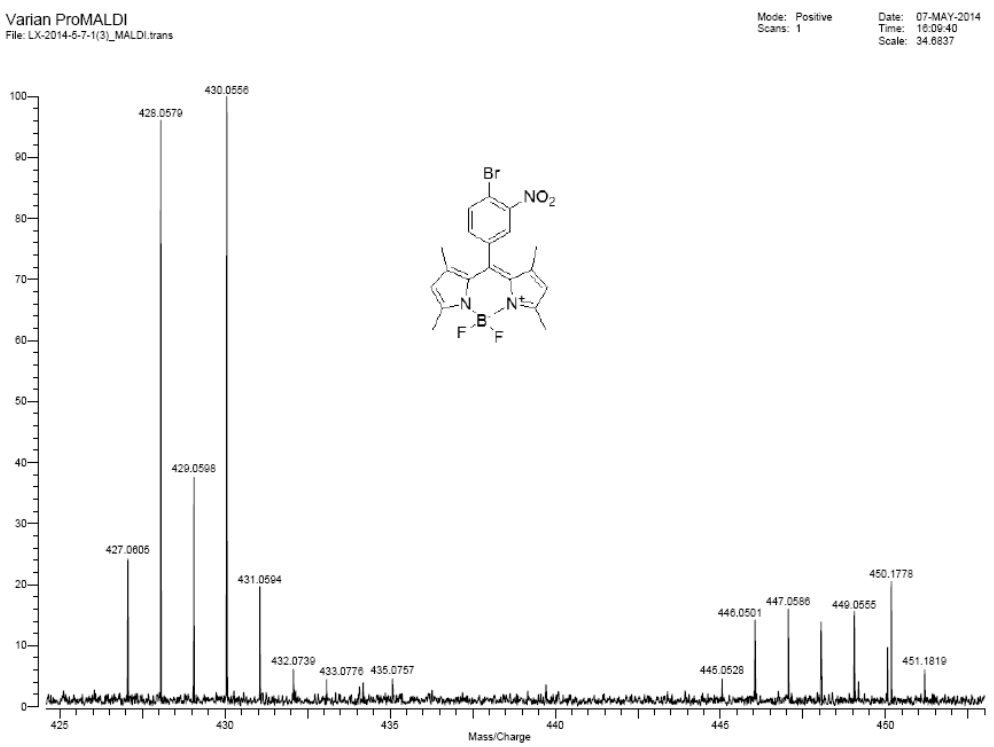


Figure S3 HRMS chart of compound **4**.

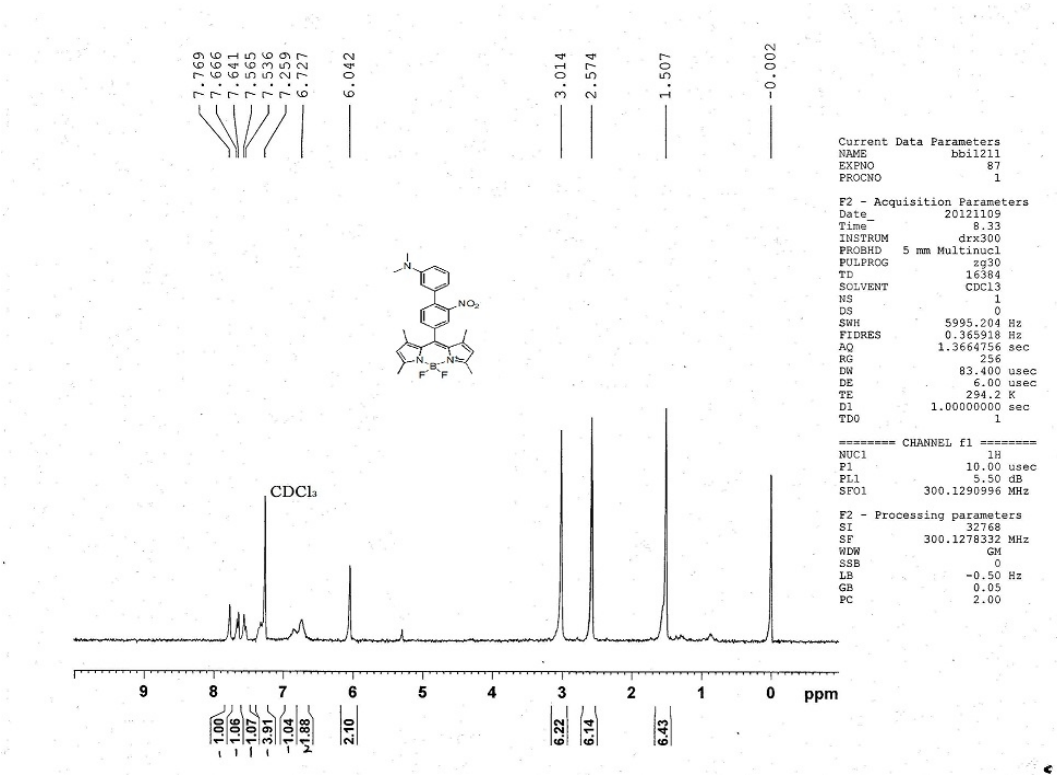


Figure S4 ^{1}H NMR chart of compound **3** ($CDCl_3$, 300 MHz).

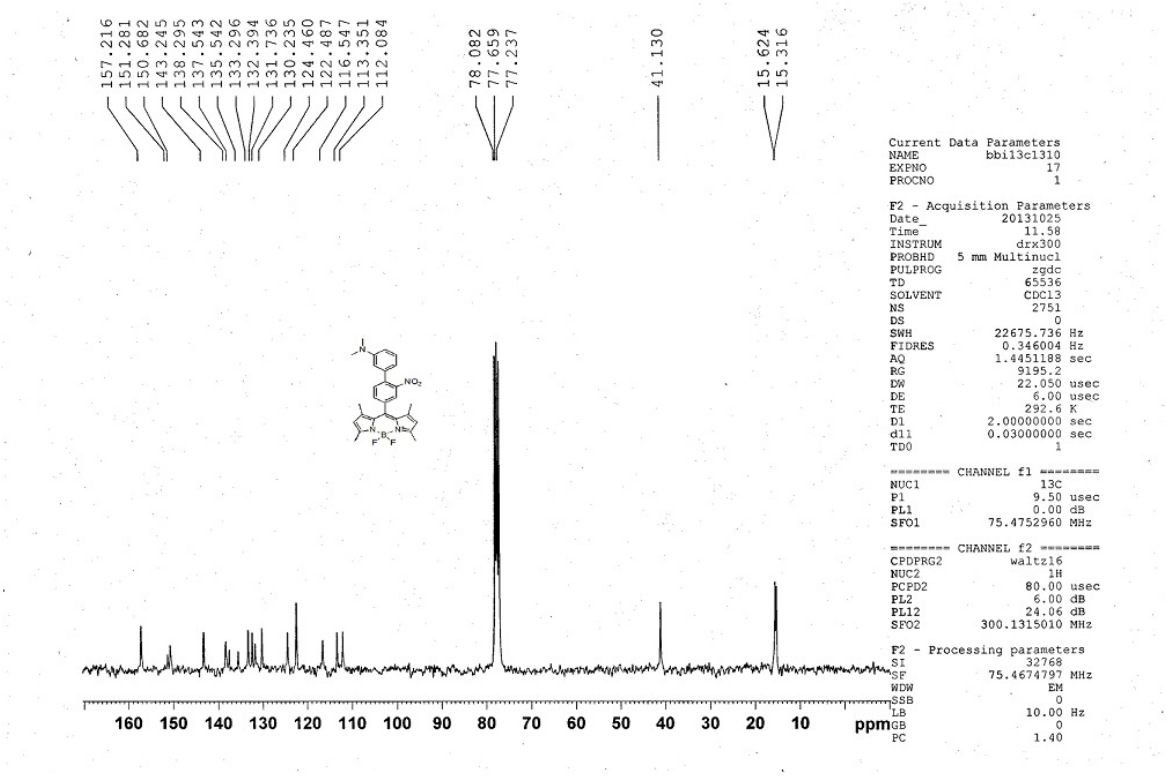


Figure S5 ^{13}C NMR chart of compound **3** ($CDCl_3$, 75 MHz).

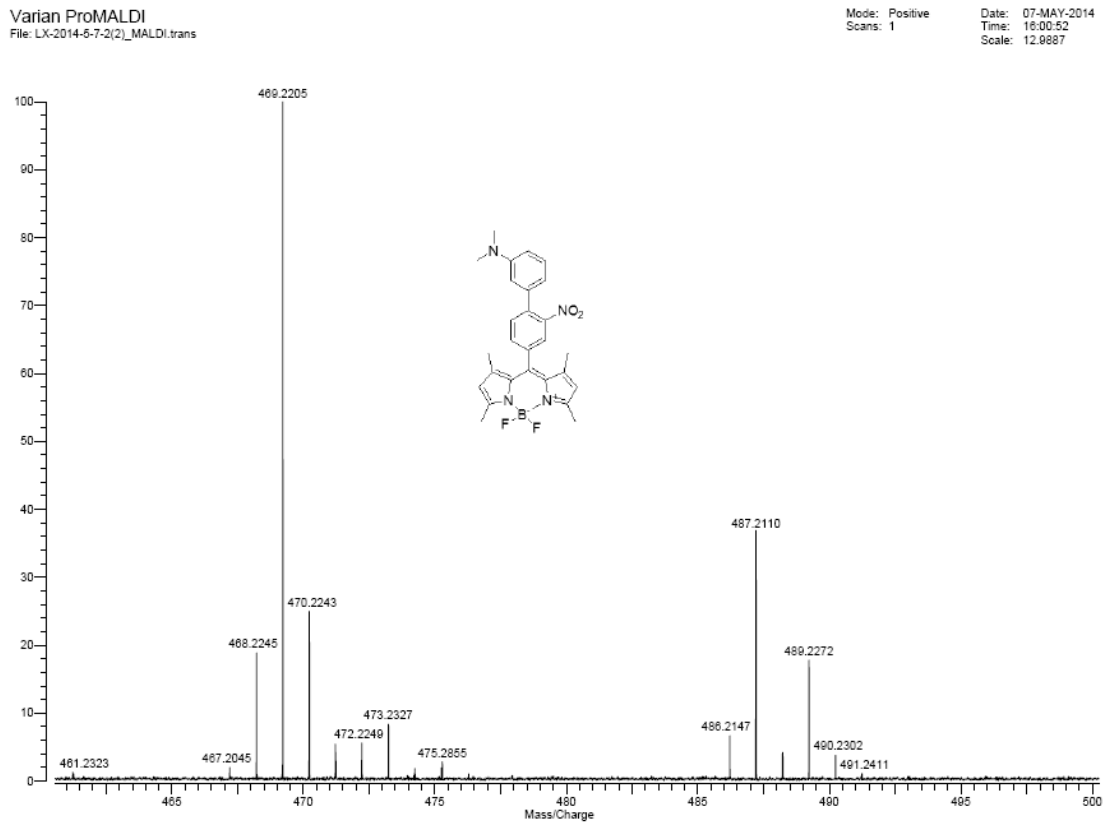


Figure S6 HRMS chart of compound **3**.

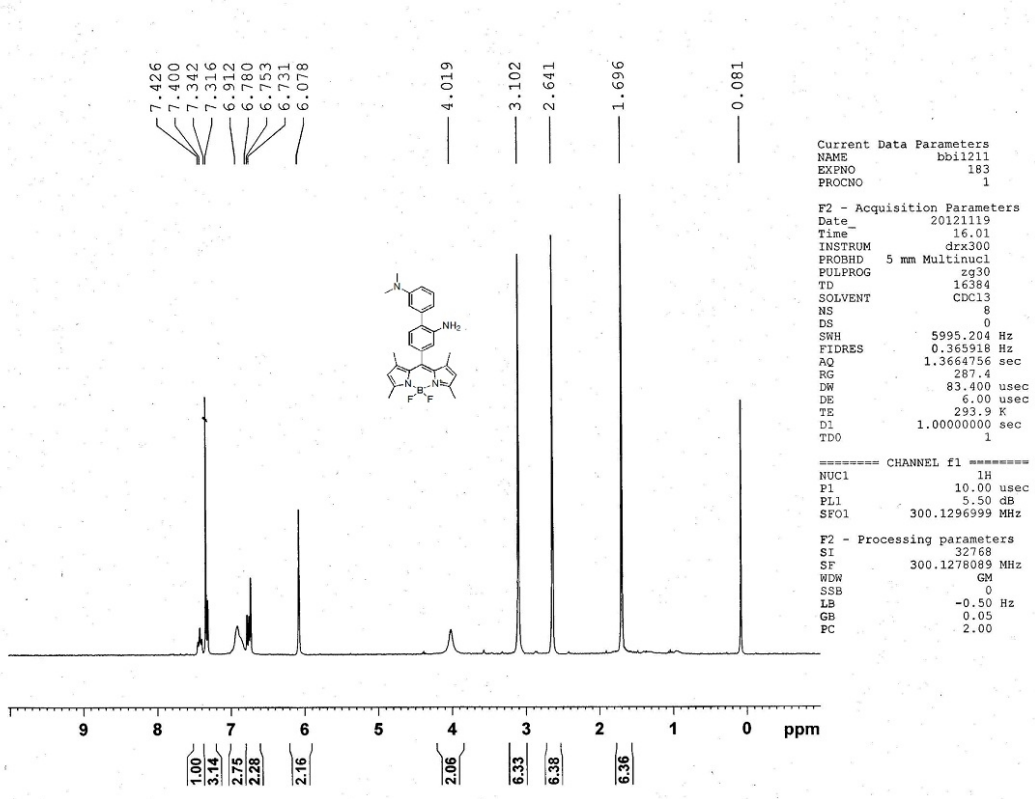


Figure S7 ^{1}H NMR chart of probe **1** ($CDCl_3$, 300 MHz).

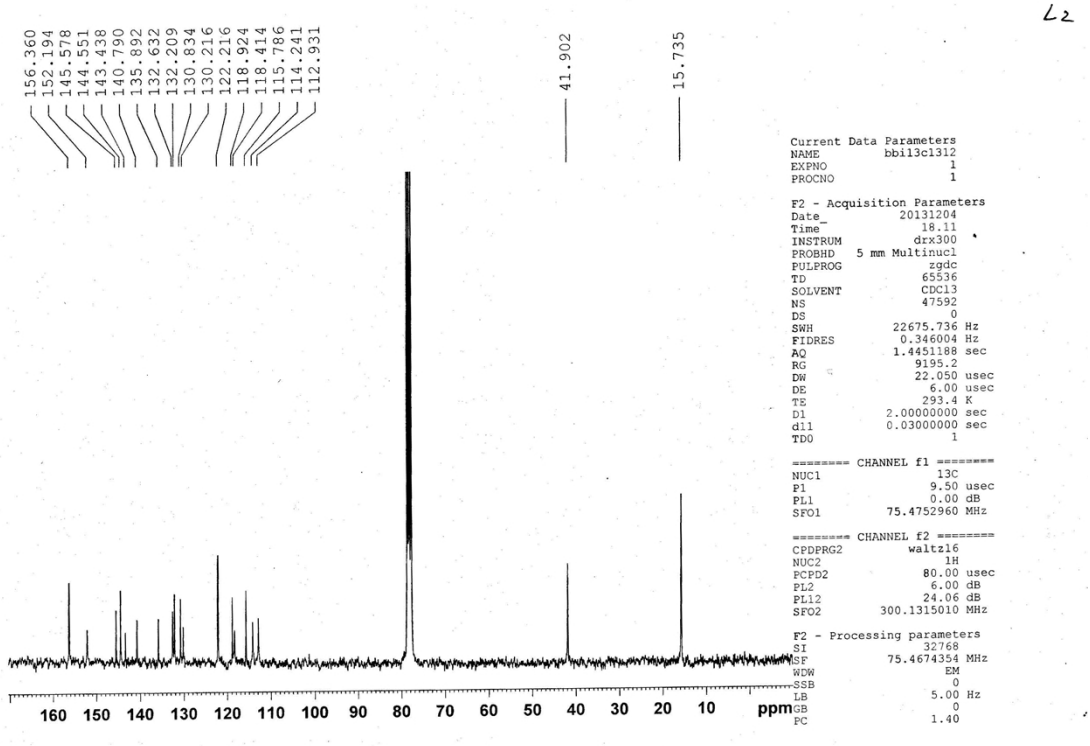


Figure S8 ^{13}C NMR chart of probe **1** ($CDCl_3$, 75 MHz).

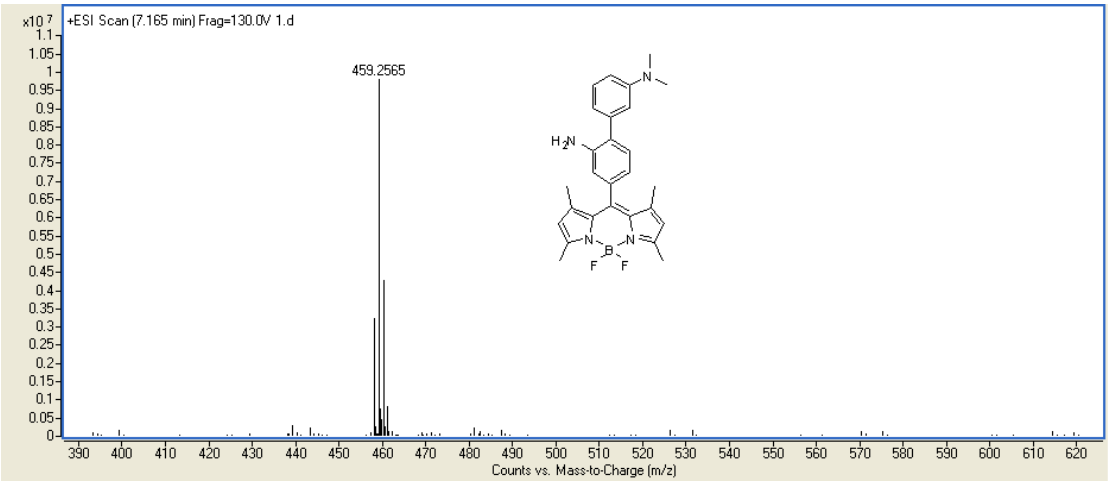


Figure S9 HRMS charts of probe **1**.

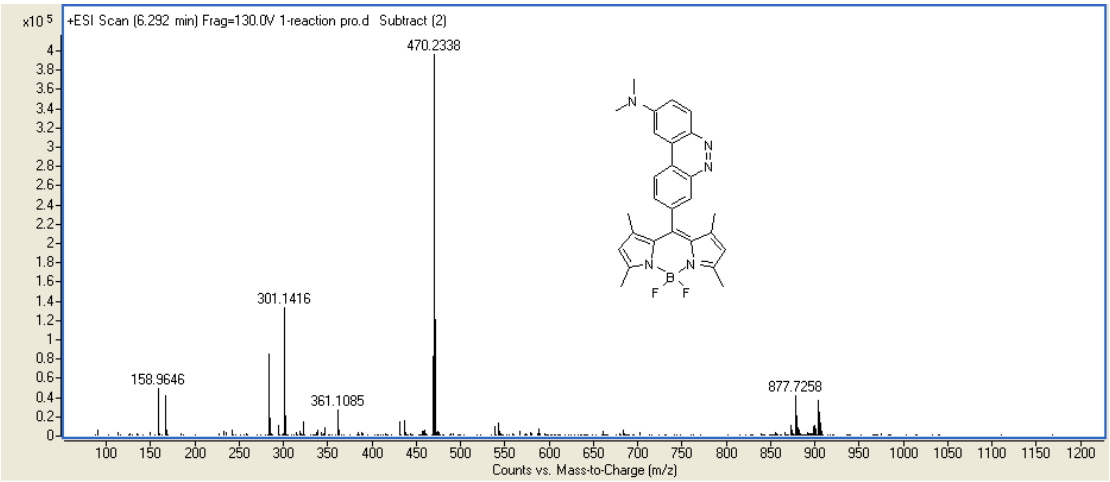


Figure S10 HRMS charts of product **2**.