Supporting Information

Boronic Acid-Linked Fluorescent and Colorimetric Probes for Copper Ions

K. M. K. Swamy,[a] Sung-Kyun Ko,[b] Soo Kyung Kwon,[a] Ha Na Lee,[a] Chun Mao,[a] Joung-Min Kim,[c] Keun-Hyeung Lee,[c] Jinheung Kim,[a] Injae Shin[b]* and Juyoung Yoon[a]*

[a]*Division of Nano Sciences and Department of Chemistry, Ewha Womans University, Seoul 120-750, Korea*
[b]*Department of Chemistry, Yonsei University, Seoul 120-749, Korea*
[c]*Department of Chemistry, Inha University, Incheon 402-751, Korea*
jyoon@ewha.ac.kr; injae@yonsei.ac.kr

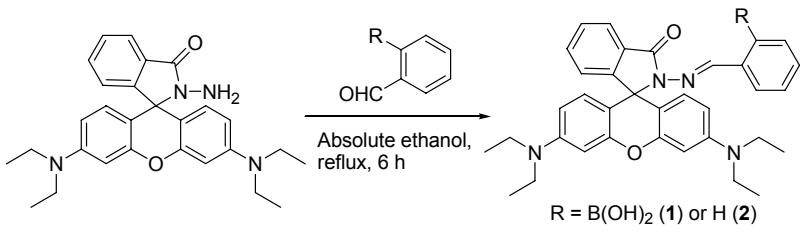
Experimental Section

General methods. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Flash chromatography was carried out on silica gel 60 (230-400 mesh ASTM; Merck). Thin layer chromatography (TLC) was carried out using Merck 60 F_{254} plates with a thickness of 0.25 mm. Preparative TLC was performed using Merck 60 F_{254} plates with a thickness of 1 mm. Transferrin was purchased from Sigma as an iron free from.

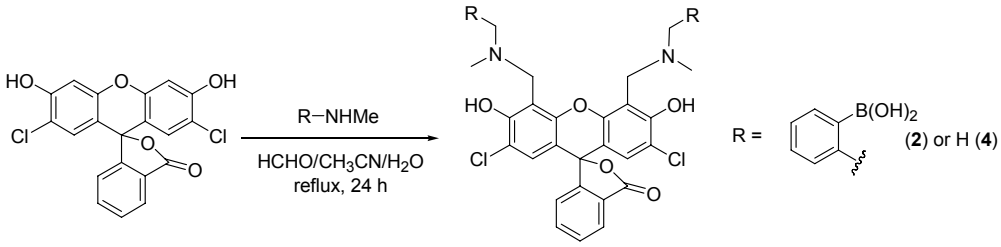
Melting points were measured using a Büchi 530 melting point apparatus, and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded using Bruker 250 or Varian 500. Chemical shifts were expressed in ppm and coupling constants (*J*) in Hz. Mass spectra were obtained using a JMS-HX 110A/110A Tandem Mass Spectrometer (JEOL). UV absorption spectra were obtained on UVIKON 933 Double Beam UV/VIS Spectrometer. Fluorescence emission spectra were obtained using RF-5301/PC Spectrofluorophotometer (Shimadzu). The observed pH was measured as $-\log[H^+]$ using an Istek 720P pH meter equipped with a glass electrode calibrated with standard buffer solutions. Compound **2**[1] and **4**[2] were prepared using the reported procedure. Pyrene-1-boronic acid was purchased from Aldrich.

---

[1] Y. Xiang, A. Tong, P. Jina and Y. Ju, *Org. Lett.* 2006, **8**, 2863.
[2] S. Kou, H. N. Lee, D. v. Noort, K. M. K. Swamy, S. H. Kim, J. H. Soh, K. Lee, S. Nam, J. Yoon and S. Park, *Angew. Chem. Int. Ed.* 2008, **47**, 872.



Compound 1. Rhodamine hydrazide[3] (0.46 g, 1 mmol) was dissolved in 20 mL absolute ethanol. An excessive 2-formylphenylboronic acid (4 mmol) was added then the mixture was refluxed in an air bath for 6 h. Ethanol was evaporated completely and purified the crude product on column (neutral allumina; MC + MeOH; 98 + 2) to get 0.280 g of **1** (47%) as pink solid: mp 195-197 ^{o}C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.13 (s, 1H), 8.11 (m, 1H), 7.94 (m, 1H), 7.85 (2, 2H, B-(O**H**)$_2$), 7.39 (m, 2H), 7.24 (m, 2H), 7.01 (m, 2H), 3.22 (q, 8H, J = 7.1 Hz), 1.06 (t, 12H, J = 7.1 Hz); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 165.2, 152.9,152.7, 149.2, 138.4, 138.2, 133.8, 133.3, 130.1, 129.6, 129.2, 128.4, 128.2, 127.6, 123.7, 123.6, 108.4, 104.5, 97.7, 65.1, 44.3, 12.6; FAB Mass m/z = 571.3 $(M\text{-}H_2O+H)^+$, calc. for $C_{35}H_{36}BN_4O_3$= 571.3.



Compound 2. 2,7-Dichlorofluoroscein (0.8 g, 2.00 mmol) and paraformaldehyde (0.47g, 16.00 mmol) were dissolved in 15 mL of CH_3CN and stirred. 2-Methylaminomethyllboronic acid **5**[4] (1.3 g, 8.00 mmol) in 20 mL of CH_3CN was added and followed by 15 mL of H_2O. The reaction mixture was refluxed for 24 h. The solvent was then removed *in vacuo* and purified the residue by column chromatography using acetone (100%). Eluted product was again purified by column chromatography using ethyl acetate-methanol (9:1, v/v) to yield 24 % of the pure product: mp 124-126 ^{o}C; ^{1}H NMR (250 MHz, CD_3OD) δ 8.16 (m, 1H), 7.91 (s, 1H), 7.65-7.70 (m, 2H), 7.39-7.49 (m, 8H), 7.22 (m, 2H), 4.42 (m, 4H), 4.34 (s, 4H), 2.73 (s, 6H); ^{13}C NMR (62.5 MHz, CD_3OD) δ174.3, 172.9, 157.7, 155.8, 140.2, 134.6, 131.9, 131.8, 131.2, 131.1, 131.0,

[3] V. Dujols, F. Ford and Czarnik, A. W. *J. Am. Chem. Soc.* 1997, **119**, 7386.
[4] K. Secor and T. E. Glass, *Org. Lett.* 2004, **6**, 3727.

130.9, 130.5, 130.3, 128.2, 112.1, 108.7, 105.9, 79.5, 60.8, 52.4, 50.0, 49.7, 49.3, 49.0, 48.7, 48.3, 48.0, 40.4; FAB Mass m/z = 755.19 $(M+H)^+$, calc. for $C_{38}H_{35}B_2Cl_2N_2O_9$= 754.19.

Preparation of fluorometric metal ion titration solutions. Stock solutions (1 mM) of the metal perchlorate salts were prepared using doubly distilled demineralized water. Stock solutions of **1** and **2** were also prepared in acetonitrile. The solutions were used on the day of preparation. Test solutions were prepared by placing 40 μL of the probe stock solution into a test tube, adding an appropriate aliquot of each metal stock (0- μL), and diluting the solution to 4 mL with pH 7.4 20 mM HEPES buffer. For all measurements, excitation was at 556 or 504 nm. Both excitation and emission slit widths were either 1.5 or 3 nm.

Imaging of mammalian cells incubated with copper ions and 1: Murine P19 cells were seeded in a 6-well plate at a density of 10^4 cells per well in culture media (Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS)). After 24 h, P19 cells were incubated with 0.5 - 1mM $Cu(ClO_4)_2$ in culture media for 30 min. After washing with PBS to remove the remaining copper ions, the cells were incubated to 50 μM **1** for 30 min at 37 ^oC.
Murine 3T3-L1 fibroblasts were seeded in a 6-well plate at a density of 10^4 cells per well in culture media. After 24 h, 3T3-L1 cells were incubated with 0.5 - 1mM $Cu(ClO_4)_2$ in culture media for 30 min. After washing with PBS to remove the remaining copper ions, the cells were incubated to 50 μM **1** for 30 min at 37 ^oC.
Murine C2C12 cells were seeded in a 6-well plate at a density of 10^4 cells per well in culture media. After 24 h, C2C12 cells were incubated with 0.5 - 1mM $Cu(ClO_4)_2$ in culture media for 30 min. After washing with PBS to remove the remaining copper ions, the cells were incubated to 50 μM **1** for 30 min at 37 ^oC.
Human HCT-15 colon cancer cells were seeded in a 6-well plate at a density of 10^4 cells per well in culture media. After 24 h, the cells were incubated with 0.5 - 1mM $Cu(ClO_4)_2$ in culture media for 30 min. After washing with PBS to remove the remaining copper ions, the cells were incubated to 50 μM **1** for 30 min at 37 ^oC. The cells incubated with both copper ions and **1** were imaged by fluorescence microscopy (Stemi 2000-C, ZAISS, Germany).

Imaging of human muscle cells incubated with copper ions and 1. The human muscle biopsy was generously provided by Dr. Hyun Woo Kim (Yonsei University

College of Medicine). Human muscle fibers were isolated and cultured by the procedure developed by Bonavaud, S. et al. (*In Vitro Cell Dev. Biol. Anim.* **2002**, *38*, 66-72). The muscle sample was digested in a Petri dish containing 0.2% collagenase (Sigma) in Ham's F10 media (Gibco) at 37 °C for 90 min and then washed with PBS several time. Most of the human muscle fibers were released from the tissue after enzymatic digestion. The single muscle fibers were isolated by repeatedly triturating the muscle fragments with a very wide-mouthed Pasteur pipette. Isolated muscle fibers were finally plated in one drop of medium Ham's F10 media into matrigel (Collaborative Biomedical Products, 1 mg/mL) coated 6-well plates (BioCoat), and were allowed to attach for at least 4 h before adding plating media (10% FBS, 1% chick embryo extract (CEE, Sera Laboratories International) in Ham's-F10 media). After 4−5 days, the muscle precursor cells began to migrate from the fiber. The cells were counted by Trypsinisation and cultured in DMEM supplemented with 10% FBS, 1% CEE, 50 unit/mL of penicillin and 50 μg/mL of streptomycin.

Human muscle cells were seeded in a 6 well plate at a density of 10^3 cells per well in culture media (DMEM supplemented with 10% FBS, 50 unit/mL of penicillin and 50 μg/mL of streptomycin) and incubated with 0.5 - 1mM $Cu(ClO_4)_2$ in culture media for 30 min. After washing with PBS to remove the remaining copper ions, the cells were incubated with 50 μM **1** in culture media for 30 min at 37 ºC. The cells incubated with both copper ions and **1** were imaged by fluorescence microscopy.

Imaging of zebrafish incubated with copper ions and 1. Zebrafish was kept at 28 ^oC and maintained at optimal breeding conditions. For mating, male and female zebrafish was maintained in one tank at 28 □ on a 12 h light/12 h dark cycle and then the spawning of eggs were triggered by giving light stimulation in the morning. Almost all the eggs were fertilized immediately. The 5-day old zebrafish was maintained in E3 embryo media (15 mM NaCl, 0.5 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 0.15 mM KH_2PO_4, 0.05 mM Na_2HPO_4, 0.7 mM $NaHCO_3$, 10^{-5}% methylene blue; pH 7.5). The 5-day old zebrafish was incubated with 0.5 - 1mM $Cu(ClO_4)_2$ in E3 media for 30 min at 28 ºC. After washing with PBS to remove the remaining copper ions, the zebrafish was further incubated with 50 μM **1** for 30 min at 28 ºC. Zebrafish was imaged by fluorescence microscopy.

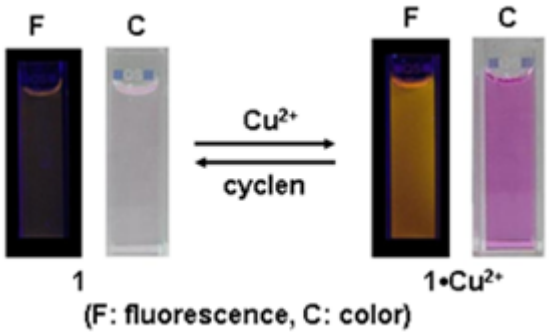


Figure-S1. Fluorescent (left) and colorimetric changes (right) of **1** (10 μM) upon addition of Cu^{2+} (1 mM) in 20 mM HEPES (0.5% CH_3CN) at pH 7.4.

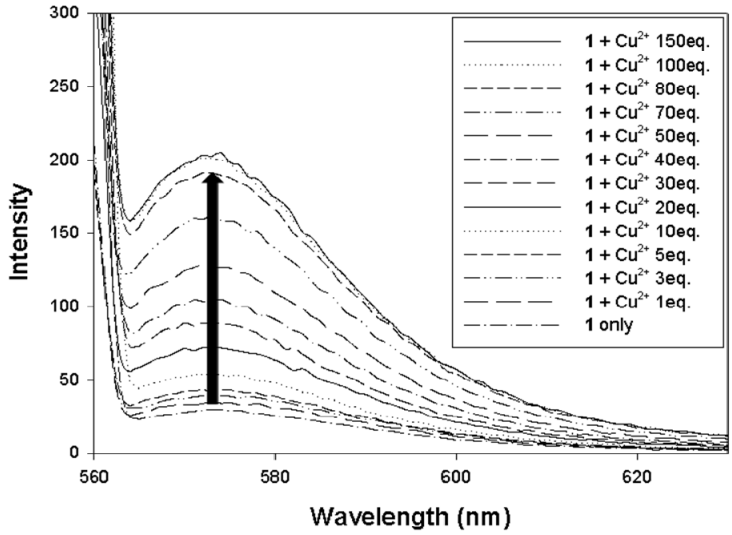


Figure-S2. Fluorescent titrations of chemosensor **1** (3 μM) with various concentrations of Cu^{2+} at pH 7.4 in 20 mM HEPES (0.5% CH_3CN) at pH 7.4.

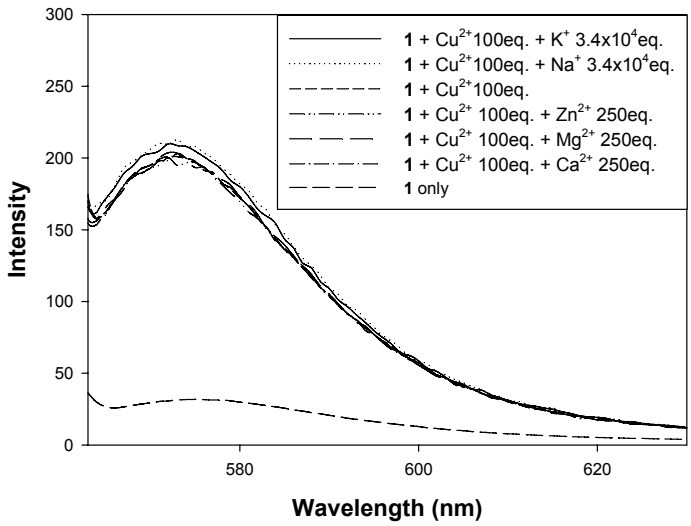


Figure-S3. Fluorescent changes of chemosensor **1** (3 μM) with Cu^{2+} (100 eq.) in the presence of excess metal ions (250eq. and possible physiological concentration 100mM) at pH 7.4 in 20 mM HEPES (0.5% CH_3CN) at pH 7.4.

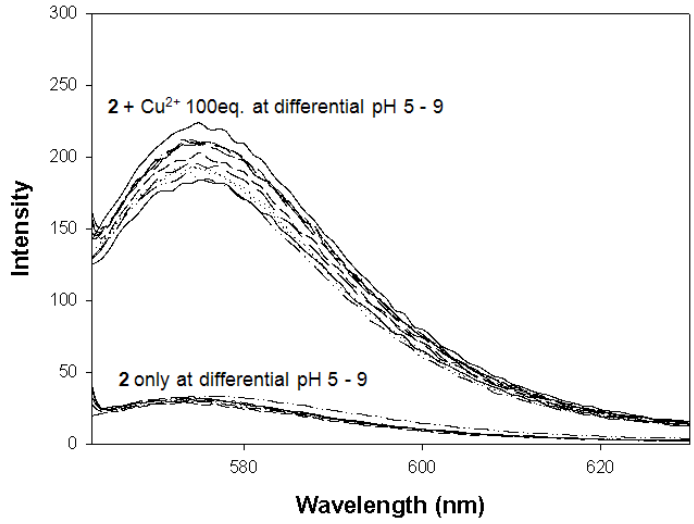


Figure-S4. Fluorescent changes of chemosensor **1** (3 μM) with Cu^{2+} (100 eq.) at differential pH in 20 mM HEPES (0.5% CH_3CN).

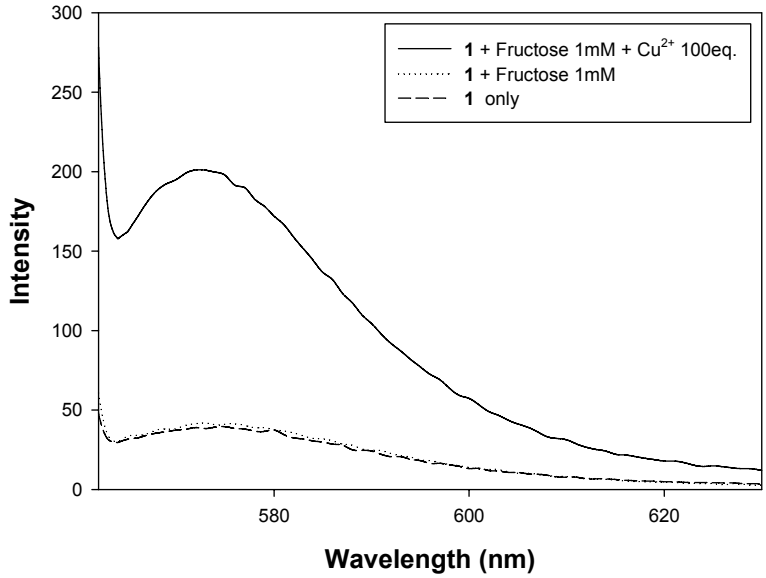


Figure-S5. Fluorescent changes of chemosensor **1** (3 μM) with Cu^{2+} (100 eq.) in presence of fructose 1mM in 20 mM HEPES (0.5% CH_3CN).

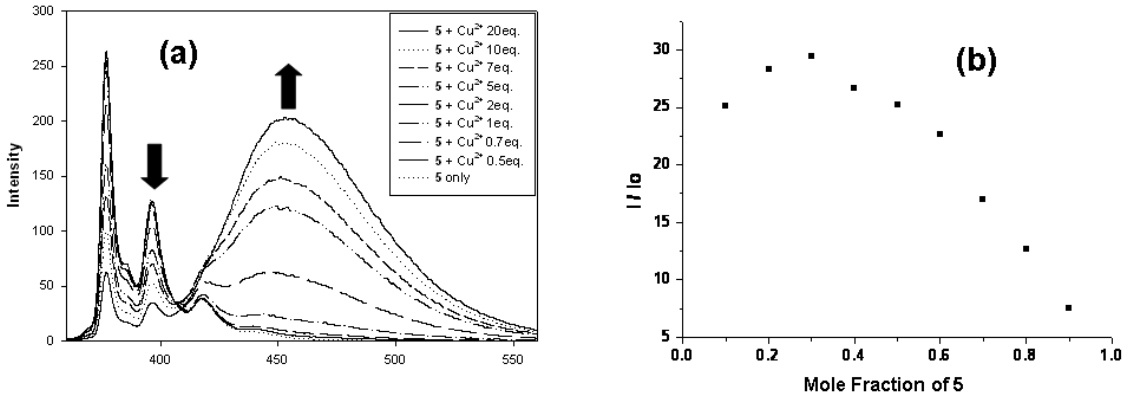


Figure-S6. (a) Fluorescent changes of **5** upon addition of Cu^{2+} in CH_3CN (excitation at 342 nm). (b) A Job's plot of **5** and Cu^{2+} in CH_3CN (excitation at 342 nm and emission at 416 nm).

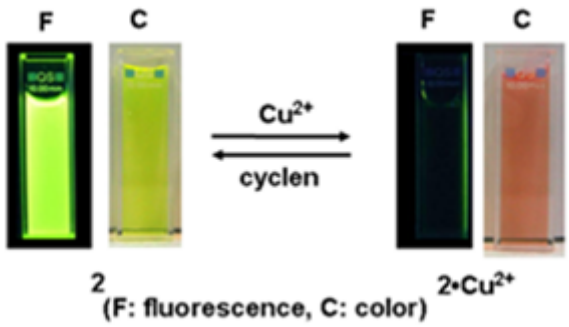


Figure-S7. Fluorescent (left) and colorimetric (right) changes of **2** (10 μM) upon addition of Cu^{2+} (1 mM) in 20 mM HEPES (0.5% CH_3CN) at pH 7.4

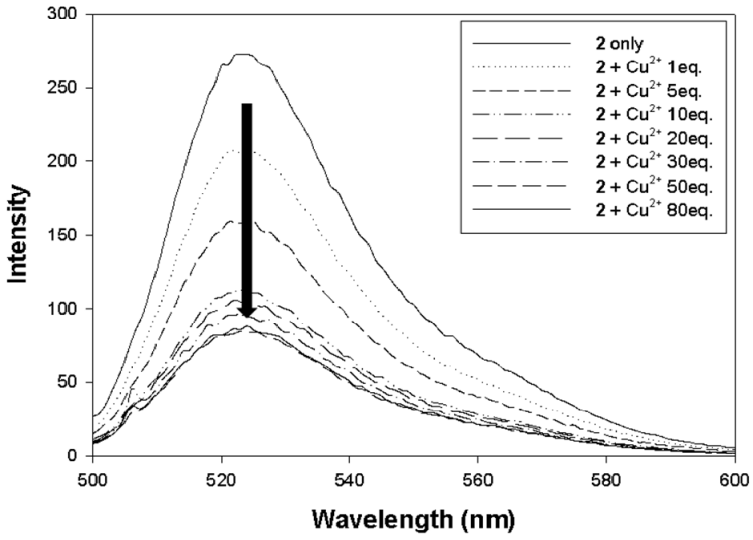


Figure-S8. Fluorescence spectra of **2** (1 μM) upon addition of various concentrations of Cu^{2+} at pH 7.4 in 20 mM HEPES (0.5% CH_3CN) at pH 7.4. (excitation at 504 nm).

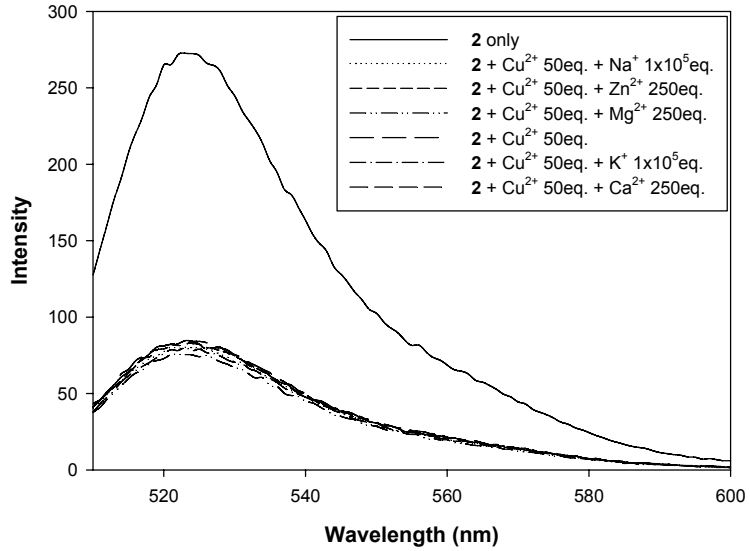


Figure-S9. Fluorescent changes of chemosensor **2** (1 μM) with Cu^{2+} (50 eq.) in the presence of excess metal ions (250eq. and possible physiological concentration 100mM) at pH 7.4 in 20 mM HEPES (0.5% CH_3CN) at pH 7.4.

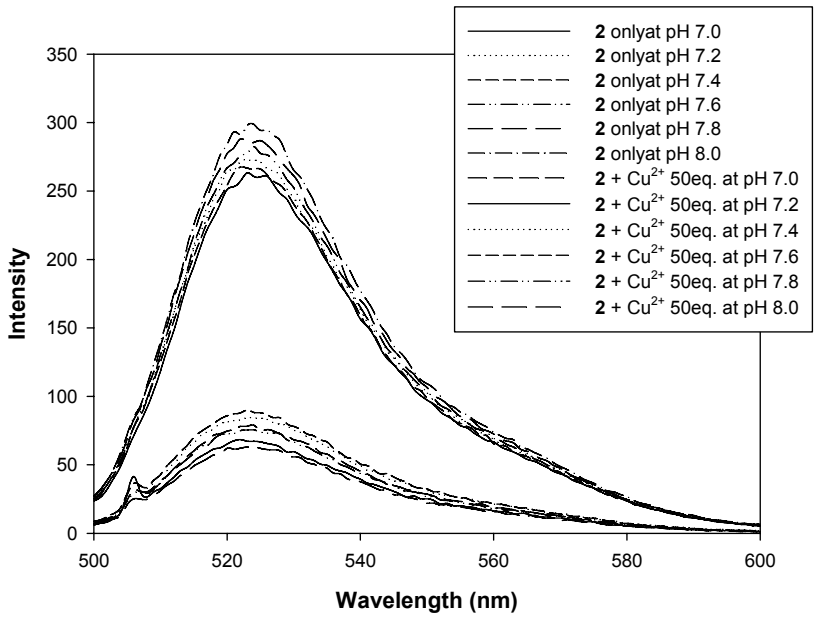


Figure-S10. Fluorescent changes of chemosensor **2** (1 μM) with Cu^{2+} (50 eq.) at differential pH in 20 mM HEPES (0.5% CH_3CN).

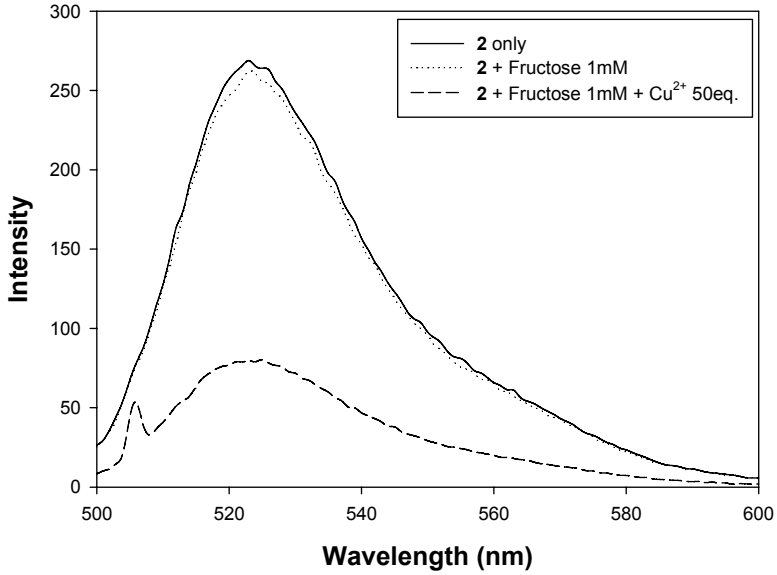


Figure-S11. Fluorescent changes of chemosensor **2** (1 μM) with Cu^{2+} (50 eq.) in presence of fructose (1 mM) in 20 mM HEPES (0.5% CH_3CN).

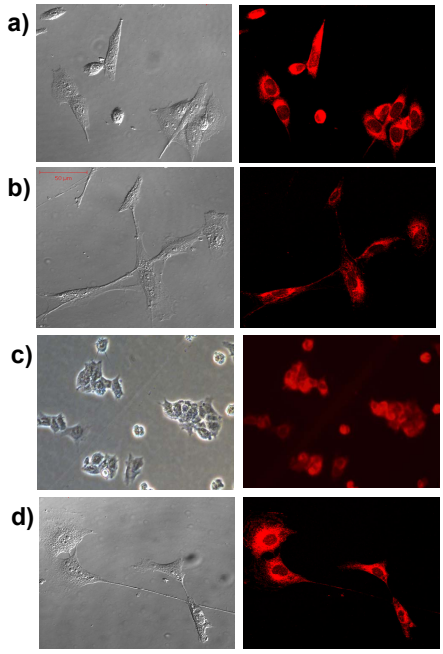


Figure-S12. Cells incubated with **1** and Cu^{2+}. Cells were treated with Cu^{2+} (0.5 - 1 mM) for 30 min, washed with PBS to remove the remaining copper ions, and incubated with **1** (50 μM) for 30 min. Images of a) C2C12 myoblasts, b) human muscle cells, c) colon cancer cells (HCT-15) and d) 3T3-L1 fibroblasts treated with both **1** and copper ions.

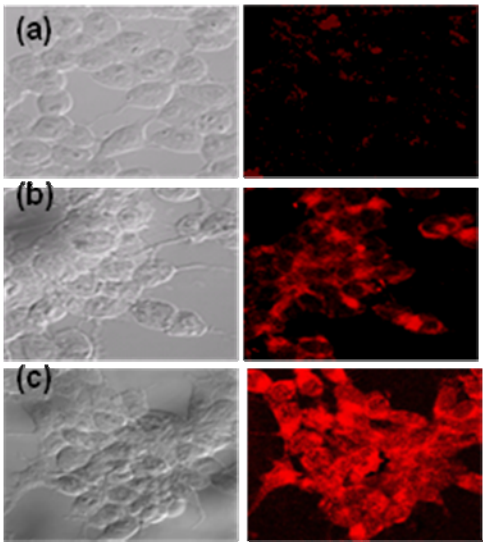


Figure-S13. Images of P19 cells incubated with 50 μM **1** (0.5% CH_3CN) and (a) 0, (b) 200 μM and (c) 300 μM $Cu(ClO_4)_2$

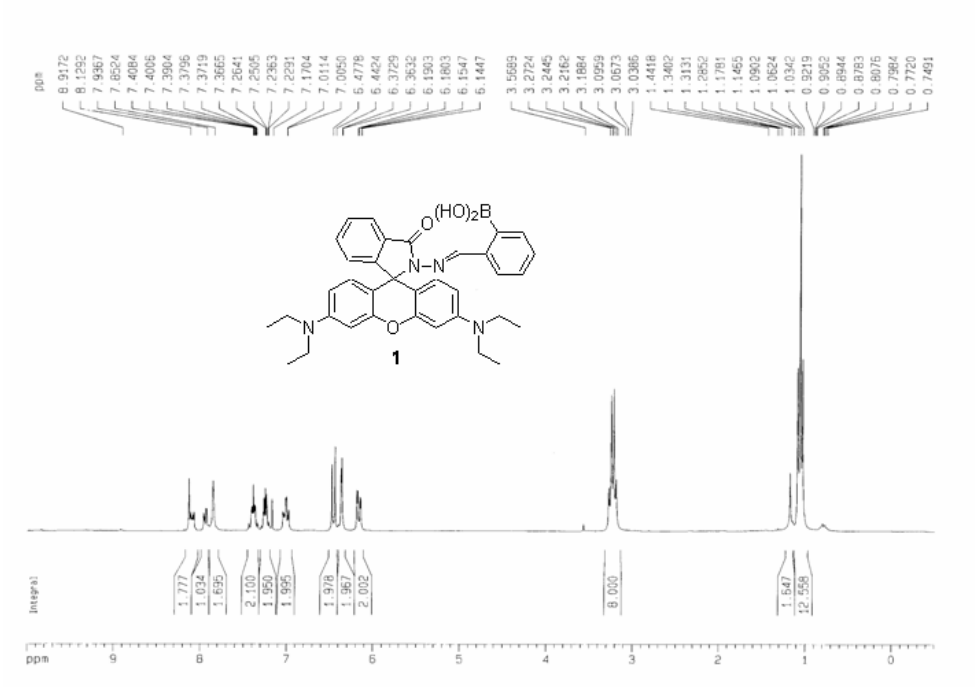


Figure-S14. ^{1}H NMR (250 MHz) of compound **1** in $CDCl_3$.

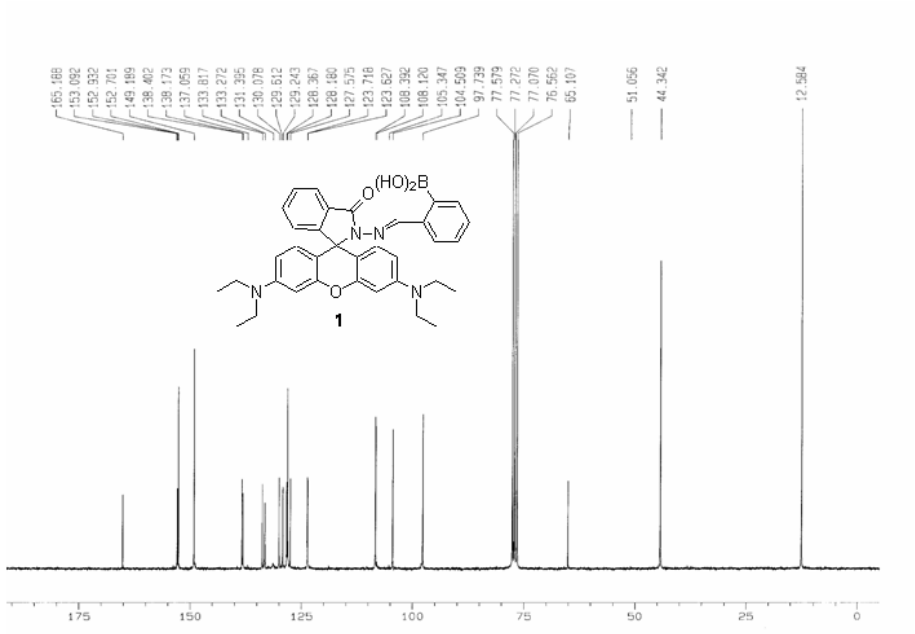


Figure-S15. ^{13}C NMR (62.5 MHz) of compound **1** in $CDCl_3$.

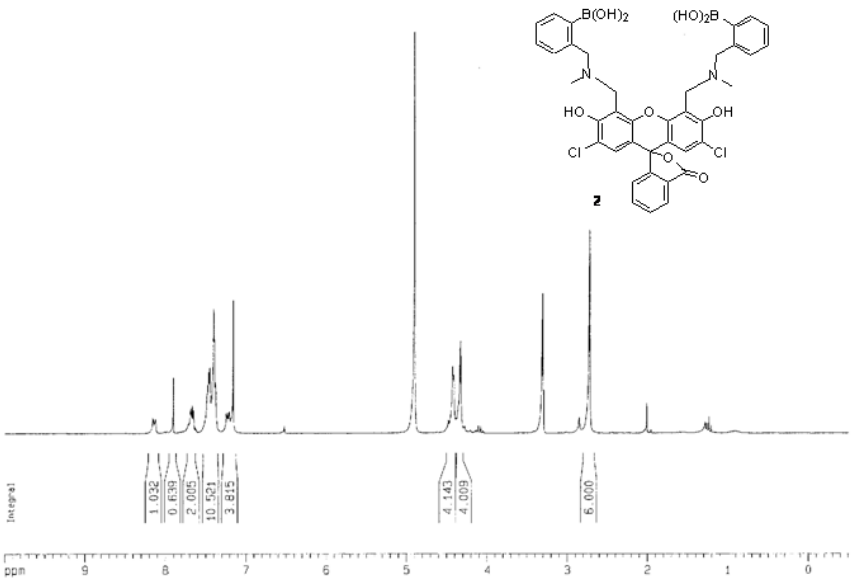


Figure-S16. ^{1}H NMR (250 MHz) of compound **2** in CD_3OD.

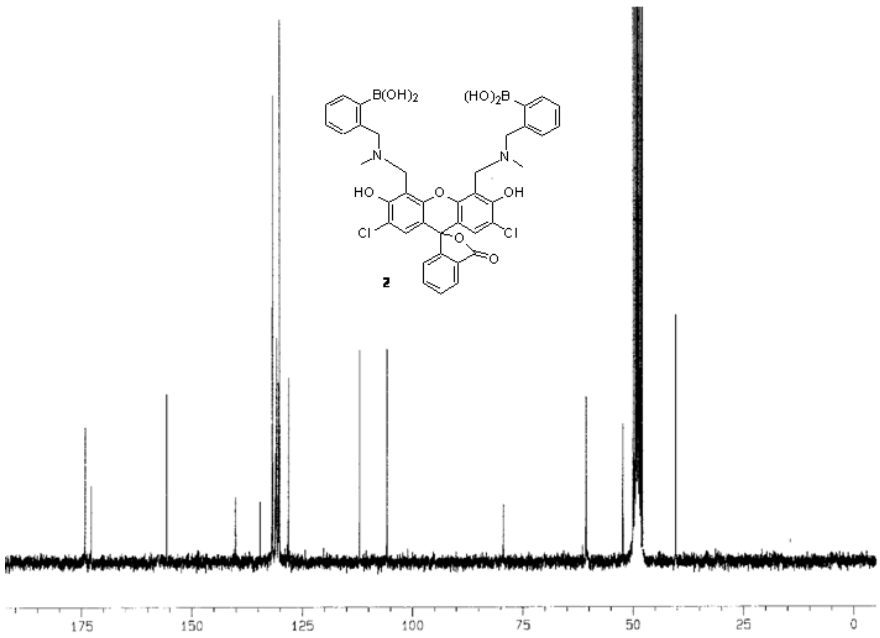


Figure-S17. ^{13}C NMR (62.5 MHz) of compound **2** in CD_3OD.