Supporting Information

A rapid response ‘‘Turn–On’’ fluorescent probe for nitroreductase detection and its application in hypoxic tumor cell imaging

Jian Xu [a,b], Shaobo Sun [c], Qian Li [a], Ying Yue [a], Yingdong Li [c,*], Shijun Shao [a,*]

[a] Key Laboratory of Chemistry of Northwestern Plant Resources and Key Laboratory for Natural Medicine of Gansu Province, Lanzhou institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000, P. R. China.

[b] University of Chinese Academy of Sciences, Beijing 100039, P. R. China.

[c] Institute of Integrated Traditional and Western Medicine, Gansu University of Chinese Medicine, Lanzhou 730000, P. R. China.

Table of Contents:

1. Absorption and emission spectra of probe 1 toward nitroreductase.

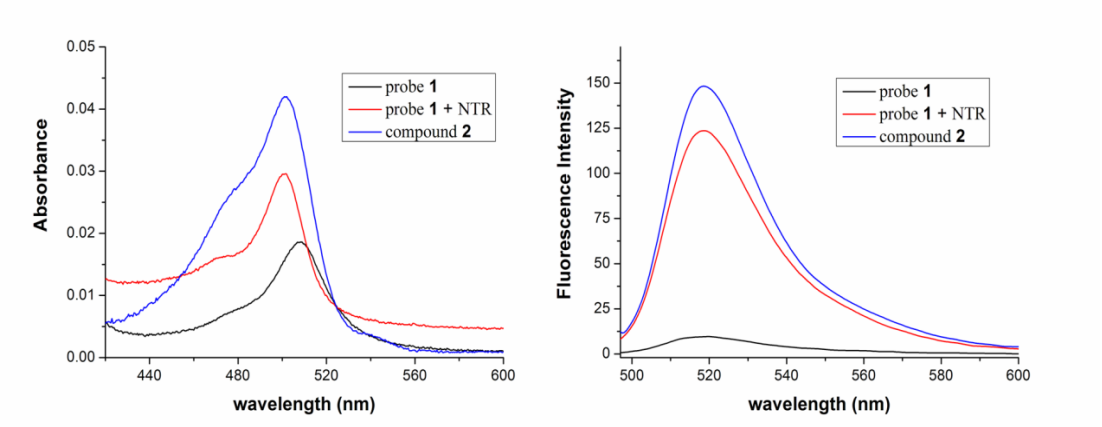

Fig. S1. UV-vis absorption spectra and fluorescent emission spectra of probe **1** (5 μM, black line), compound **2** (5 μM, blue line) and the reaction mixture (red line) of probe **1** (5 μM) with nitroreductase (2.5 μg/ mL), in the presence of 50 μM NADH for 5 min. All measurements were acquired at 37 °C in 10 mM PBS, pH 7.4, with excitation at 470 nm.

2. Time response of probe 1 to nitroreductase.

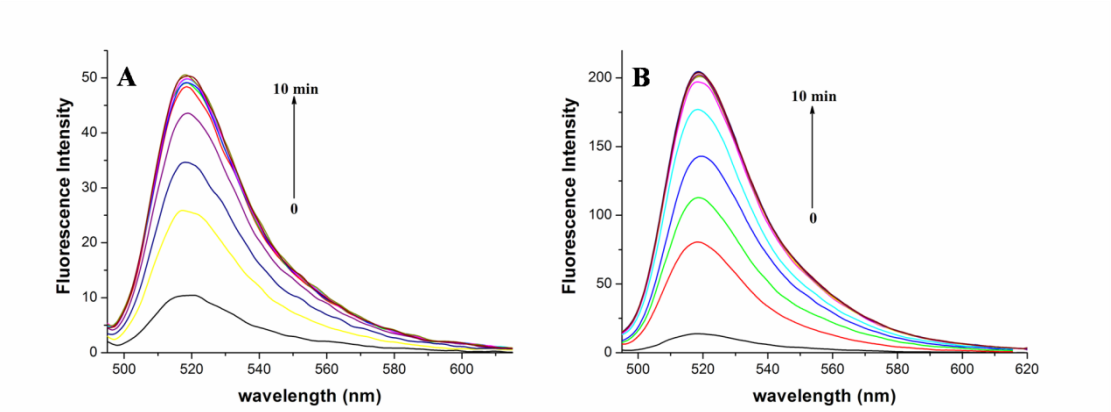

Fig. S2. Fluorescence turn-on response of probe **1** (5 μM) to (A) 0.5 μg/ mL nitroreductase and (B) 2.5 μg/ mL nitroreductase in the presence of 50 μM NADH at 37 °C. Spectra shown were acquired before nitroreductase addition and 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 min after nitroreductase was added. All measurements were acquired at 37 °C in 10 mM PBS, pH 7.4, with excitation at 470 nm.

3. Comparison of various fluorescent probes for NTR detection.

Table S1

Comparison of various fluorescent probes for nitroreductase detection.

Fluorescent NTR probe	Response time (min)	Linear range (ng/mL)	Detection limit (ng/mL)	Reference
Resorufin based probe	30	15-300	0.27	*Anal. Chem.* 2013, 85, 3926-3932.
Resorufin based probe	20	5-300	0.1	*Chem. Commun.* 2013, 49, 5859-5861.
1,8-naphthalimide based probe	10	Not mentioned	Not mentioned	*Org. Lett.* 2011, 13, 928-931.
Tricarbocyanine based probe	15	3000-13000	77	*Chem. Commun.* 2013, 49, 2554-2556.
Coumarin based probe	120	Not mentioned	Not mentioned	*Biosens. Bioelectron.* 2011, 26, 3511-3516.
BODIPY based probe	5	100-1000	9.6	this work

4. Lineweaver-Burk plot for the enzyme-catalyzed reaction.

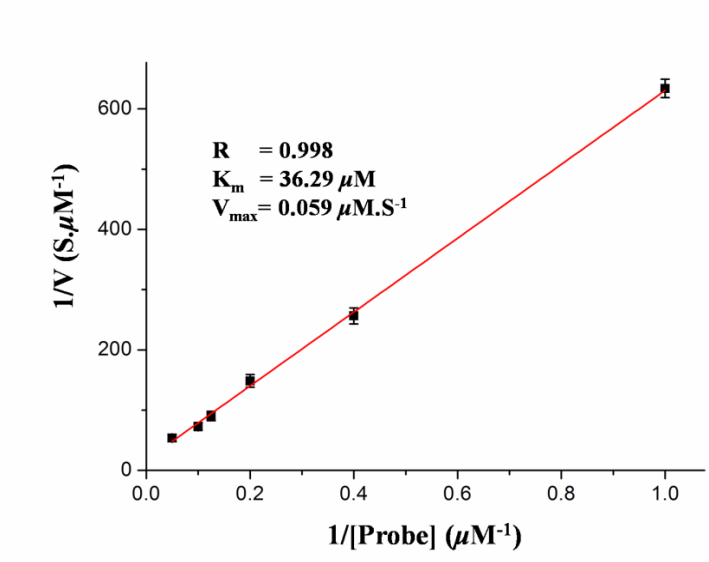

Fig. S3. Lineweaver-Burk plot for the enzyme-catalyzed reaction. The Michaelis-Menten equation was described as: $V = V_{max}$ [probe] / (K_m + [probe]), where V is the reaction rate, [probe] is the probe concentration (substrate), and K_m is the Michaelis constant. Conditions: 0.50 μg/mL nitroreductase, 50 μM NADH, 1 - 20 μM of probe **1**, $\lambda_{ex}/_{em}$ = 470/520 nm. Reaction at each probe concentration was repeated three times, and the error bars represent standard deviations. Points were fitted using a linear regression model (correlation coefficient R = 0.998).

5. Effects of pH and temperature on the reaction system.

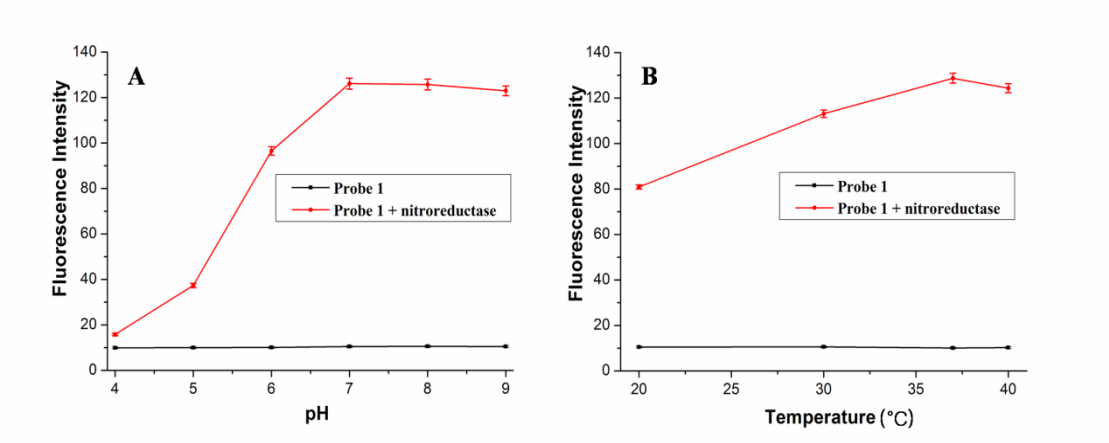

Fig. S4. Effects of (A) pH and (B) temperature on the fluorescence of probe **1** (5 μM) reacting with nitroreductase (1 μg/mL) in the presence of 50 μM NADH. All measurements were acquired in 10 mM PBS, pH 7.4, with excitation at 470 nm. Every data point was the mean of three measurements. The error bars are the standard deviation.

6. HRMS proof for the sensing mechanism.

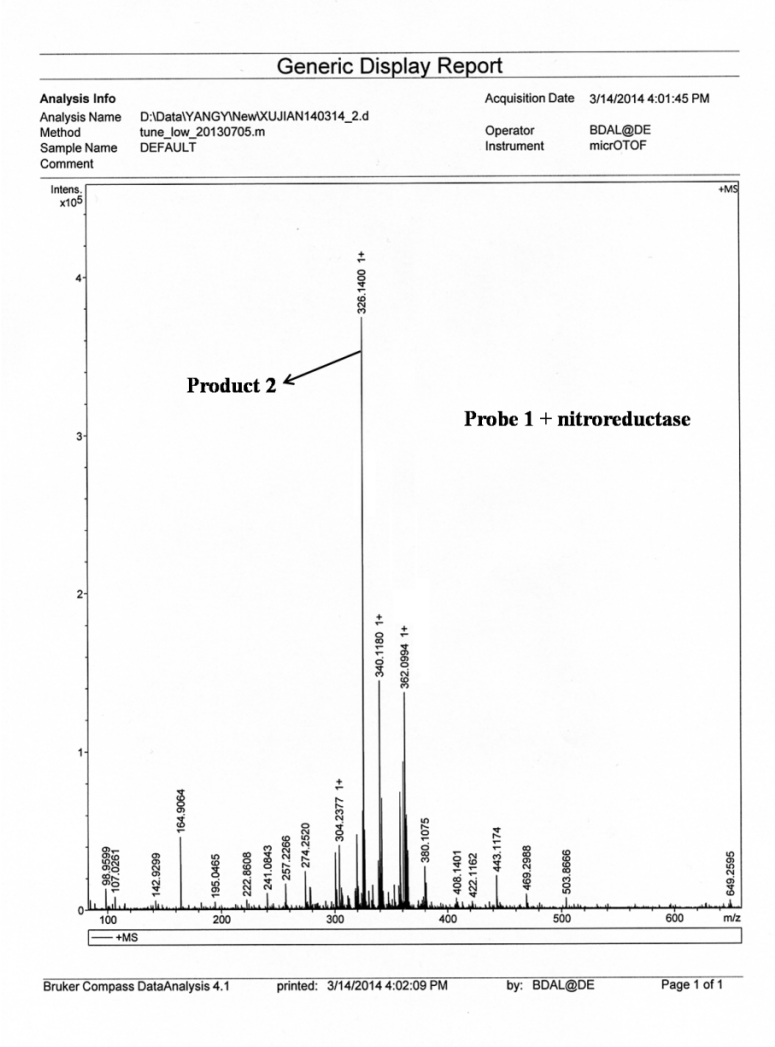

Fig. S5. HRMS spectra of the reaction solution of probe **1** (100 μM) with nitroreductase (5 μg/ mL).

7. HPLC analysis for the reaction system.

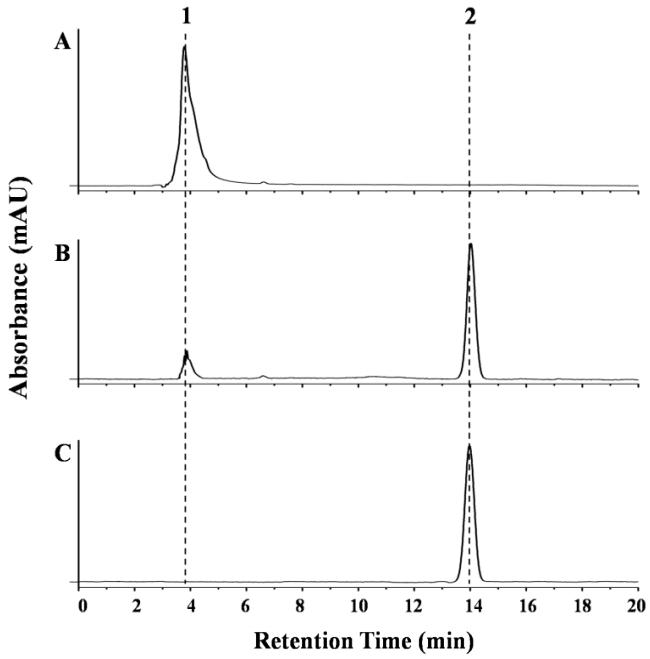

Fig. S6. Chromatograms of different reaction systems. (A) 100 μM probe **1**; (B) the reaction products of 100 μM probe **1** with 5 μg/ mL nitroreductase in the presence of 500 μM NADH for 5 min; (C) 100 μM 4-pyridinyl BODIPY **2**. The assignments of the peaks: (1) 3.79 min, probe **1**; (2) 14.00 min, 4-pyridinyl BODIPY **2**. Mobile phase: methanol–water, 70:30 (v/v). Detection: UV-vis (500 nm) detector. Flow rate: 1mL/min. T: 20 °C. Injection volume: 10 μL.

8. HRMS spectra of the reaction of probe 1 with *Escherichia coli.*

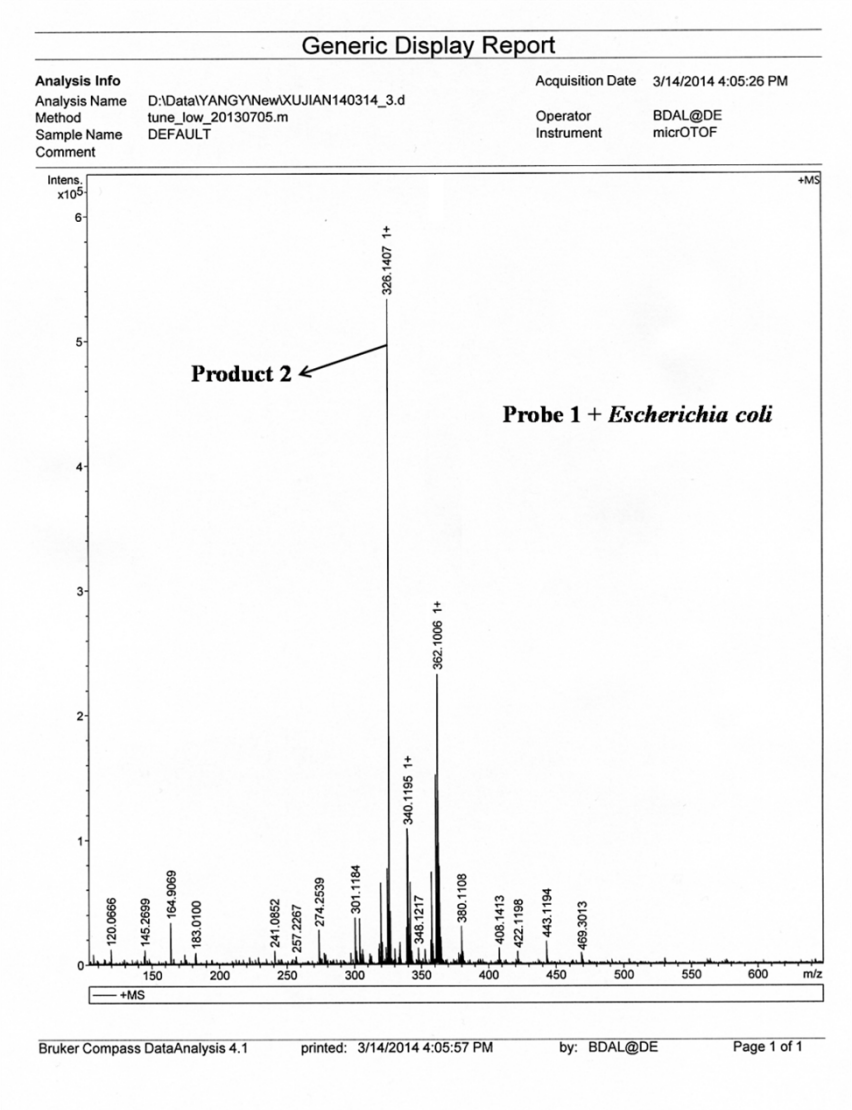

Fig. S7. HRMS spectra of the reaction solution of probe **1** (100 μM) with *Escherichia coli* (OD_{600} =1.0).

9. Real-time detection of nitroreductase produced by *Escherichia coli*

Table S2

Real-time detection of nitroreductase produced by *Escherichia coli* (DH5α) with an initial OD_{600} of 0.045.

Growth time (h)	0	1	2	3	4	5	6
Concentration of nitroreductase in the culture media[a] (μg/mL)	0.01±0.001	0.07±0.003	0.212±0.004	0.415±0.007	0.709±0.011	0.675±0.009	0.672±0.008

[a] Mean of three determinations±standard deviation.

10. ^{1}H NMR, ^{13}C NMR and MS spectra of compound 1-2.

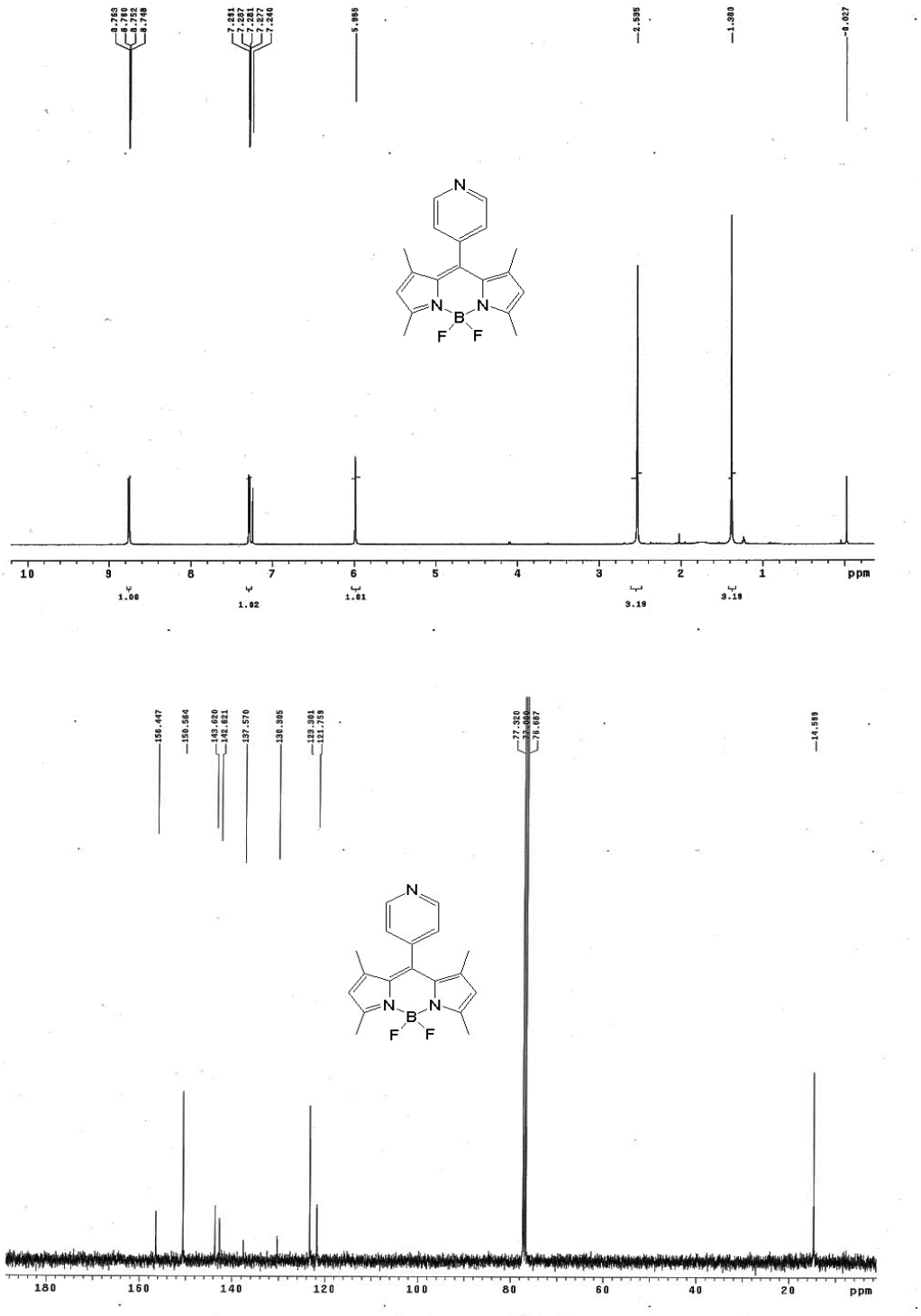

Fig. S8. ^{1}H NMR and ^{13}C NMR spectra of compound **2** in $CDCl_3$.

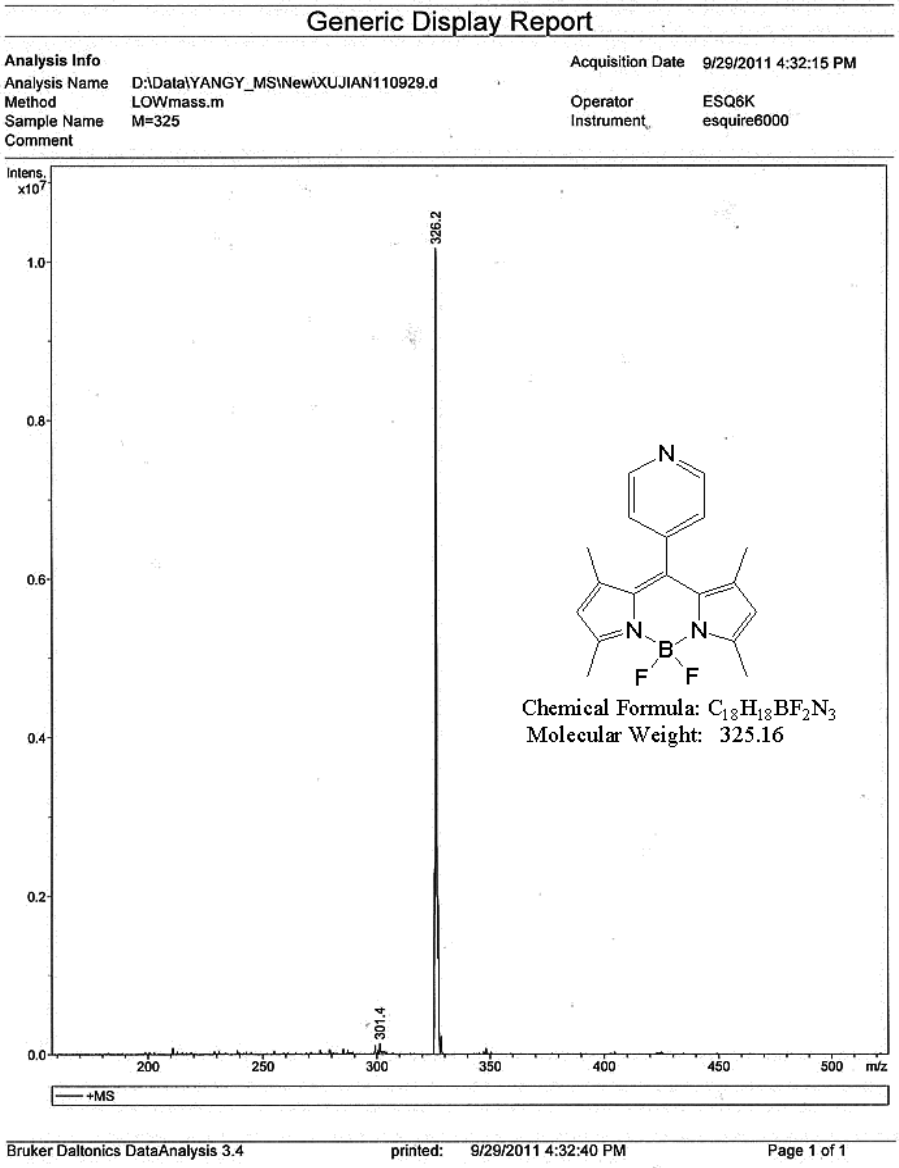

Fig. S9. ESI-MS spectra of compound **2**.

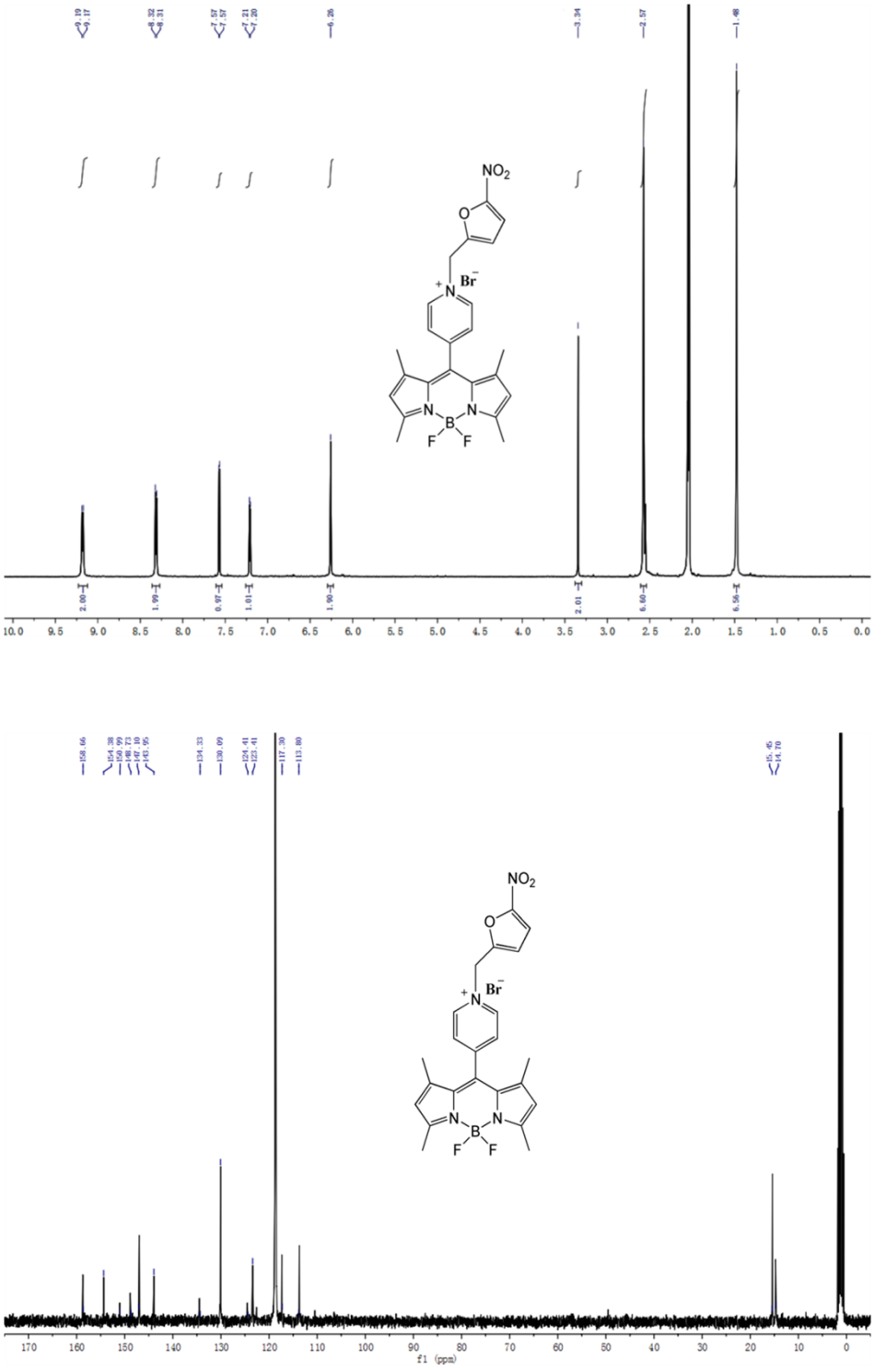

Fig. S10. ^{1}H NMR and ^{13}C NMR spectra of probe **1** in CD_3CN.

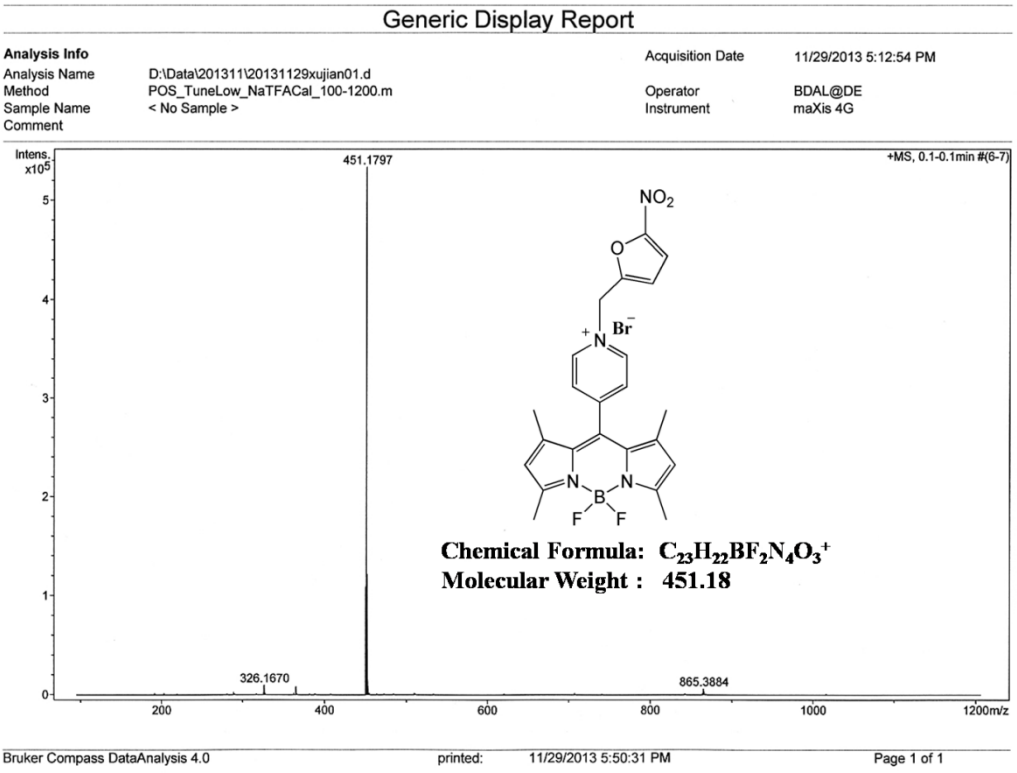

Fig. S11. ESI-MS spectra of probe **1**.