Supporting Information for

A novel colorimetric and far-red emission ratiometric fluorescent probe for the highly selective and ultrafast detection of hypochlorite in water and its application in bioimaging

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1. Preparation of various analytes

The solutions of various analytes, including K^+, Ca^{2+}, Na^+, Fe^{2+}, Fe^{3+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, ClO_4^-, Cl^-, Br^-, I^-, SCN^-, SO_3^{2-}, HCO_3^-, NO_3^-, NO_2^-, HS^-, S^{2-}, Hcy, Cys, GSH, $^{\bullet}OH$, NO, $tBuO^{\bullet}$, H_2O_2, $^1O_2$, O_2^-, $ONOO^-$, and TBHP were prepared according to the following methods. The solutions of ions (1×10^{-2} M) were prepared by dissolving corresponding salt including KCl, $CaCl_2$, $NaClO_4 \cdot H_2O$, $Fe(ClO_4)_2$, $Fe(ClO_4)_3$, $Mg(ClO_4)_2$, $Zn(ClO_4)_2 \cdot 6H_2O$, $Cu(ClO_4)_2 \cdot 6H_2O$, $NaClO_4$, NaCl, KBr, KI, KSCN, Na_2SO_3, $NaHCO_3$, $NaNO_3$, $NaNO_2$, NaHS, and Na_2S in deionized water. Amino acids including Cys, Hcy and GSH (1×10^{-2} M) were prepared in deionized water. $^{\bullet}OH$ was prepared by the Fenton reaction of Fe^{2+} with H_2O_2. [1] NO was generated from sodium nitroprusside. O_2^- was prepared from KO_2 in DMSO. $tBuO^{\bullet}$ was generated from the reaction of Fe^{2+} with t-BuOOH. $^1O_2$ was obtained from OCl^- and H_2O_2. TBHP was prepared by diluting tert-butyl hydroperoxide aqueous solution.

2. Determination of the detection limit

The detection limit (DL) is calculated on the basis of the fluorescence titration using Eq. (1):

$$DL = 3\sigma/k \qquad (1)$$

Where σ is the standard deviation of eleven blank measurements and k is the slope of the linear equation between the fluorescence intensities at 637 nm and the concentrations of OCl^-.[2]

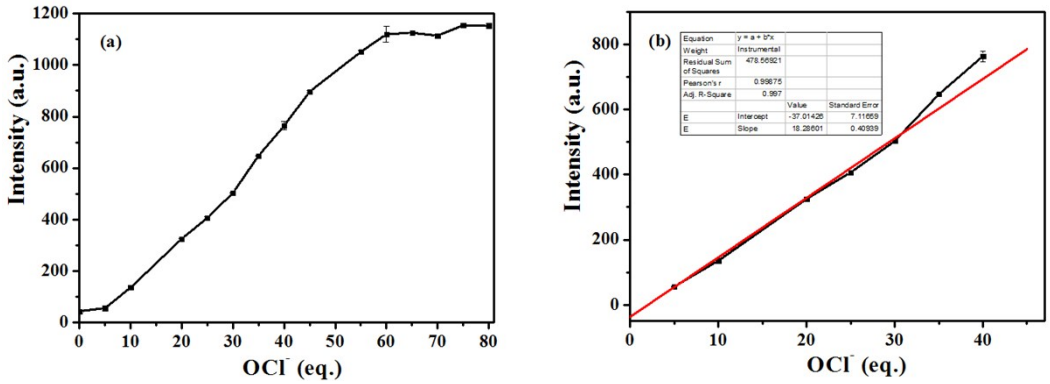


Fig. S1 (a) Fluorescence intensity of **DCOH-FR-OCl** (10 μM) in the presence of different concentrations of OCl^- (0-80.0 equiv.) in a 100% aqueous solution at 25 °C; (b) fluorescence intensity of **DCOH-FR-OCl** (10 μM) at 637 nm as a function of OCl^-.

3. Photostability studies

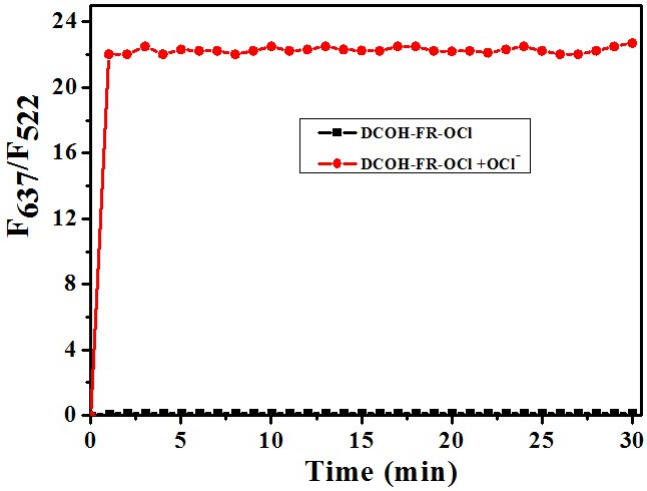
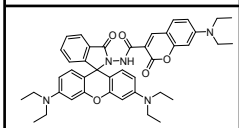
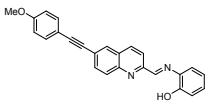
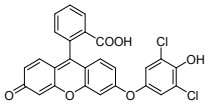
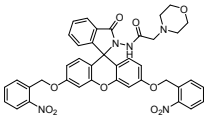
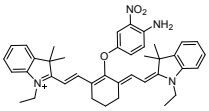
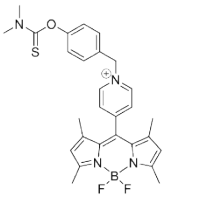
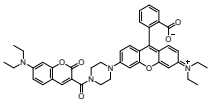
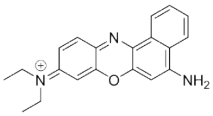
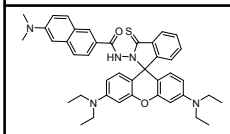
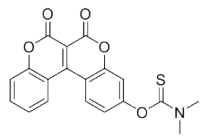
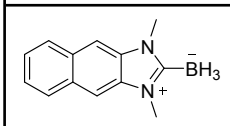
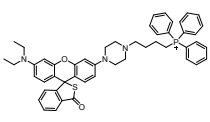


Fig. S2 The stability of **DCOH-FR-OCl** and further addition of 60.0 equiv. OCl^- in a 100% aqueous solution.

4. Comparison the performances of DCOH-FR-OCl and reported OCl^- probes

Probe	Colorimetric detection	Ratiometric fluorescence detection	Far-infrared emission and imaging	λ (nm)	Reaction medium (v:v)	Response time	Stokes shift	Reference
	–	Yes	No	$\lambda_{ex}/\lambda_{em}$=410, 554/501,578	DMF:PBS = 4:6	within 1 min	–	Ref. **[3]**

	No	No (fluorescence turn-on)	No	$\lambda_{ex}/\lambda_{em}$=370/495	DMSO:PBS = 9:1	within 10 min	125 nm	**4**
	No	No (fluorescence turn-on)	No	$\lambda_{ex}/\lambda_{em}$=490/527	Aqueous solution	within 1 min	37 nm	**5**
	No	No (fluorescence turn-on)	No	$\lambda_{ex}/\lambda_{em}$=480/525	DMF:H_2O = 2:8	100 s	45 nm	**6**
	—	No (fluorescence turn-on)	Yes	$\lambda_{ex}/\lambda_{em}$=543/625	HEPES 37 ℃	15 min	82 nm	**7**
	No	No (fluorescence turn-on)	No	$\lambda_{ex}/\lambda_{em}$=498/516	PBS	10 min	18 nm	**8**
	—	Yes	No	$\lambda_{ex}/\lambda_{em}$=410/470, 580	DMF:NaH_2PO_4 = 4:6	Within 100 s	170 nm	**9**
	No	No (fluorescence turn-off)	Yes	$\lambda_{ex}/\lambda_{em}$=600/672	PBS	1 min	72 nm	**10**
	—	Yes	No	$\lambda_{ex}/\lambda_{em}$=350/440, 585	EtOH:Na_2HPO_4 = 3:7	2 min	235 nm	**11**
	No	No (fluorescence turn-on)	No	$\lambda_{ex}/\lambda_{em}$=460/540	PBS	Within 3 s	80 nm	**12**
	No	Yes	No	$\lambda_{ex}/\lambda_{em}$=326/361, 450	CH_3CN:H_2O = 1:9	within 1 min	124 nm	**13**
	Yes	No (fluorescence turn-on)	No	$\lambda_{ex}/\lambda_{em}$=553/580	DMSO:PBS = 1:99	1 min	27 nm	**14**

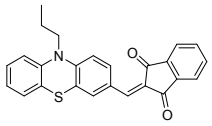
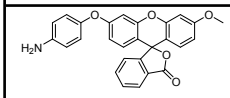
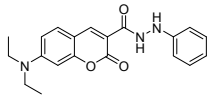
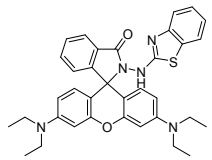
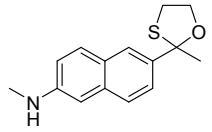
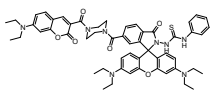
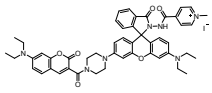
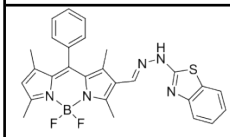
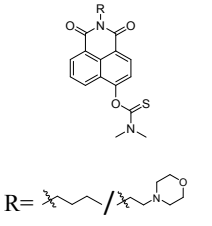
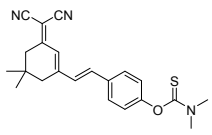
	Yes	No (fluorescence turn-on)	No	$\lambda_{ex}/\lambda_{em}$=440/551	DMSO: PBS = 9:1	within 1 min	111 nm	**15**
	Yes	No (fluorescence turn-on)	No	$\lambda_{ex}/\lambda_{em}$= —/515	EtOH:H_2O = 1:19	300 s	—	**16**
	—	No (fluorescence turn-on)	No	$\lambda_{ex}/\lambda_{em}$= 398/468	PBS buffer (pH 7.4, containing 20% CH_3CN	5 min	70 nm	**17**
	Yes	No (fluorescence turn-on)	No	$\lambda_{ex}/\lambda_{em}$= 550/575	CH_3CN:PBS= 3:7	40 min	25 nm	**18**
	No	No (fluorescence turn-on)	No	$\lambda_{ex}/\lambda_{em}$= 375/500	EtOH:H_2O = 1:1	Within 10 s	125 nm	**19**
	Yes	Yes	No	$\lambda_{ex}/\lambda_{em}$= 414/473, 594	DMF:PBS= 1:1	—	180 nm	**20**
	Yes	Yes	No	$\lambda_{ex}/\lambda_{em}$= 420/483, 570	PBS buffer (pH 7.4, containing 0.5% EtOH	Within 1 min	150 nm	**21**
	Yes	No (fluorescence turn-on)	No	$\lambda_{ex}/\lambda_{em}$= 480/508	PBS:MeOH= 1:1	30 min	28 nm	**22**
	No	No	No	$\lambda_{ex}/\lambda_{em}$= 410/547	PBS: EtOH = 1:1	2 min	137 nm	**23**
	Yes	Yes	Yes	$\lambda_{ex}/\lambda_{em}$= 479/522, 637	Aqueous solution	within 3 s	158 nm	**This work**

Table S1. A comparison between the probe **DCOH-FR-OCl** and other reported probes for

OCl^-.

5. Effects of pH on fluorescent response of DCOH-FR-OCl for OCl^-

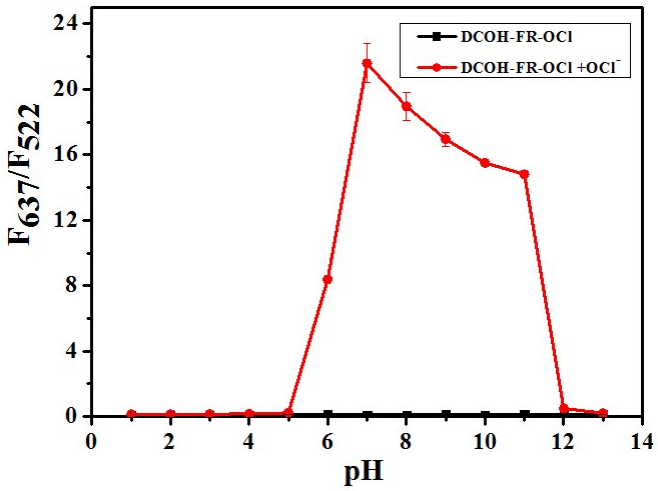


Fig. S3 The effects of pH on fluorescence response of **DCOH-FR-OCl** to OCl^- in a 100% aqueous solution.

6. HPLC analyses for the proposed reaction mechanism.

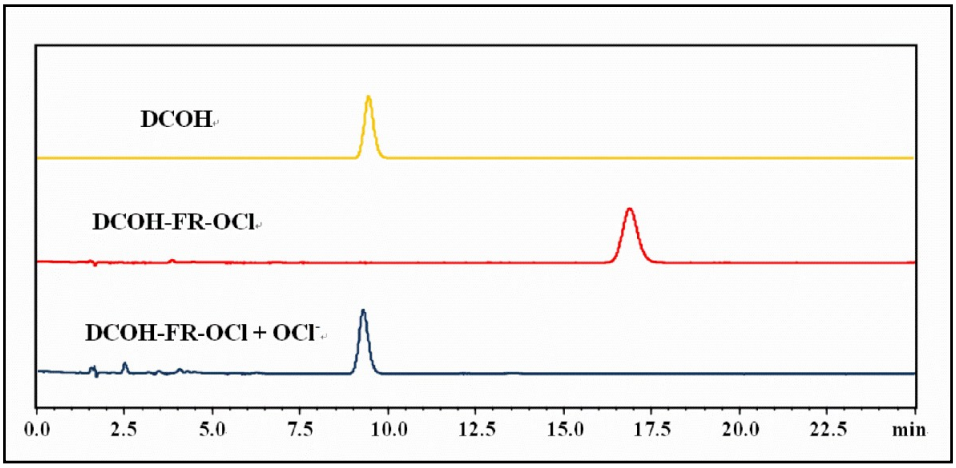


Fig. S4 HPLC analyses of the **DCOH-FR-OCl, DCOH** and the reaction solution of the **DCOH-FR-OCl** and OCl^-.

7. The optimized structures of DCOH-FR-OCl and DCOH

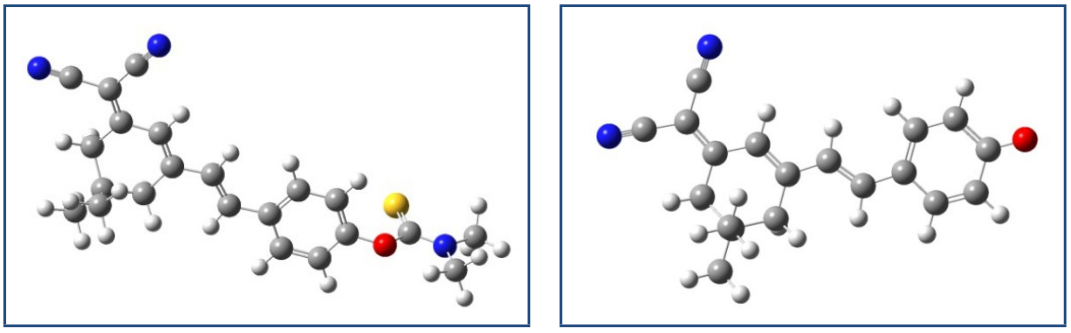


Fig. S5 The optimized structures of **DCOH-FR-OCl** and **DCOH** at the B3LYP/6-31G (d) basis set by using Gaussian 16.

8. Absorption and fluorescence spectra of probe DCOH-FR-OCl with OCl^- and those of fluorophore DCOH

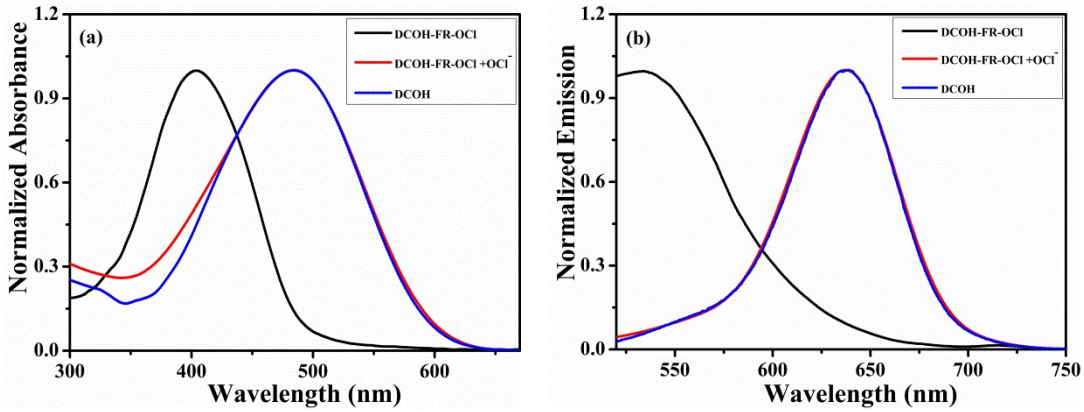


Fig. S6 The normalized absorption (a) and fluorescence spectra (b) of probe **DCOH-FR-OCl** (10 μM) in the absence and presence of OCl^- (60.0 equiv.) and those of **DCOH** (10 μM), λ_{ex} =479 nm.

9. Fluorescence imaging

(1) Fluorescence imaging of exogenous OCl^- in RAW 264.7 cells.

Two groups of experiments were set for imaging exogenous OCl^-. In the control group, the cells were cultured with 10 μM of **DCOH-FR-OCl** for 30 min in an atmosphere of 5% CO_2 at 37 °C, and washed with PBS three times. Then, the fluorescence imaging of the cells was carried out. In the test group, the cells were first treated with 10 μM of **DCOH-FR-OCl** for 30 min, followed by incubation with 600 μM OCl^- for another 30 min. After washing with PBS three times, the fluorescence imaging of the cells was carried out. Fluorescence images were obtained on Leica Microsystems D-35578 confocal laser-scanning microscope with a 40× objective lens with the excitation wavelengths of 405 nm and 488 nm. The fluorescence emissions were collected at 500–600 nm (green channel) and 600–700 nm (red channel), respectively.

(2) Fluorescence imaging of endogenous OCl^- in RAW 264.7 cells.

Three groups of experiments were set for imaging endogenous OCl^-. In the control group, the cells were cultured with 10 μM of **DCOH-FR-OCl** for 30 min in an atmosphere of 5% CO_2 at 37 °C, and washed with PBS three times. Then, the fluorescence imaging of the cells was carried out. The cells were first treated with 200 μM 4-aminobenzoic acid hydrazide (ABAH, an inhibitor of MPO) for 60 min, followed by incubation with **DCOH-FR-OCl** for 30 min. After washing with PBS three times, the fluorescence imaging of the cells was carried out. The cells were first treated with 1.0 μg mL^{-1} phorbol 12-myristate 13-acetate (PMA, a ROS stimulant) for 60 min, followed by incubation with **DCOH-FR-OCl** for 30 min. After washing with PBS three times, the fluorescence imaging of the cells was carried out. The excitation wavelengths were 405 nm and 488 nm. The fluorescence emissions were collected at 500−600 nm (green channel) and 600−700 nm (red channel), respectively.

10. ^{1}H NMR, ^{13}C NMR and HRMS analyses of compounds

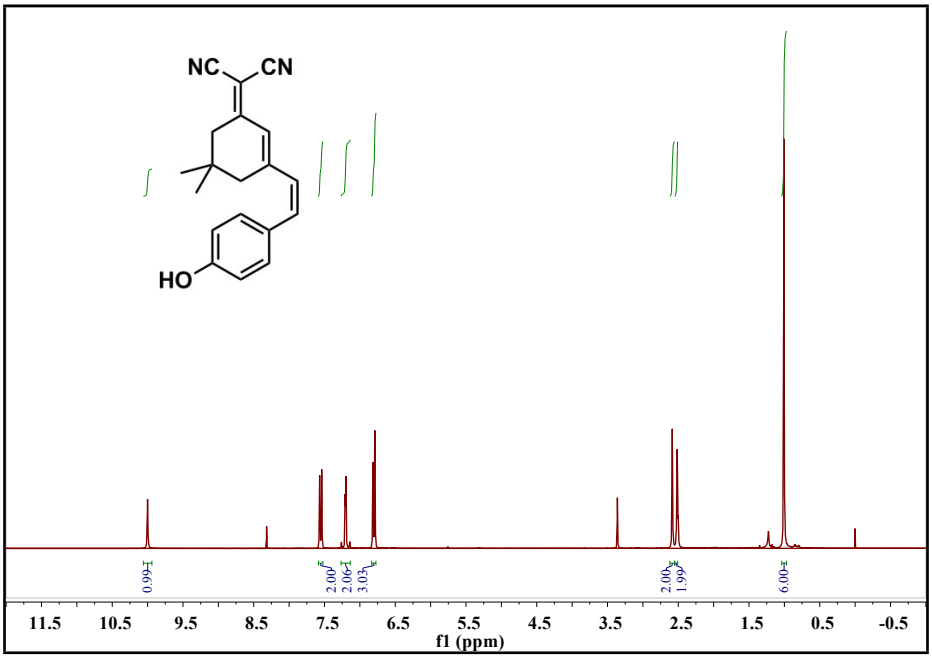


Fig. S7 The ^{1}H NMR spectra of **DCOH** in DMSO-d_6.

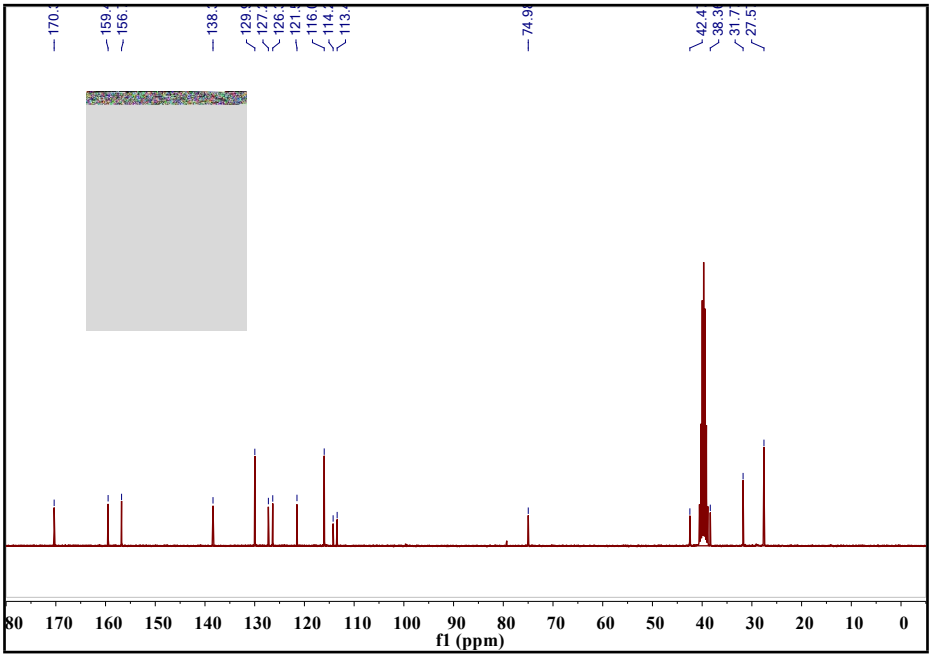


Fig. S8 The ^{13}C NMR spectra of **DCOH** in DMSO-d_6.

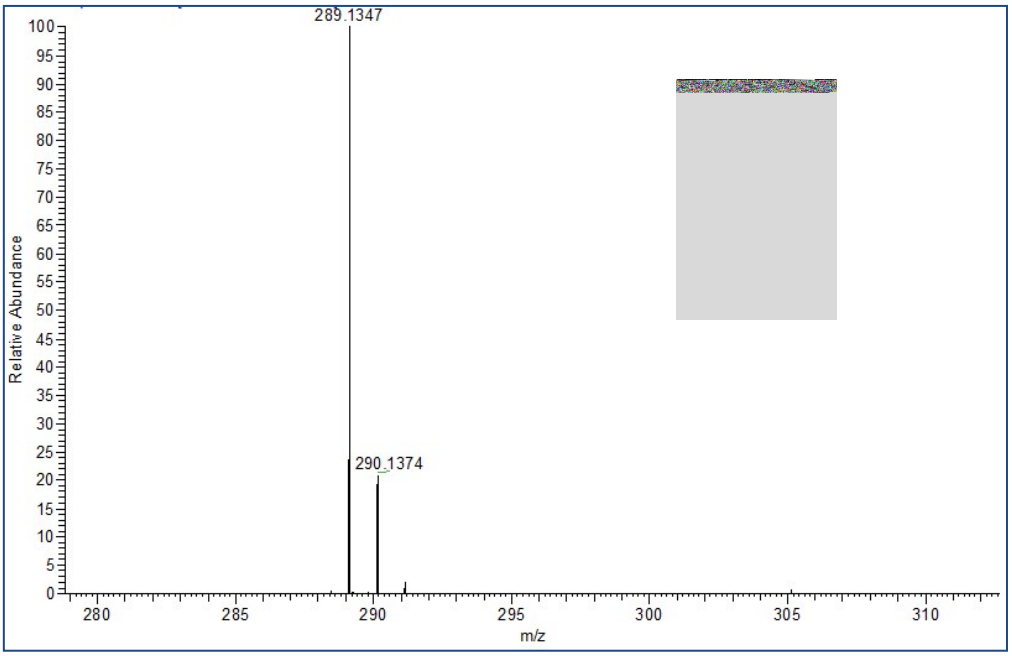


Fig. S9 High resolution mass spectra of **DCOH.**

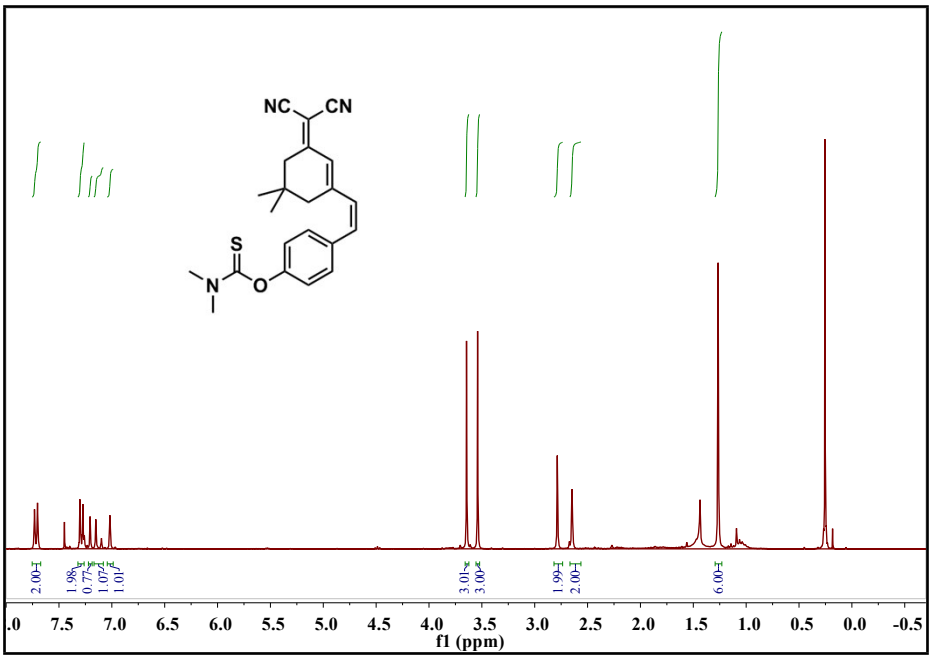


Fig. S10 The ^{1}H NMR spectra of **DCOH-FR-OCl** in $CDCl_3$.

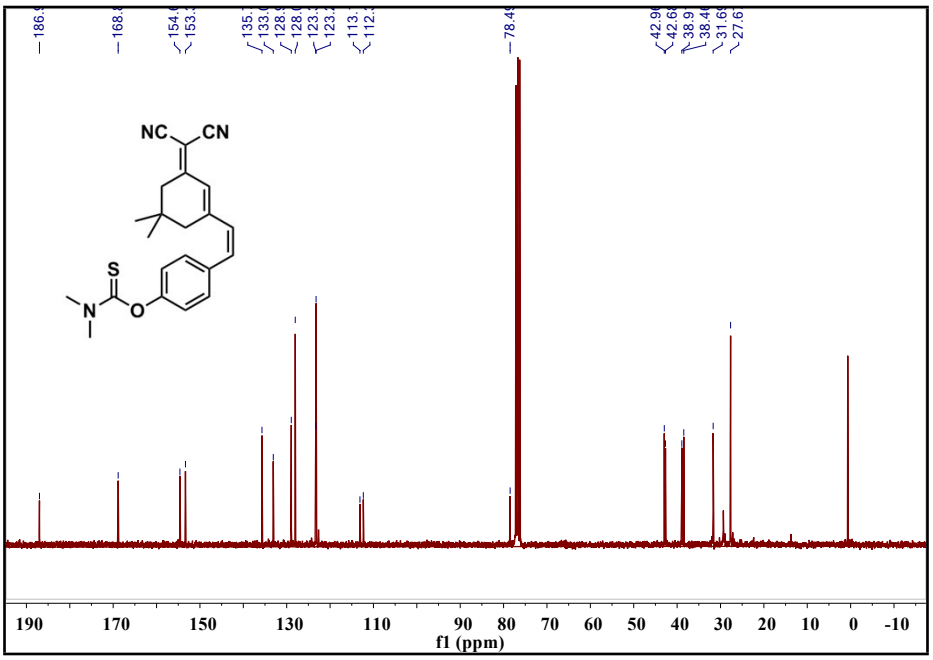


Fig. S11 The ^{13}C NMR spectra of **DCOH-FR-OCl** in $CDCl_3$.

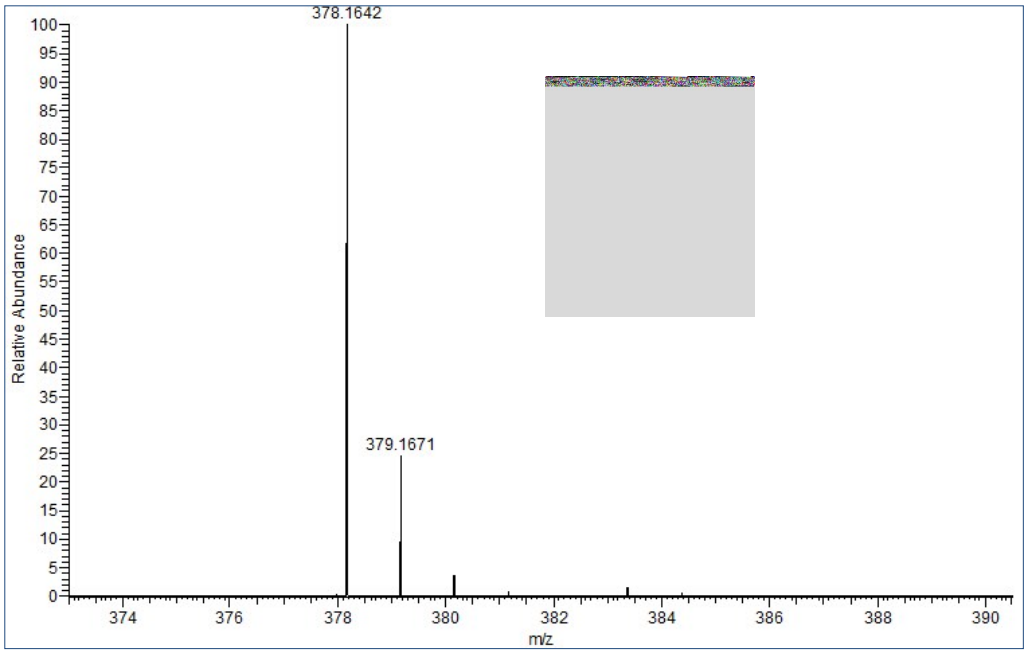


Fig. S12 High resolution mass spectra of **DCOH-FR-OCl.**

References

1 Y.-K. Yang, H. J. Cho, J. Lee, I. Shin and J. Tae, *Org. Lett.*, 2009, **11**, 859-861.

2 M. Shyamal, P. Mazumdar, S. Maity, S. Samanta, G. P. Sahoo and A. Misra, *ACS Sens.*, 2016, **1**, 739-747.

3 L. Long, D. Zhang, X. Li, J. Zhang, C. Zhang and L. Zhou, *Anal. Chim. Acta.*, 2013, **775**, 100-105.

4 X. Wang, X. Wang, Y. Feng, M. Zhu, H. Yin, Q. Guo and X. Meng, *Dalton Trans.,* 2015, **44**, 6613-6619.

5 J. J. Hu, N.-K. Wong, M.-Y. Lu, X. Chen, S. Ye, A. Q. Zhao, P. Gao, R. Y.-T. Kao, J. Shen and D. Yang, *Chem. Sci.*, 2016, **7**, 2094-2099.

6 M. Ren, Z. Li, J. Nie, L. Wang and W. Lin, *Chem. Commun.*, 2018, **54**, 9238-9241.

7 Y. Huang, N. He, Y. Wang, L. Zhang, Q. Kang, Y. Wang, D. Shen, J. Choo and L. Chen, *J. Mater. Chem. B*, 2019, **7**, 2557-2564.

8 D. Ji, G. Li, S. Zhang, M. Zhu, C. Li and R. Qiao, *Sens. Actuator B-Chem.*, 2018, **259**, 816-824.

9 Y.-R. Zhang, X.-P. Chen, S. Jing, J.-Y. Zhang, Q. Yuan, J.-Y. Miao and B.-X. Zhao, *Chem. Commun.*, 2014, **50**, 14241-14244.

10 B. Zhu, L. Wu, M. Zhang, Y. Wang, C. Liu, Z. Wang, Q. Duan and P. Jia, *Biosens. Bioelectron.*, 2018, **107**, 218-223.

11 Y.-R. Zhang, N. Meng, J.-Y. Miao and B.-X. Zhao, *Chem. Asian J.,* 2015, **21**, 19058-19063.

12 B. Zhu, L. Wu, H. Zhu, Z. Wang, Q. Duan, Z. Fang, P. Jia, Z. Li and C. Liu, *Sens. Actuator B-Chem.*, 2018, **269**, 1-7.

13 Y. L. Pak, S. J. Park, G. Song, Y. Yim, H. Kang, H. M. Kim, J. Bouffard and J. Yoon, *Anal. Chem.*, 2018, **90**, 12937-12943.

14 J. Zhou, L. Li, W. Shi, X. Gao, X. Li and H. Ma, *Chem. Sci.*, 2015, **6**, 4884-4888.

15 H. Feng, Y. Wang, J. Liu, Z. Zhang, X. Yang, R. Chen, Q. Meng and R. Zhang, *J. Mater. Chem. B*, 2019, **7**, 3909-3916.

16 C. Zhang, Q. Nie, I. Ismail, Z. Xi and L. Yi, *Chem. Commun.*, 2018, **54**, 3835-3838.

17 L. Long, Y. Wu, L. Wang, A. Gong, F. Hu and C. Zhang, *Chem. Commun.*, 2015, **51**, 10435-10438.

18 Z. Tang, X.-L. Ding, Y. Liu, Z.-M. Zhao and B.-X. Zhao, *RSC Adv.*, 2015, **5**, 99664-99668.

19 L. Yuan, L. Wang, B. K. Agrawalla, S.-J. Park, H. Zhu, B. Sivaraman, J. Peng, Q.-H. Xu and Y.-T. Chang, *J. Am. Chem. Soc.*, 2015, **137**, 5930-5938.

20 L. Yuan, W. Lin, Y. Xie, B. Chen and J. Song, *Chem. Asian J.,* 2012, **18**, 2700-2706, S2700/2701-S2700/2721.

21 S.-L. Shen, X. Zhao, X.-F. Zhang, X.-L. Liu, H. Wang, Y.-Y. Dai, J.-Y. Miao and B.-X. Zhao, *J. Mater. Chem. B*, 2017, **5**, 289-295.

22 W.-C. Chen, P. Venkatesan and S.-P. Wu, *Anal. Chim. Acta.*, 2015, **882**, 68-75.

23 B. Zhu, P. Li, W. Shu, X. Wang, C. Liu, Y. Wang, Z. Wang, Y. Wang and B. Tang, *Anal. Chem.*, 2016, **88**, 12532-12538.