Supplementary Information

An Aqueous Red Emitting Fluorescent Fluoride Sensing Probe Having a Large Stokes Shift and Its Application in Cell Imaging

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Materials and instruments:

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments. NMR spectra were recorded on a BRUKER 400 spectrometer, using TMS as an internal standard. All accurate mass spectrometric experiments were performed on a micrOTOF-Q II mass spectrometer (BrukerDaltonik, Germany). UV-Vis absorption spectra were measured using a Shimadzu UV-2450 spectrophotometer. Emission spectra were recorded at room temperature using a HITACHI F4600 fluorescence spectrophotometer with both the excitation and emission slit widths set at 5.0 nm. Cell imaging was performed with a Nikon C1si inverted microscope. TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from Qingdao Ocean Chemicals.

Preparation of test solutions:

The stock solution of probe **1** was prepared at 0.1 mM in acetonitrile. The solutions of various tested analytes were prepared by dissolving NaCl, NaBr, NaI, tetrabutylammonium cyanide, $NaNO_3$, $NaHSO_4$, $NaClO_4$, CH_3COONa, NaSCN, NaN_3, Cys, BSA, Na_2CO_3, Na_2SO_4, GSH and NaF in twice-distilled water. The test solution of the probe **1** (10 μM) in 3 mL 35 mM HEPES buffer (pH 7.4) was prepared by placing 0.3 mL stock solution of probe **1** (10 μM) and 0.6 mL acetonitrile in 2.1 mL HEPES buffer. The resulting solution was shaken well and incubated with appropriate testing species for 10 min at room temperature before recording the spectra. Unless otherwise noted, for all measurements, the excitation wavelength was 470 nm, the excitation slit widths were 5.0 nm, and emission slit widths were 5.0 nm.

Cell culture and fluorescence imaging:

HaCaT cells were seeded in a 6-well plate in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin. The cells were incubated under an atmosphere of 5% CO_2 and 95% air at 37°C for 24 h. Before the experiments, cells were washed with HEPES buffered solution. HaCaT cells were treated with a solution of probe **1**(5 μM in HEPES buffer containing 1% DMSO) and incubated at 37°C for 10 min. After washing three times with HEPES buffered solution, the pretreated HaCaT cells were incubated with NaF (500 μM) at 37°C for 20 min. Fluorescence imaging was performed after washing the cells three times with HEPES buffered solution.

Synthesis of 1, 4-diethyl-7-(*tert*-butyldimethylsilyloxy)-

1, 2, 3, 4-tetrahydroquinoxalin-6-carbaldehyde.

To a round-bottom flask (50 mL) equipped with a magnetic stirrer was added 1, 4-diethyl-1, 2, 3, 4-tetrahydro-7-hydroquinoxalin-6-carbaldehyde[1] (23.4 mg, 0.1 mmol), DMAP (16 mg, 0.13 mmol) and one drop of triethylamine in dry CH_2Cl_2 (10 mL). After stirring the reaction mixture for 5 min, *tert*-butyl dimethylchlorosilane (20 mg, 0.13 mmol) was added and the resulting solution was stirred at room temperature for 1 hour. Next, 30 mL water was added and the organic layer was separated and dried over anhydrous Na_2SO_4. The drying agent was removed by filtration and the solvent was removed by distillation. The obtained residue was purified by silica gel column chromatography (3:1 petroleum: ethyl acetate as eluent) to yield the product as a yellow oil (30 mg, 86%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 10.14 (d, *J* = 7.2 Hz, 1H), 6.99 (d, *J* = 27.2 Hz, 1H), 5.91 (d, *J* = 15.7 Hz, 1H), 3.35 (t, *J* = 65.5 Hz, 8H), 1.20 (dt, *J* = 15.9, 7.1 Hz, 6H), 1.03 (s, 9H), 0.25 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 187.31, 154.98, 143.22, 129.54, 116.14, 107.95, 100.02, 47.78, 45.53, 45.23, 44.83, 29.70, 25.77, 18.34, 10.68, 10.06. HRMS (EI) m/z calcd for $[C_{19}H_{32}N_2O_2Si + H]^+$: 349.2311, Found : 349.2310.

Synthesis of probe 1.

To a stirred solution of 1, 4-diethyl-7-(*tert*-butyldimethylsilyloxy)-1, 2 , 3, 4-tetrahydro-quinoxalin-6-carbaldehyde (34.8 mg, 0.1 mmol) and malononitrile (6.6 mg, 0.1mmol) in 5 mL ethanol was added one drop of piperidine. The reaction mixture was allowed to stir at room temperature for 30 min. The solvent was evaporated under reduced pressure. The obtained residue was purified by silica gel column chromatography (1:1 petroleum: dichoromethane as eluent) to yield the product as a yellow solid (21 mg, 53%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (s, 1H), 7.43 (s, 1H), 5.92 (s, 1H), 3.59 – 3.52 (m, 2H), 3.35 (dq, *J* = 25.8, 7.1 Hz, 4H), 3.23 – 3.17 (m, 2H), 1.35 – 1.13 (m, 6H), 1.01 (s, 9H), 0.24 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.80, 150.59, 144.68, 129.78, 117.58, 116.38, 112.19, 107.15, 99.13, 66.89, 48.18, 46.02, 45.44, 44.59, 29.71, 25.75, 18.38, 10.88, 9.84. HRMS (EI) m/z calcd for $[C_{22}H_{32}N_4OSi]^+$: 396.2340, Found : 396.2345.

Reference:

1. Amit R. Jagtap, Vijay S. Satam, Rajkumar N. Rajule, Vinod R. Kanekar. *Dyes and pigments*, 2009, 82, 84-89.

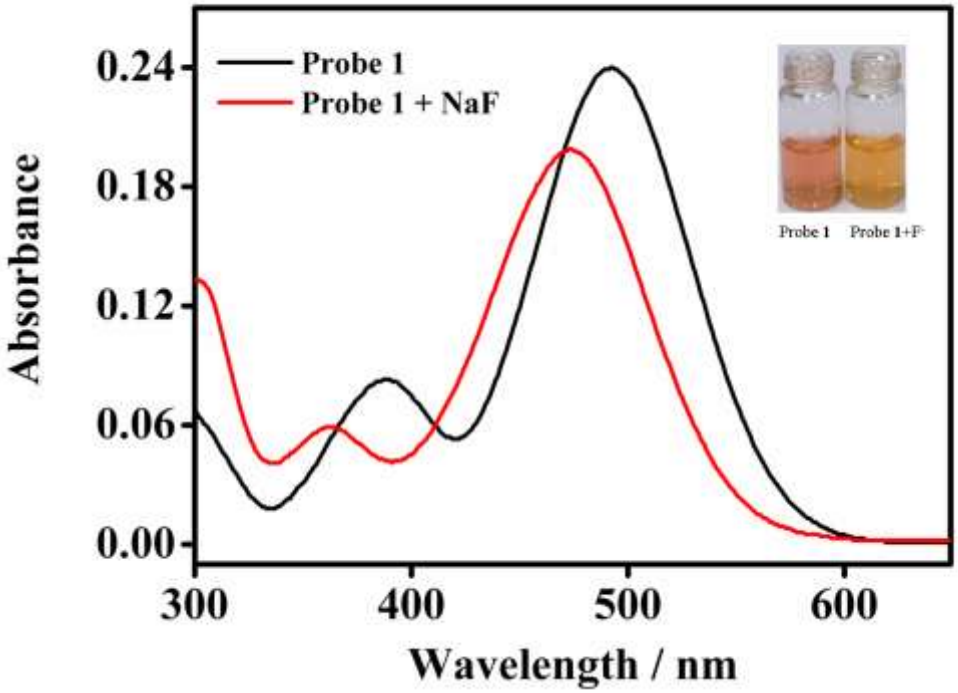

Figure S1. UV-Vis absorption spectra of probe **1** (10 μM) and the reaction product of probe **1** with NaF (1 mM) in 7.4 HEPES buffer (pH = 7.4, containing 30% CH_3CN, v/v).

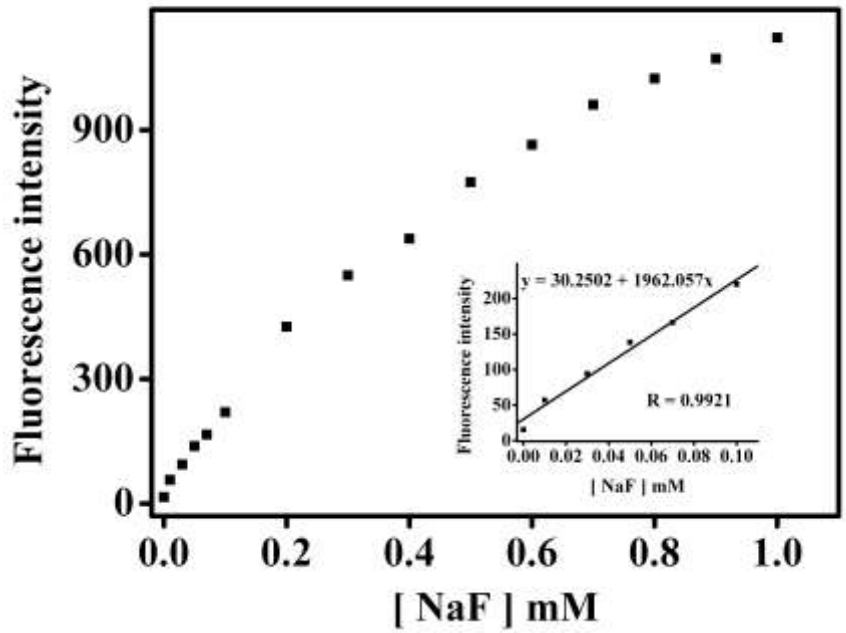

Figure S2.The changes of fluorescence intensity of probe **1** (10 μM) with different amount of NaF (0.0-1.0 mM) in 7.4 HEPES buffer (pH = 7.4, containing 30% CH_3CN, v/v) after incubated for 10 min. Insert: Enlarged display of the linear relationship between fluorescence intensity and the low concentrations of NaF (0.0-0.1 mM).

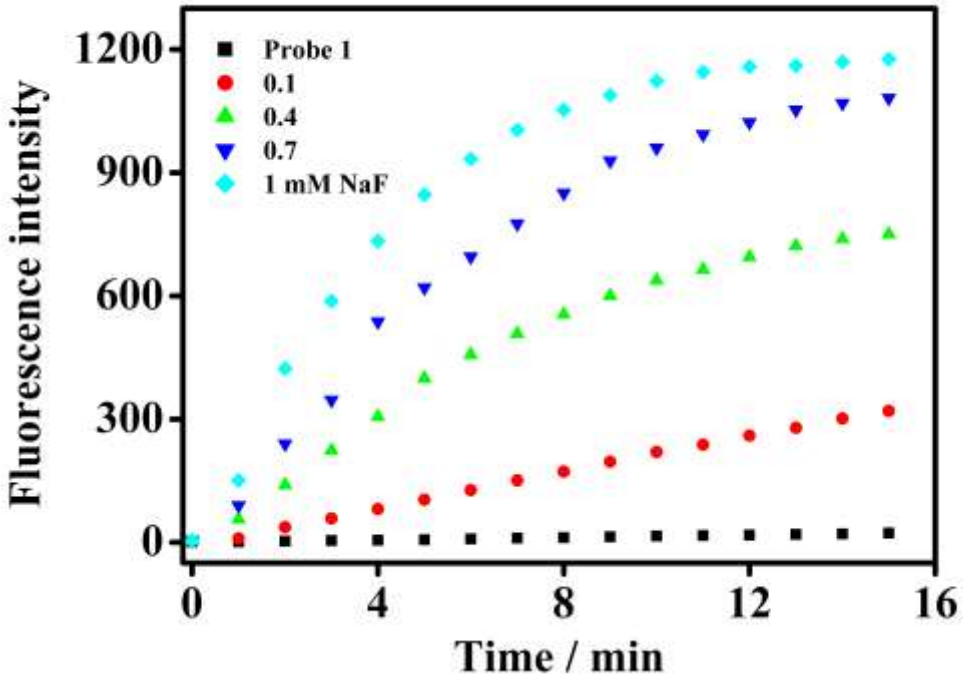

Figure S3 Kinetics of fluorescence enhancement rate at 616 nm for probe **1** (10 μM) with different concentrations of NaF (0.0-1.0 mM) in HEPES buffer (pH = 7.4, containing 30% CH_3CN, v/v).

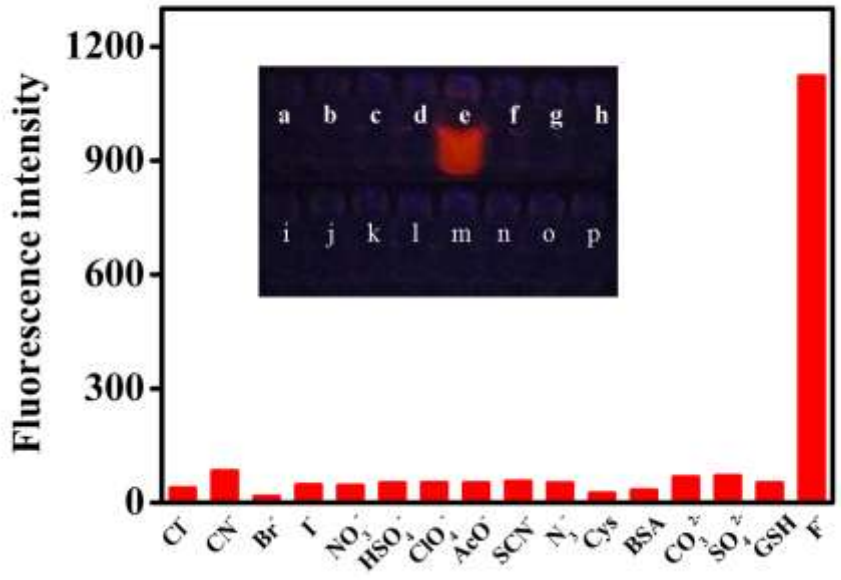

Figure S4. The changes of fluorescence intensity of probe **1** (10 μM) upon the addition various tested analytes (1.0 mM) (a. Cl-, b. CN^-; c. Br^-; d. I^-; e. F^-; f. NO_3^-; g. HSO_4^-; h. ClO_4^-; i. AcO^-; j. SCN^-; k. N_3^-; l. Cys; m. BSA; n. CO_3^{2-}; o. SO_4^{2-}; p. GSH) in 7.4 HEPES buffer (pH = 7.4, containg 30% CH_3CN, v/v) after incubated for 10 min. Insert: the changes of fluorescence color for probe **1** with various tested analytes under UV light irradiation.

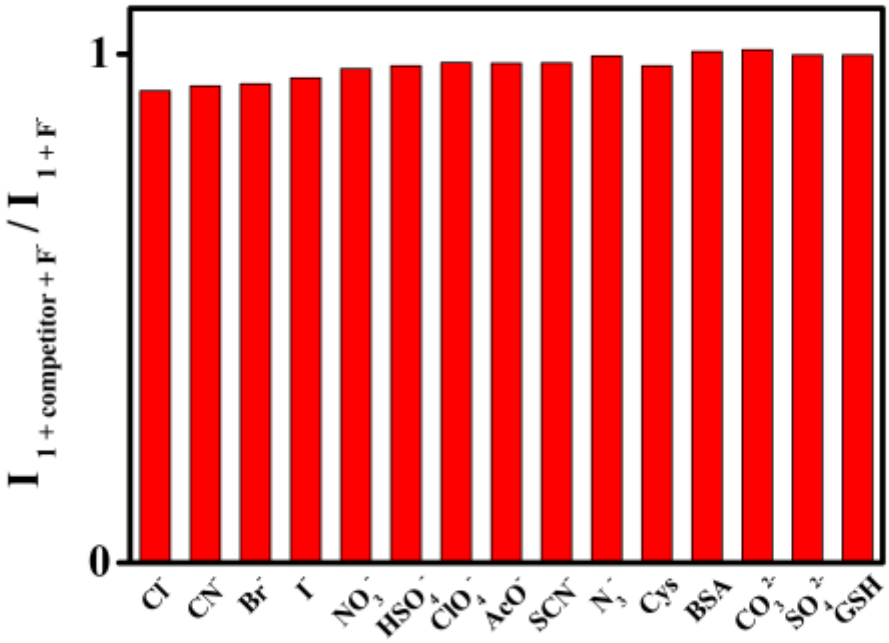

Figure S5. Interfering effect of various tested analytes on the fluorescence intensity of probe **1** (10 μM) in response to NaF (1 mM) in 7.4 HEPES buffer (pH = 7.4, containg 30% CH_3CN, v/v) after incubated for 10 min.

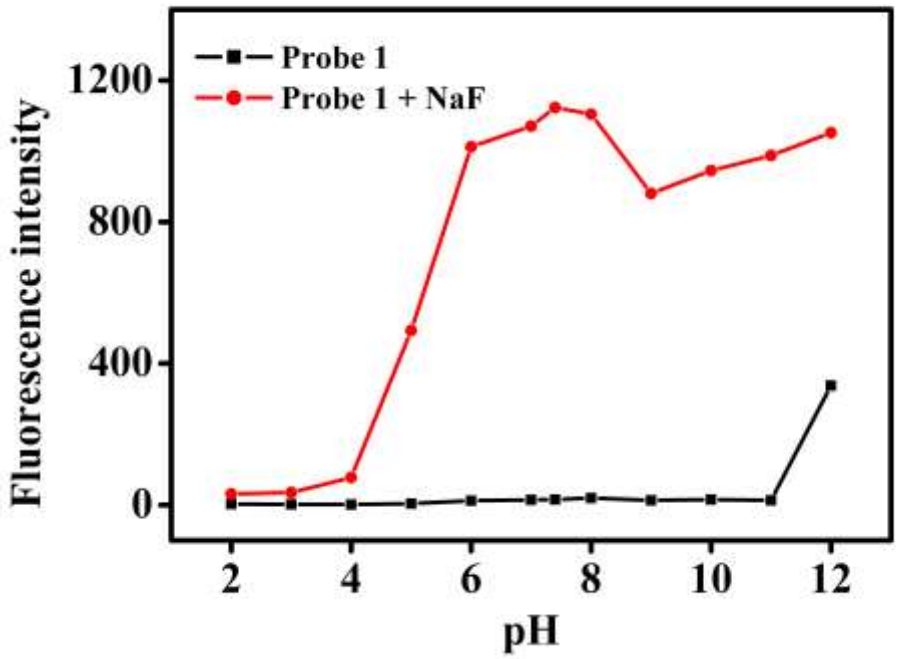

Figure S6. pH effect on the fluorescence intensity at 616 nm of probe **1**(1 mM) and probe **1** (1 mM) with NaF (1 mM) in 7:3 H_2O-CH_3CN solution (v/v) after incubated for 10 min.

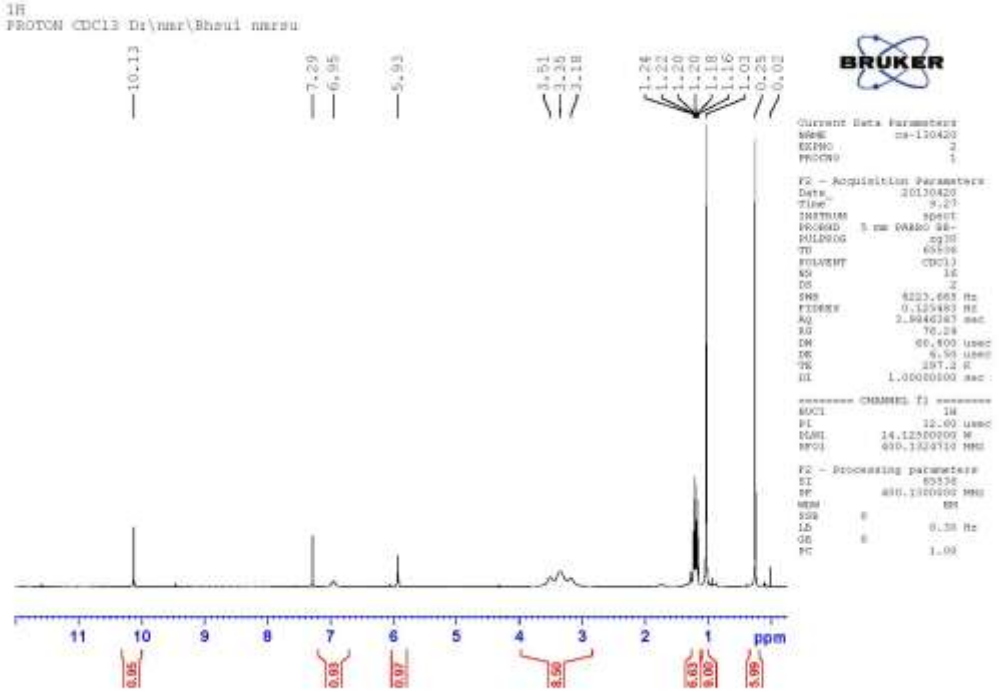

Figure S7. ^{1}H NMR spectrum of 1, 4-diethyl-7-(*tert*-butyldimethylsilyloxy)-1, 2, 3, 4-tetrahydro-quinoxalin-6-carbaldehyde in $CDCl_3$.

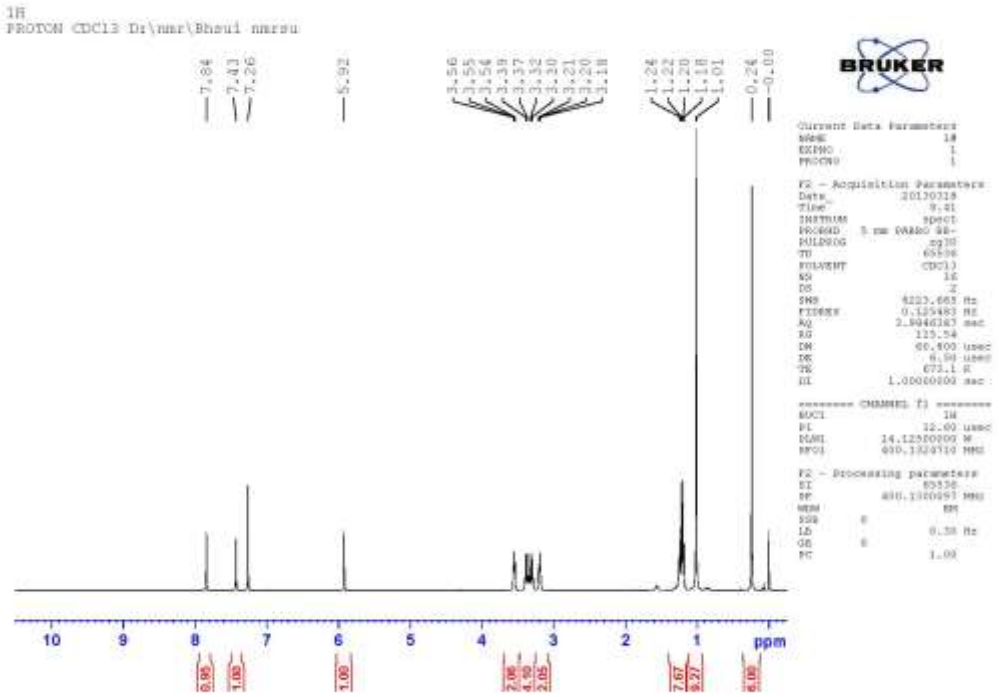

Figure S8. ^{1}H NMR spectrum of probe **1** in $CDCl_3$.

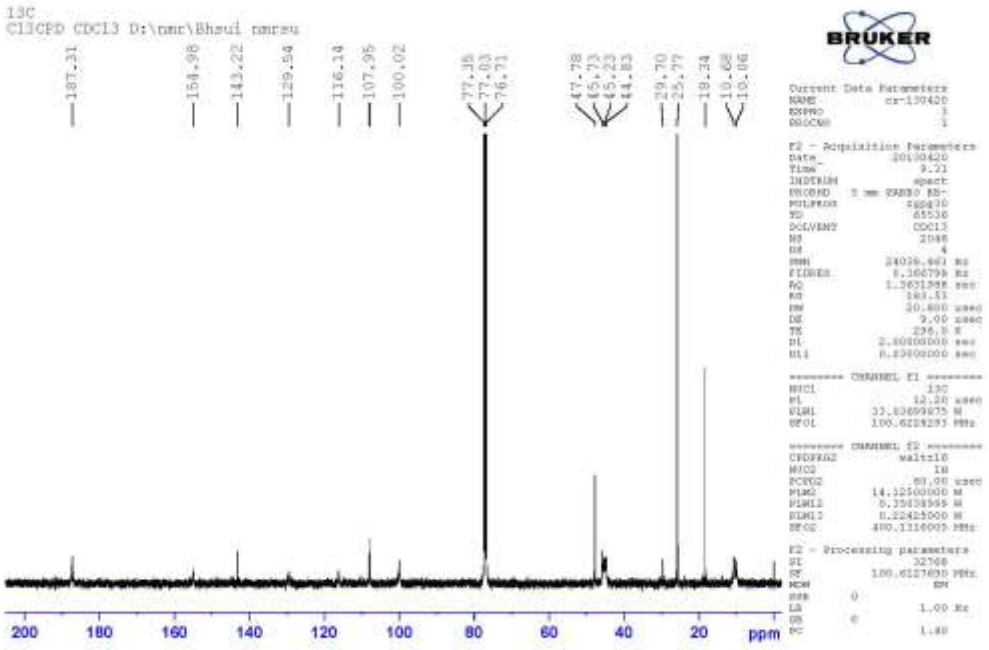

Figure S9. ^{13}C NMR spectrum of 1, 4-diethyl-7-(*tert*-butyldimethylsilyloxy)-1, 2, 3, 4-tetrahydro-quinoxalin-6-carbaldehyde in $CDCl_3$.

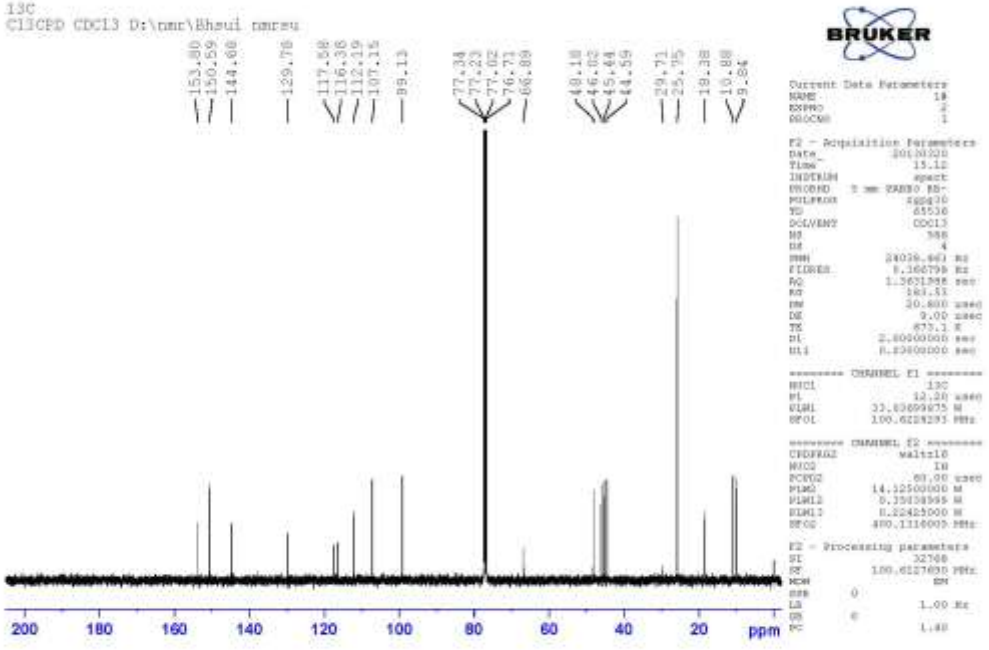

Figure S10. ^{13}C NMR spectrum of probe **1** in $CDCl_3$.

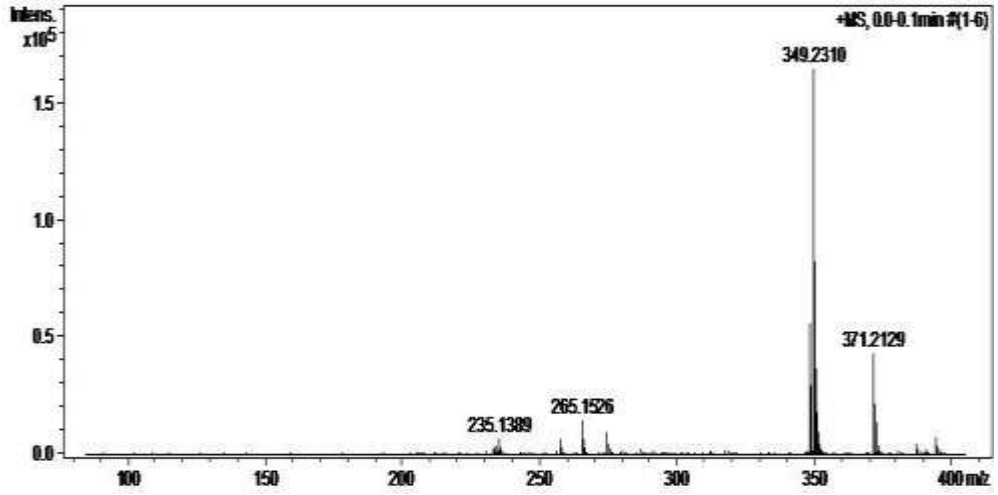

Figure S11. Mass spectrum of 1, 4-diethyl-7-(*tert*-butyldimethylsilyloxy)-1, 2, 3, 4-tetrahydro-quinoxalin-6-carbaldehyde.

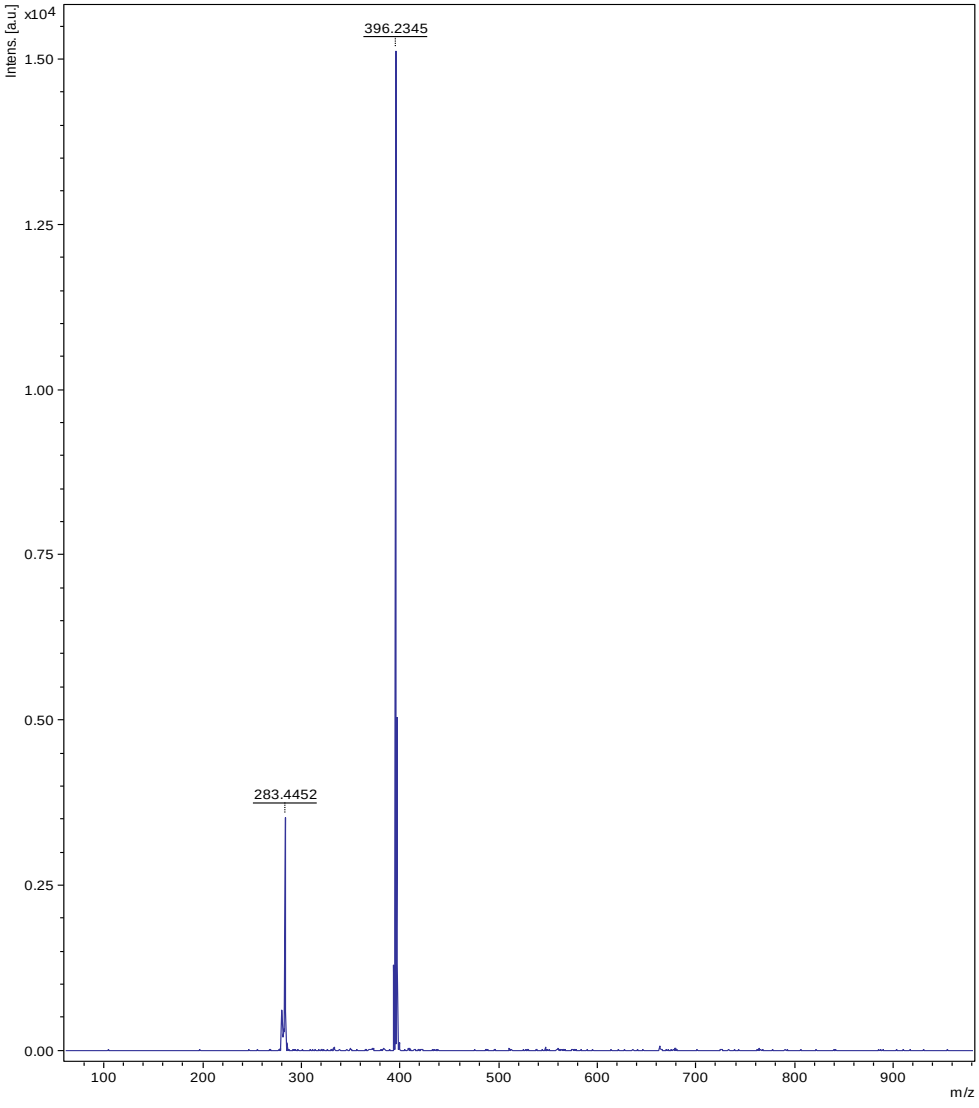

Figure S12. Mass spectrum of probe **1**.

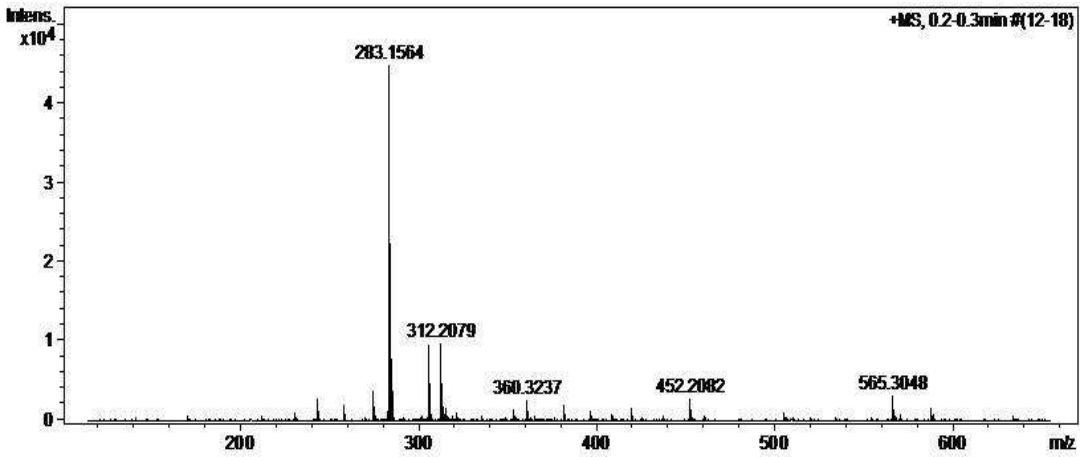

Figure S13. Mass spectrum of the reaction product of probe **1** with F^-.

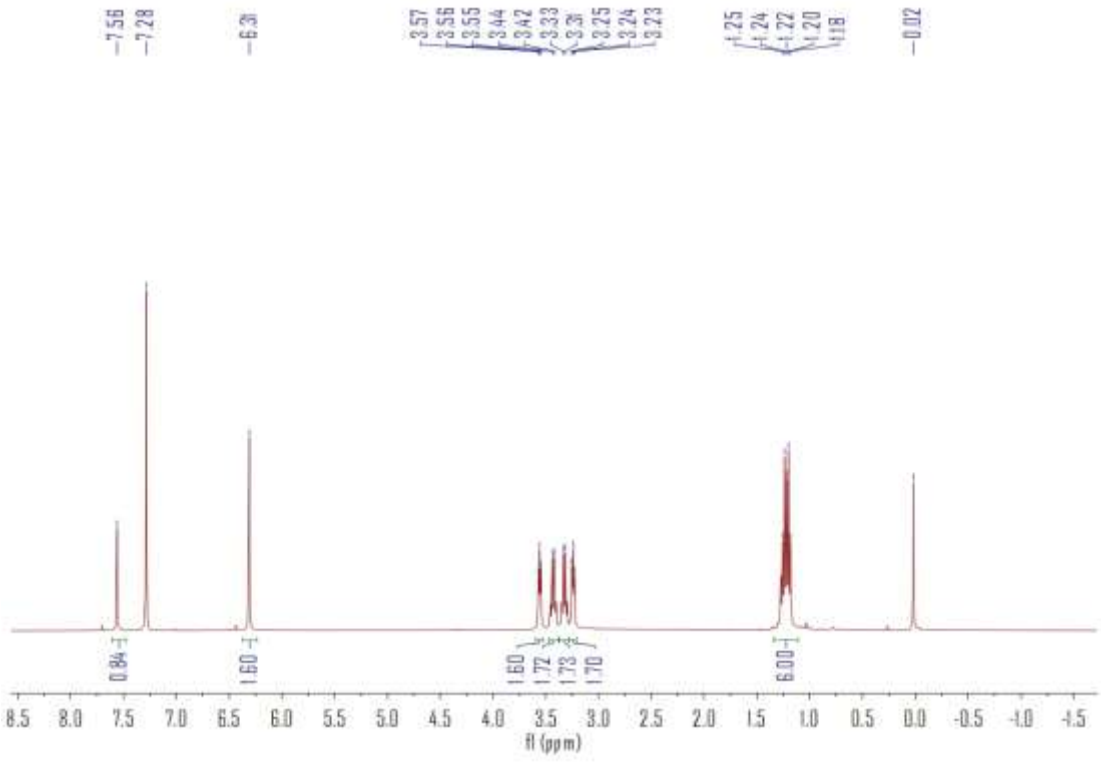

Figure S14. ^1H NMR spectrum of dye **2** in $CDCl_3$.