Electronic Supplementary Information (ESI)

Fluorescence imaging for Fe^{3+} in *Arabidopsis* by using simple naphthalene-based ligands

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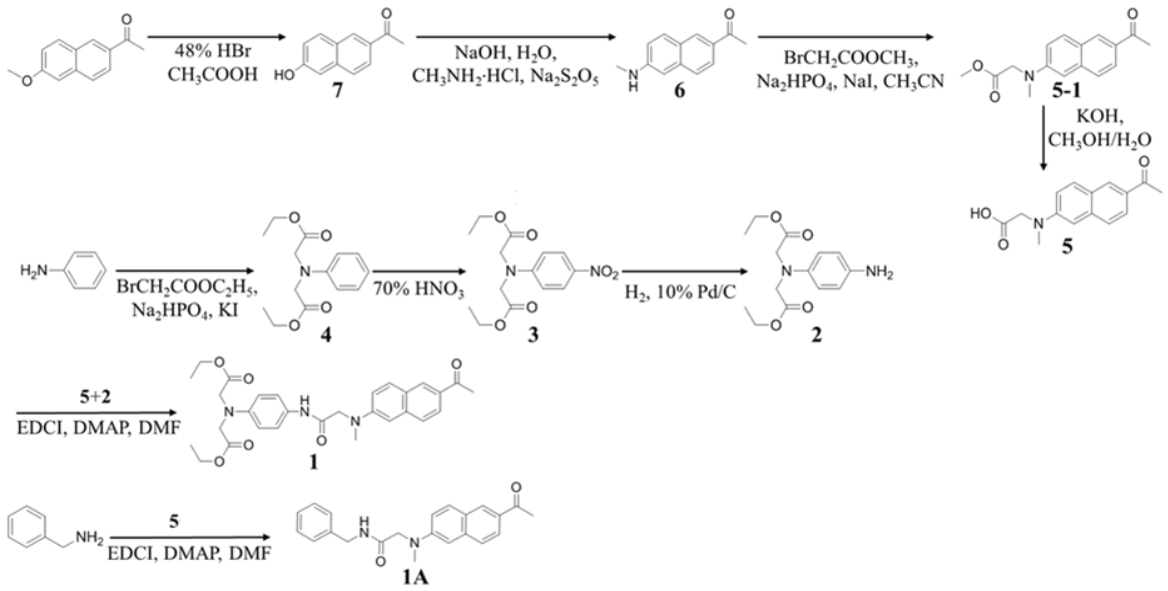


Scheme S1. Synthesis for ligands of **1** and **1A** with fluorophore of naphthalene.

Compound 4. Ethyl bromoacetate (5.56 ml, 50 mmol) was added slowly to the mixture of aniline (0.91 ml, 10 mmol), sodium phosphate dibasic (2.84 g, 20 mmol) and potassium iodide (1.66 g, 10 mmol) in anhydrous acetonitrile (10 ml). Next, the reaction mixture was refluxed for 12 hours 80℃ under nitrogen atmosphere. In a process of work-up, the organic layer was extracted with water and chloroform. Sodium sulfate was used and the organic solvents were evaporated under reduced pressure. The product was purified by column chromatography at condition of hexane : ethyl acetate = 10:2. A brown-colored viscous liquid was obtained (0.60 g, 22 %). R_f = 0.35 (hexane : ethyl acetate = 10 : 2) ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.24 (m, 2H), 6.80 (t, *J* = 7.32, 7.32 Hz, 1H), 6.64 (d, 2H), 4.24 (q, 4H), 4.16 (s, 4H), 1.30 (t, 6H).

Compound 3. The compound **4** (2.90 g, 10 mmol) was taken into 30 ml of acetic acid in one neck flask. It was stirred in a bath containing acetone, water and ice under nitrogen atmosphere at 0 ℃. Next, 3 ml of 60 % nitrate acid was dropped slowly and it proceeded to react about 15 minutes. It was poured in the container with ice water. After filtered, resulting solid was recrystallized from ethanol, and the organic solvent was evaporated clearly under reduced

pressure. A greenish product was obtained (1.43 g, 42 %). R_f = 0.51 (hexane : ethyl acetate = 2 : 1). Mp = 160-162 ◦C MS (ES+) *m*/*z* = 310 (M+). Anal. calc. for $C_{14}H_{18}N_2O_6$: C, 54.19; H, 5.85; N, 9.03. Found: C, 54.12; H, 5.85; N, 8.98%. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H (ppm): 8.15 (d, 2H), 6.61 (d, 2H), 4.26(q, 4H), 4.22 (s, 4H), 1.31 (t, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm): 13.74, 53.00, 61.29, 110.82, 125.55, 138.52, 152.23, 168.86. FT-IR (KBr, cm^{-1}): 3474, 3121, 3098, 2976, 2908, 2696, 2614, 2426, 2231, 1918, 1893, 1751, 1591, 1516, 1420, 1272, 1117, 1026, 961, 918, 871, 828, 757, 736, 696, 632, 586, 535, 559.72.

Compound 2. The compound **3** (0.1 g, 0.32 mmol) and palldium-10 % carbon (0.007 g) were dried in a vacuum for 5 hours. After anhydrous methanol (5 ml) was injected into the two neck flask. The solution was hydrogenated for 3 hours with balloons of three layer which were filled with hydrogen gas at 1 atm. A speed to be stirred was controlled between fast and slow to get reaction proceed well. After the reaction, the catalyst was filtered off using celite and the solvent was removed by evaporating under reduced pressure. A brown colored liquid was obtained (0.09 g, 90 %). R_f=0.41 (dichloromethane : ethyl acetate = 7 : 3). MS (ES+) *m*/*z* = 281 (MH)+. Anal. calc. for $C_{14}H_{20}N_2O_4$: C, 59.99; H, 7.19; N, 9.99. Found: C, 59.25; H, 7.03; N, 9.84%. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H (ppm): 6.68 (d, J = 8.60 Hz, 1H), 6.55 (d, 2H), 4.20 (q, 4H), 4.10 (s, 4H), 1.26 (t, J = 7.14, 7.14 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C (ppm): 170.88, 140.94, 138.02, 116.26, 114.54, 60.49, 53.68, 13.79. IR (NaCl, cm^{-1}): 3330, 2981, 2930, 2355, 1733, 1616, 1519, 1448, 1412, 1347, 1255, 1188, 1097, 1025, 974, 918, 817, 729, 521.

Compound 7. 2-acetyl-6-methoxynaphtalene (0.4 g, 2 mmol) in glacial acid (25 ml), 48% hydrobromic acid (6 ml, 0.24 mol) was added. The mixture was stirred for 20 hours at 90 °C. After the reaction, excess acid was removed using diluted sodium bicarbonate and brine and the residue taken up in ethyl acetate. Magnesium sulfate was used and the organic solvent was evaporated under reduced pressure. The product was purified by column chromatography at condition of hexane : ethyl acetate = 7 : 3. A white colored product was obtained (0.08 g, 19 %). R_f=0.36 (hexane : ethyl acetate = 7 : 3). Mp. = 173 °C. IR (KBr, cm^{-1}): 3362, 1664. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H (ppm): 8.43 (d, *J* = 1.15 Hz, 1H), 8.02 (dd, 1H), 7.90 (d, *J* = 8.70 Hz, 1H), 7.74 (d, 1H), 7.20 (d, 1H), 7.18 (dd, 1H), 5.55 (broad s, 1H), 2.73 (s, 3H). Anal. Calcd for $C_{12}H_{10}O_2$: C, 77.40; H, 5.41. Found: C, 77.52; H, 5.46.

Compound 6. Methylamine hydrochloride (1.1 g, 16 mmol), sodium metabisulfite (0.97 g, 11 mmol), sodium hydroxide (0.65 g, 16 mmol) and the compound **7** (0.50 g, 2.7 mmol) in water (20 ml) was put in a steel bomb reactor. The mixture was stirred by high temperature at 140 ℃ and pressure for 48 hours. After the organic layer was extracted from the mixture with water and ethyl acetate. The product was purified by column chromatography at condition of chloroform : ethyl acetate = 70 : 3. A yellowish brown-colored product was obtained (0.29 g, 54 %). R_f=0.60 (chloroform : ethyl acetate = 70 : 3). Mp. = 181 °C. IR (KBr, cm^{-1}): 3347, 1663. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.31 (d, 1H), 7.96 (dd, 1H), 7.75 (d, 1H), 7.67 (d, 1H), 6.98 (dd, 1H), 6.87 (d, 1H), 4.17 (broad s, 1H), 3.02 (s, 3H), 2.70 (s, 3H). Anal. Calcd. for $C_{13}H_{13}NO$: C, 78.36; H, 6.58 N, 7.03. Found: C, 78.32; H, 6.56; N, 7.08.

Compound 5-1. Mthyl bromoacetate (1 ml, 11 mmol) was added slowly to the mixture of sodium iodide (0.78 g, 5 mmol), sodium phosphate dibasic (1.03 g, 7.3 mmol) and the compound **6** (0.2 g, 1 mmol) in anhydrous acetonitrile (3 ml). Next, the reaction mixture was refluxed overnight at 80 ℃ under nitrogen atmosphere. In a process of work-up, the organic layer was extracted from the filtrate with brine and ethyl acetate. Sodium sulfate was used. The organic solvents were evaporated under reduced pressure. The product was purified by column chromatography using condition of dichloromethane: ethyl acetate = 100 : 2 by gradient elution. A yellow-colored product was obtained (0.14 g, 52 %). R_f = 0.33 (dichloromethane : ethyl acetate = 100 : 2). Mp = 92 ℃. IR (KBr, cm^{-1}): 1754, 1671. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H (ppm): 8.33 (d, J = 1.56 Hz, 1H), 7.94 (dd, 1H), 7.82 (d, 1H), 7.66 (d, 1H), 7.11 (dd, J = 9.09, 2.64 Hz, 1H), 6.90 (d, 1H), 4.23 (s, 2H), 3.75 (s, 3H), 3.22 (s, 3H), 2.68 (s, 3H). Anal. Calcd for $C_{16}H_{17}NO_3$: C, 70.83; H, 6.32; N, 5.16; Found: C, 70.82; H, 6.30; N, 5.17.

Compound 5. The compound **5-1** (0.10 g, 0.37 mmol) in methanol (3 ml) and potassium hydroxide (0.07 g, 1.4 mmol) in water (0.5 ml) were put into flask. Next, it was refluxed for 6 hours at 60 ℃. The resultant solution was poured in a beaker with ice-water and 35 % hydrochloride acid was dropped slowly below 5 ℃ until pH = 3. The resulting precipitate was collected through a sintered glass filter, washed with water and further purified by recrystallization from methanol. A brown colored product was obtained (0.09 g, 93 %). ^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ_H (ppm): 8.45 (d, J = 1.38 Hz, 1H), 7.89 (d, 1H), 7.80 (dd, 1H), 7.68 (d, 1H), 7.22 (dd, 1H), 6.95 (d, J = 2.38 Hz, 1H), 4.27 (s, 2H), 3.11 (s, 3H), 2.62 (s, 3H).

Compound 1. The compound **5** (0.26 g, 1.0 mmol), the compound **2** (0.23 g, 0.82 mmol), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (0.16 g, 1.0 mmol) and 4-dimethylamino pyridine (0.016 g, 0.13 mmol) was added in anhydrous dimethylformamide (4 ml). It was stirred for 19 hours at room temperature under nitrogen atmosphere. The organic layer was extracted with water and ethyl acetate from the reaction mixture. The product was purified by column chromatography at condition of dichloromethane : ethyl acetate = 8 : 2. A greenish-gray colored product was obtained (0.24 g, 52 %). R_f = 0.41 (dichloromethane : ethyl acetate = 8 : 2). ^{1}H NMR (300 MHz, $CDCl_3$) δ_H (ppm): 8.36 (d, 1H), 8.10 (s, 1H), 7.88 (d, 1H), 7.71 (d, J = 8.74 Hz, 1H), 7.34 (d, 1H), 7.16 (dd, 1H), 6.56 (d, 1H), 4.19 (q, 4H), 4.11 (s, 2H), 4.10 (s, 4H), 3.24 (s, 3H), 2.70 (s, 3H), 1.26 (t, 6H). ^{13}C NMR (500 MHz, CD_3OD) δ_C (ppm): 199.3, 173.2, 149.8, 138.2, 130.9, 130.9, 130.8, 126.4, 125.7, 124.1, 116.0, 105.5, 53.5, 38.7, 25.4. Anal. Calcd. for $C_{15}H_{15}NO_3$: C, 70.02; H, 5.88; N, 5.44. Found: C, 70.08; H, 5.79; N, 5.45.

Compound 1A. The compound 5 (0.10 g, 0.40 mmol), a benzylamine hydrochloride (0.08g, 0.58 mmol), sodium bicarbonate (0.047g, 0.58 mmol), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (0.093 g, 0.48 mmol) and dimethylaminopyridine (0.01 g, 0.08 mmol) was added in anhydrous dimethylformamide (4 ml). It was stirred for 30 hours at room temperature under nitrogen atmosphere. In a process of work-up, the organic layer was extracted with water and ethyl acetate from the reaction mixture and evaporated under reduced pressure and further purified by recrystallization from methanol. Next, a white colored product (0.08 g, 47 %) was obtained by column chromatography at condition of chloroform : ethylacetate = 1 : 1. R_f = 0.60 (chloroform : ethyl acetate = 1 : 1). ^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ_H(ppm): 2.62 (s, 3H), 3.17 (s, 3H), 4.17 (s, 2H), 4.30 (d, *J* = 5.98 Hz, 2H), 6.94 (d, *J* = 2.34 Hz, 1H), 7.31-7.16 (m, 6H), 7.66 (d, *J* = 8.77 Hz, 1H), 7.83 (dd, *J* = 8.68, 1.76 Hz, 1H),

7.93 (d, J = 9.15 Hz, 1H), 8.46 (s, 1H), 8.57 (t, J = 5.99, 5.99 Hz, 1H). ^{13}C NMR (500 MHz, $CDCl_3$) δ_C (ppm): 197.7, 169.7, 148.8, 137.8, 137.2, 131.9, 131.2, 130.1, 128.7, 127.6, 127.5, 127.4, 126.6, 126.2, 124.9, 116.2, 107.0, 58.4, 43.2, 40.1, 26.5. Anal. Calcd. for $C_{22}H_{22}N_2O_2$: C, 76.28; H, 6.40; N, 8.09; O, 9.24. IR (KBr, cm^{-1}): 3212, 2923, 1672, 1650, 1620, 1528, 1493, 1328, 1384, 1200, 1177, 1115, 1071, 1024, 940, 954, 904, 847, 802, 750, 704, 668, 471. MS (ES+) m/z = 714$(M + M + 2Na)^+$, 347$(M+H)^+$.

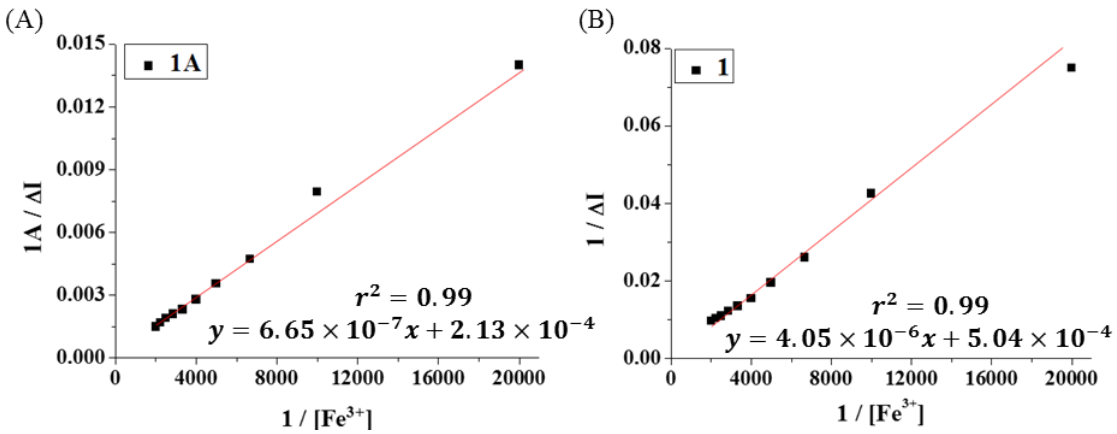
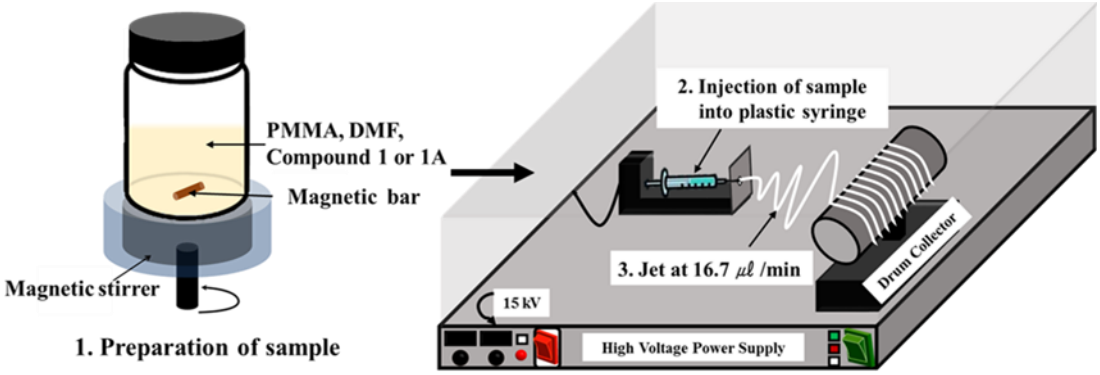


Fig. S1 Benesi-Hildebrand plots of (A) **1**-Fe^{3+} complex and (B) **1A**-Fe^{3+} complex. An emission wavelength was selected 495 nm about **1** and **1A**, respectively, for plots. Excitation wavelength of both **1** and **1A** was 356 nm.



Scheme 2. Illustration for the preparation of **1** or **1A**-embedded electrospun nanofibrous films. A composition of samples for NF-1 or NF-1A; **1** or **1A** was prepared for sample on conditioning of DMF 4 ml, PMMA 0.5 g (molecular weight = 350,000 $gmol^{-1}$), **1** or **1A** 0.01 g, respectively, solution flow rate 1 ml/hour, voltage 15 kV, drum speed 215 rpm, working distance 15 cm, and metal needle size 25 gage.

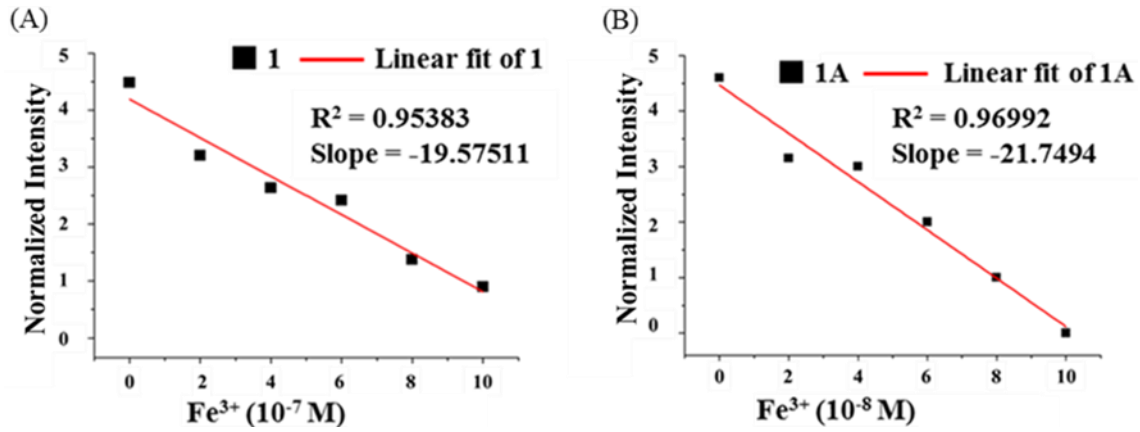


Fig. S2 Calibration plot for limit of detection of (A) **1**-Fe^{3+} and (B) **1A**-Fe^{3+} into water-methanol (10:1, v/v, pH 7). The monitored maximum photoluminescence emission wavelength was 430 nm. Each intensity according to concentration of Fe^{3+} was mean value in the measurement of 10 times. The detection of limit was calculated using following equation (a).

Detection of limit = $3\times \sigma/s$ (a)

σ is a standard deviation of the blank measurements.

s is a slope of the calibration plot. The slope was used as an absolute value.

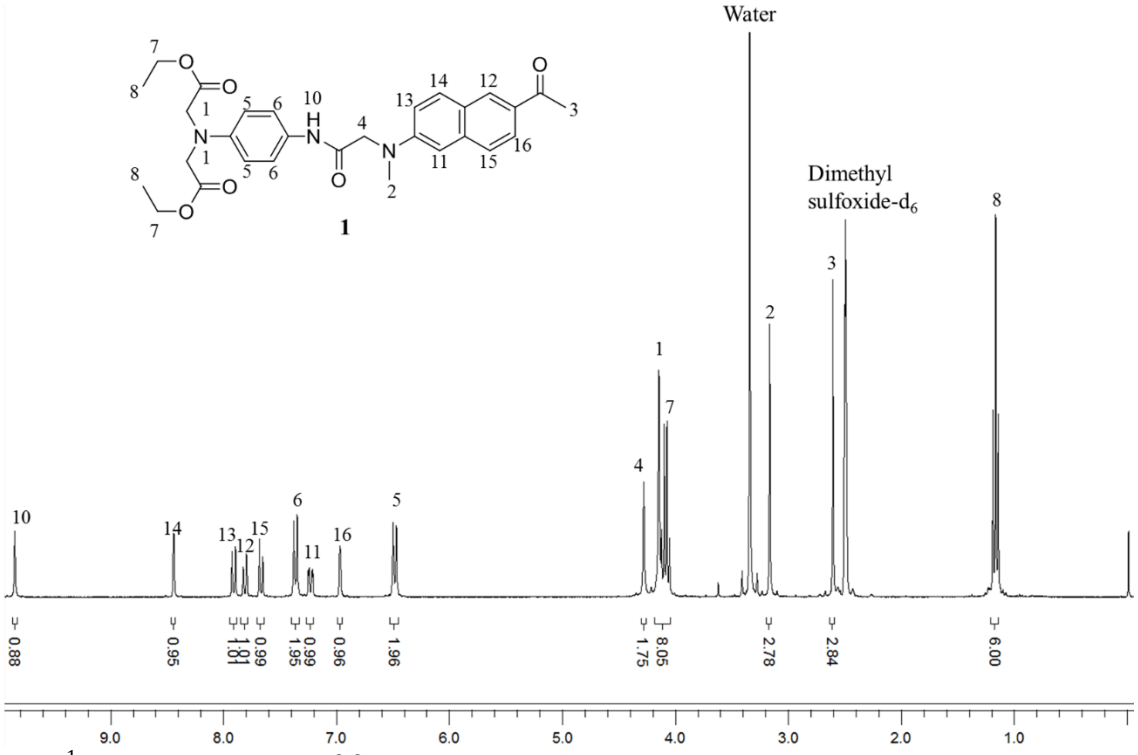


Fig. S3 ^{1}H NMR spectrum of fluorescence probe 1 (300 MHz).

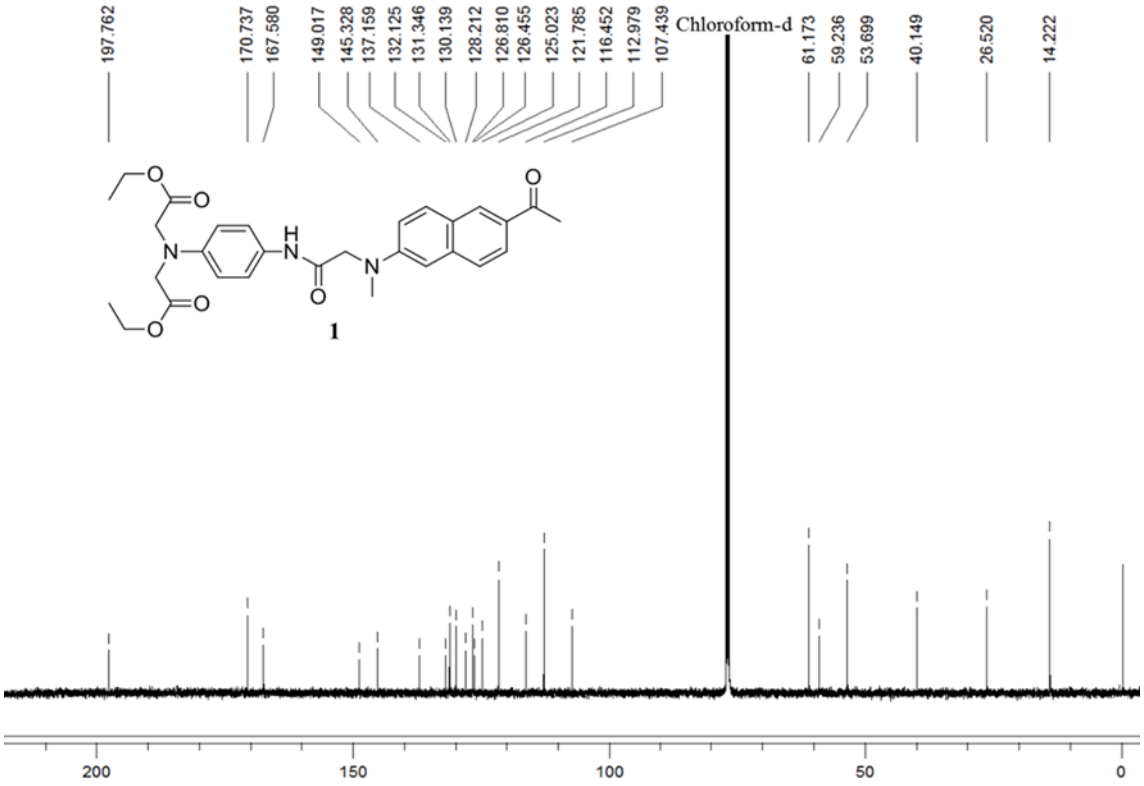


Fig. S4 ^{13}C NMR spectrum of fluorescence probe **1** (500 MHz).

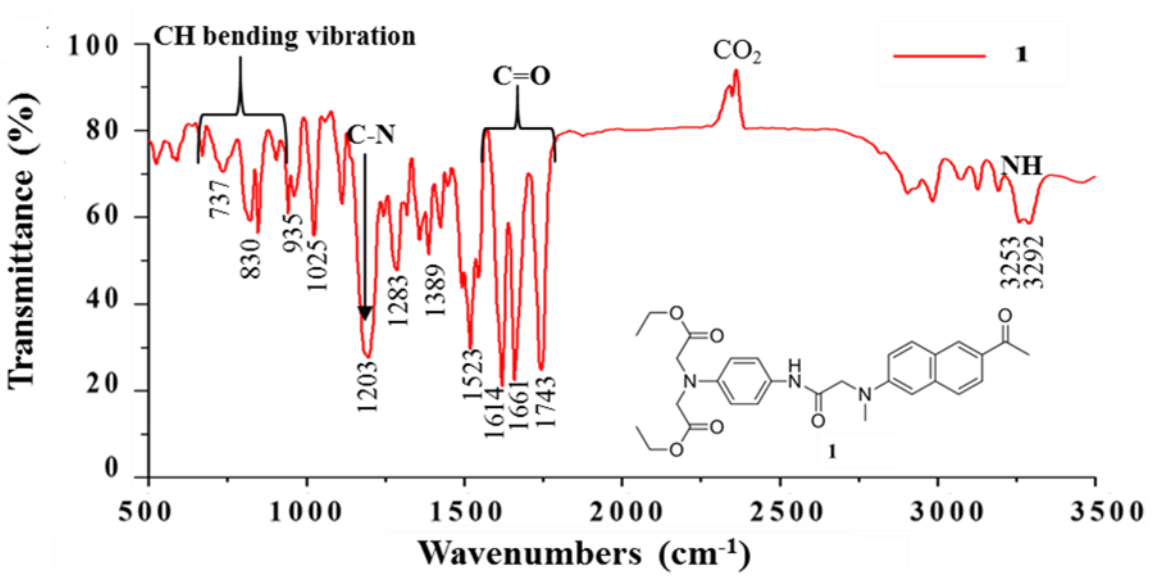


Fig. S5 IR Spectrum of fluorescence probe **1**.

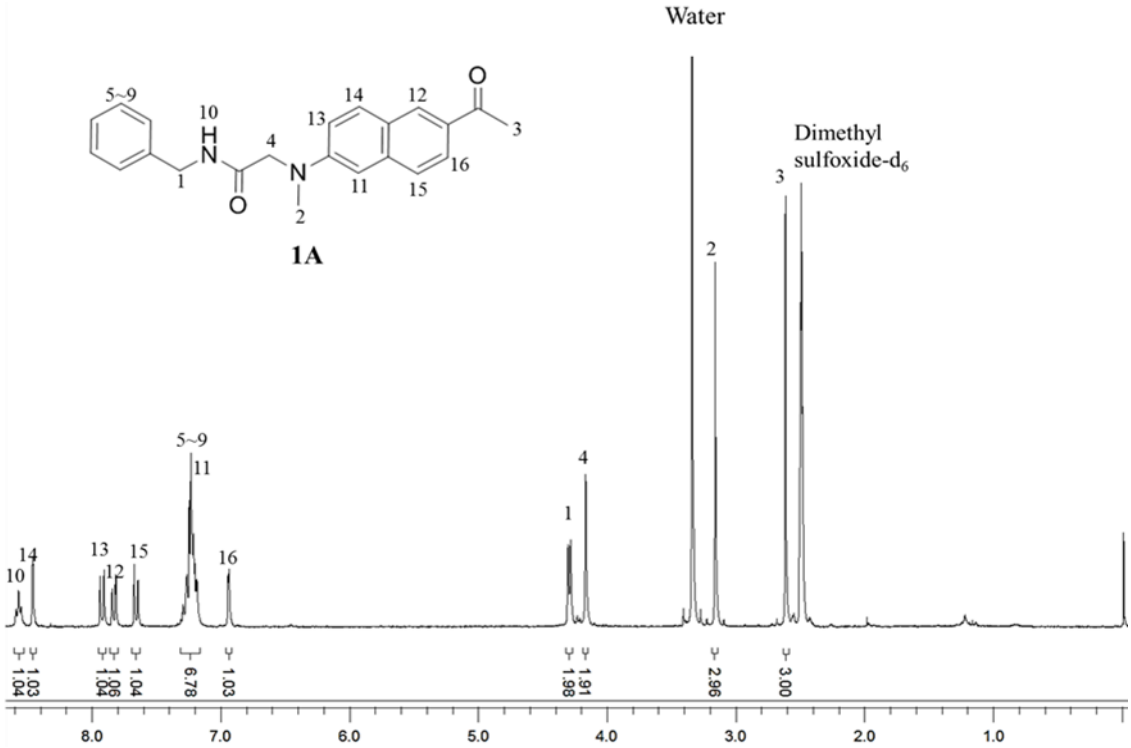


Fig. S6 ^{1}H NMR spectrum of fluorescence probe **1A** (300 MHz).

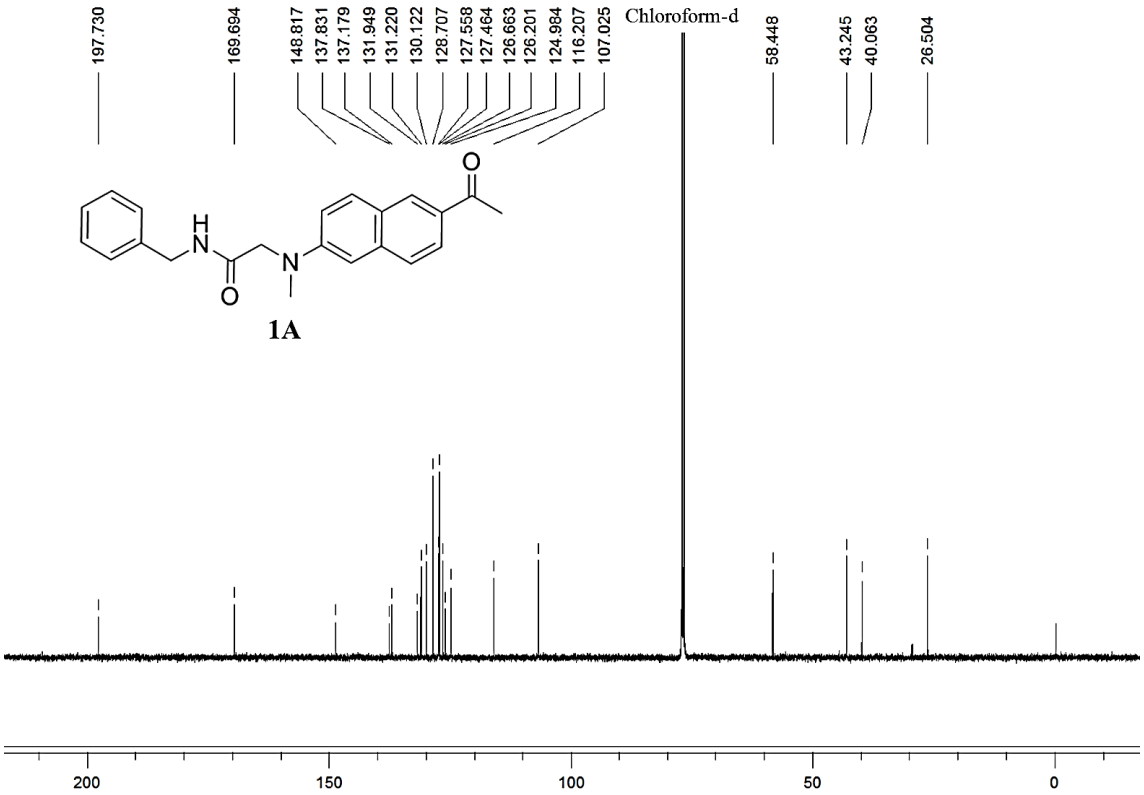


Fig. S7 ^{13}C NMR Spectrum of fluorescence probe **1A** (500 MHz).

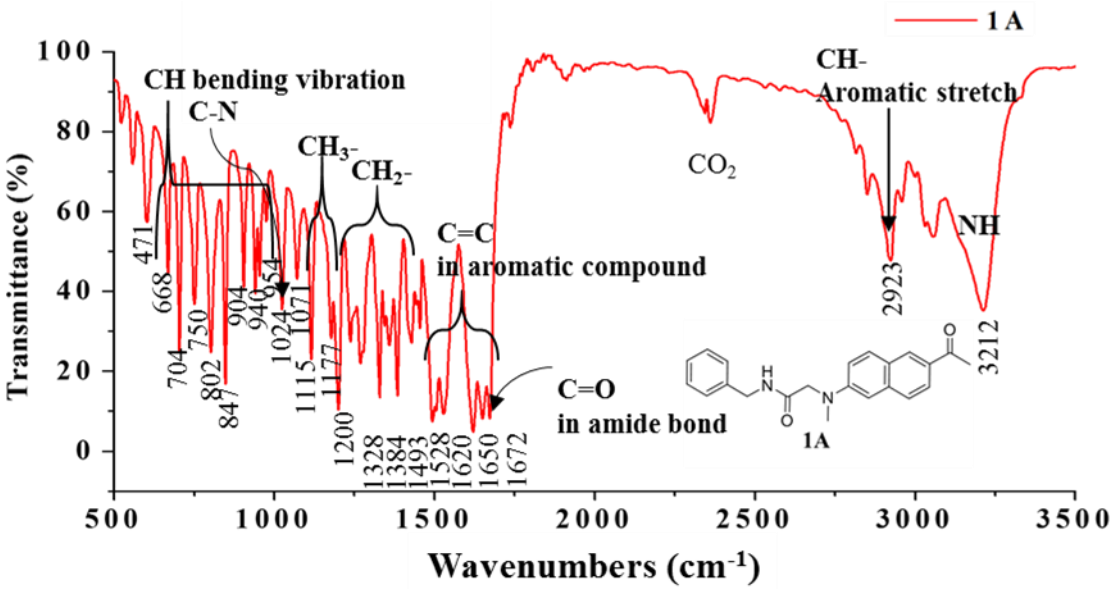


Fig. S8 IR Spectrum of fluorescence probe **1A**.

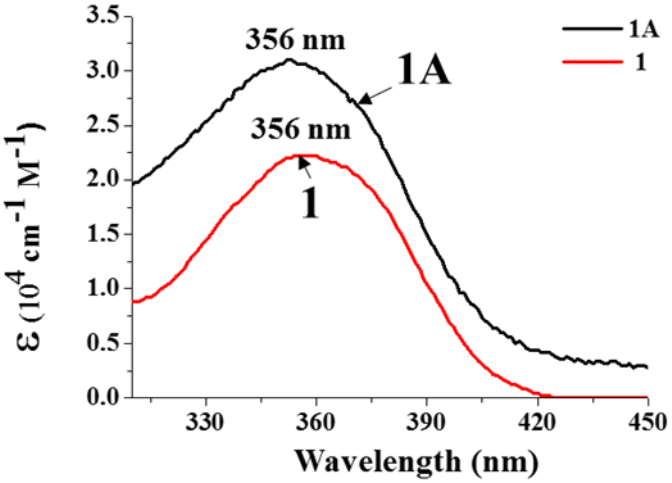


Fig. S9 Molar absorption coefficient of the fluorescence probe **1** (5 μM, in water-methanol (1:1, v/v, pH 7)) or **1A** (5 μM, in water-methanol (1:1, v/v, pH 7)). The excitation wavelength of both **1** and **1A** in solution was measured at 356 nm.

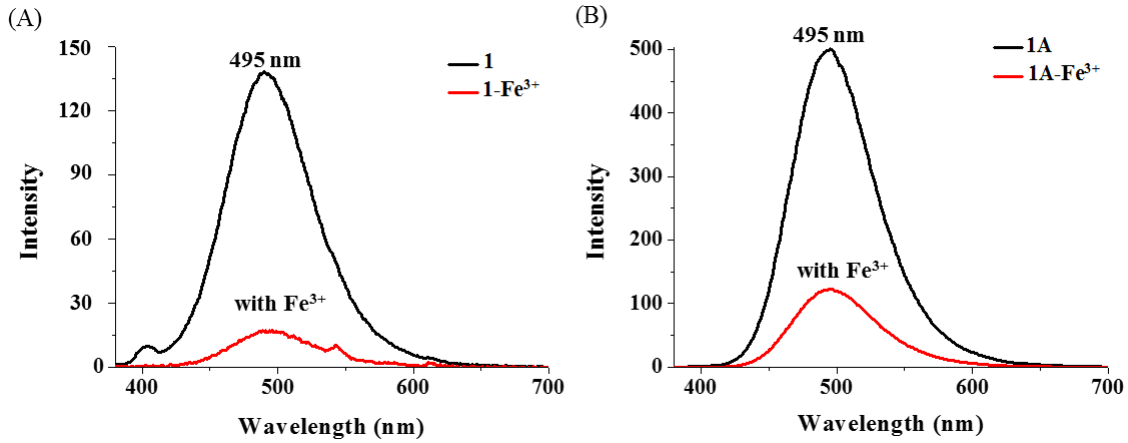


Fig. S10 Fluorescence spectra of (A) the fluorescence probe **1** (5 μM, in water-methanol (1:1, v/v, pH 7)) or with Fe^{3+} about **1** (200 equivalent, in water-methanol (1:1, v/v, pH 7)) and (B) the fluorescence probe **1A** (5 μM, in water-methanol (1:1, v/v, pH 7)) with Fe^{3+} about **1A** (100 equivalent, in water-methanol (1:1, v/v, pH 7)). A slit width of **1** is excitation wavelength 5 nm and emission wavelength 5 nm. That of **1A** is excitation wavelength 3 nm and emission wavelength 3 nm.

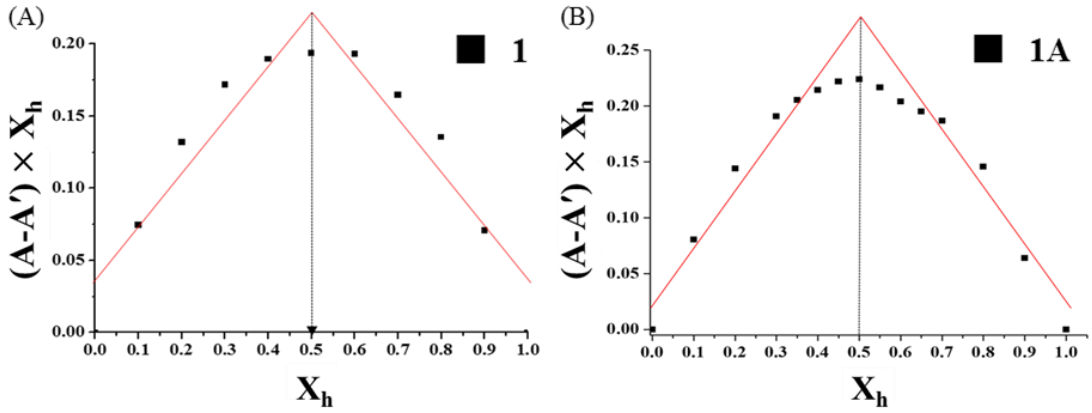


Fig. S11 Job's plots with Fe^{3+} of both (A) **1** and (B) **1A**. Measured absorbance of **1** and **1A** were obtained by maintaining total concentration 50 μM, in water-methanol (1:1, v/v, pH 7) and 356 nm of excitation wavelength and using width of 1 cm cell at the room temperature. In plot of (A-A´) × X_h *versus* X_h, A means the absorbance in complex of each **1** and **1A** with Fe^{3+} according to volume ratio, A´ is the absorbance in the presence only of metal solution and X_h is mole fraction as to ligand of each **1** and **1A** in solution of complex with **1**-Fe^{3+} and **1A**-Fe^{3+}, respectively.

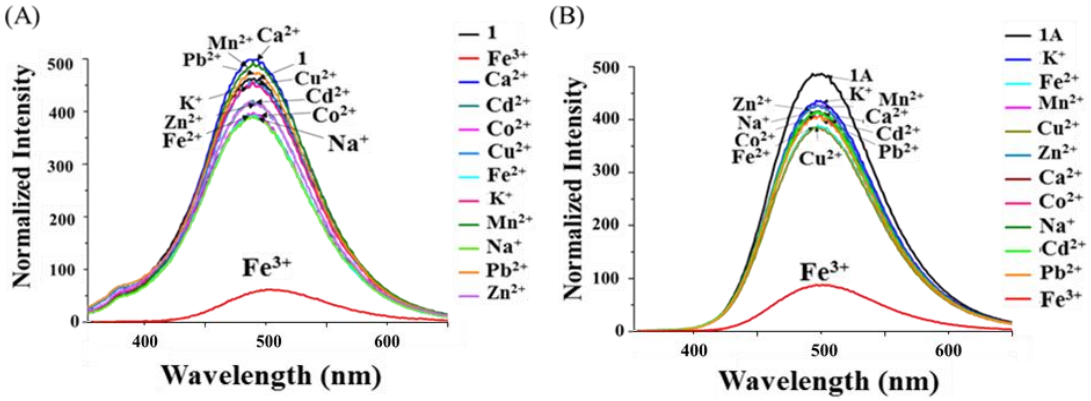


Fig. S12 Fluorescence spectra with each metal cation of (A) **1** (5 μM, in water-methanol (1:1, v/v, pH 7)) and (B) **1A** (5 μM, in water-methanol (1:1, v/v, pH 7)). The metal cations were Na^{+}, K^{+}, Zn^{2+}, Pb^{2+}, Mn^{2+}, Cu^{2+}, Co^{2+}, Ca^{2+}, Fe^{2+}**,** Fe^{3+} and Cd^{2+} including nitrate ions in water-methanol (1:1, v/v, pH 7). At both (A) and (B), all metal ions added as 200 equivalent in methanol as to **1** (5 μM) and (B) as 100 equivalent in water-methanol (1:1, v/v, pH 7) as to **1A** (5 μM).

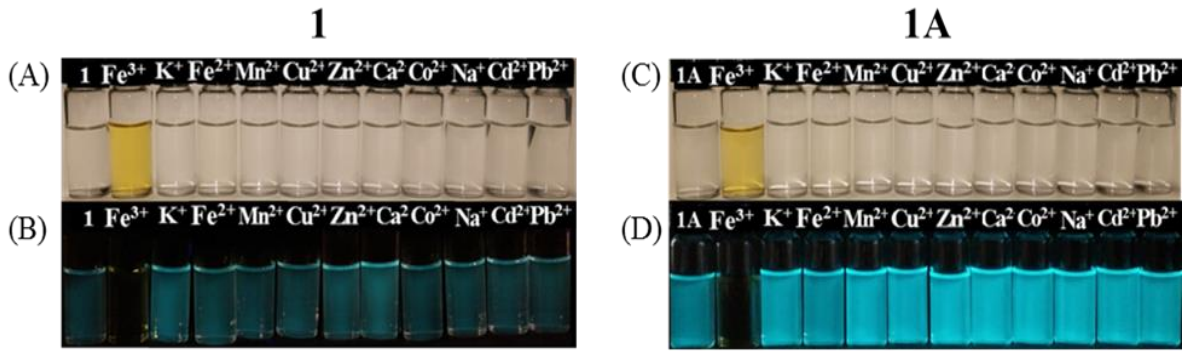


Fig. S13 Photograph of changes in color of (A) and (B) about **1** (5 μM) in water-methanol (1:1, v/v, pH 7) in the addition as 200 equivalent of each metal ions like Na^{+}, K^{+}, Zn^{2+}, Pb^{2+}, Mn^{2+}, Cu^{2+}, Co^{2+}, Ca^{2+}, Fe^{2+}, Fe^{3+} and Cd^{2+}. In the same way, photograph of changes in color of (C) and (D) about **1A** (5 μM) in water-methanol (1:1, v/v, pH 7) in the addition as of 100 equivalent of each metals ion like Na^{+}, K^{+}, Zn^{2+}, Pb^{2+}, Mn^{2+}, Cu^{2+}, Co^{2+}, Ca^{2+}, Fe^{2+}, Fe^{3+} and Cd^{2+}. The (A) and (C) were taken pictures indoors without 365 nm UV lamp, by contrast, (B) and (C) were taken pictures under 365 nm UV lamp.

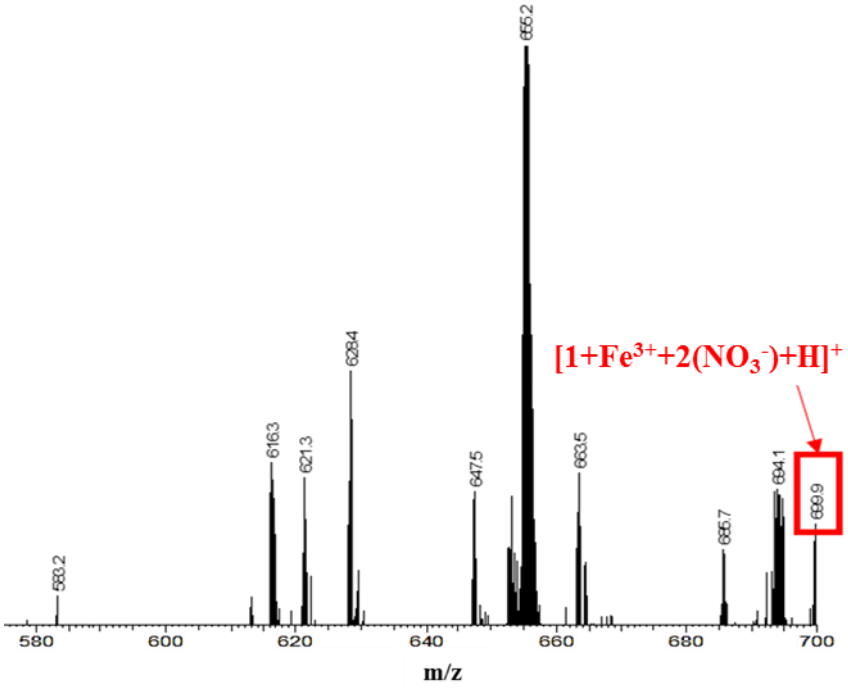


Fig. S14 ESI mass spectra (positive) of **1** (0.05 μM) in the presence of $Fe(NO_3)_3$ (0.05 μM), indicating the formation of 1:1 complex.

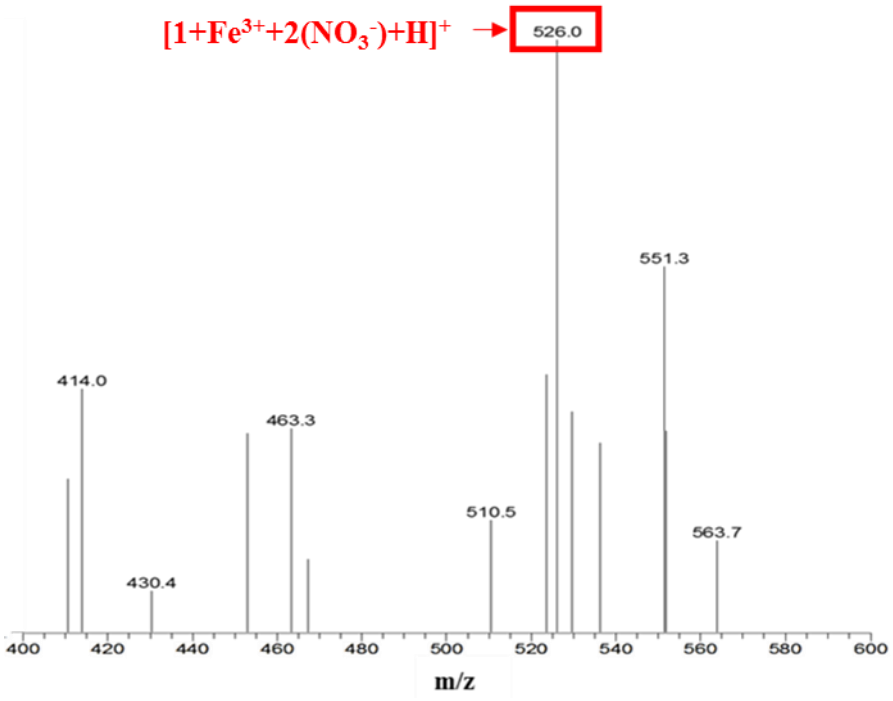


Fig. S15 ESI mass spectra (positive) of **1A** (0.005 μM) in the presence of $Fe(NO_3)_3$ (0.005 μM), indicating the formation of 1:1 complex.

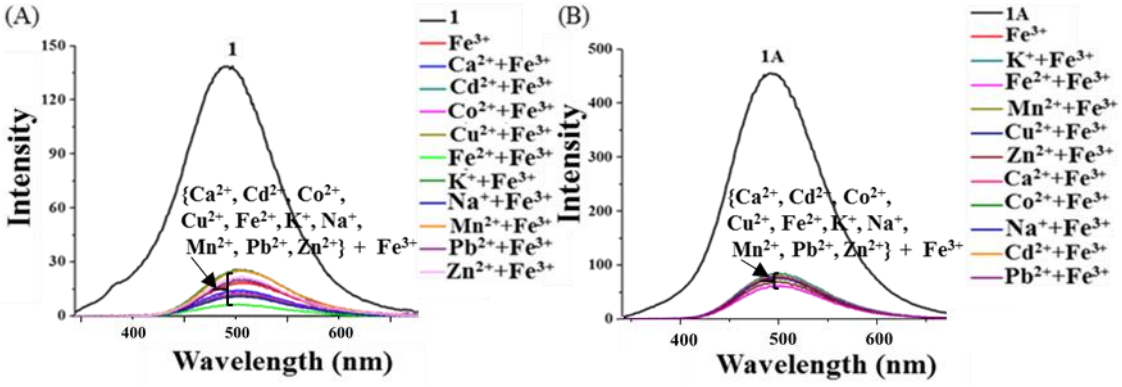


Fig. S16 Under presence of a competitive metal ions, fluorescence spectra with Fe^{3+} in the presence of other metal cations of (A) **1** (5 μM) and (B) **1A** (5 μM). The metal cations were Na^{+}, K^{+}, Zn^{2+}, Pb^{2+}, Mn^{2+}, Cu^{2+}, Co^{2+}, Ca^{2+}, Fe^{2+}, Fe^{3+} and Cd^{2+} including nitrate anions in water-methanol (1:1, v/v, pH 7). Also, the fluorescence spectra were measured by adding 200 equivalent of all metal cations in **1** and 100 equivalent of those in **1A**. A slit width of **1** is excitation wavelength 5 nm and emission wavelength 5 nm. That of **1A** is excitation wavelength 3 nm and emission wavelength 3 nm.

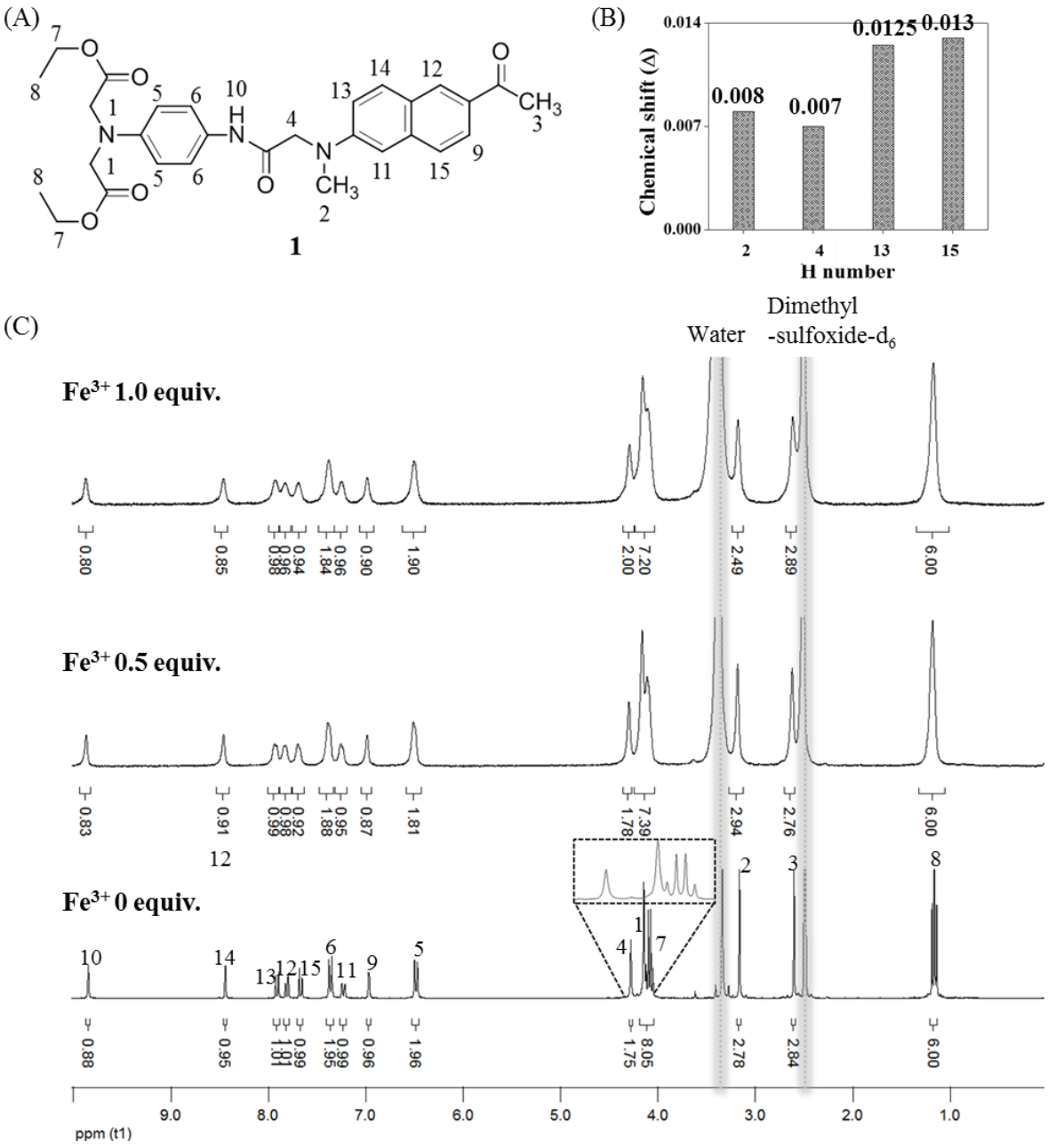


Fig. S17 ^{1}H NMR spectra of **1** (0.01 M, dimethylsulfoxide)-Fe^{3+} (deuterium oxide) complex. (A) Hydrogen-numbered compound **1**. (B) Plot of chemical shifts by difference **1** (0 equivalent) between **1**-Fe^{3+} of 1.0 equivalent. (C) ^{1}H NMR titration spectra according to addition of Fe^{3+} as 0.5 and 1.0 equivalent.

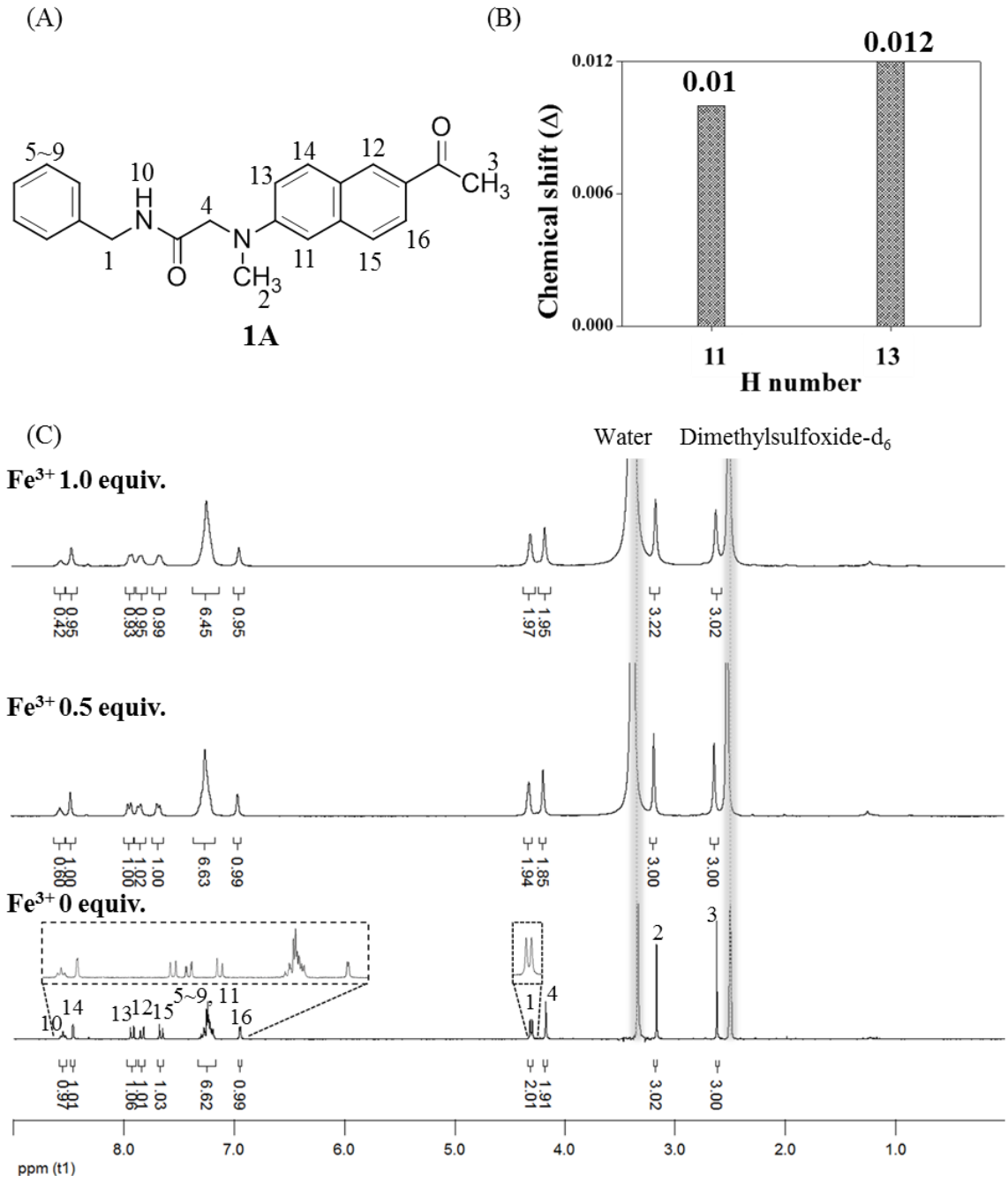


Fig. S18 ^{1}H NMR spectra of **1A** (0.01 M, dimethylsulfoxide)-Fe^{3+} (deuterium oxide) complex. (A) Hydrogen-numbered compound **1A**. (B) Plot of chemical shifts by difference **1A** (0 equivalent) between **1A**-Fe^{3+} of 1.0 equivalent. (C) ^{1}H NMR titration spectra according to addition of Fe^{3+} as 0.5 and 1.0 equivalent.

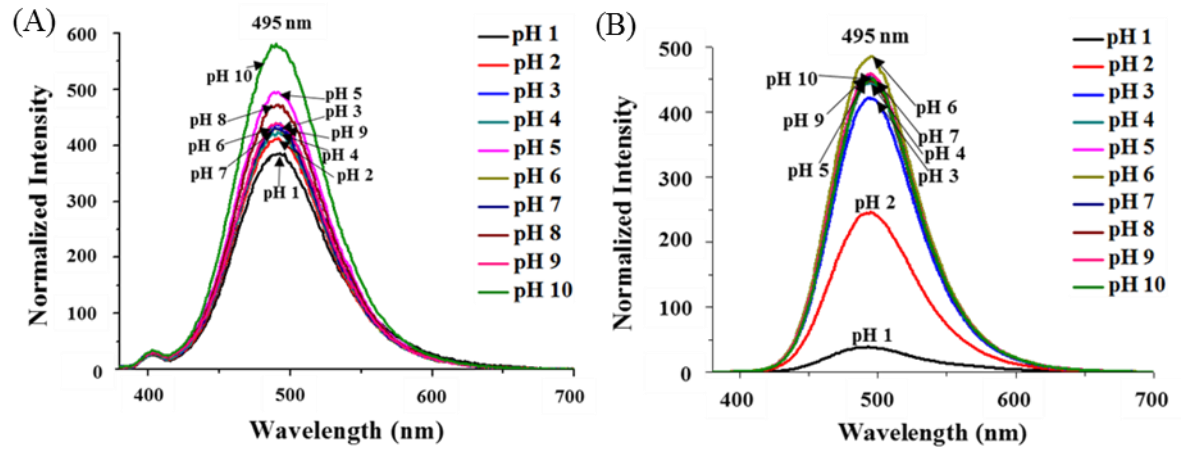


Fig. S19 Fluorescence spectra of **1** (5 μM, water-methanol (1:1, v/v)) (A) and **1A** (5 μM, water-methanol (1:1, v/v)) (B) without Fe^{3+} at different pH condition from 1 to 10.

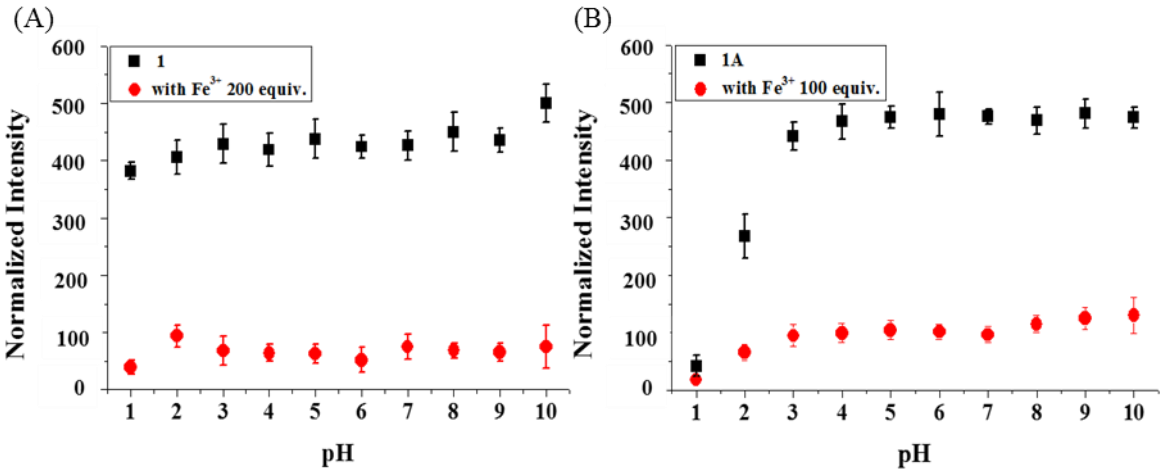


Fig. S20 Plot of fluorescence intensity at 495 nm about effect of pH of **1** (5 μM, water-methanol (1:1, v/v)) and **1**-Fe^{3+} of 200 equivalent (A) at different pH condition from 1 to 10. The plot of **1A** (5 μM, water-methanol (1:1, v/v)) and **1A**-Fe^{3+} of 100 equivalent (B) at different pH condition from 1 to 10.

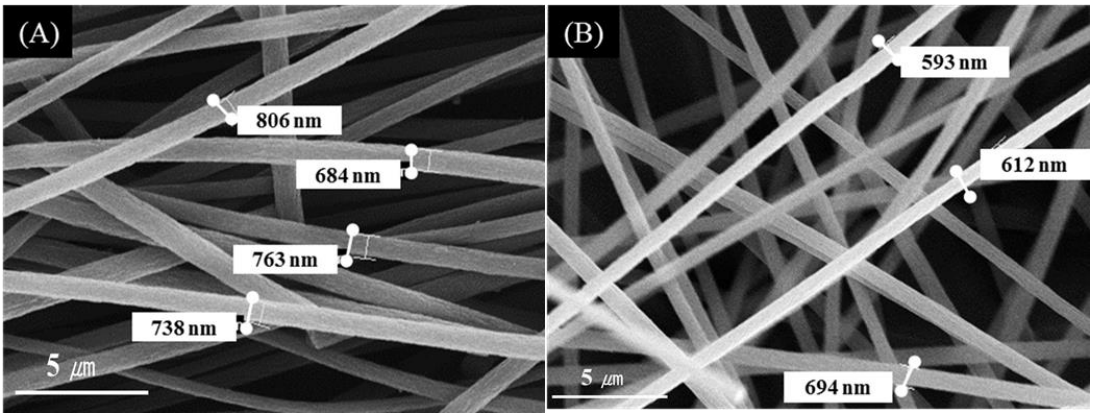


Fig. S21 SEM images of nanofibrous flims (A) NF-**1** and (B) NF-**1A** at same magnification of 5000 times.

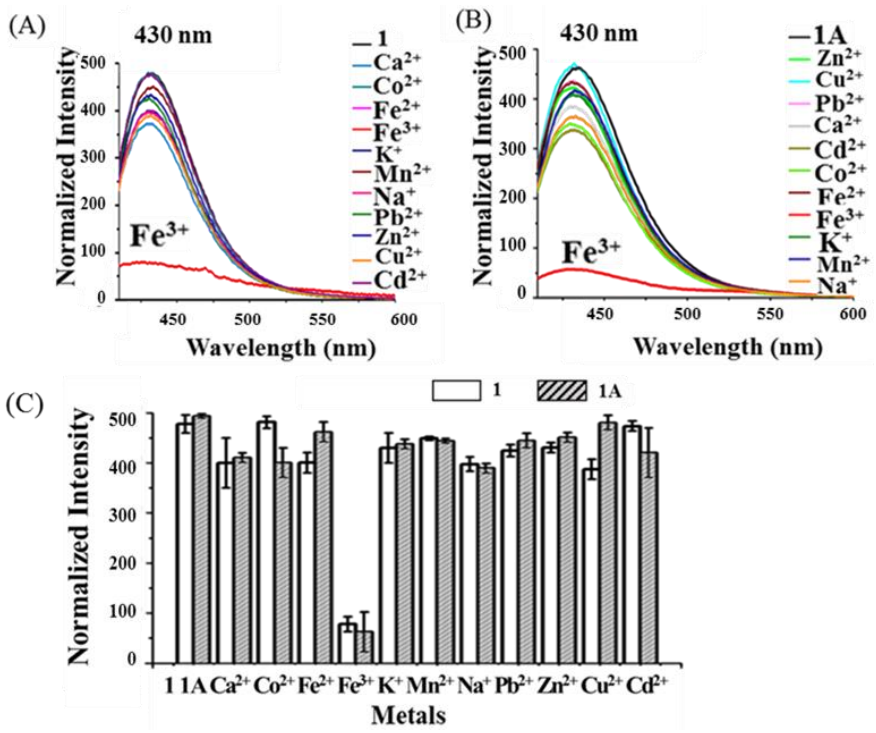


Fig. S22 Fluorescence spectra according to addition of each metal cation about (A) NF-**1** and (B) NF-**1A**. The metal cations such as Na^{+}, K^{+}, Zn^{2+}, Pb^{2+}, Mn^{2+}, Cu^{2+}, Co^{2+}, Ca^{2+}, Fe^{2+}**,** Fe^{3+} and Cd^{2+} were immersed into water-methanol (1:1, v/v, pH 7) solution for 24 hours at 0.1 M with following setup: excitation wavelength 380 nm, emission wavelength 430 nm, and slit width of 1.5 nm excitation wavelength and that of 3 nm emission wavelength using width of 1mm cell. (C) Sensing ability about various metal cations of each NF-**1** and NF-**1A**. These histograms were plotted at 430 nm wavelength.

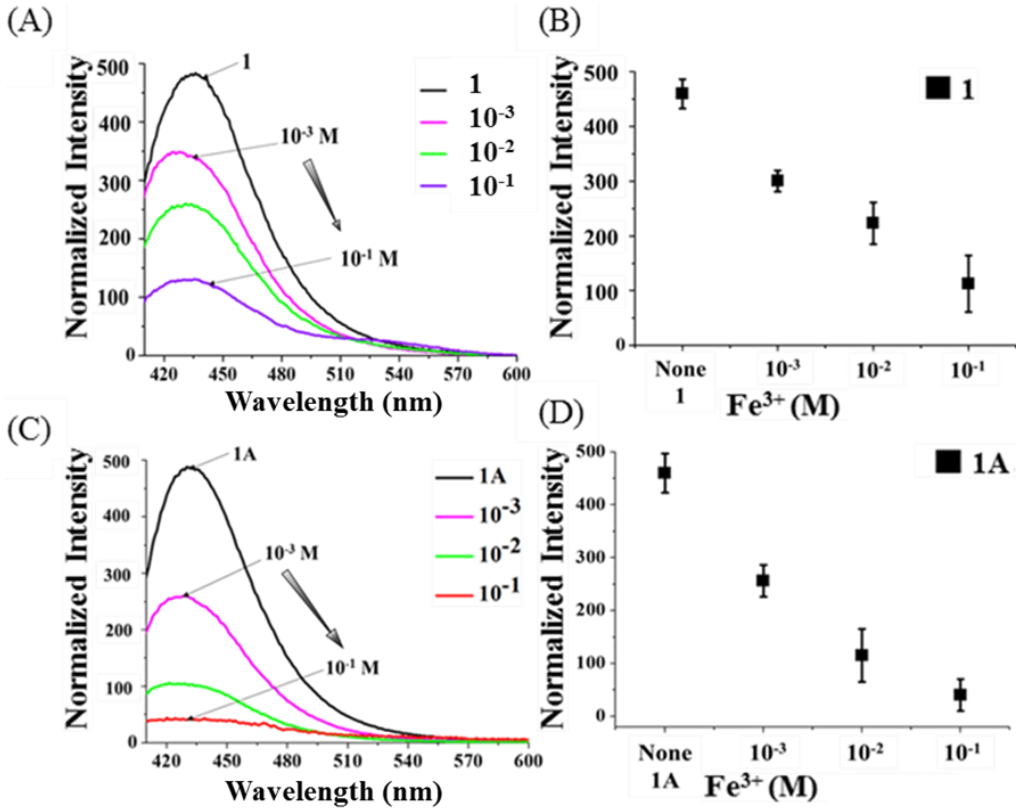


Fig. S23 Fluorescence spectra of nanofibrous films of (A) NF-**1** and (C) NF-**1A**. The plots of both (B) and (D) were graphed at 430 nm emission wavelength and the excitation wavelength was 380 nm in both NF-**1** and NF-**1A**. On the size of 0.8 × 1.5 (cm^2), using width of 1mm cell, each intensity was measured by immersing the films into water-methanol (1:1, v/v, pH 7) solution for 24 hours according to Fe^{3+} concentration like 0.001, 0.01, 0.1 M.

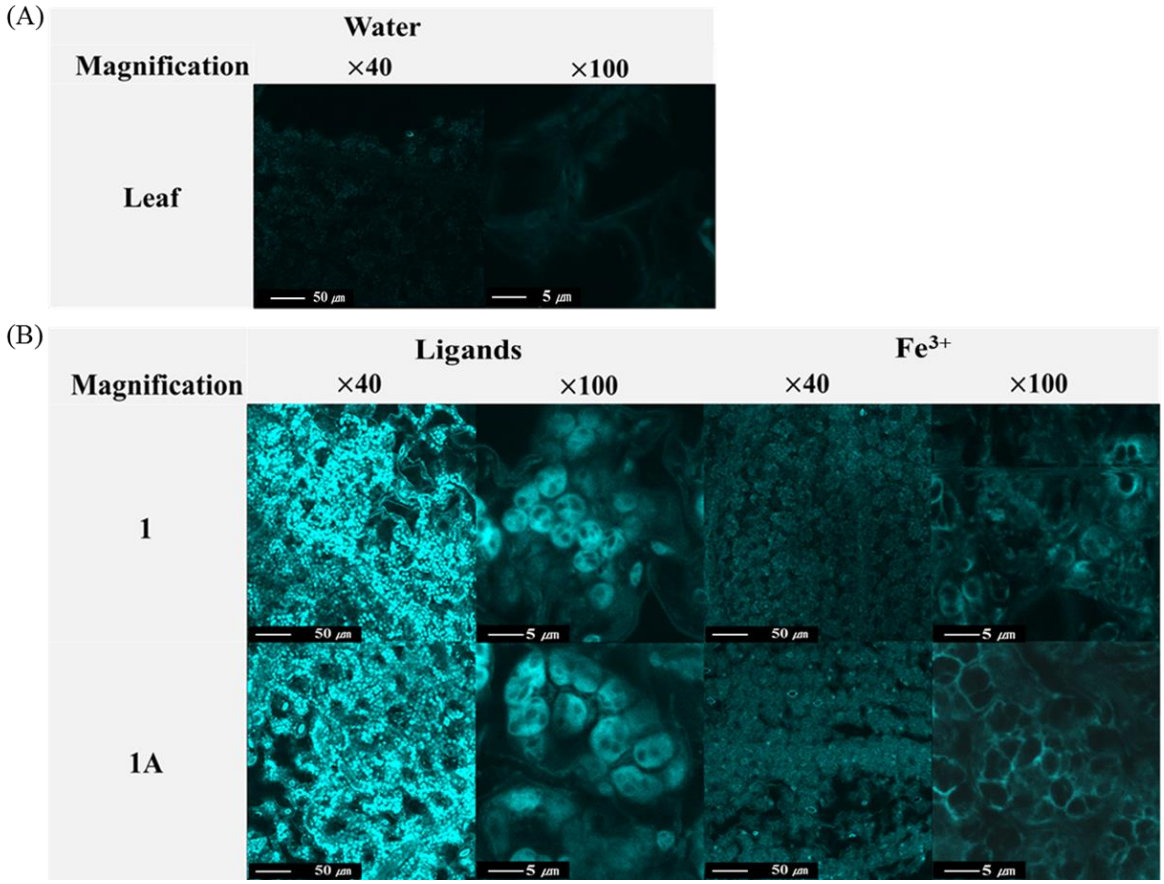


Fig. S24 Fluorescence images into the leaf parts in *Arabidopsis* of (A) addition only water without **1**-Fe^{3+} or **1A**-Fe^{3+} complex and (B) before and after addition of Fe^{3+} (300 μM) in leaf parts treated with each **1** (5 μM) and **1A** (5 μM).