Supporting Information for Manipulation of Fluorescent and Colorimetric Changes of Fluorescein Derivatives and Applications for Sensing Silver Ion

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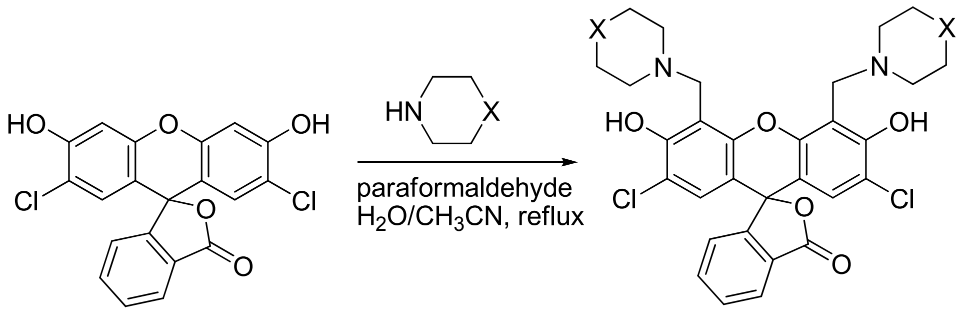
Experimental section

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.

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. 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).



1: X = O, 81 %
2: X = S, 84 %
3: X = NCH_3, 90%

2-[2,7-Dichloro-6-hydroxy-3-oxo-4,5-bis(morpholin-N-ylmethyl)-3*H*-xanthen-9-yl] benzoic acid (1). Morpholine (0.69 g, 7.97 mmol) and paraformaldehyde (0.22 g, 7.47 mmol) were combined in 20 mL of CH_3CN and refluxed for 30 min. 2,7-Dichlorofluoroscein (1 g, 2.49 mmol) in 30 mL of CH_3CN/H_2O (1:1) was added to the above reaction mixture and continued reflux for another 24 h. The solvents were removed completely under vacuum by repeated addition of $CHCl_3$ and evaporations. Flash chromatography on silica gel (98:2 CH_2Cl_2/MeOH) yielded the product as off-white solid which slowly turned light pink on keeping for several days (1.2 g, 80.5%): mp 300 °C, decompose; ^{1}H NMR ($CDCl_3$) δ 8.06 (d, 2H, J = 6.7 Hz), 7.72 (m, 4H), 7.20 (d, 2H, J = 6.0 Hz), 6.63 (s, 2H), 5.30 (s, OH), 4.04 (d, 4H, J = 4.1 Hz), 3.84 (brs, 8H), 2.75 (brs, 8H); ^{13}C NMR ($CDCl_3$) δ 168.6, 155.9, 151.1, 147.9, 135.4, 130.4, 127.7, 125.6, 123.9, 117.2, 110.6, 108.2, 82.6, 66.5, 55.0, 53.2; HRMS (FAB) m/z = 621.1183 $(M+Na)^+$, calc. for $C_{30}H_{28}Cl_2N_2O_7Na$ = 621.1171.

2-[2,7-Dichloro-6-hydroxy-3-oxo-4,5-bis(thiomorpholin-N-ylmethyl)-3*H*-xanthen-9-yl]benzoic acid (2). This compound (84% yield) was prepared following the similar method given for **1**: mp 300 °C, decompose.; ^{1}H NMR ($CDCl_3$) δ 8.06 (d, 2H, J =

6.6 Hz), 7.72 (m, 4H), 7.19 (d, 2H, *J* = 6.1 Hz), 6.63 (s, 2H), 5.30 (s, OH), 4.00 (d, 4H, *J* = 5.7 Hz), 2.93 (brs, 8H), 2.80 (brs, 8H); ^{13}C NMR ($CDCl_3$) δ 168.6, 156.1, 151.2, 147.9, 135.4, 130.4, 127.1, 125.6, 123.9, 117.2, 110.5, 108.3, 82.6, 55.5, 54.8, 27.8; HRMS (FAB) m/z = 653.0698 $(M+Na)^+$, calc. for $C_{30}H_{28}Cl_2N_2O_5S_2Na$ = 653.0714.

2-[2,7-Dichloro-6-hydroxy-3-oxo-4, 5-bis (1-methylpiperazine-N-ylmethyl)-3*H*-xanthen-9-yl]benzoic acid (3). 2,7-Dichlorofluoroscein (1.00 g, 2.49 mmol) and paraformaldehyde (0.22 g, 7.47 mmol) were combined in 35 mL of CH_3CN. 1-Methylpiperazine (0.75 g, 7.97 mmol) is added slowly under stirring at room temperature followed with 15 mL of water. The reaction mixture was refluxed for 12 h. The solvents were removed completely under vacuum by repeated addition of $CHCl_3$ and evaporation. Flash column chromatography on silica gel (98:2 CH_2Cl_2/MeOH) yielded the product as a salmon pink solid (1.4g, 90%): mp 300 °C, decompose.; ^{1}H NMR (D_2O) δ 7.60 (d, 1H, *J* = 7.7 Hz), 7.43 (t, 1H, *J* = 7.57 Hz), 7.18 (t, 1H, *J* = 7.53 Hz), 7.05 (s, 2H), 6.41 (d, 1H, *J* = 7.47 Hz), 3.87 (s, 4H), 2.87 (brs, 16H), 2.53 (brs, 6H); ^{13}C NMR (D_2O) δ 174.2, 173.7, 157.4, 155.1, 139.6, 130.4, 129.9, 129.4, 129.2, 128.6, 127.8, 110.7, 107.9, 52.1, 50.0, 49.5, 43.1; HRMS (FAB) m/z = 647.1804 $(M+Na)^+$, calc. for $C_{32}H_{34}Cl_2N4O_5Na$ = 647.1803.

Preparation of fluorometric metal ion titration solutions.

Stock solutions (1 mM) of the perchlorate salts of Ag^+, Ca^{2+}, Cd^{2+}, Co^{2+}, Cs^+, Cu^{2+}, Hg^{2+}, K^+, Li^+, Mg^{2+}, Mn^{2+}, Na^+, Ni^{2+}, Rb^+ and Zn^{2+} in double distilled water were prepared. Stock solution of host (0.1 mM) was also prepared in DMSO. Test solutions were prepared by placing the probe stock solution into a test tube, adding an appropriate aliquot of each metal stock, and diluting the solution to 4 mL with 0.01 M HEPES buffer.

For all measurements, excitation was at 504 or 507 nm. Both excitation and emission slit widths were 1.5 nm.

X-ray crystallography.

The diffraction data for **1** and **2** were collected at 293 K on a Bruker SMART AXS diffractometer equipped with a monochromator in the Mo Kα ($\lambda = 0.71073$ Å) incident beam. Crystals were mounted on a glass fiber. The CCD data were integrated and scaled using the Bruker-SAINT software package, and the structure was solved and refined using SHEXTL V6.12[1]. Hydrogen atoms were located in the calculated positions. O1S for **1** and O1S, O2S, C1S, and C2S for **2** are refined isotropically. There is a mirror plane through the C8/O1/C1/C2/C3/C4/C5/C6/C7 ring and a water solvent molecule for **2**. *Crystal data of **1***: *Monoclinic*, *C2/m*, Z = 4, $a = 21.357(2)$ Å, $b = 16.3275(15)$ Å, $c = 8.9215(8)$ Å, $\beta = 101.689(2)°$, $V = 3046.3(5)$ Å^3, $\mu = 0.264$ mm^{-1}, $d_{\mathrm{calc}} = 1.346$ g/cm^3, $R_1 = 0.0659$, $wR_2 = 0.1683$ for 2745 unique reflections, 212 variables. *Crystal data of **2***: *Monoclinic*, *$P2_1/m$*, Z = 2, $a = 8.7630(18)$ Å, $b = 14.817(3)$ Å, $c = 13.295(3)$ Å, $\beta = 92.06(3)°$, $V = 1725.1(6)$ Å^3, $\mu = 0.361$ mm^{-1}, $d_{\mathrm{calc}} = 1.339$ g/cm^3, $R_1 = 0.0641$, $wR_2 = 0.1499$ for 3105 unique reflections, 219 variables. CCDC numbers are **707407** and **707406** for **1** and **2**, respectively.

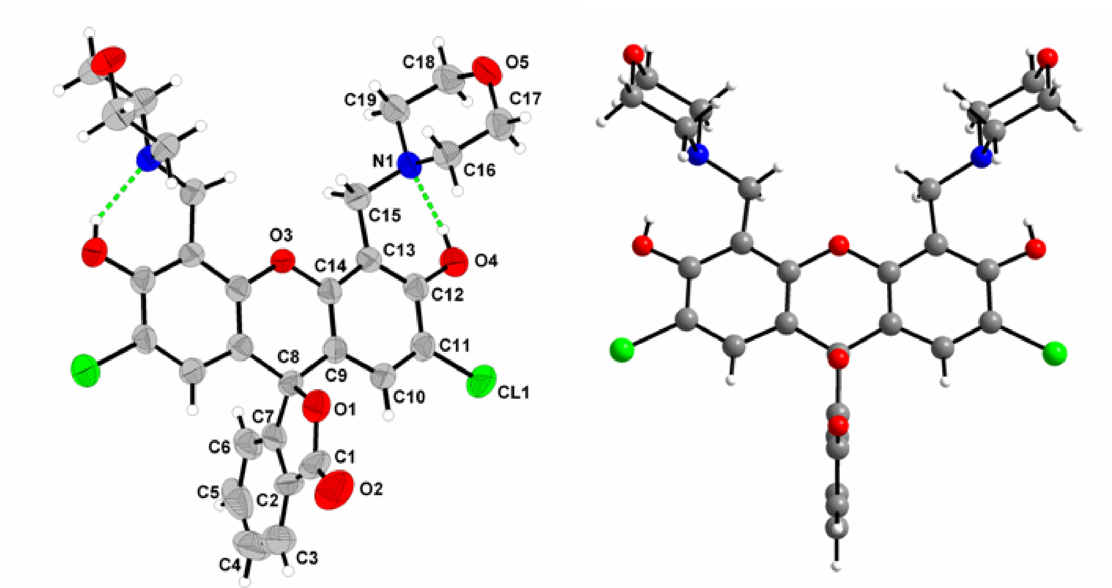


Figure S1. Crystal structure of **1**. (O4-H4···N1: 1.89 Å)

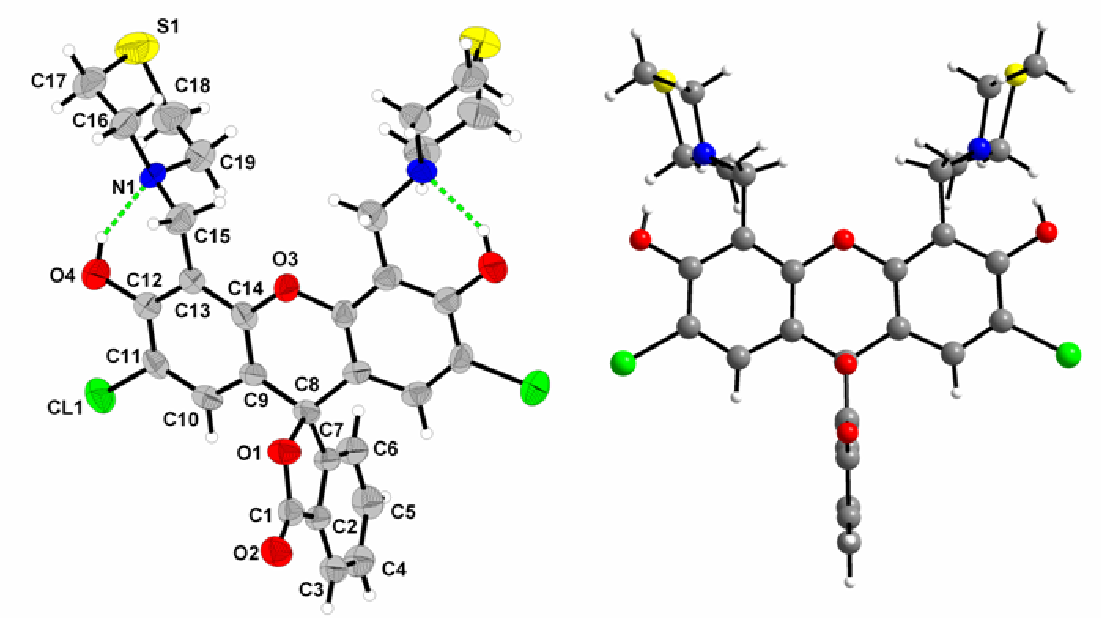


Figure S2. Crystal structure of **2**. (O4-H4···N1: 1.89 Å)

Table S1. Crystallographic data for compounds **1** and **2**.

	1	**2**
Empirical formula	$C_{30}H_{30}Cl_2N_2O_8$	$C_{30}\ H_{36}\ Cl_2\ N_2\ O_7\ S_2$

Formula weight	617.46	695.65
Temperature	293 K	293 K
Wavelength	0.71073 Å	0.71073 Å
Space group	C2/m	P 2_1/m
a	21.357(2) Å	8.7630(18) Å
b	16.3275(15) Å	14.817(3) Å
c	8.9215(8) Å	13.295(3) Å
α	90.000°	90.000°
β	101.698(2)°	92.06(3)°
γ	90.000°	90.000°
Volume	3046.3(5) $Å^3$	1725.1(6) $Å^3$
Z	4	2
Calculated density	1.346 Mg/m^3	1.339 Mg/m^3
Absorp.coefficient	0.265 mm^{-1}	0.357 mm^{-1}
F(000)	1288	728
Crystal size	0.08 x 0.03 x 0.03 mm^3	0.15 x 0.08 x 0.02 mm^3
Independent reflections	2745 [R(int) = 0.0520]	3105 [R(int) = 0.0782]
Data/restraints/parameters	2745 / 2 / 212	3105 / 0 / 219
Goodness-of-fit[a] on F^2	0.975	0.871
Final R indices [I>2σ(I)]	R_1^b = 0.0659, wR_2^c = 0.1683	R_1^b = 0.0641, wR_2^c = 0.1499
R indices (all data)	R_1^b = 0.1187, wR_2^c = 0.1996	R_1^b = 0.1659, wR_2^c = 0.1674
Largest diff. peak and hole	0.333 and –0.310 e.$Å^{-3}$	0.303 and -0.321 e.$Å^{-3}$

Table S2. Hydrogen bond distances and angles for compounds **1** and **2**.

	H···N	O···N	O-H···N
1	1.89 Å	2.599(4) Å	143.4°
2	1.89 Å	2.625(7) Å	147.7°

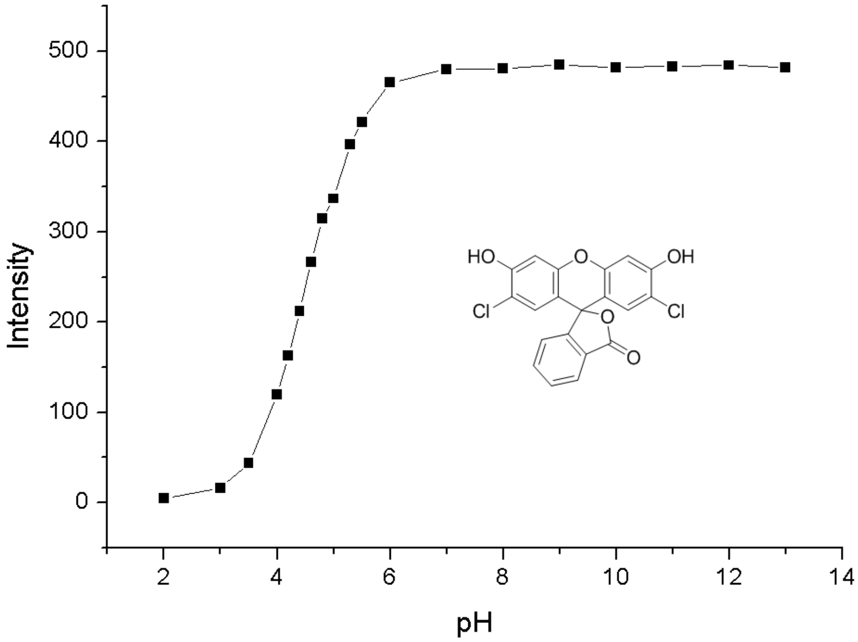


Figure S3. Fluorescent pH titration of 2,7-dichlorofluorescein.

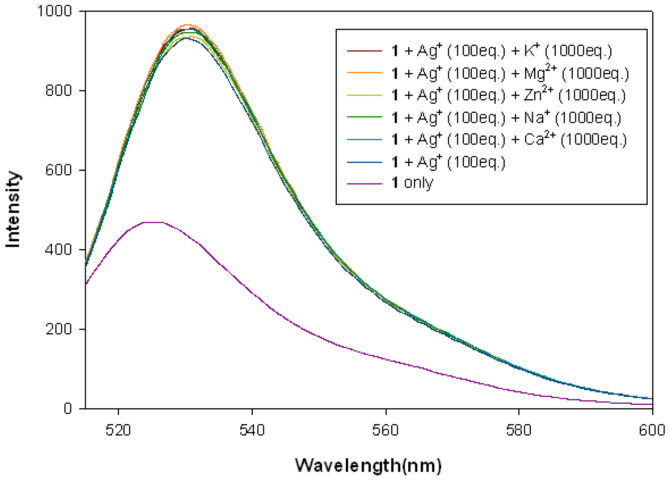


Figure S4. Fluorescent changes of **1** (3 μM) with Ag^+ (100 eq.) in the presence of 1000 eq. of K^+, Na^+, Ca^{2+}, Mg^{2+} and Zn^{2+} at pH 7.4 (0.01 M HEPES: DMSO = 95:5, v/v) (excitation = 504 nm).

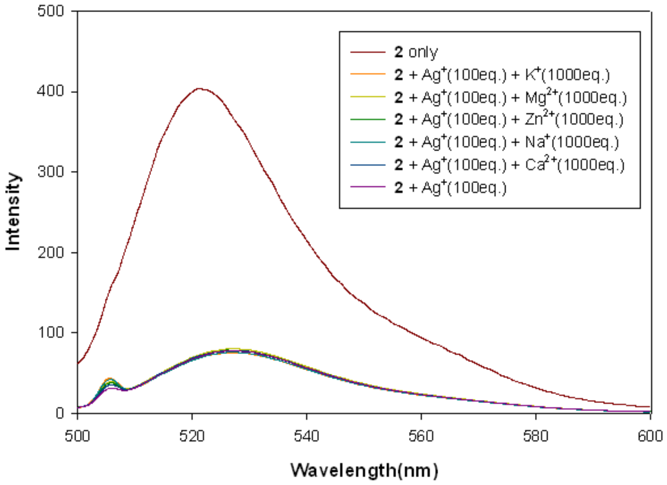


Figure S5. Fluorescent changes of **2** (1 µM) with Ag^+ (100 eq.) in the presence of 1000 eq. of K^+, Na^+, Ca^{2+}, Mg^{2+} and Zn^{2+} at pH 7.4 (0.01 M HEPES: DMSO = 95:5, v/v) (excitation = 507 nm).

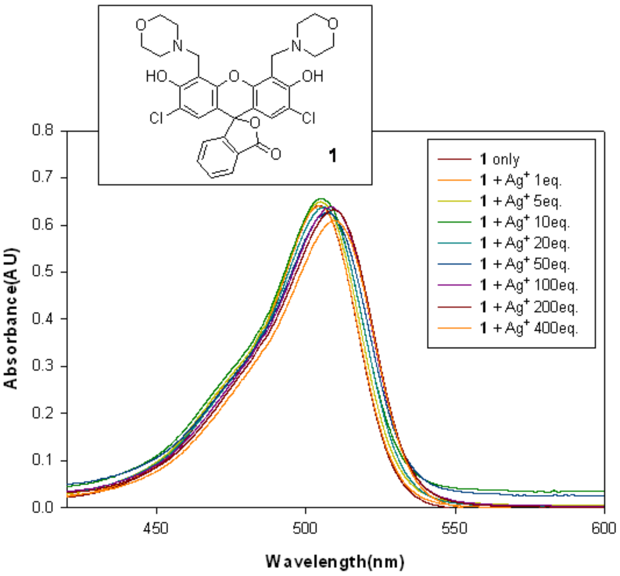


Figure S6. UV/vis changes of **1** (10 µM) upon addition of Ag^+ ion at pH 7.4 (DMSO : 0.01M HEPES = 5 : 95, v/v).

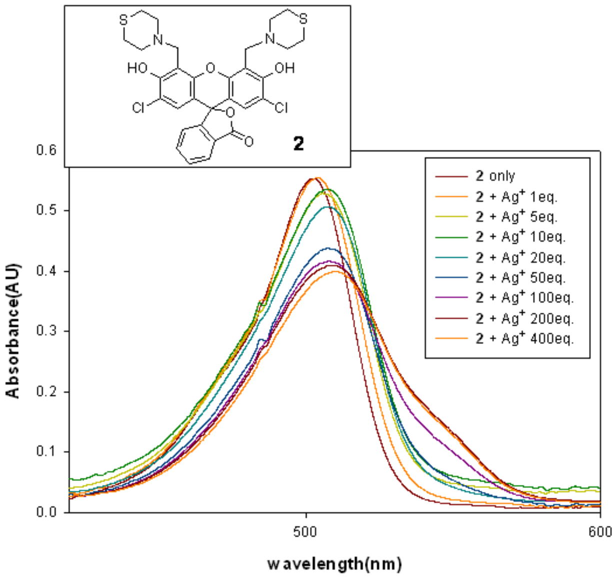


Figure S7. UV/vis changes of **2** (10 µM) upon addition of Ag^+ ion at pH 7.4 (DMSO : 0.01M HEPES = 5 : 95, v/v)**.**

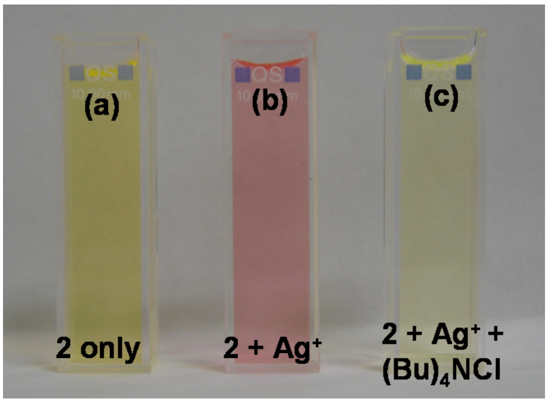


Figure S8. Colorimetric changes of **2** (20 µM) at pH 7.4 (0.01 M HEPES: DMSO = 95:5, v/v): (a) **2** (20 µM); (b) upon the addition of Ag^+ (300 eq.); (c) filtrate after the addition of excess tetrabutylammonium chloride.

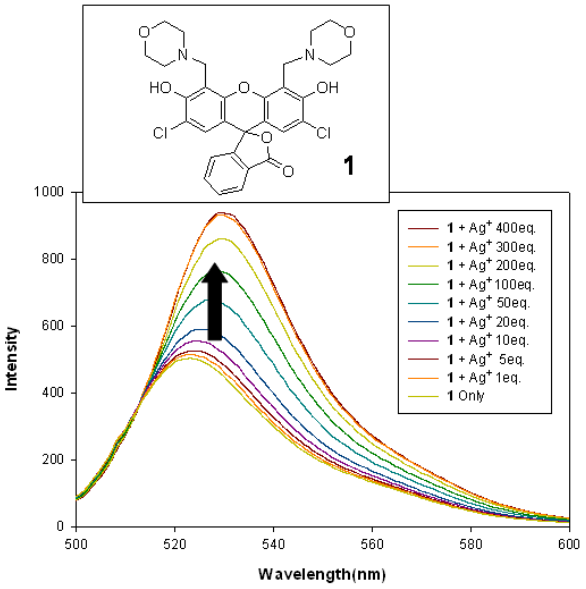


Figure S9. Fluorescent titrations of **1** (1 μM) with Ag$^+$ at pH 7.4 (DMSO : 0.01M HEPES = 5 : 95, v/v) (excitation = 507 nm).

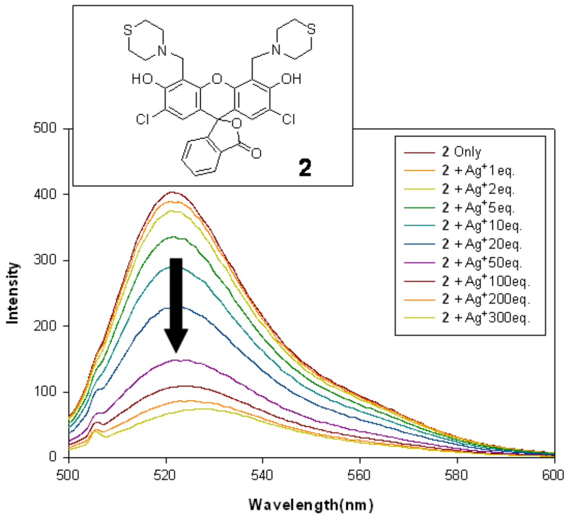


Figure S10. Fluorescent titrations of **2** (1 μM) with Ag$^+$ at pH 7.4 (DMSO : 0.01M HEPES = 5 : 95, v/v) (excitation = 504nm).

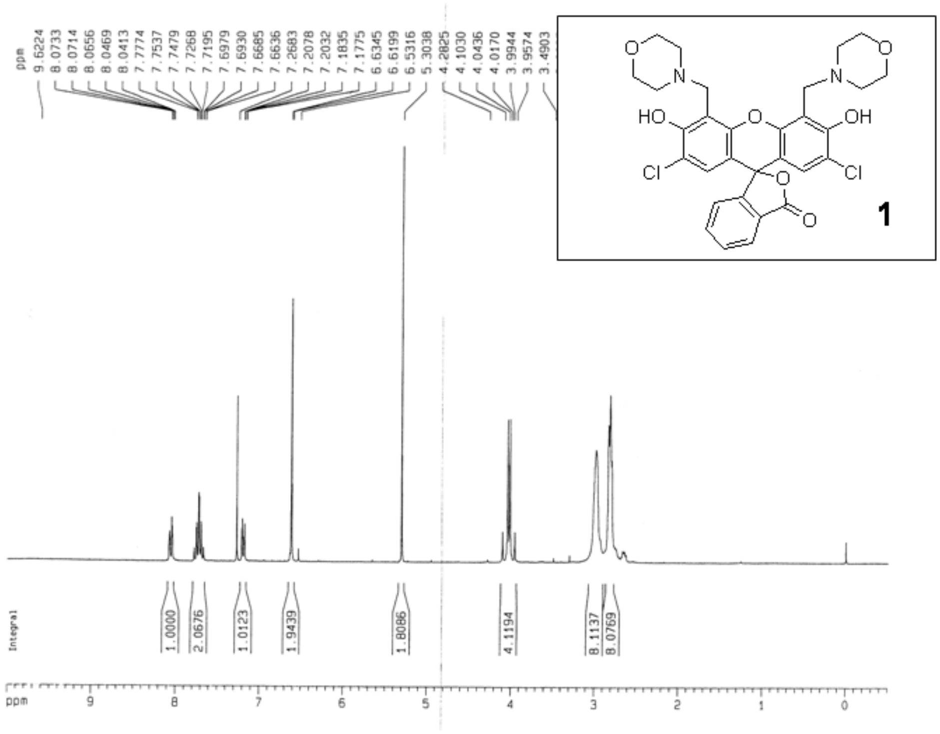


Figure S11. ^{1}H NMR (250 MHz) of compound **1** in $CDCl_3$.

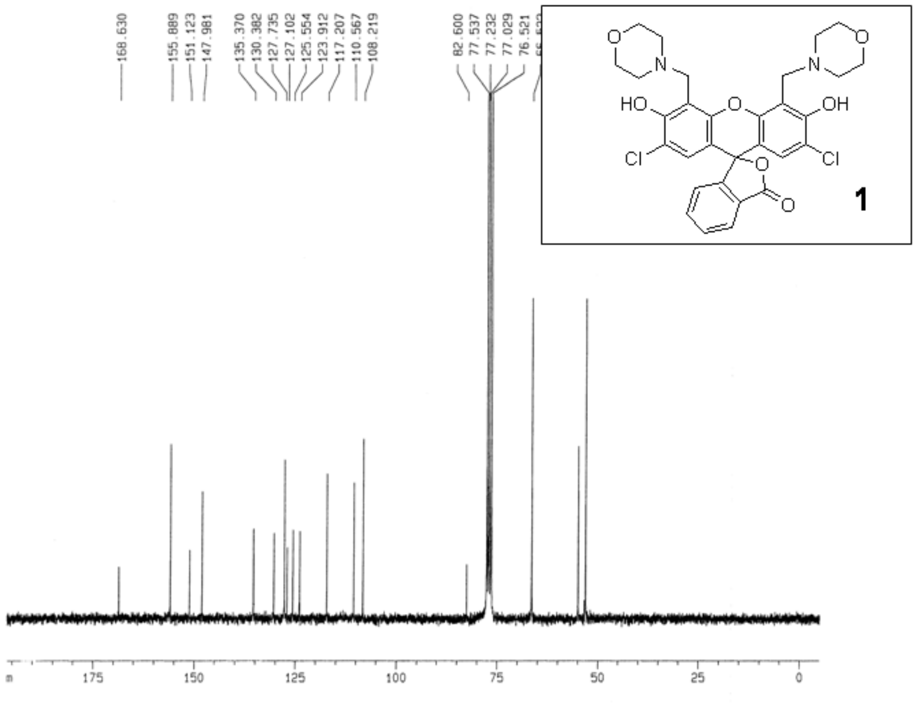


Figure S12. ^{13}C NMR (62.5 MHz) of compound **1** in $CDCl_3$.

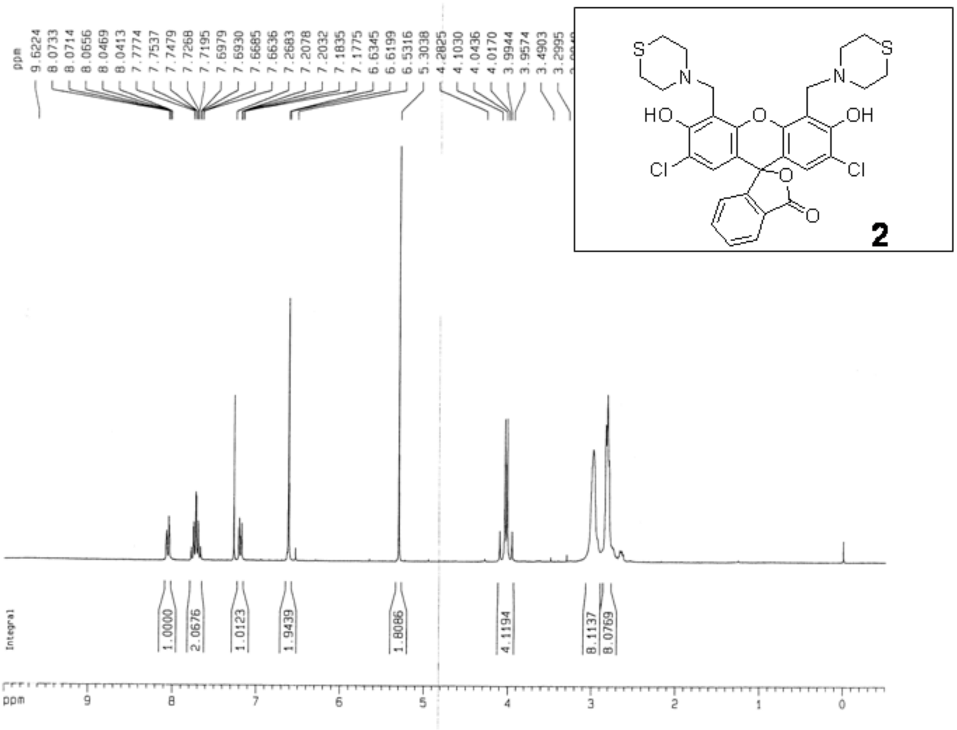


Figure S13. ^{1}H NMR (250 MHz) of compound **2** in $CDCl_3$.

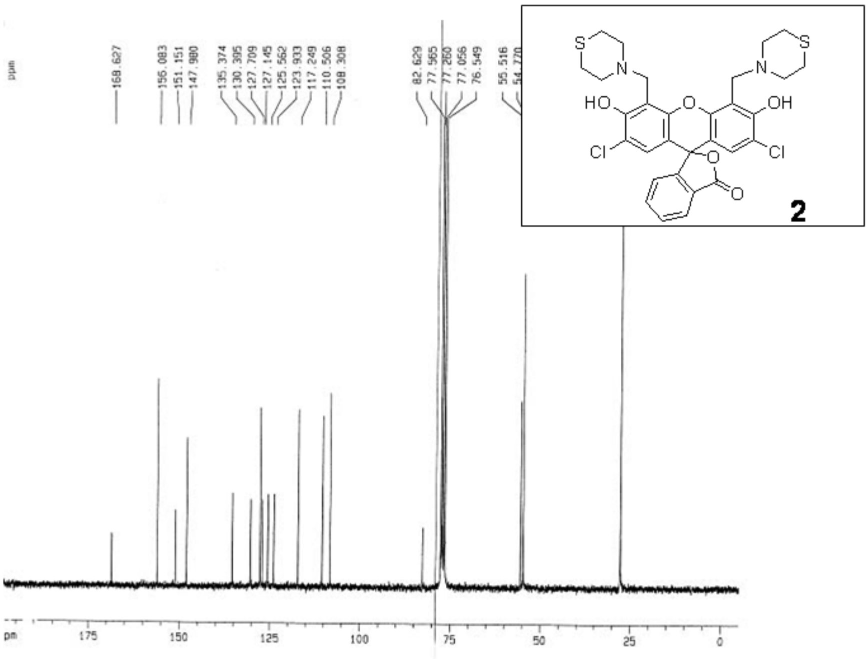


Figure S14. ^{13}C NMR (62.5 MHz) of compound **2** in $CDCl_3$.

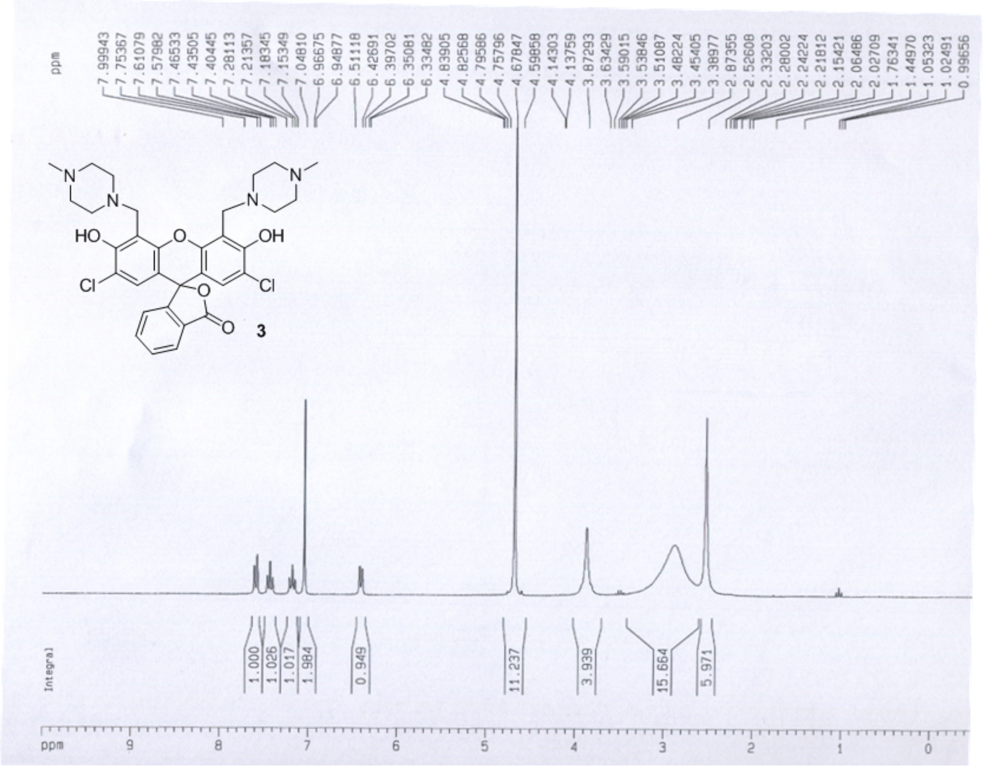


Figure S15. ^{1}H NMR (250 MHz) of compound **3** in $CDCl_3$.

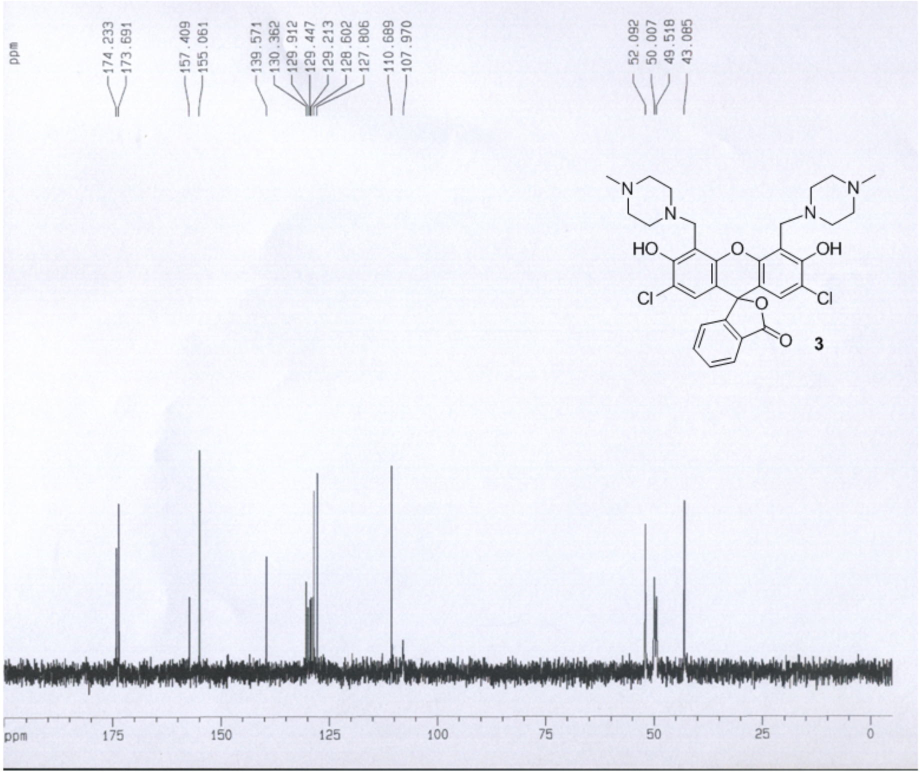


Figure S16. ^{13}C NMR (62.5 MHz) of compound **3** in $CDCl_3$.

References:

1) G. M. Sheldrick, SHELXTL/PC. Version 6.12 for Windows XP. 2001, Bruker AXS Inc., Madison, Wisconsin, USA.