

Electronic Supplementary Information (ESI)

**“Turn-on” Fluorescent Probe for Hypochlorous Acid:
Convenient Synthesis, Good Sensing Performance, and New
Design Strategy by the Removal of C=N Isomerization**

*Xiaohong Cheng, Huizhen Jia, Teng Long, Jun Feng, Jingui Qin, and Zhen Li**

*Department of Chemistry, Hubei Key Lab on Organic and Polymeric Opto-Electronic
Materials, Wuhan University, Wuhan, 430072, China. Fax: 86 27 68756757; Tel: 86
27 62254108; E-mail: lizhen@whu.edu.cn or lichemlab@163.com.*

Experimental Section

Materials and Instrumentations

Chloroform was dried over and distilled from CaH₂ under an atmosphere of dry nitrogen. All other reagents were of analytical reagent grade and used without further purification. Doubly distilled water was used in all experiments. The ¹H and ¹³C NMR spectra were measured on Varian Mercury300 spectrometer using tetramethylsilane (TMS; δ = 0 ppm) as internal standard. Photoluminescence spectra were performed on a Hitachi F-4500 fluorescence spectrophotometer. The ESI mass spectra were measured on a Finnigan LCQ advantage mass spectrometer. The pH values were determined by using a DELTA 320 PH dollar. Compound **Flu-0** was prepared according to the literature procedure.¹

Synthesis of Flu-1

Compound **Flu-0** (0.216 g, 0.6 mmol) and hydroxylamine hydrochloride (0.085 g, 1.2 mmol) in ethanol (5 mL) were stirred at room temperature overnight. After the

reaction, the solvent was removed under reduced pressure. The resultant residue was purified by silica gel column chromatography (dichloromethane / ethyl acetate / methanol = 35 / 5 / 2) to afford the light yellow solid (0.108 g, 48%). ¹H NMR (300 MHz, *d*₆-DMSO): δ = 6.55-6.71 (m, 4H, Ar-H), 6.85 (s, 1H, Ar-H), 7.28-7.31 (d, 1H, Ar-H), 7.70-7.82 (m, 2H, Ar-H), 7.99-8.01 (d, 1H, Ar-H), 8.86 (s, 1H, -CH=N), 10.23 (s, 1H, -OH), 11.10 (s, 1H, -N-OH), 11.95 (s, 1H, -OH). ¹³C NMR (75 MHz, DMSO-*d*₆): δ = 169.3, 160.2, 159.3, 152.9, 152.0, 149.6, 146.1, 136.3, 130.9, 130.5, 129.6, 126.7, 125.4, 124.7, 113.8, 113.3, 110.6, 109.9, 105.6, 103.3, 83.2 ppm. MS (ESI), *m/z* [M+H]⁺: 376.3, calcd, 376.1.

Preparation of Solutions of Anions

1 mmol of inorganic salt (NaOAc·3H₂O, NaNO₂, NaNO₃, Na₂SO₃, NaF, NaHCO₃, Na₂SO₄, Na₃PO₄, NaCl, NaIO₃, KClO₃, KBr, Na₂HPO₄·12H₂O, NaHSO₃, NaHSO₄, KI, Na₂S₂O₃·5H₂O, KSCN, NaClO) and H₂O₂ were dissolved in distilled water (10 mL) to afford 1×10⁻¹ mol/L aqueous solution. The stock solutions were diluted to desired concentrations with water when needed.

Fluorescence Titration of Flu-1 with ClO⁻

A solution of **Flu-1** (1×10⁻⁵ mol/L) was prepared in DMSO-H₂O solution (1:9, v/v, 10 mM HEPES, pH 7.05). Then 3.0 mL of the solution of **Flu-1** was placed in a quartz cell (10.0 mm width) and the fluorescence spectrum was recorded. The NaClO solution was introduced in portions and fluorescence intensity changes were recorded at room temperature each time (Excitation wavelength: 454 nm).

Fluorescence Intensity Changes of Flu-1 with Other Anions

A solution of **Flu-1** (1×10^{-5} mol/L) was prepared in DMSO-H₂O solution (1:9, v/v, 10 mM HEPES, pH 7.05). Then 3.0 mL of the solution of **Flu-1** was placed in a quartz cell (10.0 mm width) and the fluorescence spectrum was recorded. Different anion solutions were introduced and the changes of the fluorescence intensity were recorded at room temperature each time (Excitation wavelength: 454 nm).

Reference

- 1 W. Wang, O. Rusin, X. Xu, K. K. Kim, J. O. Escobedo, S. O. Fakayode, K. A. Fletcher, M. Lowry, C. M. Schowalter, C. M. Lawrence, F. R. Fronczek, I. M. Warner, R.M. Strongin, *J. Am. Chem. Soc.*, 2005, **127**, 15949-15958.

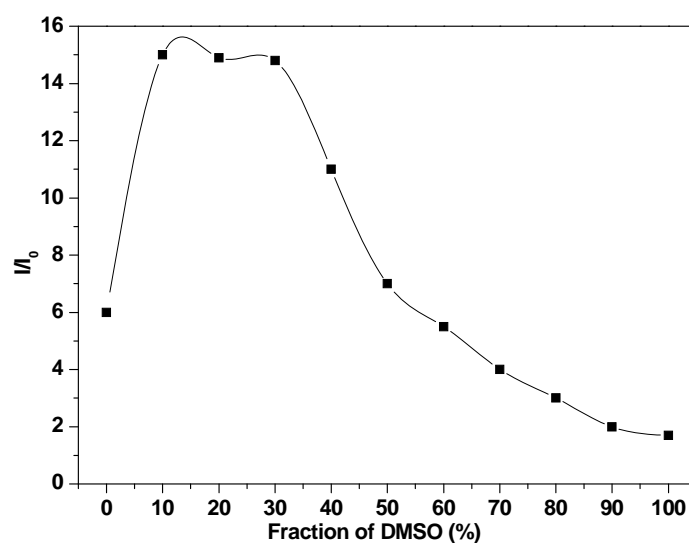


Figure S1. Effect of fraction of DMSO on the reaction of **Flu-1** (10 μ M) with OCl⁻ (20 μ M). Fluorescence intensity was measured at $\lambda_{\text{ex/em}} = 454/530$ nm.

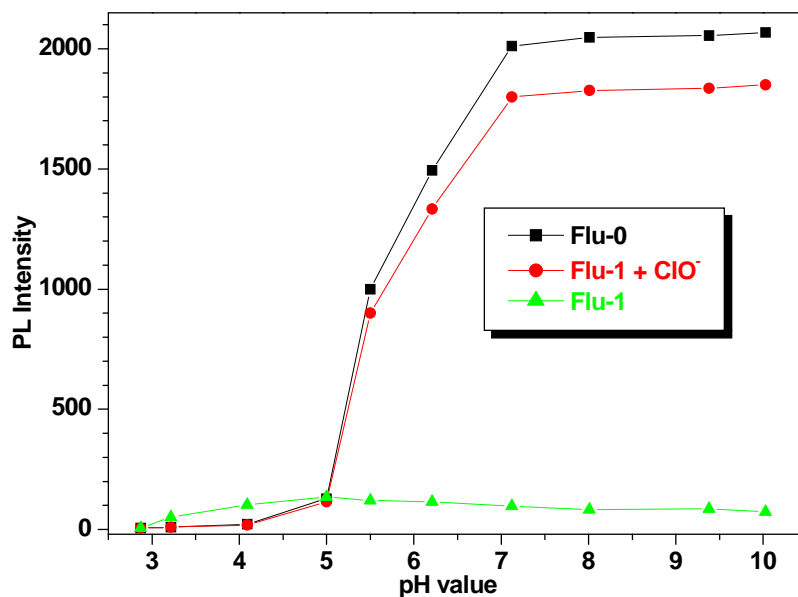


Figure S2. Effect of pH on the reaction of **Flu-1** (10 μM) with OCl^- (20 μM). Fluorescence intensity was measured at $\lambda_{\text{ex/em}} = 454/530$ nm.

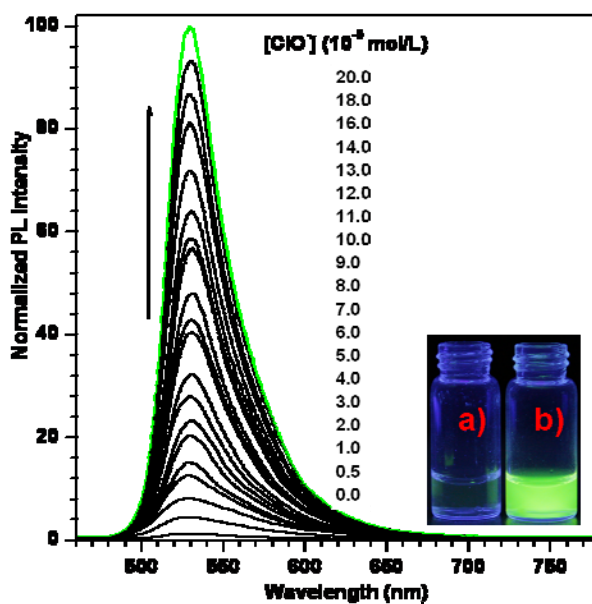


Figure S3. Normalized emission spectra of **Flu-1** (10 μM) in the presence of increasing concentration of OCl^- (the black line), with that of **Flu-0** (the green line) for comparison. Inset: Fluorescence photograph of **Flu-1** (a) and **Flu-1** + OCl^- (20 eq., b).

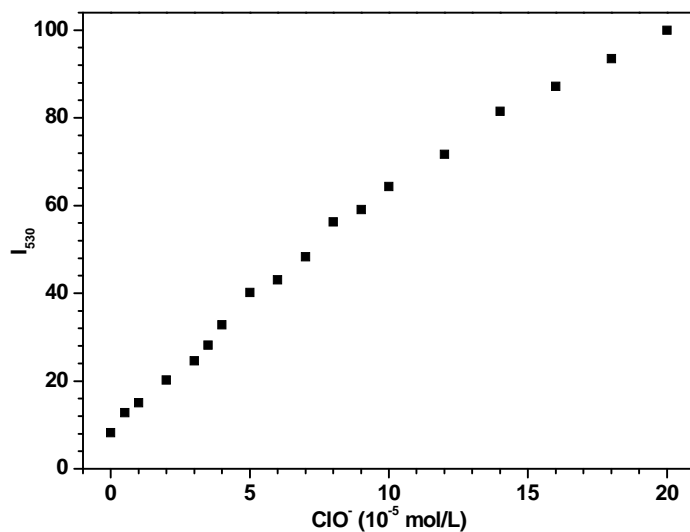


Figure S4. Plot of fluorescence intensity of Flu-1 as a function of the concentration of OCl⁻.

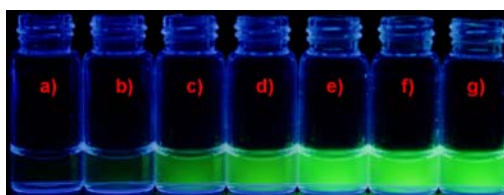


Figure S5. Fluorescence photograph of Flu-1 (10 μM) in the presence of increasing concentration of OCl⁻. From a to g (× 10⁻⁵ mol/L): 0, 2, 4, 6, 8, 10, and 20.

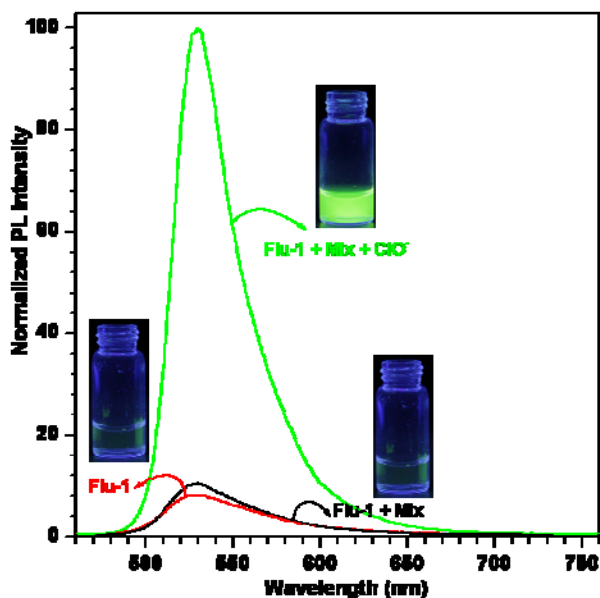


Figure S6. Normalized fluorescence intensity of Flu-1, Flu-1 + OCl⁻ and Flu-1 + mixture of anions + OCl⁻.

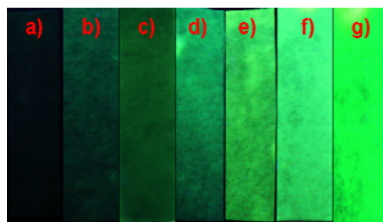


Figure S7. Fluorescence photographs of test strips of **Flu-1** (10^{-3} mol/L, a) and **Flu-1** + OCl⁻ (b: 1×10^{-5} mol/L; c: 5×10^{-5} mol/L; d: 10^{-4} mol/L; e: 3×10^{-4} mol/L; f: 6×10^{-4} mol/L; g: 9×10^{-4} mol/L).

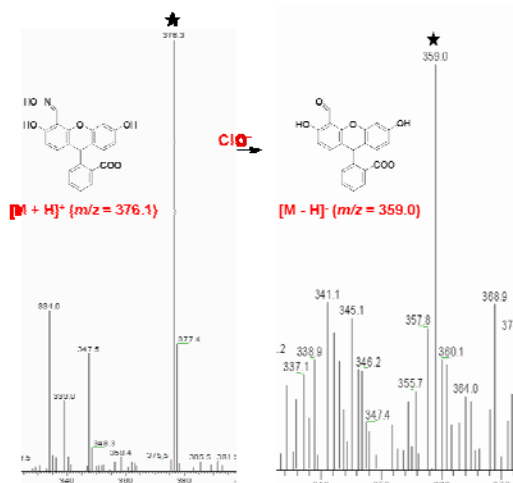


Figure S8. ESI-MS spectra of **Flu-1** (left) and the product of the reaction between **Flu-1** and OCl⁻ (right).

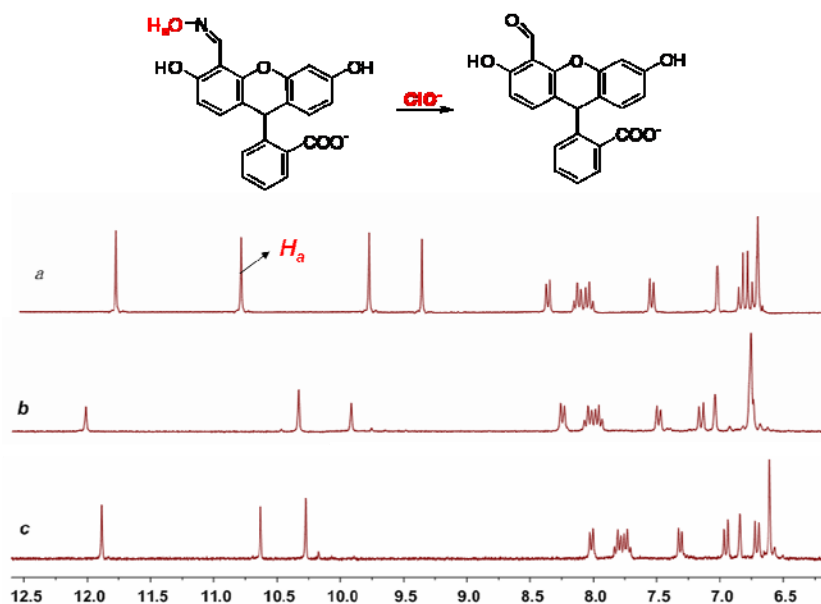


Figure S9. ¹H NMR spectra of **Flu-1** (a), **Flu-0** (b), and the isolated resultant compound **Flu-1-OCl⁻** (c), in d_6 -DMSO.