Electronic Supplementary Information (ESI)

Target-Triggered Deprotonation of 6-Hydroxyindole-based BODIPY Specially Switch on NIR Fluorescence upon Selectively Binding to Zn^{2+}

Jian Cao,[a,b] Chunchang Zhao,*[,a] Xuzhe Wang,[a] Yanfen Zhang,[a] Weihong Zhu*[,a]

[a]Shanghai Key Laboratory of Functional Materials Chemistry, Key Laboratory for Advanced Materials and Institute of Fine Chemicals, East China University of Science & Technology, Shanghai 200237, P. R. China. E-mail: zhaocchang@ecust.edu.cn; whzhu@ecust.edu.cn

[b]Shanghai University of Engineering Science, Shanghai 201620, P. R. China.

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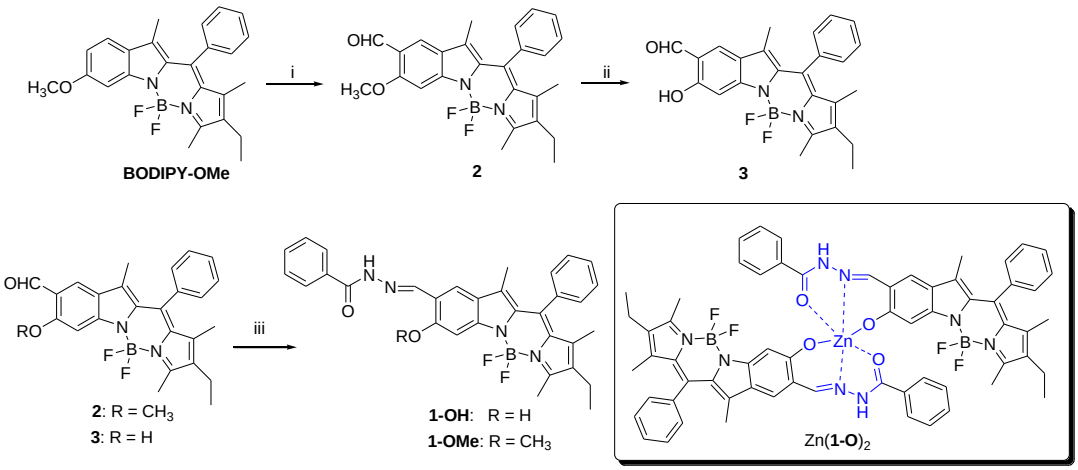
1. General Methods

All chemical reagents and solvents for synthesis were purchased from commercial suppliers and were used without further purification. Anhydrous CH_2Cl_2 were dried over $CaCl_2$ and distillated immediately prior to use. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AV-400 spectrometer with chemical shifts reported in ppm at room temperature. Mass spectra were measured on a HP 1100 LC-MS spectrometer.

UV-vis absorption spectra were recorded on a Varian Cary 100 spectrophotometer. Fluorescence spectra were measured with a Varian Cary Eclipse Fluorescence spectrophotometer. Spectral-grade solvents were used for measurements of UV-vis absorption and fluorescence. For absorption or fluorescence measurements, compounds were dissolved in CH_3CN to obtain stock solutions (5.0 mM). These stock solutions were diluted with aqueous solutions to the desired concentration.



Scheme S1. Synthesis of **1-OH** and **1-OMe**.

2. Synthesis of 2.

A mixture of DMF (3.0 mL) and $POCl_3$ (5.5 mL) was stirred in an ice bath for 5 min under argon. After being warmed to room temperature, it was stirred for additional 1 h. A solution of **BODIPY-OMe** (0.3 g, 0.72 mmol) in 10 mL CH_2Cl_2 was then added, and the resulting mixture was refluxed for 2 h. The reaction mixture was cooled to room temperature, and slowly poured into saturated aqueous Na_2CO_3 under ice-cold conditions. After being warmed to room temperature, the reaction mixture was further stirred for 30 min and washed with water. The organic layers were dried over anhydrous Na_2SO_4, and evaporated in vacuo. The crude product was further purified using column chromatography (silica gel, EtOAc/hexane=1:3) to give **2** (0.22 g, 68%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 1.03-1.06 (t, J = 7.6 Hz, 3H, -$CH_2C\boldsymbol{H}_3$), 1.40 (s, 3H, -CH_3), 1.61 (s, 3H, -CH_3), 2.36-2.41 (q, J = 7.6 Hz, 2H, -$C\boldsymbol{H}_2CH_3$), 2.70 (s, 3H, -CH_3), 4.04 (s, 3H, -CH_3), 7.15 (s, 1H)，7.33-7.35 (m, 2H), 7.55-7.56 (m, 3H), 8.04 (s, 1H), 10.40（s, 1H, -CHO）; ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 11.2, 12.3, 14.1, 17.2, 29.7, 56.0, 94.7, 121.2, 123.7, 126.2, 127.9, 128.2, 129.4, 129.5, 133.9, 134.7, 141.5, 148.7, 162.3, 164.4, 189.8; HRMS (ESI): calcd for $C_{26}H_{27}BF_2N_2O_2$ [M- H]$^-$: 445.1899; found: 445.1898.

3. Synthesis of 3.

To a solution of **2** (0.22 g, 0.49 mmol) in anhydrous CH_2Cl_2 was added BBr_3 (0.4 mL, 4.3 mmol) at -15 °C. The reaction mixture was further stirred for 1 h at -15 °C, warmed to room temperature, quenched with H_2O, extracted with CH_2Cl_2, washed with H_2O. The combined organic extracts were dried with Na_2SO_4, and evaporated in vacuo. The crude product was further purified using column chromatography (silica gel, EtOAc/hexane=1:3) to give **3** (85 mg, 40%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 1.02-1.06 (t, J = 8 Hz, 3H, -$CH_2C\boldsymbol{H}_3$), 1.40 (s, 3H, -CH_3), 1.62 (s, 3H, -CH_3), 2.36-2.41 (q, J = 7.6 Hz, 2H, -$C\boldsymbol{H}_2CH_3$), 2.71 (s, 3H, -CH_3), 7.21(s, 1H), 7.33-7.36 (m, 2H), 7.56(s, 1H), 7.57 (s, 1H), 7.57(s, 1H), 7.69(s, 1H), 9.82 (s, 1H), 11.02 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 11.1, 12.3, 13.7, 14.0, 17.2, 99.8, 117.9, 124.0, 126.6, 128.0, 128.2, 129.4, 129.5, 129.7, 130.3, 130.8, 132.5, 134.7, 138.6, 141.0, 142.8, 148.5, 161.1, 165.6, 195.5; HRMS (ESI): calcd for $C_{25}H_{24}BF_2N_2O_2$ $[M + H]^-$: 433.1899; found: 433.1899.

4. Synthesis of 1-OH.

A mixture of benzoyl hydrazine (80 mg, 0.6mmol) and **3** (85 mg, 0.2 mmol) in EtOH (10 mL) was refluxed for 2 h, and then the reaction was cooled to room temperature. The residue was poured into pure water and extracted with CH_2Cl_2, the organic layers were combined, dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by flash chromatography and then recrystallization to afford **1-OH** 55 mg (50%). ^{1}H NMR (400 MHz, d_6-DMSO, ppm): δ 0.98-1.02 (t, J = 7.6 Hz, 3H, -$CH_2C\boldsymbol{H}_3$), 1.38 (s, 3H, -CH_3), 1.61 (s, 3H, -CH_3), 2.37-2.42 (q, J = 7.6 Hz, 2H, -$C\boldsymbol{H}_2CH_3$), 2.64 (s, 3H, -CH_3), 6.95 (s, 1H), 7.47-7.49 (m, 2H), 7.53-7.57 (m, 2H), 7.60-7.65 (m, 4H), 7.86 (s, 1H), 7.93 (s, 1H), 7.95 (s, 1H), 8.68 (s, 1H), 11.70 (s, 1H), 12.11 (s, 1H); ^{13}C NMR (100 MHz, d_6-DMSO, ppm): δ 11.4, 12.3, 14.4, 14.5, 17.0, 98.9, 116.7, 123.7, 126.3, 128.1, 128.7, 129.0, 129.0, 129.7, 129.9, 130.0, 130.1, 132.3, 132.7, 133.3, 133.5, 134.6, 135.9, 137.9, 141.8, 142.3, 146.5, 148.7, 159.5, 163.3, 163.4; HRMS (ESI): calcd for $C_{32}H_{28}BF_2N_4O_2$ $[M-H]^-$: 549.2273; found: 549.2270.

5. Synthesis of 1-OMe.

The same procedure as for **1-OH**. The crude product was purified by flash chromatography to afford 50 mg (80%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 0.96-1.00 (t, J = 7.6 Hz, 3H, -$CH_2C\boldsymbol{H}_3$), 1.31 (s, 3H, -CH_3), 1.61 (s, 3H, -CH_3), 2.21-2.23 (d, J = 7.6 Hz, 2H, -$C\boldsymbol{H}_2CH_3$), 2.60 (s, 3H, -CH_3), 4.00 (s, 3H, -CH_3), 7.05(s, 1H), 7.38-7.42 (m, 4H), 7.47-7.49 (d, 1H), 7.55-7.56 (d, 3H), 7.81 (s, 2H), 7.94 (s, 1H), 8.38 (s, 1H), 9.39 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 10.0, 10.6, 11.7, 12.6, 15.8, 54.2, 92.4, 115.7, 119.7, 125.0, 125.1, 125.7, 126.6, 126.7, 126.8, 127.7, 127.8, 127.9, 128.0, 130.0, 131.5, 132.0, 132.5, 133.0, 133.3, 134.1, 135.2, 140.3, 140.5, 143.8, 145.7, 158.4, 160.2, 161.4; HRMS (ESI): calcd for $C_{33}H_{32}BF_2N_4O_2$ $[M+ H]^-$: 565.2586; found: 565.2589.

6. Preparation of cells.

Breast Cancer MCF-7 cells were cultured in RPMI-1640 supplemented with 10% FCS at 37 °C in a 5/95 CO_2/air incubator for 24 h. For fluorescence imaging, cells were incubated in glass bottom dishes for 24 h. $Zn(ClO_4)_2$ uptake experiments were performed in the same medium for 30 min at 37 °C. Then cells were washed with PBS buffer, incubated with 5.0 μM **1-OH** in culture medium for 30 min at 37 °C, washed with PBS. The confocal cell images were collected using Nikor AIR with a 60 × oil objective. The samples were excited with 638 nm and observation between 662-737 nm.

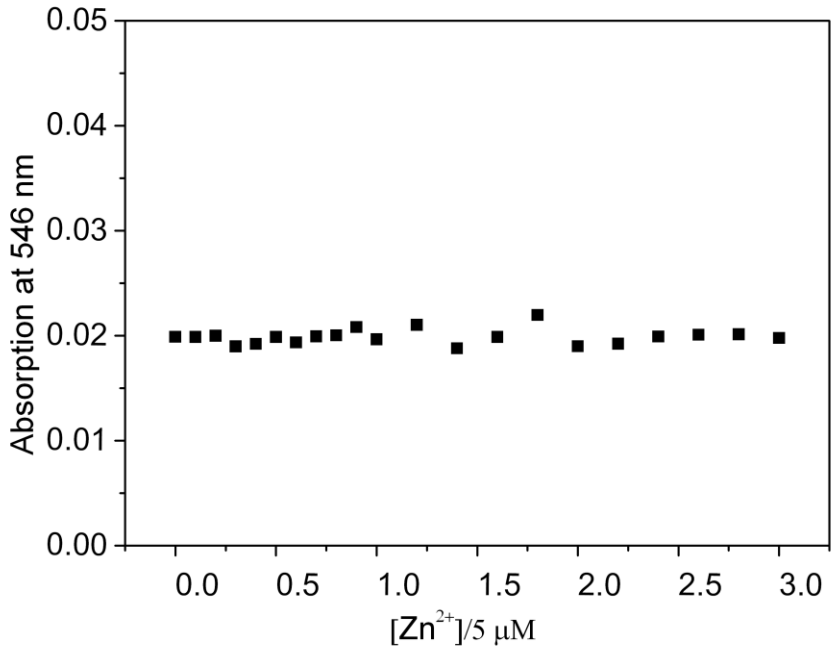
7. Calculation the binding constant

From the emission data, binding constants were also obtained using equation as follows (B. Valeur, *Molecular Fluorescence: Principles and Applications.* 2001 Wiley-VCH, Verlag GmbH.):

$$Y = \frac{X}{\varepsilon K_f \{[D]_0^2 - 4[D]_0 \frac{X}{\varepsilon} + \frac{4}{\varepsilon^2} X^2\}} + \frac{X}{\varepsilon}$$

Where Y is the concentration of $[Zn^{2+}]$ added, X is the optical density at 617 nm, $[D_0]$ is the concentration of **1-OH**, in this paper it is 5×10^{-6} M.

8. The response of 1-OMe to Zn^{2+}.



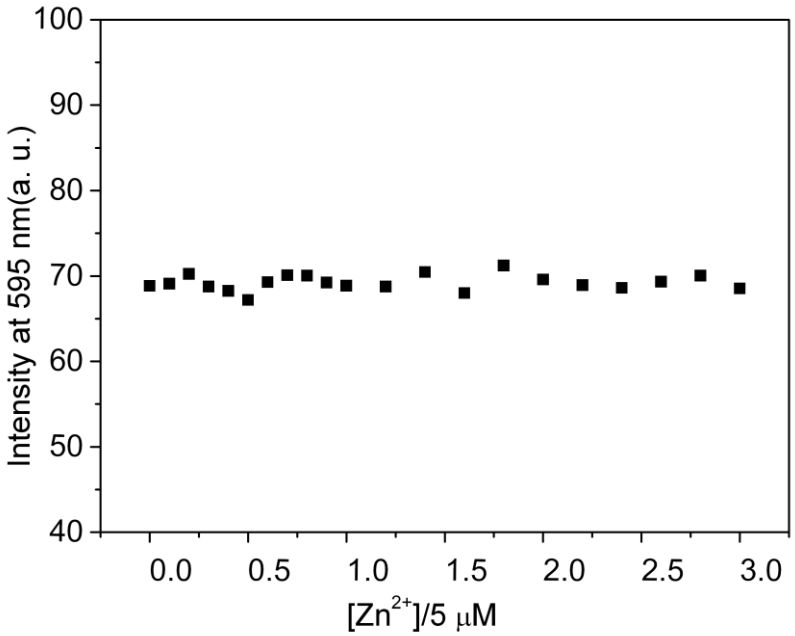


Fig. S1 Changes in Absorption (a) and fluorescence (b) of **1-OMe** (5.0 μM) in the presence of different concentrations of Zn^{2+}.

9. ^{1}H NMR titration experiment of 1-OH with Zn^{2+}

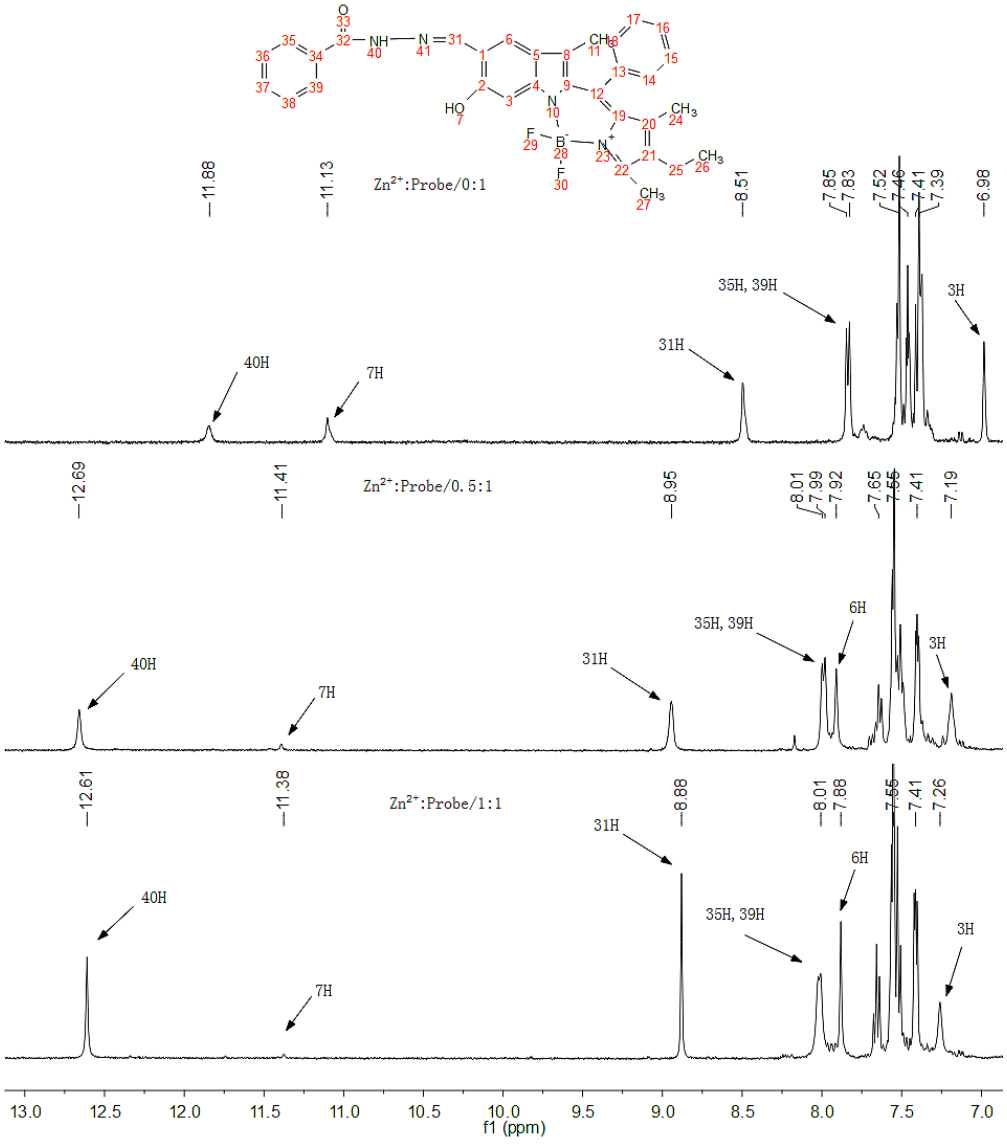


Fig. S2 ^{1}H NMR titration experiment of **1-OH** with Zn^{2+} in CD_3CN.

10. Ms spectrum of 1-OH + Zn^{2+}

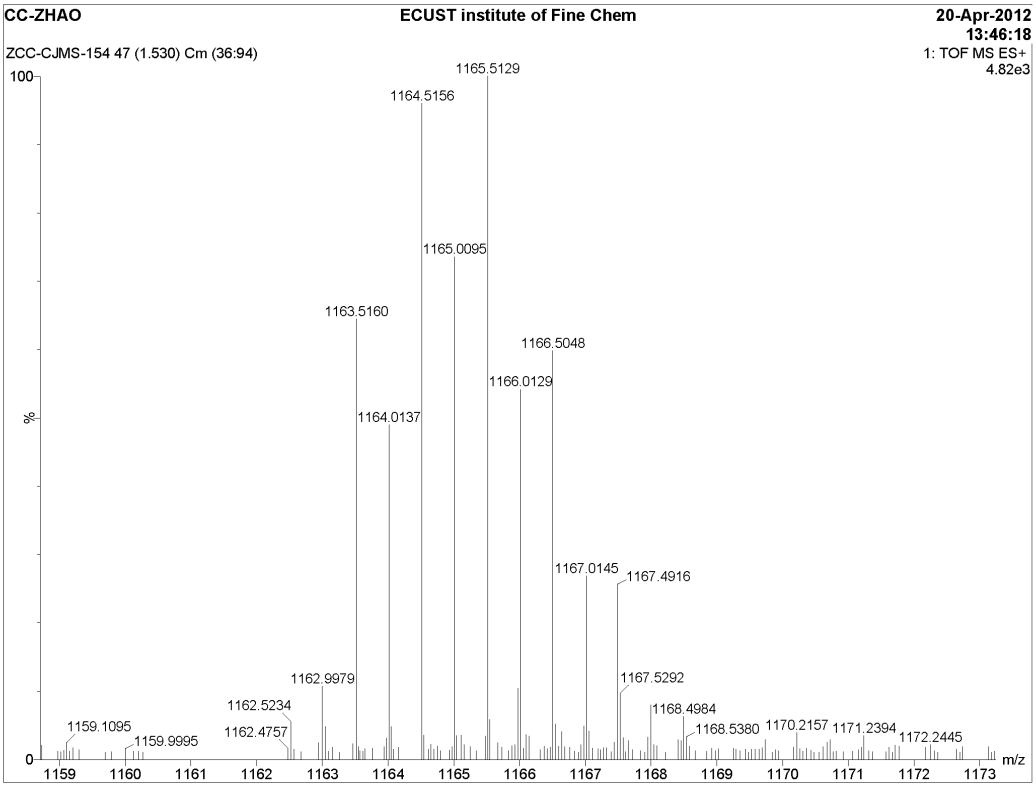


Fig. S3 Ms spectrum of **1-OH** + Zn^{2+} in CH_3CN.

11. Fluorescence of 1-OH in CH_3CN

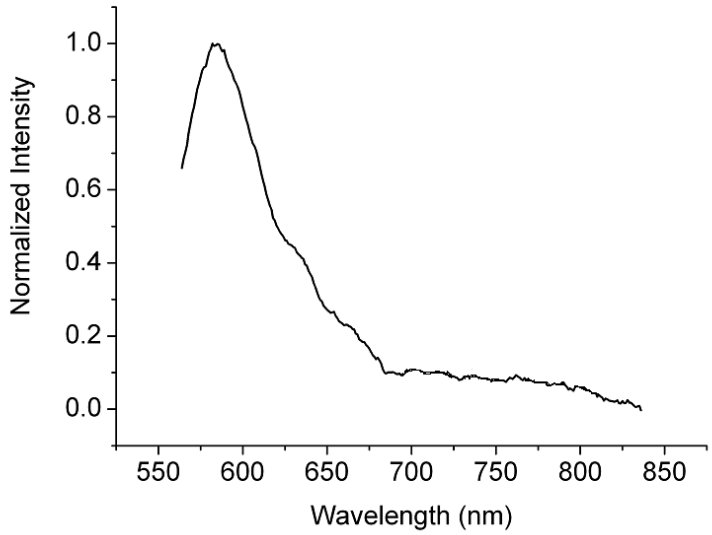
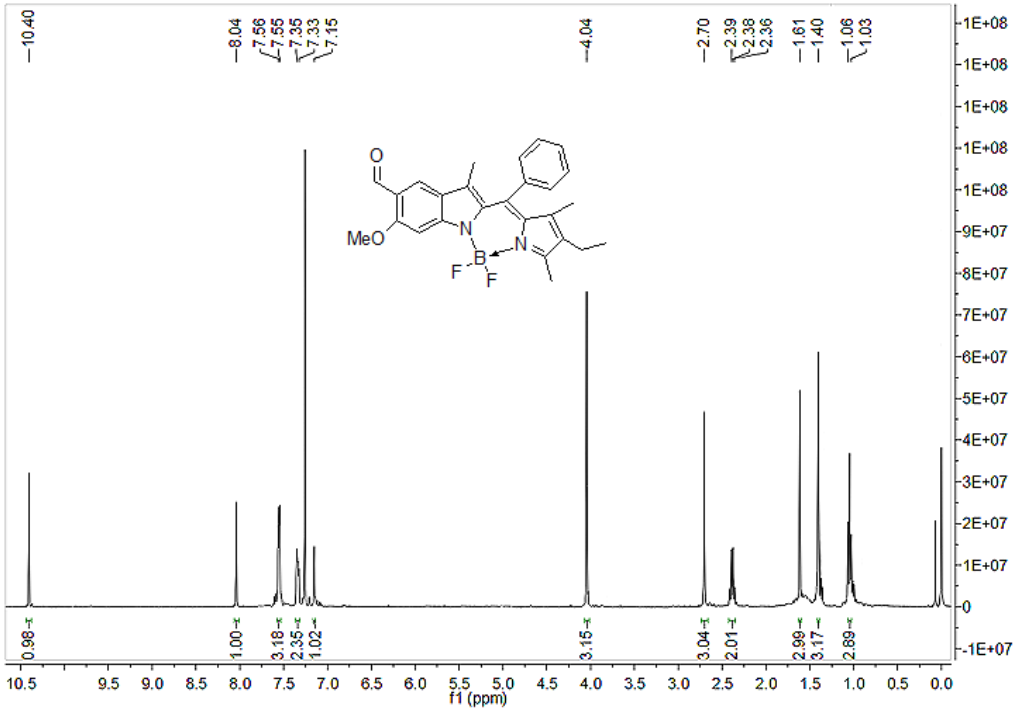
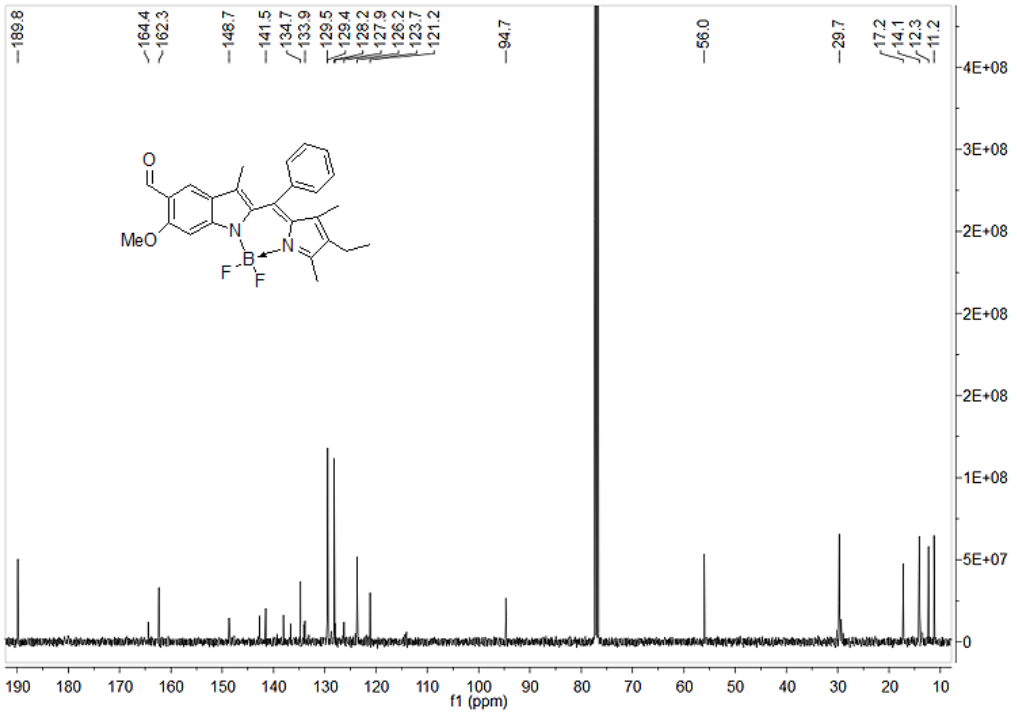
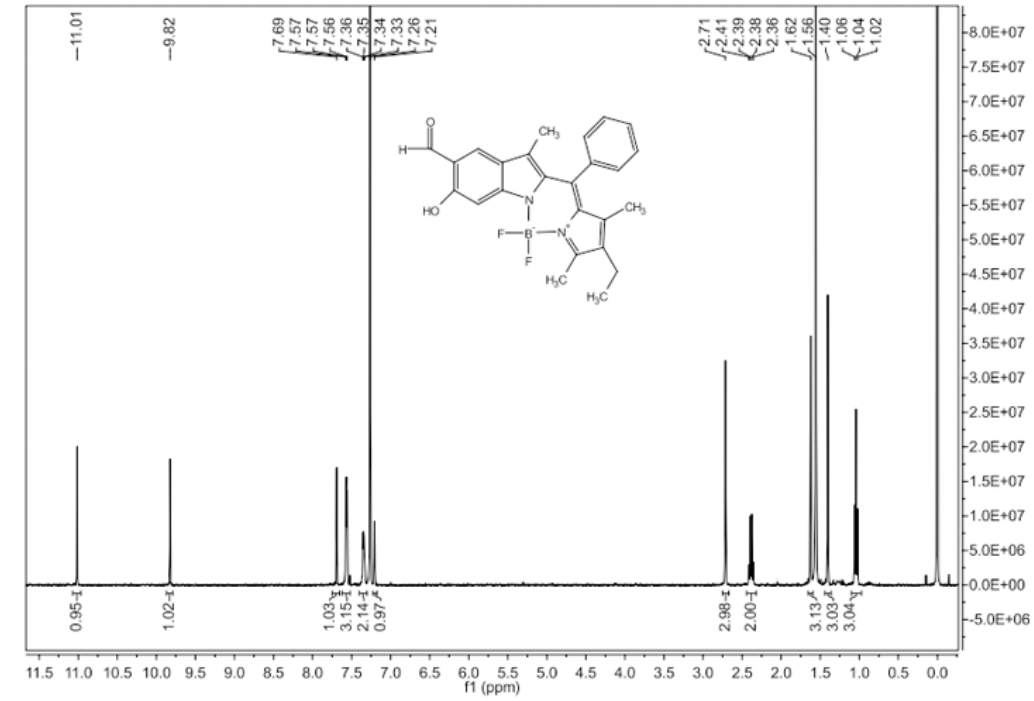


Fig. S4 Emission spectrum of **1-OH** in CH_3CN upon excitation at 545 nm. Note: the resulting emission is very weak due to the structural flexibility.

12. ^{1}H NMR and ^{13}C NMR spectra



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1.02
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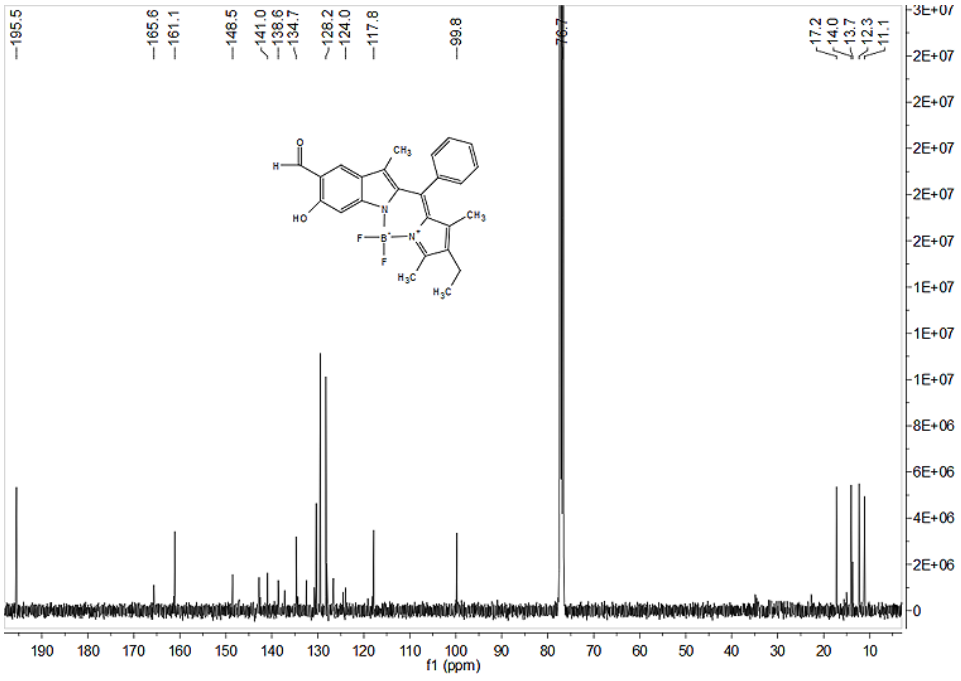
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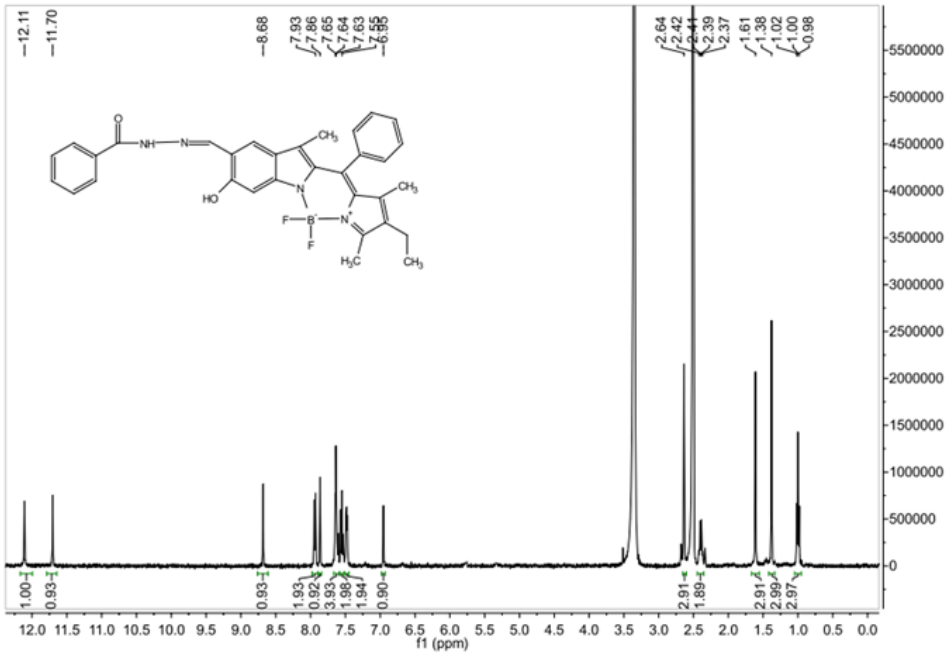
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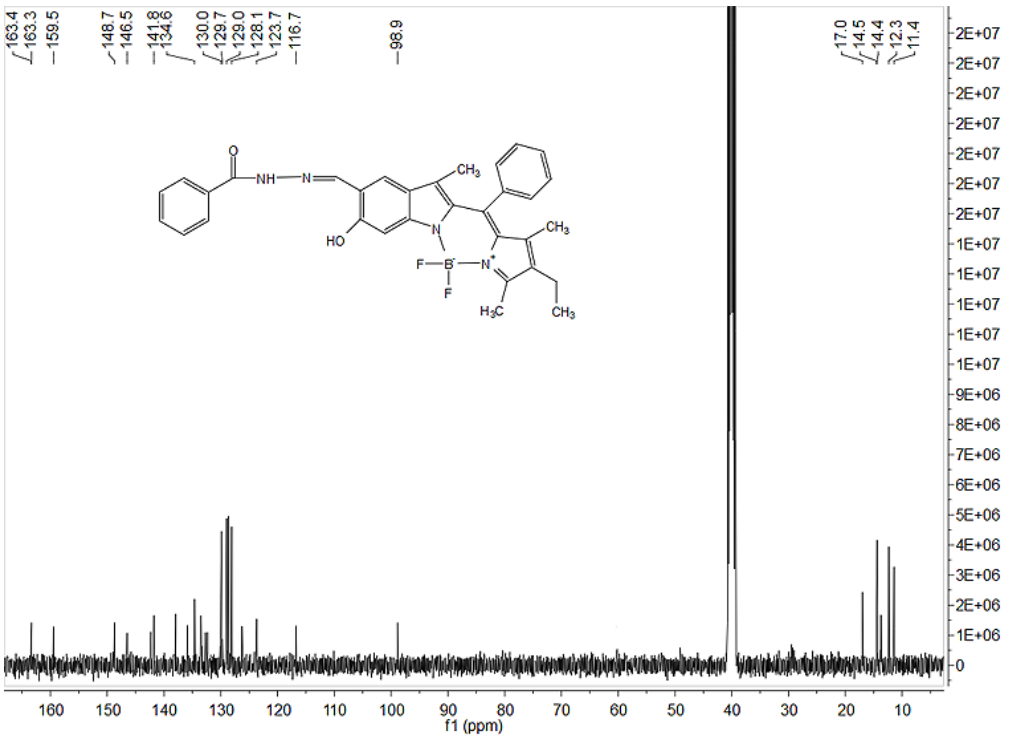
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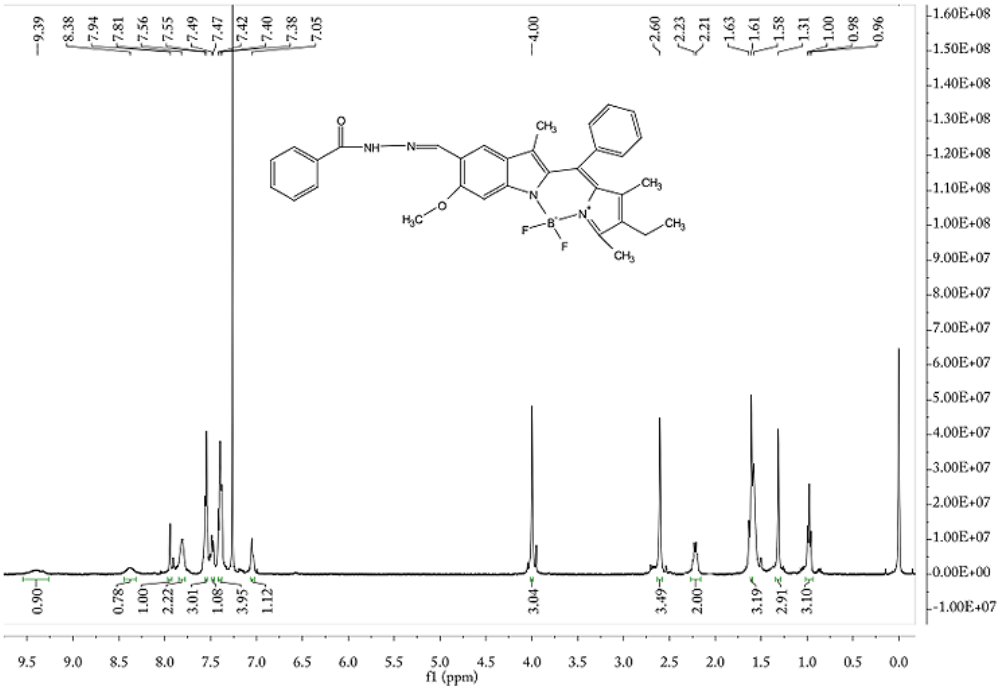
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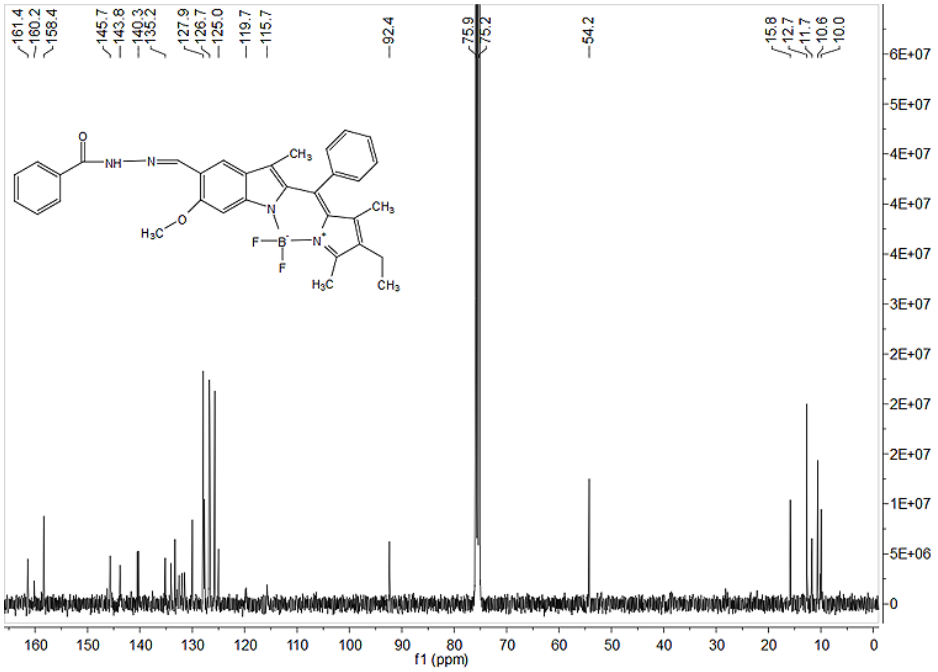
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