Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information for

Analyte-Directed Formation of Emissive Excimers for the Selective Detection of Polyamines

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Experimental

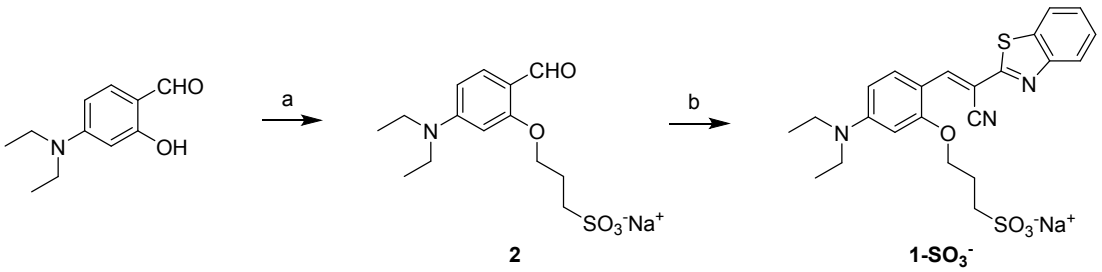
Materials

All reagents were of the highest commercial quality and used as received without further purification. All solvents were spectral grade unless otherwise noted. Anhydrous THF was obtained as a sure-seal bottle from Aldrich Co. Inc. (Milwaukee, WI). Silica gel (40 μm) was obtained from Merck Inc. Aqueous solutions were freshly prepared with deionized water from a water purification system (Human Corp. Korea).

General methods, instrumentation and measurements

Synthetic manipulations that required an inert atmosphere (where noted) were carried out under argon using standard Schlenk techniques. NMR (^{1}H, ^{13}C) spectra were recorded on Bruker Advance 500 MHz spectrometer. The ^{1}H and ^{13}C chemical shifts were reported as δ in units of parts per million (ppm), referenced to the residual solvent. Splitting patterns are denoted as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). High-resolution electrospray ionization (ESI) mass spectra were obtained at the Korean National Center for Inter-University Research. Absorption spectra were obtained on a Shimadzu UV-2501 spectrophotometer. Fluorescence measurements were recorded on a Hitachi F-7000 fluorescence spectrophotometer using quartz cuvettes with a path length of 1 cm. Fluorescence quantum yields were determined by standard methods, using fluorescein (Φ_F = 0.95 in 0.1 N NaOH) as a standard.[1] Life-time measurements were performed using a MicroTime-200 instrument at the Korea Basic Science Institute (KBSI), Daegu Center, South Korea. Dynamic light scattering (DLS) measurement was obtained on Malvern particle analyzer ZEN1690.

1. Synthesis of Probe 1-SO_3^-



Scheme S1. Synthetic scheme of probe **1-SO_3^-**, reagents and conditions a) propane sulton, NaH, THF, overnight, RT; b) 2-(benzo[*d*]thiazol-2-yl)acetonitrile, piperidine, EtOH, 24 h, RT, 63%.

Sodium (*E*)-3-(2-(2-(benzo[*d*]thiazol-2-yl)-2-cyanovinyl)-5-(diethylamino)phenoxy)propane-1-sulfonate (Probe 1-SO_3^-). To a stirred mixture of 4-(diethylamino)-salicylaldehyde (50 mg, 0.26 mmol) and NaH (60% dispersion in oil, 12.5 mg, 0.31 mmol) in 5 mL of dry THF at room temperature under an argon atmosphere were added propane sulton (47.6 mg, 0.39 mmol). After the resulting mixture was stirred at room temperature for overnight, the reaction was quenched with H_2O (3 mL), and the solvent was evaporated under reduced pressure. The crude mixture was redissolved in MeOH and precipitated with diethyl ether. The precipitate was isolated by careful centrifugation to give solid **2**. The product **2** was used for the next step without further purification. To a solution of **2** (50 mg, 0.14 mmol) and 2-(benzo[*d*]thiazol-2-yl)acetonitrile (38 mg, 0.22 mmol) in EtOH (10 mL) at room temperature under argon atmosphere was added one drop of piperidine. The resulting solution was stirred at room temperature for 24 hours. The solvent was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel using progressively more polar 30:1 to 10:1 MeOH:CH_2Cl_2. The product was further subjected to reverse-phase C-18 column chromatography (Waters 10 g RPC 18 cartridge) eluting

with H_2O to afford probe **1-SO_3^-** as a red solid (80 mg, 63 % yield).; ^{1}H-NMR (500 MHz, CD_3OD): 8.27 (s, 1H), 8.24 (d, *J* = 9 Hz, 1H), 7.87 (t, *J* = 8.5 Hz, 2H), 7.41 (t, *J* = 8.5 Hz, 1H), 7.30 (t, *J* = 8 Hz, 1H), 6.30 (m, 1H), 6.03 (d, *J* = 2 Hz, 1H), 4.20 (t, *J* = 6.25 Hz, 2H), 3.30 (m, 4H), 3.14 (t, *J* = 7.5 Hz, 2H), 2.40 (t, *J* = 7.25 Hz, 2H), 1.13 (t, *J* = 7.25 Hz, 6H). ^{13}C-NMR (125 MHz, CD_3OD): δ 166.8, 160.7, 153.4, 153.2, 141.0, 133.8, 129.7, 126.2, 124.7, 121.8, 121.3, 117.9, 109.1, 105.0, 93.8, 93.4, 66.7, 44.4, 24.9, 11.7. HR-MS (ESI): calcd. for $C_{23}H_{25}N_3O_4S_2Na$ $[M+Na]^+$ 494.1184, found 494.1184.

2. Studies of the Photophysical Properties of Probe 1-SO_3^-

(a) Absorption and fluorescence emission spectra of probe 1-SO_3^-

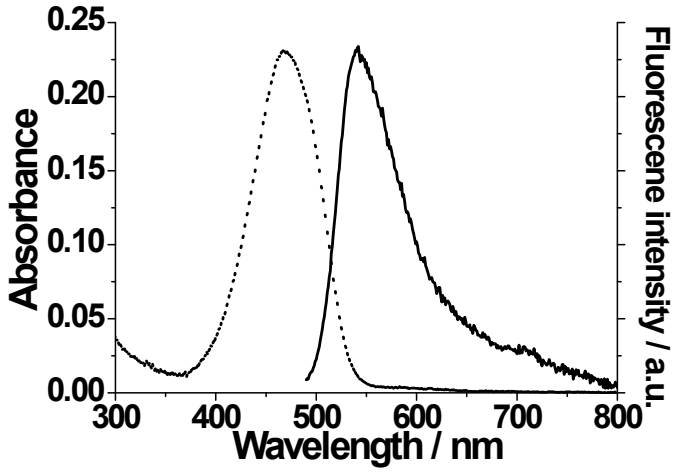


Figure S1. Absorption (dotted line) and emission spectra (solid line) of probe **1-SO_3^-** in HEPES buffer (10 mM, pH 7.4) at 25 °C. Excited at 470 nm. [**1-SO_3^-**] = 5 μM.

(b) Concentration-dependent emission spectra of probe 1-SO_3^-

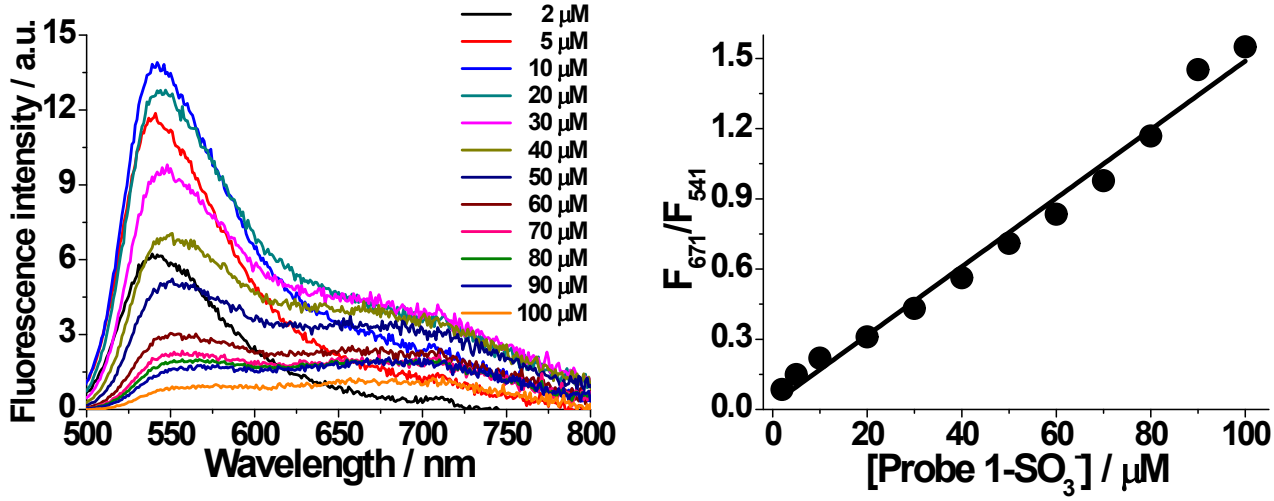


Figure S2. (left) Fluorescence emission spectra of probe **1-SO_3^-** at various concentrations (2 – 100 μM) in HEPES buffer (10 mM, pH 7.4) at 25 °C. Excited at 470 nm. (right) Plot of the fluorescence intensity ratio (F_{671}/F_{541}) vs. the concentration of probe **1-SO_3^-** (2 – 100 μM).

(c) Emission spectra of probe 1-SO_3^- as a function of pH

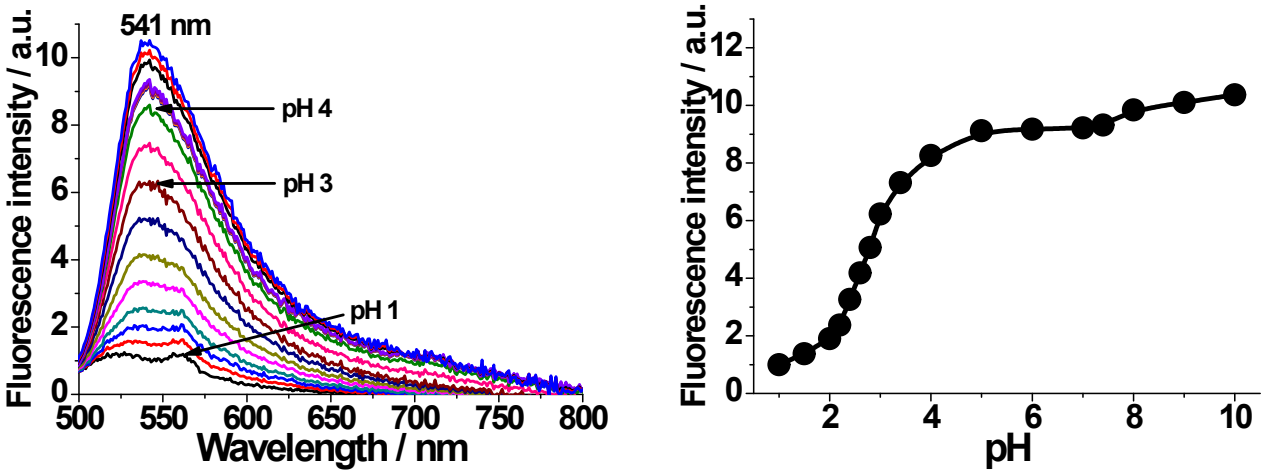


Figure S3. (left) Fluorescence emission spectra of probe **1-SO_3^-** in different pH buffer systems at 25 °C. (right) Relative fluorescence intensity at 541 nm of probe **1-SO_3^-** in different pH conditions. [**1-SO_3^-**] = 5 μM. Excited at 470 nm. pK_a of probe **1-SO_3^-** is estimated to be 2.35 ± 0.05.

3. Stability Studies of Probe 1-SO_3^-

To investigate the chemical stability of probe **1-SO_3^-**, fluorescence spectra of probe in aerated assay solution (10 mM HEPES buffer, pH 7.4) at 25 °C was recorded every two hour for 12 hours. The chemical stability of probe **1-SO_3^-** was quantified by monitoring the fluorescence intensity at 541 nm as a function of incubation time.

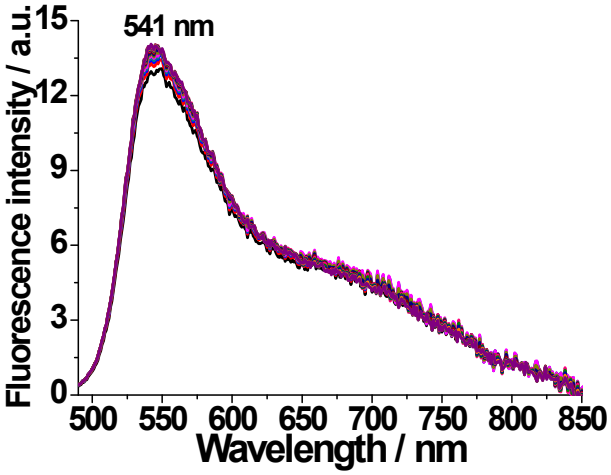


Figure S4. Chemical stability of probe **1-SO_3^-** in HEPES buffer (10 mM, pH = 7.4) at 25 °C. Excited at 470 nm. Fluorescence emission spectra were obtained every 2 hour (0 – 12 hours). [**1-SO_3^-**] = 20 μM.

4. Fluorescence Sensing Studies of Probe 1-SO_3^- toward Amine Analytes.

Amine analyte (i.e., spermine) was dissolved in HEPES buffer (10 mM, pH 7.4) and diluted with the buffer solution to make different spermine concentrations. Spermine solution (20 μL) was mixed with the probe **1-SO_3^-** dissolved in HEPES buffer solution (10 mM, pH 7.4, 1980 μL). Final concentration of probe **1-SO_3^-** in the solution was 20 μM, and the spermine concentration was 0–100 μM. The change in fluorescence intensity at 600 nm was recorded for different periods of incubation time at 25 ℃.

To find optimized fluorimetric assay conditions, effects of probe concentration, incubation time, pH, and assay media on fluorescence turn-on response were investigated.

(a) Effect of concentration of probe 1-SO_3^- on fluorescence turn-on responses toward spermine

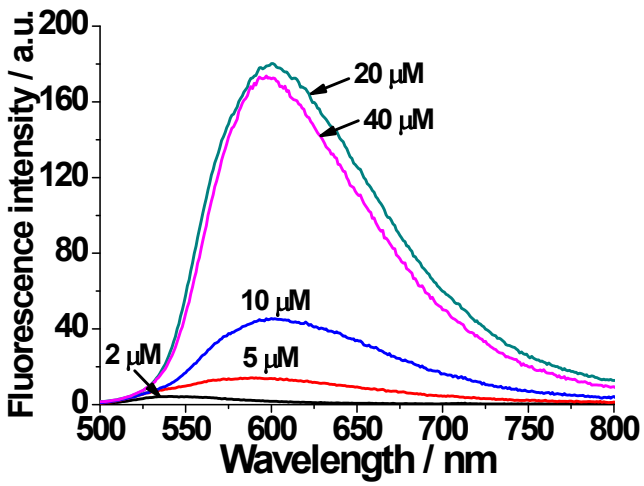


Figure S5. Comparison of fluorescence turn-on response of probe **1-SO_3^-** at different concentrations (2, 5, 10, 20, 40 μM) upon incubation with spermine (20 μM) in HEPES buffer (10 mM, pH = 7.4) at 25 °C. The emission spectra were obtained 20 min after the addition of spermine. Excited at 470 nm.

(b) Time-dependent fluorescence responses of probe 1-SO_3^- toward spermine at different concentrations

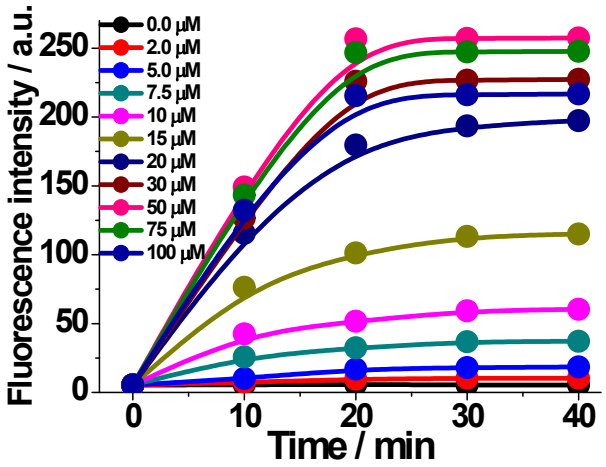


Figure S6. Time-dependent fluorescence intensity of probe **1-SO_3^-** (20 μM) upon incubation with a different amount of spermine (0-100 μM) in HEPES buffer (10 mM, pH = 7.4) at 25 °C. The spectra were obtained every 10 min (0 – 40 min) and fluorescence intensity at 600 nm was recorded. Excited at 470 nm.

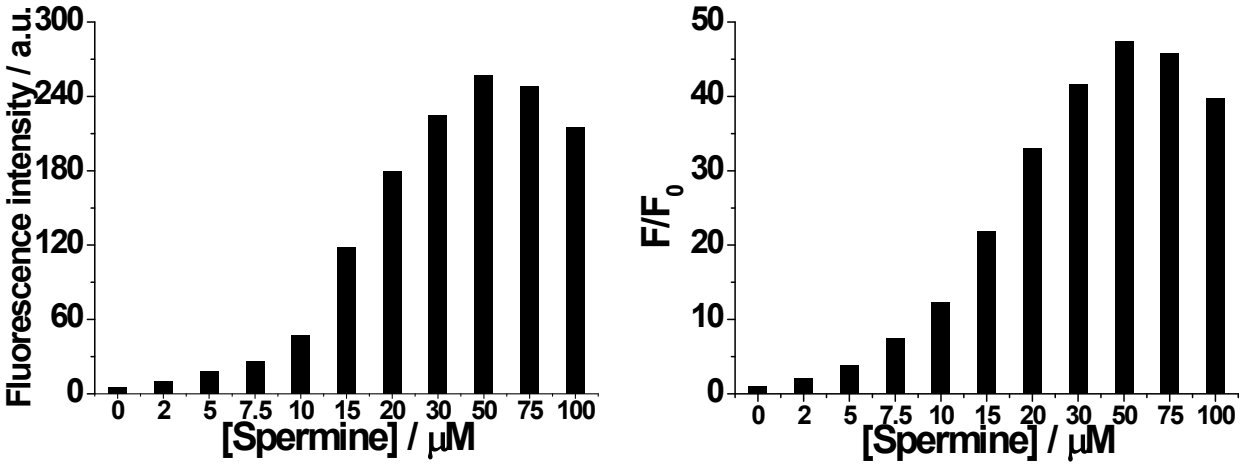


Figure S7. Relative fluorescence intensity of probe **1-SO_3^-** (20 μM) upon incubation with a different amount of spermine (0-100 μM) in HEPES buffer (10 mM, pH = 7.4) at 25 °C. The emission spectra were obtained 20 min after addition of spermine, and fluorescence intensity was measured at 600 nm. Excited at 470 nm.

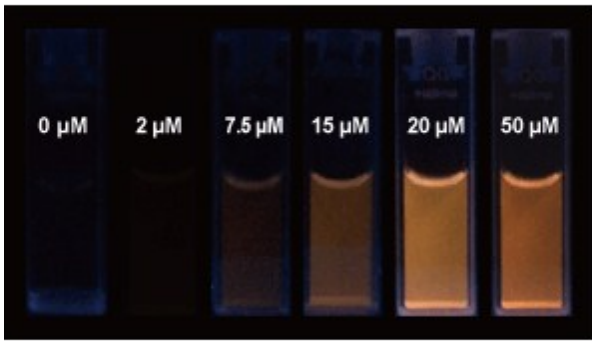


Figure S8. Photographs of probe **1-SO_3^-** (20 μM) in the presence of spermine at different concentrations (left to right: 0, 2 μM, 7.5 μM, 15 μM, 20 μM, 50 μM) after 30 min incubation under UV light (365 nm) illumination.

(c) Effect of pH on fluorescence turn-on responses of probe 1-SO_3^- toward spermine

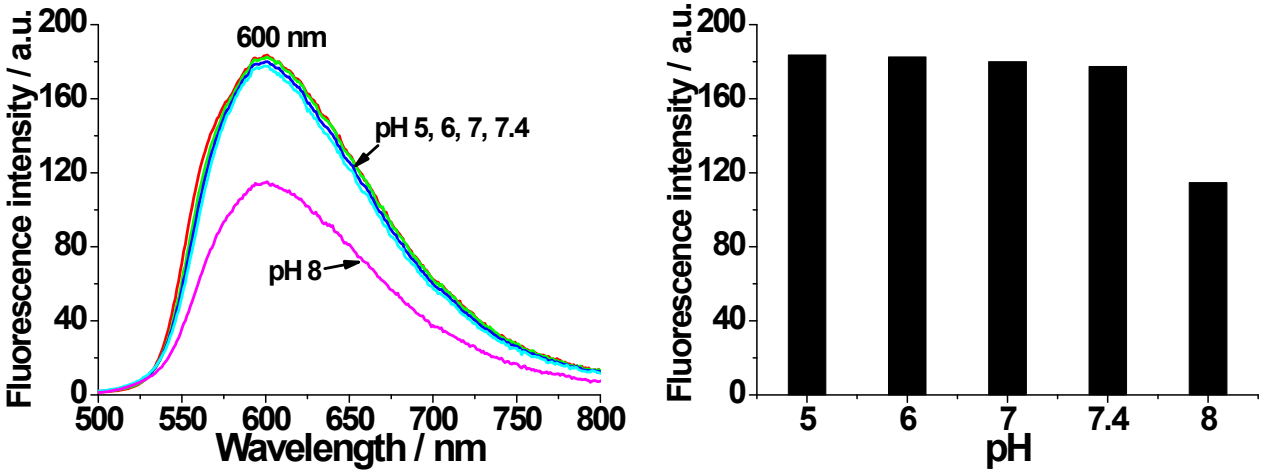


Figure S9. Fluorescence turn-on response of probe **1-SO_3^-** (20 μM) toward spermine (20 μM) in buffer system at different pH conditions (5, 6, 7, 7.4, 8) at 25 °C. The emission spectra were obtained 20 min after the addition of spermine, and fluorescence intensity was measured at 600 nm. Excited at 470 nm.

(d) Comparison of fluorescence turn-on responses of probe 1-SO_3^- in various assay media

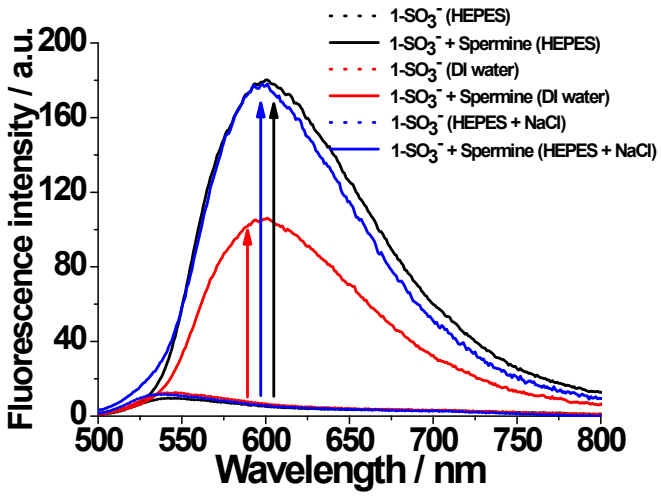


Figure S10. Comparison of fluorescence turn-on response of probe **1-SO_3^-** upon incubation with spermine (red) in deionized water, (black) in HEPES buffer (10 mM, pH = 7.4), and (blue) in HEPES buffer (10 mM, pH = 7.4) containing 150 mM NaCl at 25 °C. The emission spectra were obtained 20 min after the addition of spermine. Excited at 470 nm. [**1-SO_3^-**] = 20 μM. [spermine] = 20 μM.

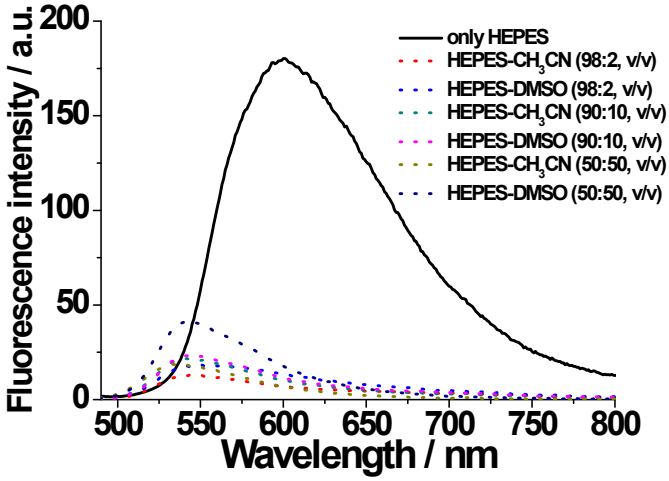


Figure S11. Comparison of fluorescence turn-on response of probe **1-SO_3^-** upon incubation with spermine (black solid line) in HEPES buffer (10 mM, pH = 7.4) and (dot lines) in HEPES buffer (10 mM, pH = 7.4) containing organic solvents (CH_3CN 2%, 10%, 50%; DMSO 2%, 10%, 50%) at 25 °C. The emission spectra were obtained 20 min after the addition of spermine. Excited at 470 nm. [**1-SO_3^-**] = 20 μM. [spermine] = 20 μM.

(e) Time-resolved photoluminescence decay dynamics of 1-SO_3^- without and with spermine

Fluorescence lifetime decays were measured using an inverted-type scanning confocal microscope (MicroTime-200, Picoquant, Germany) with a 20x objective. A 470 nm single-mode pulsed diode laser (~100 ps pulse width) was used as an excitation source. A dichroic mirror (490 DCXR, AHF), a long-pass filter (HQ500lp, AHF), a 50 μm pinhole, and a single photon avalanche diode (PDM series, MPD) were used to collect emissions from the samples. Time-correlated single-photon counting technique was used to obtain fluorescence decay curves as a function of time with a resolution of 16 ps. Exponential fittings for the obtained fluorescence decays were performed by the iterative least-squares deconvolution fitting using the Symphotime software (version 5.3).

Table S1. Fluorescence decay of **1-SO_3^-** only vs. **1-SO_3^-** with spermine in HEPES buffer (10 mM, pH 7.4)

1-SO_3^- without or with spermine	λ_{em} [nm]	τ_1 (f_1)[a] [ns]	τ_2 (f_2)[a] [ns]	τ_3 (f_3)[a] [ns]	τ_{avg}[b] [ns]	χ^2[c]
Only **1-SO_3^-**	542	0.07 (0.816)	0.39 (0.175)	1.89 (0.009)	0.14	1.841
1-SO_3^- + spermine	600	0.097 (0.613)	0.553 (0.331)	2.43 (0.056)	0.38	1.571

[a]Lifetime (τ) and fraction (A) of shorter (1) or longer (2 and 3) lived species. [b]The weighted mean lifetime. [c]Reduced χ^2 value.

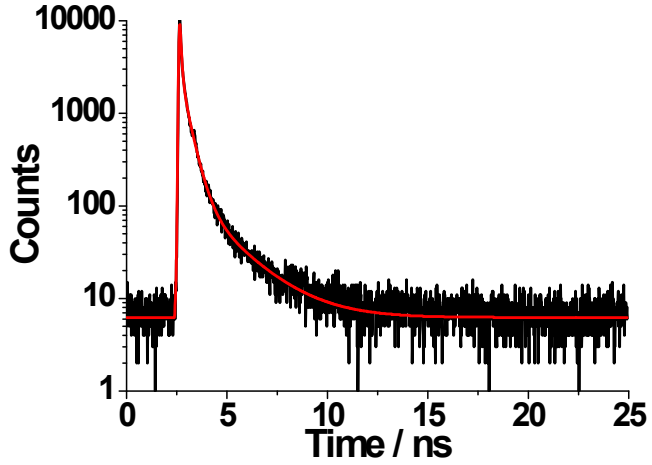


Figure S12. Time-resolved emission decays of probe **1-SO_3^-** in HEPES buffer (10 mM, pH 7.4). Probe **1-SO_3^-** tri-exponential decays gave lifetimes of 0.391 ns (17.5%), 0.07 ns (81.6%) and 1.89 ns (0.9%). Average lifetime of 0.14 ns. [**1-SO_3^-**] = 20 μM.

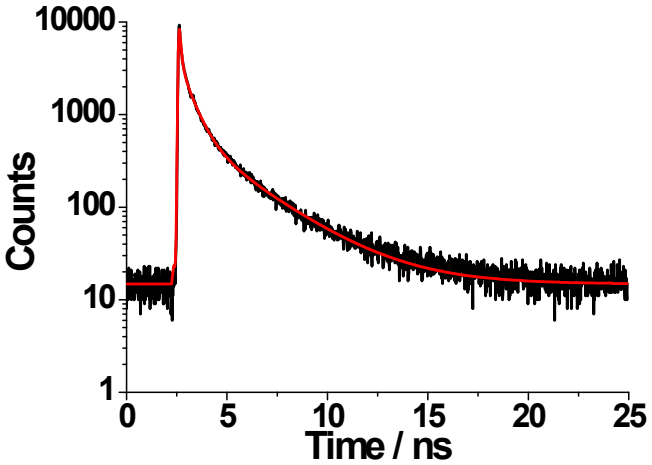


Figure S13. Time-resolved emission decays of probe **1-SO_3^-** upon addition of spermine in HEPES buffer (10 mM, pH 7.4). Probe **1-SO_3^-**-spermine tri-exponential decays gave lifetimes of 0.553 ns (33.1%), 0.097 ns (61.3%) and 2.43 ns (5.6%). Average lifetime of 0.38 ns. [**1-SO_3^-**] = 20 μM. [spermine] = 20 μM. Incubation time = 20 min.

(f) Determination of detection limit of probe 1-SO_3^- toward spermine

The fluorescence emission spectra of probe **1-SO_3^-** in HEPES buffer (10 mM, pH 7.4) were collected for 25 times to determine the background noise σ. Then fluorescence turn-on response of probe **1-SO_3^-** upon treatment with a different amount of spermine were monitored. The value were obtained 20 min after the addition of spermine. A linear regression curve was then fitted (R^2=0.998) according to fluorescence intensities at 600 nm as a function of amount of spermine in the range of 0 to 5 μM, and the slope of the curve was obtained. The detection limit (3σ/slope) was then determined to be 0.6 μM for probe **1-SO_3^-**.

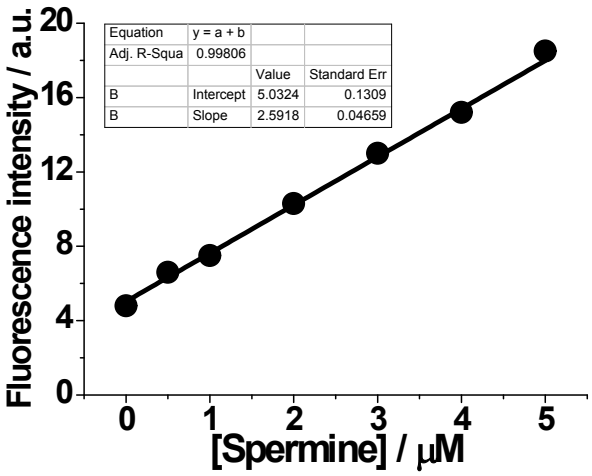


Figure S14. A linear relationship between fluorescence intensity at 600 nm and amounts of spermine (0–5 μM). Fluorescence intensity of probe **1-SO_3^-** (20 μM) was measured 20 min after the addition of spermine in HEPES buffer (10 mM, pH 7.4) at 25 °C. Excited at 470 nm. Detection limit = 0.6 μM for spermine.

(g) Fluorescence turn-on responses of probe 1-SO_3^- toward spermidine at different concentrations

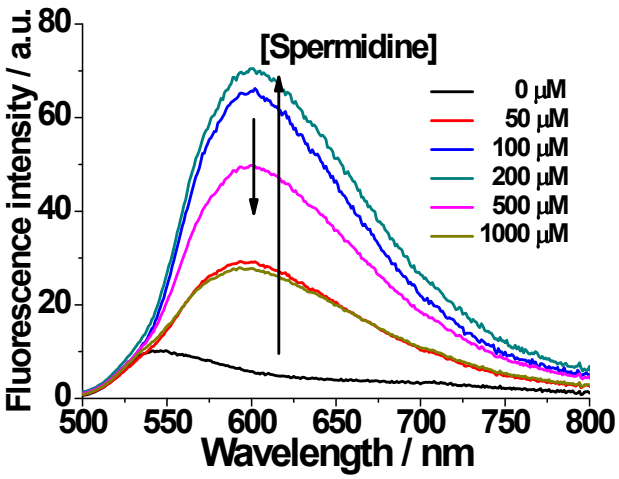


Figure S15. Fluorescence turn-on response of probe **1-SO_3^-** (20 μM) upon incubation with spermidine at different concentrations (0, 50, 100, 200, 500, 1000 μM) in 10 mM HEPES buffer (10 mM, pH = 7.4) at 25 °C. The emission spectra were obtained 20 min after the addition of spermidine. Excited at 470 nm.

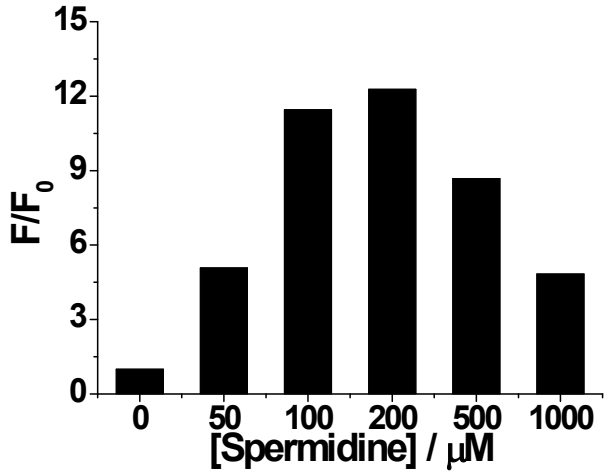


Figure S16. Relative fluorescence intensity of probe **1-SO_3^-** (20 μM) upon incubation with spermidine at different concentrations (0, 50, 100, 200, 500, 1000 μM) in HEPES buffer (10 mM, pH = 7.4) at 25 °C. The spectra were obtained 20 min after addition of spermidine, and fluorescence intensity was measured at 600 nm. Excited at 470 nm.

(h) Selectivity studies of probe 1-SO_3^- toward various amines

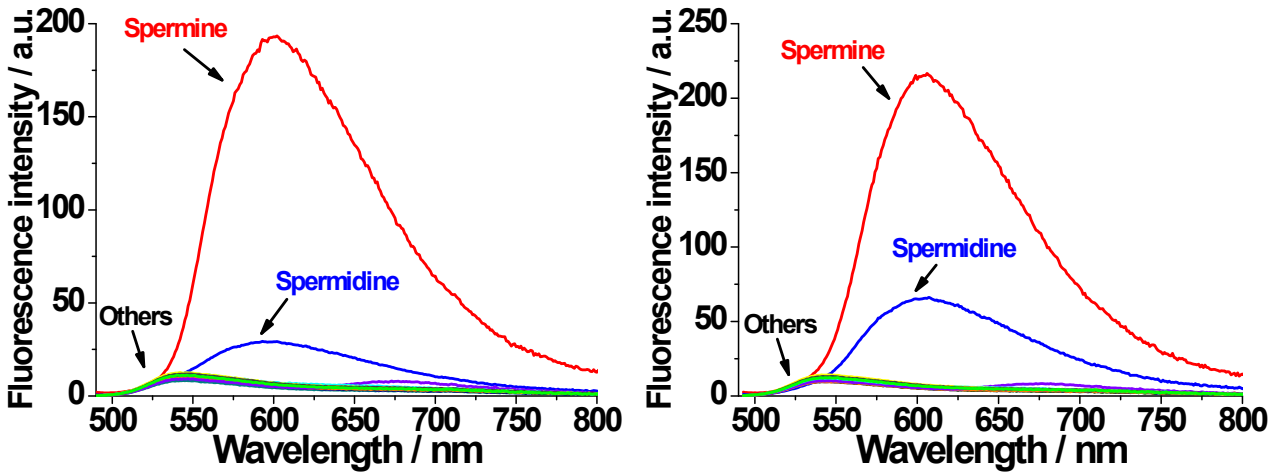


Figure S17. Fluorescence spectra of probe **1-SO_3^-** (20 μM) upon incubation with various amine analytes (left 50 μM; right 100 μM). Fluorescence spectra were obtained 20 min after the addition of each analyte (spermine, spermidine, 1,2-ethylenediamine, 1,3-propanediamine, 1,4-butanediamine, 1,8-octanediamine, 1-propanamine, 1-butanamine, 1-hexanamine, 1-octanamine, lysine, benzylamine, serotonin, dopamine, tyramine, creatinine, sarcosine) to **1-SO_3^-** in HEPES buffer (10 mM, pH = 7.4) at 25 °C. Excited at 470 nm.

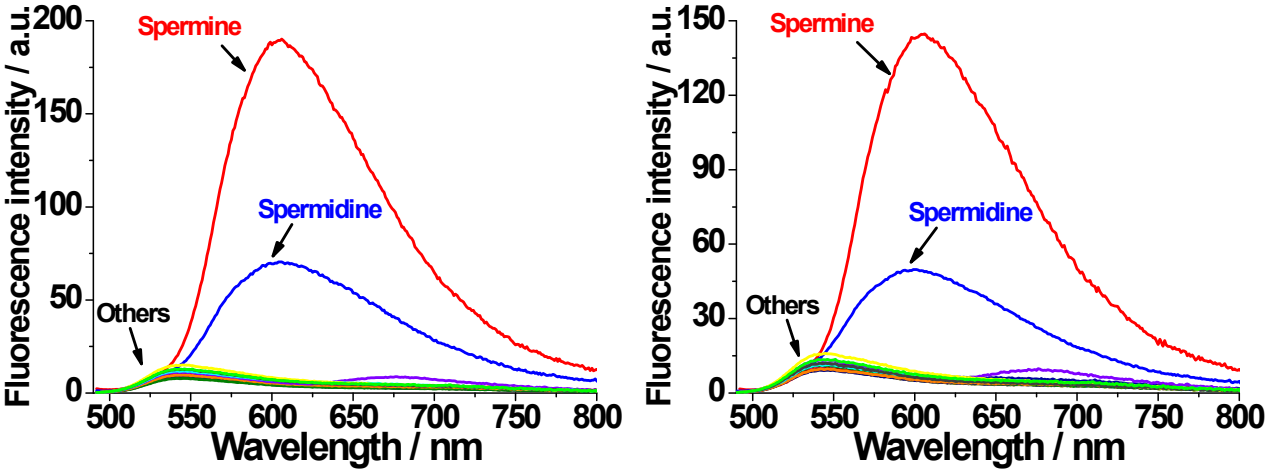


Figure S18. Fluorescence spectra of probe **1-SO_3^-** (20 μM) upon incubation with various amine analytes (left 250 μM; right 500 μM). Fluorescence spectra were obtained 20 min after the addition of each analyte (spermine, spermidine, 1,2-ethylenediamine, 1,3-propanediamine, 1,4-butanediamine, 1,8-octanediamine, 1-propanamine, 1-butanamine, 1-hexanamine, 1-octanamine, lysine, benzylamine, serotonin, dopamine, tyramine, creatinine, sarcosine) to **1-SO_3^-** in HEPES buffer (10 mM, pH = 7.4) at 25 °C. Excited at 470 nm.

(i) Interference study of spermine detection in the presence of other amines

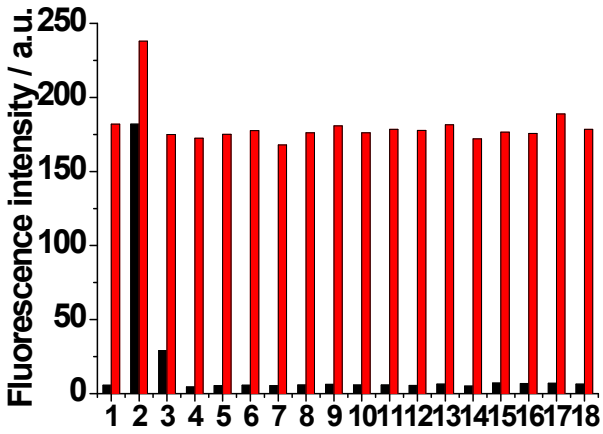


Figure S19. Relative fluorescence response of probe **1-SO_3^-** (20 μM) upon incubation with various amine analytes (20 μM for all analytes, black bar), and relative fluorescence response of **1-SO_3^-** (20 μM) upon incubation with 20 μM spermine in the presence of each amine analyte (20 μM, red bar) in HEPES buffer (10 mM, pH = 7.4) at 25 °C. Fluorescence intensity at 600 nm was measured. Excited at 470 nm. Incubation time = 20 min. Left to right: probe **1-SO_3^-** (1) spermine (2), spermidine (3), 1,2-ethylenediamine (4), 1,3-propanediamine (5), 1,4-butanediamine (6), 1,8-octanediamine (7), 1-propanamine (8), 1-butanamine (9), 1-hexanamine (10), 1-octanamine (11), lysine (12), benzylamine (13), tyramine (14), serotonin (15), dopamine (16), creatinine (17), sarcosine (18).

(j) Confirmation of aggregation formation of probe 1-SO_3^- upon addition of spermine

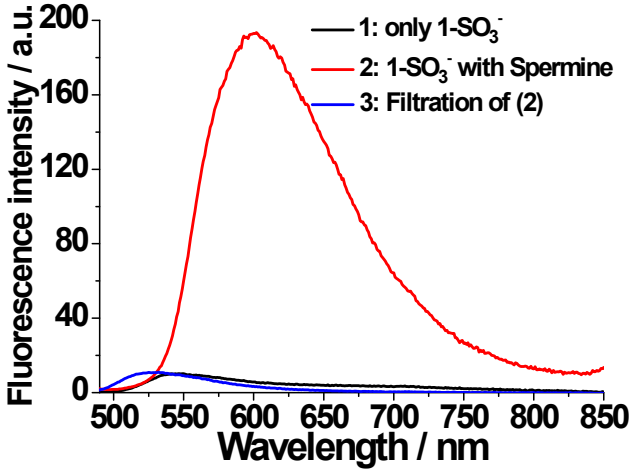


Figure S20. Fluorescence emission spectra of probe **1-SO_3^-** (black, 20 μM) upon 30 min incubation with 1 equiv. spermine before filtration (red) and after filtration (blue) in HEPES buffer (10 mM, pH 7.4) at 25 °C. Excited at 470 nm. Whatman syringe filter (pore size: 0.2 μm, diameter: 13 mm) was used for filtration.

(k) Identification of aggregation formation of probe 1-SO_3^- upon addition of spermine

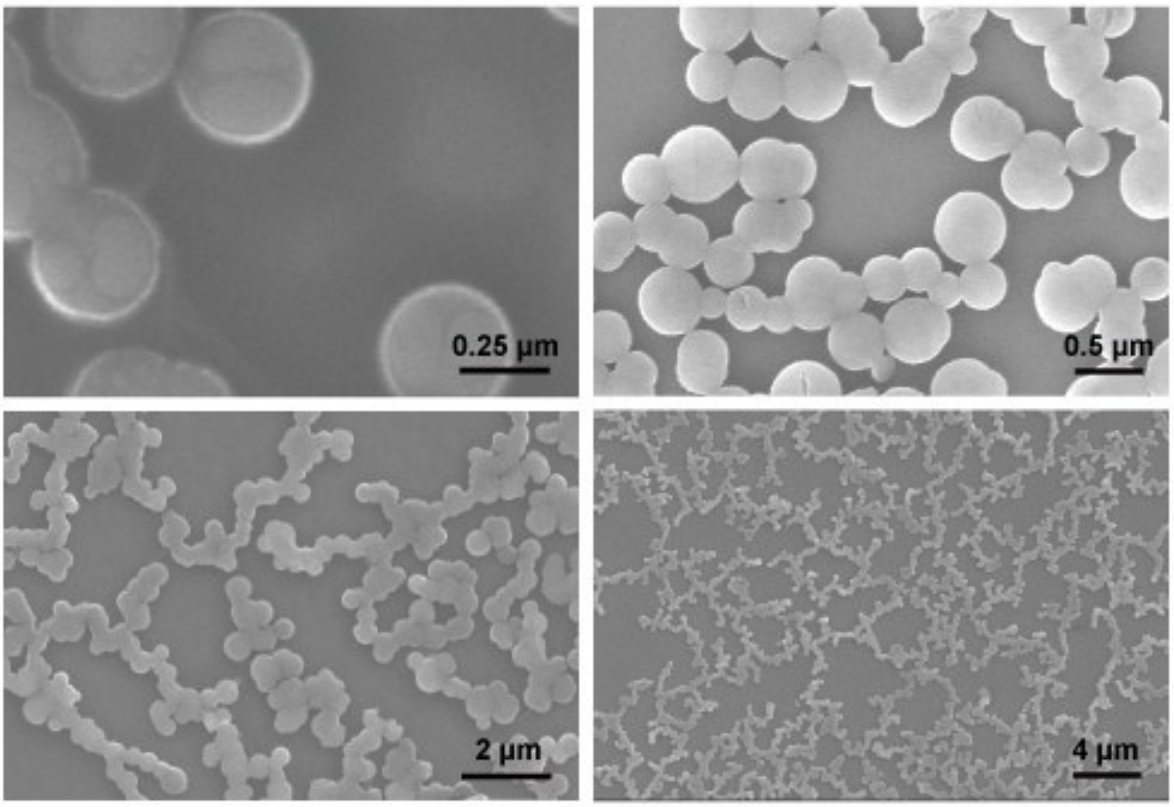


Figure S21. SEM image of the dried aggregates, formed from a solution of probe **1-SO_3^-** (20 μM) upon addition of spermine (20 μM) in HEPES buffer (10 mM, pH = 7.4) at 25 °C. Incubation time = 20 min.

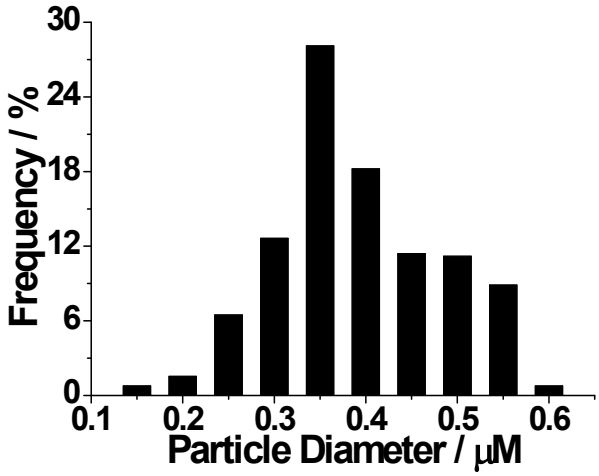


Figure S22. Size distribution of the dried aggregates, formed from a solution of probe **1-SO_3^-** (20 μM) upon addition of spermine (20 μM) in HEPES buffer (10 mM, pH = 7.4) at 25 °C. Average diameter of aggregates is 350 ± 50 nm.

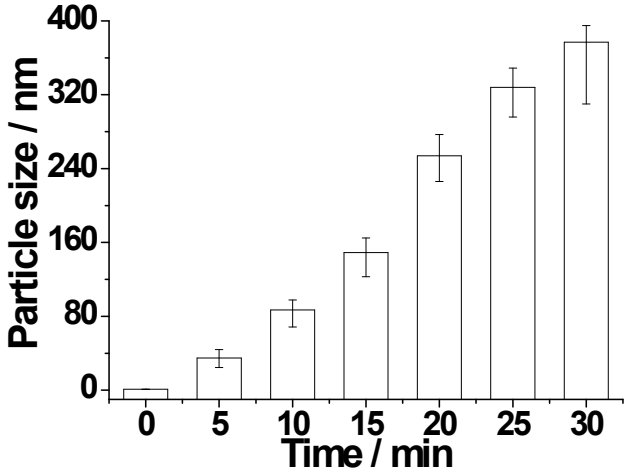


Figure S23. Time-dependent size distribution of the aggregates of probe **1-SO_3^-** (20 μM) upon addition of spermine (20 μM) in HEPES buffer (10 mM, pH = 7.4) at 25 °C by dynamic light scattering (DLS) measurement.

(l) Binding stoichiometry of probe 1-SO_3^- with spermine and spermidine

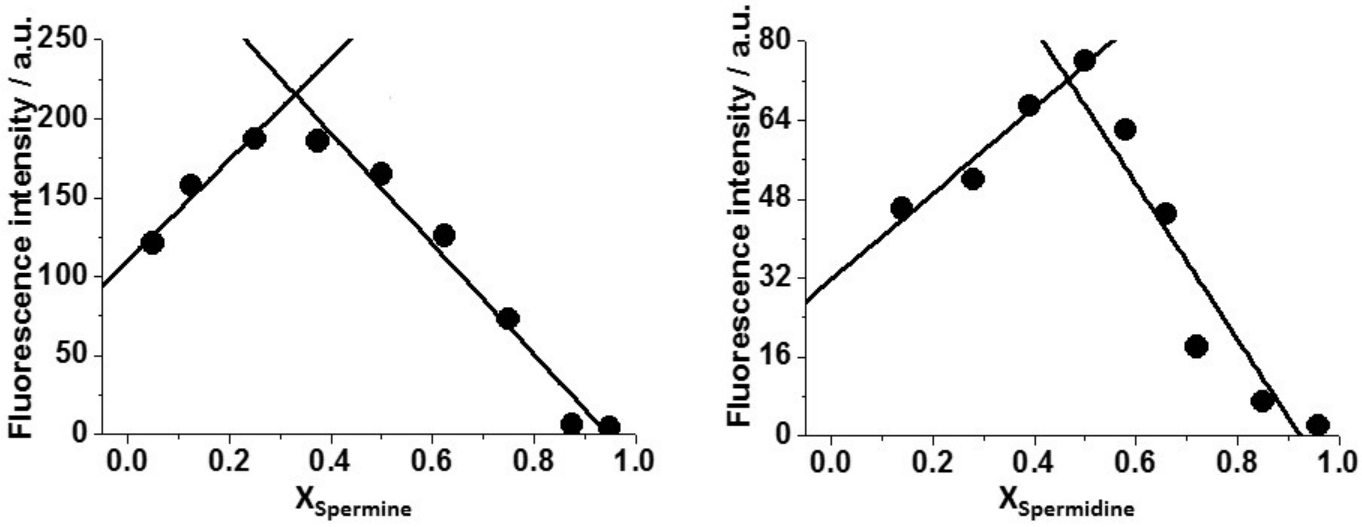


Figure S24. (left) Job's plot of a 2:1 complex of probe **1-SO_3^-** and spermine, where the fluorescence at 600 nm is plotted against the mole fraction of spermine at an invariant total concentration of 40 μM in HEPES buffer (10 mM, pH 7.4). (right) Job's plot of a 1:1 complex of probe **1-SO_3^-** and spermidine, where the fluorescence at 600 nm is plotted against the mole fraction of spermidine at an invariant total concentration of 140 μM in HEPES buffer (10 mM, pH 7.4). Incubation time = 20 min.

5. Application of Probe 1-SO_3^- with Spermine Present in Artificial Urine Sample

According to the method reported previously,[2] an artificial urine solution was prepared by mixing 0.85 g ammonium phosphate dibasic, 0.12 g calcium chloride, 0.75 g creatine, 0.25 g magnesium chloride hexahydrate, 1.01 g potassium chloride, 1.01 g sodium sulfate, and 0.38 g urea in deionized water (500 mL). The pH of the solution was adjusted to 6.0. Solutions of spermine at various concentrations (0-100 μM) were prepared in the artificial urine solution. The fluorescence turn-on responses toward spermine in artificial urine were measured 20 min after the addition of spermine.

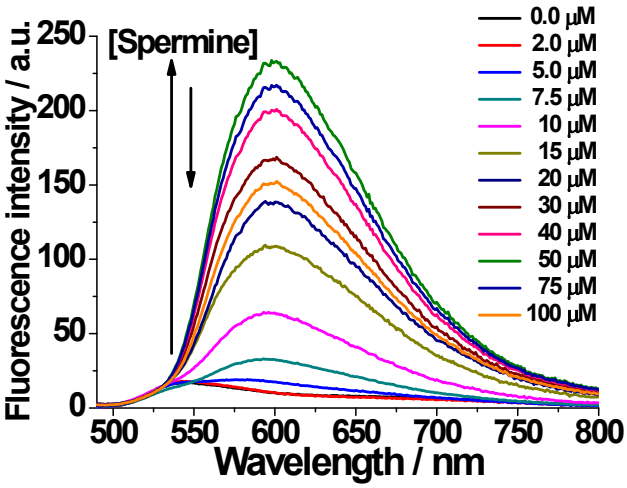


Figure S25. Fluorescence emission spectra of probe **1-SO_3^-** (20 μM) upon incubation with spermine at different concentrations (0-100 μM) in artificial urine solutions at 25 °C. The spectra were obtained after incubation for 20 min. Excited at 470 nm.

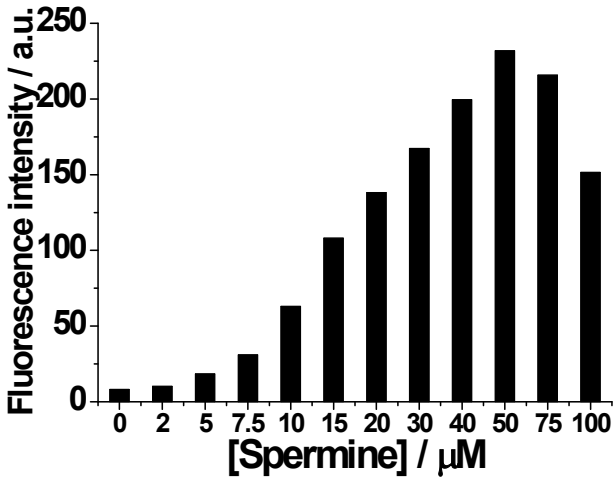


Figure S26. Relative fluorescence intensity of probe **1-SO_3^-** (20 μM) upon incubation with a different amount of spermine (0-100 μM) in artificial urine solutions at 25 °C. The spectra were obtained 20 min after addition of spermine, and fluorescence intensity was measured at 600 nm. Excited at 470 nm.

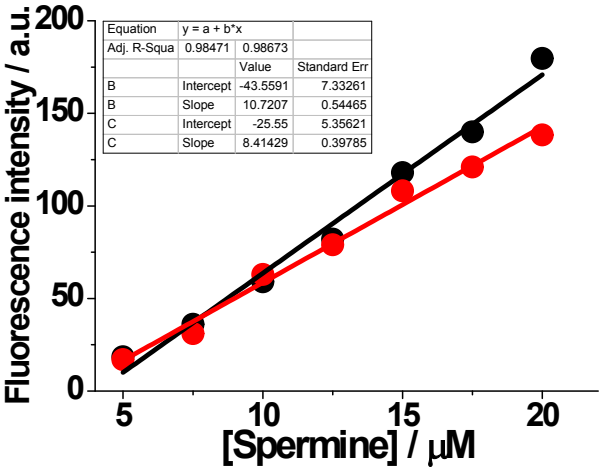
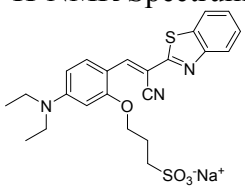
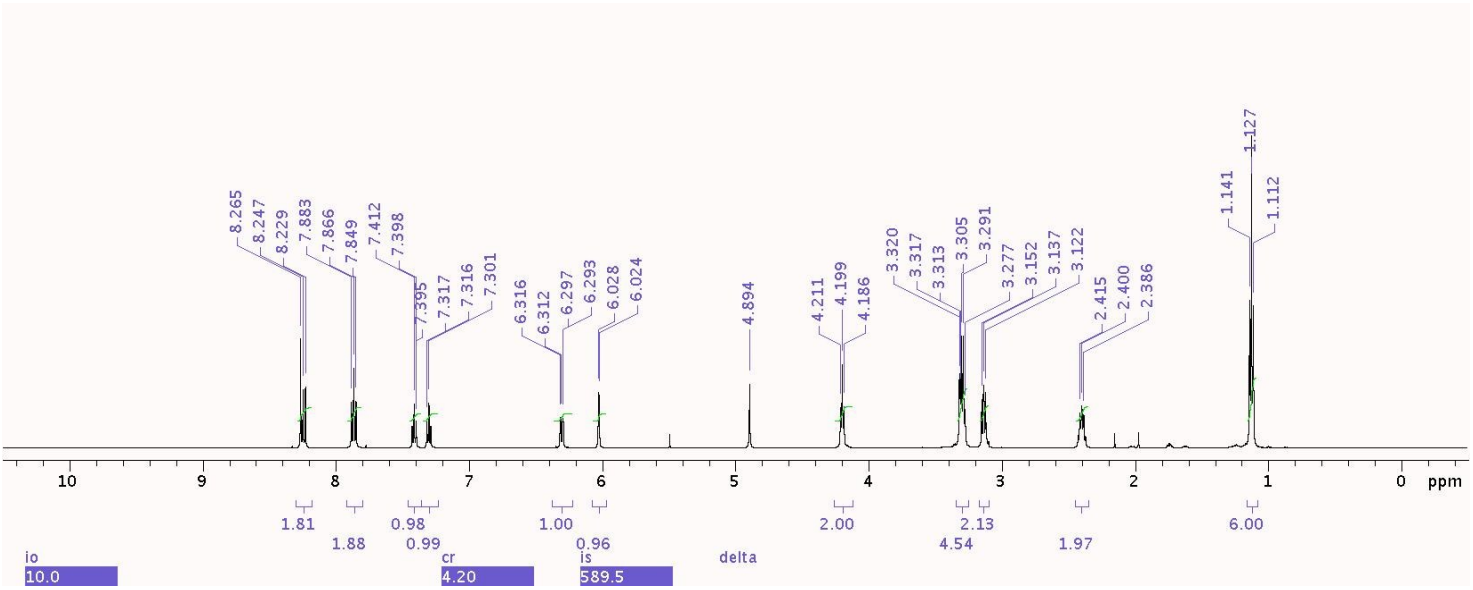


Figure S27. A linear relationship between fluorescence intensity at 600 nm and amounts of spermine (5–20 μM). Fluorescence intensity of probe **1-SO_3^-** (20 μM) was measured 20 min after the addition of spermine in 10 mM HEPES buffer (black line) and in artificial urine (red line) at 25 °C.

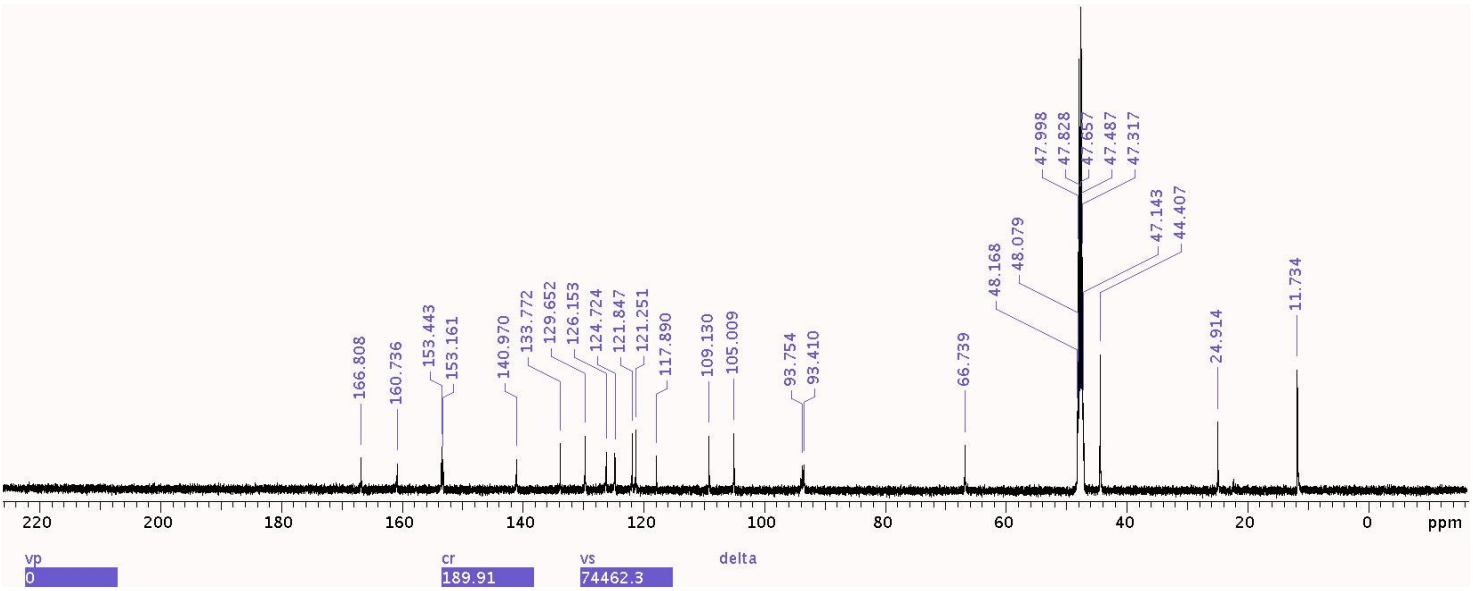
6. ^{1}H-NMR and ^{13}C-NMR Spectra

^{1}H-NMR Spectrum of probe **1-SO_3^-** in CD_3OD (500 MHz):





^{13}C-NMR Spectrum of probe **1-SO_3^-** in CD_3OD (125 MHz):



7. References

1. A. T. R. Williams, S. A. Winfield, J. N. Miller, *Analyst,* 1983, **108**, 1067.
2. J. Y. Lock, E. Wyatt, S. Upadhyayula, A. Whall, V. Nuñez, V. I. Vullev and H. Liu, *J. Biomed. Mater. Res. Part A*, 2014, **102**, 781.