

*Supporting Information*

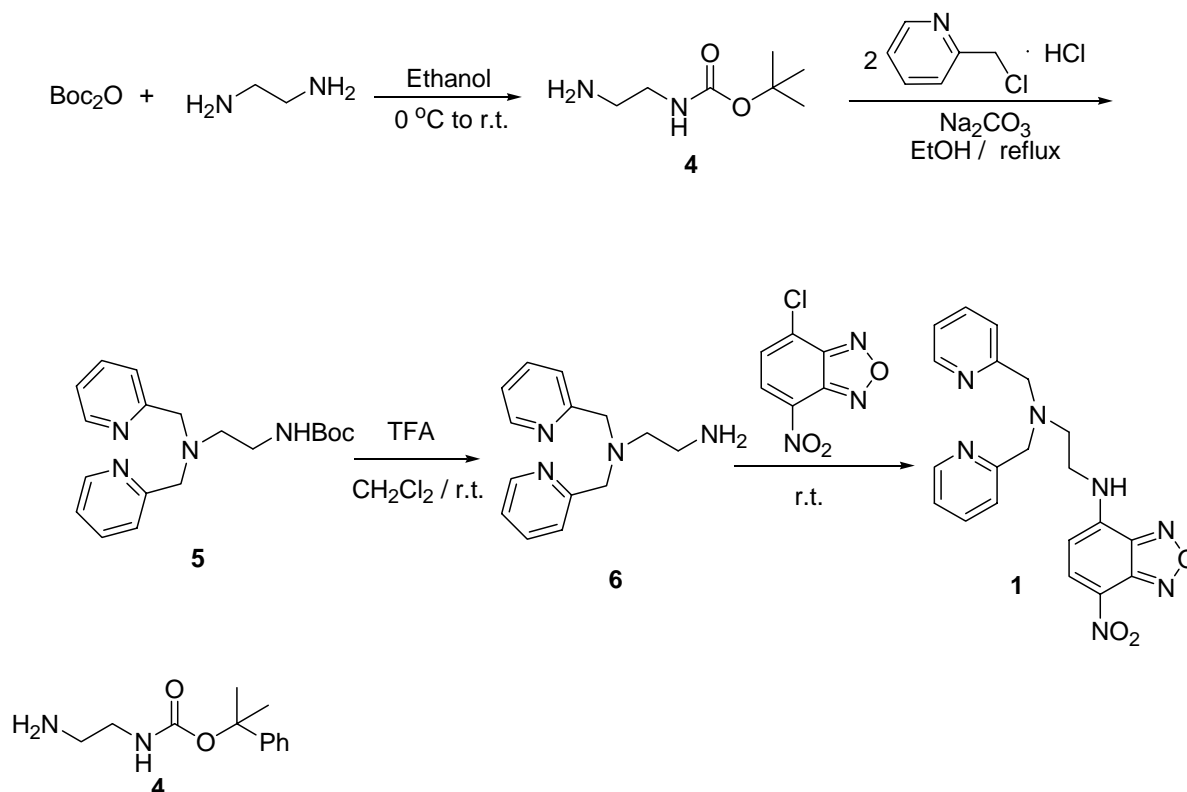
**An NBD Fluorophore-based Sensitive and Selective Fluorescent Probe  
for Zinc Ion**

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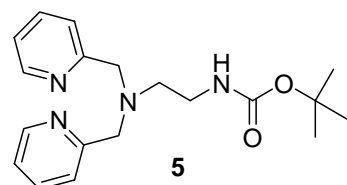
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**General Information:** Commercial reagents were used as received, unless otherwise stated. Merck 60 silica gel was used for chromatography, and Whatman silica gel plates with fluorescence F<sub>254</sub> were used for thin-layer chromatography (TLC) analysis. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on Bruker Avance 500, and tetramethylsilane (TMS) was used as a reference. Data for <sup>1</sup>H are reported as follows: chemical shift (ppm), and multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet). Data for <sup>13</sup>C NMR are reported as ppm. Mass Spectra were obtained from University of New Mexico Mass Spectral facility.

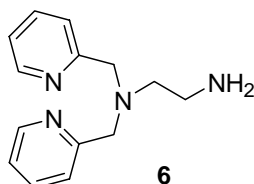


**(2-Aminoethyl)carbamic acid tert-butyl ester (4).** To a solution of ethylenediamine (1.35 mL, 20 mmol) in 10 mL ethanol was added di-*t*-butyl dicarbonate (441 mg, 2 mmol) dropwise at 0 °C. The reaction mixture was warmed up to room temperature and stirred for 24 h under a nitrogen atmosphere, then evaporated in *vacuo*. The residue was dissolved in 20 mL of dichloromethane and washed with aqueous solution of sodium hydroxide. The organic layer was dried with magnesium sulfate, filtered and evaporated in *vacuo* to give the crude product of **4** as colorless oil (315 mg, 91 % yield). <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 5.23 (s, 1H), 3.09 (d, 2H, *J* = 5.5 Hz), 2.72 (t, 2H, *J* = 6 Hz), 1.47 (s, 2H), 1.36 (s, 9H); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>): δ 156.4, 79.1, 43.4, 41.9, 28.5.

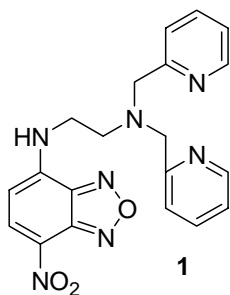


**tert-Butyl 2-(bis(pyridin-2-ylmethyl)amino)ethylcarbamate (5).** A mixture of **4** (290 mg, 1.67 mmol), 2-pyridylmethyl chloride hydrochloride (615 mg, 3.67 mmol) and sodium carbonate (467 mg, 7.34 mmol) in 20 mL of ethanol was refluxed for 12 h under a nitrogen atmosphere. Then the solvent was evaporated, the mixture was dissolved in 20 mL of aqueous solution of sodium

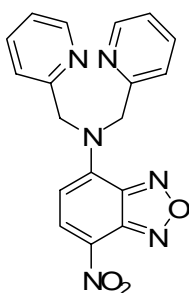
hydroxide and extracted with dichloromethane ( $3 \times 30$  mL). The combined organic layer was dried with magnesium sulfate, filtered and evaporated in *vacuo*. The crude product was purified by silica gel chromatography using triethylamine/methanol/dichloromethane (1:1:100) as the elute to afford **5** as brown oil (400 mg, 70% yield).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.55 (d, 2H,  $J = 5$  Hz), 7.64 (t, 2H,  $J = 7.5$  Hz), 7.42 (d, 2H,  $J = 7.5$  Hz), 7.16 (dd, 2H,  $J_1 = 7$  Hz,  $J_2 = 5$  Hz), 5.89 (s, 1H), 3.87 (s, 4H), 3.24 (d, 2H,  $J = 5$  Hz), 2.70 (t, 2H,  $J = 5.5$  Hz), 1.45 (s, 9H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta$  159.4, 156.3, 149.2, 136.5, 123.2, 122.2, 78.7, 60.3, 53.6, 38.6, 28.6.



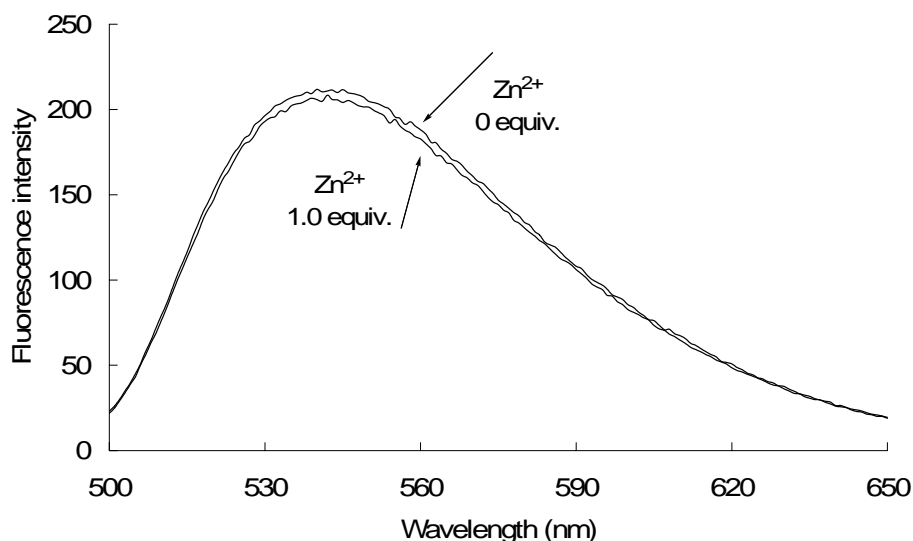
***N*-Bis(pyridin-2-yl)methylethane-1,2-diamine (6)**. To 15 mL trifluoroacetic acid was added dropwise a solution of **5** (400 mg, 1.17 mmol) in 10 mL of dichloromethane at 0 °C. The reaction was warmed up to room temperature and stirred 2 h under a nitrogen atmosphere, then evaporated in *vacuo*. The residue was dissolved in an aqueous solution of sodium hydroxide and extracted with dichloromethane ( $3 \times 20$  mL). The combined organic layer was dried with magnesium sulfate, filtered and evaporated in *vacuo* to afford **6** as brown oil (280 mg, 99% yield).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.54 (d, 2H,  $J = 4$  Hz), 7.60 (t, 2H,  $J = 7.5$  Hz), 7.32 (d, 2H,  $J = 8$  Hz), 7.14 (t, 2H,  $J = 6$  Hz), 4.62 (s, 2H), 3.86 (s, 4H), 2.92 (t, 2H,  $J = 5.5$  Hz), 2.80 (t, 3H,  $J = 5.5$  Hz);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta$  159.6, 149.2, 136.6, 123.1, 122.2, 60.7, 57.0, 39.5.



***N*-(2-(Bis(pyridin-2-yl)methyl)amino)ethyl-7-nitrobenzo[*c*][1,2,5]oxadiazol-4-amine (1)**. To a solution of **6** (243 mg, 1.0 mmol) and triethylamine (169  $\mu\text{L}$ , 1.2 mmol) in 10 mL dichloromethane was added NBD-Cl (245 mg, 1.2 mmol) dropwise at room temperature and stirred overnight, then evaporated in *vacuo*. The residue was dissolved in an aqueous solution of sodium hydroxide and extracted with dichloromethane ( $3 \times 20$  mL). The combined organic layer was dried with magnesium sulfate, filtered and evaporated in *vacuo*. The crude product was purified by silica gel chromatography using triethylamine/methanol/dichloromethane (1:1:60) as elute to afford **1** as brown solid (294 mg, 78% yield).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  10.35 (s, 1H), 8.74 (d, 2H,  $J = 3.5$  Hz), 8.44 (d, 1H,  $J = 8.5$  Hz), 7.60 (t, 2H,  $J = 7.5$  Hz), 7.33 (d, 2H,  $J = 8$  Hz), 7.20 (t, 2H,  $J = 6$  Hz), 6.01 (d, 1H,  $J = 7.5$  Hz), 4.04 (s, 4H), 3.51 (s, 2H), 3.12 (t, 2H,  $J = 5$  Hz);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta$  158.6, 149.5, 145.0, 144.5, 144.4, 137.2, 137.0, 123.1, 122.5, 122.4, 98.1, 59.5, 50.6, 42.5. HRMS: calculated 405.1549, found 406.1628 ( $\text{M} + \text{H}^+$ ).



**7-Nitro-*N,N*-bis(pyridin-2-ylmethyl)benzo[*c*][1,2,5]oxadiazol-4-amine (2).** To a solution of di-(2-picolyl)-amine (93  $\mu\text{L}$ , 0.5 mmol) and triethylamine (106  $\mu\text{L}$ , 0.75 mmol) in 10 mL dichloromethane was added NBD-Cl (123 mg, 1.2 mmol) dropwise at room temperature and stirred overnight, then evaporated in *vacuo*. The residue was dissolved in an aqueous solution of sodium hydroxide and extracted with dichloromethane ( $3 \times 20$  mL). The combined organic layer was dried with magnesium sulfate, filtered and evaporated in *vacuo*. The crude product was purified by silica gel chromatography using triethylamine/methanol/dichloromethane (1:1:50) as elute to afford the desired product as brown solid (162 mg, 90% yield).  $^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ ):  $\delta$  8.56 (d, 2H,  $J = 4.5$  Hz), 8.36 (d, 1H,  $J = 9$ Hz), 7.70 (m, 3H), 7.33 (d, 2H,  $J = 8.5$  Hz), 7.24 (dd, 2H,  $J_1 = 5$  Hz,  $J_2 = 2$  Hz), 6.35 (d, 1 H,  $J = 9$  Hz), 5.43 (s, 4H);  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ):  $\delta$  155.2, 150.1, 146., 145.0, 144.8, 137.3, 135.5, 123.7, 123.2, 122.0, 103.3, 58.9.



**Figure S1:** Emission spectra ( $\lambda_{\text{ex}} = 470$  nm) of probe **2** ( $10^{-5}$  M) after addition of with  $\text{Zn}^{2+}$  ( $1.0 \times 10^{-5}$  M) at room temperature in PBS buffer (pH 7.3).

#### Determination of $K_d$ of probe **1**<sup>1,2</sup>

The binding constant  $K_d$  was determined using the following equation:  $F = (F_{\text{max}}[\text{Zn}^{2+}] + F_{\text{min}} K_d) / (K_d + [\text{Zn}^{2+}])$ , where  $F$  is the observed fluorescence,  $F_{\text{max}}$  is the fluorescence for the  $\text{Zn}^{2+}$  : Probe (1 : 1) complex, and  $F_{\text{min}}$  is the fluorescence in the absence of  $\text{Zn}^{2+}$ . The  $K_d$  was calculated by using the by using the Excel program as reported, its value is 4.6  $\mu\text{M}$ .

**Job's plot of probe **1** and  $\text{Zn}^{2+}$**  (Fig. S2). The total concentration of probe and  $\text{Zn}^{2+}$  were kept at

constant 10  $\mu\text{M}$ . The maximum fluorescence response at 0.5 fraction of probe indicates the formation of a 1:1 probe **1**:  $\text{Zn}^{2+}$  complex (Fig. S2).

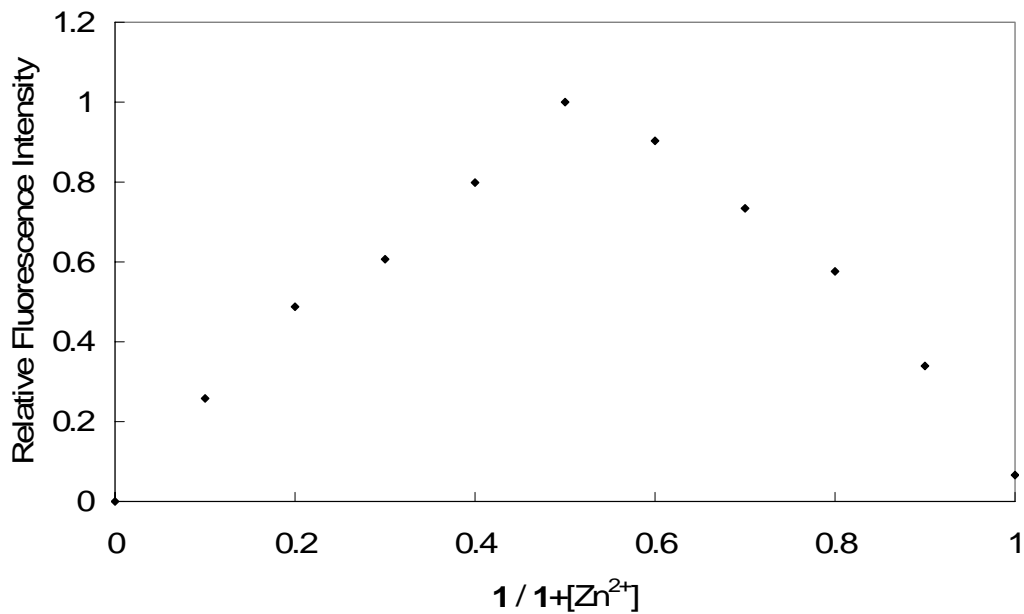


Fig. S2

**References:**

- 1 A. E. Martell, R. M. Smith *Critical Stability Constants*, Plenum Press: New York, 1989.
- 2 Q. He, E. W. Miller, A. P. Wong, C. J. Chang; *J. Am. Chem. Soc.* 2006, **128**, 9316-9317.

