Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

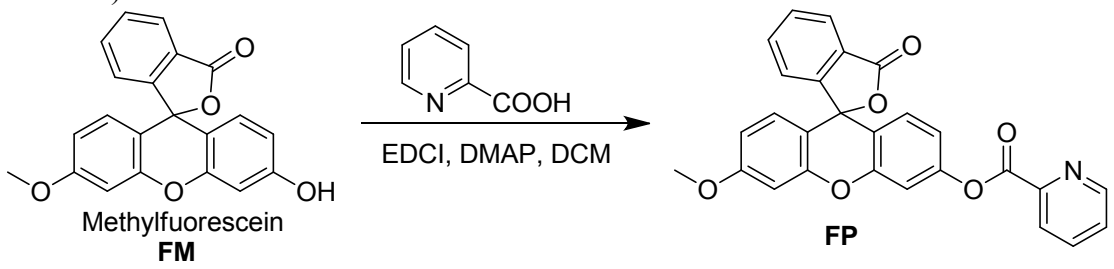
A reaction-based fluorescent turn-on probe for Cu^{2+} in complete aqueous solution

Dongjian Zhu,* Yanghe Luo,* Xiaowei Yan, Wei Xie, Wen Cai, Xing Zhong
Research Institute of Food Science & Engineering Technology, Hezhou University, Hezhou 542899, P. R. China
*To whom correspondence should be addressed. E-mail: zhudongjian@iccas.ac.cn (Dongjian Zhu), 250581250@qq.com (Yanghe Luo)

Contents

1 Synthesis of FP

General Information. All the chemicals and solvents were purchased from Energy Chemical or Aladdin Industrial Corporation, and used as received with the following exceptions. Dichloromethane (DCM) was distilled from calcium hydride. ^{1}H and ^{13}C NMR spectra were measured on Bruker Avance-400 400 MHz NMR spectrometer and referenced to solvent signals. Electrospray ionization mass spectra (ESI-MS) were measured on an LC-MS 6120 equipped with Single Quadrupole LC/MS system (Agilent, America) instrument.



Scheme S1. Synthesis of FP

A solution of methylfuorescein[S1] (346 mg, 1 mmol) in CH_2Cl_2 (20 mL) was treated with 2-picolinic acid (244 mg, 2 mmol), DMAP (73 mg, 0.6 mmol), and EDCI (383 mg, 2 mmol). The resultant solution was stirred at room temperature overnight under argon atmosphere. After completion of the reaction, the reaction was diluted with water. The organic phase was separated, and the aqueous phase was extracted with dichloromethane (2 × 20 mL). The organic phases were combined and washed with 0.1 N HCl, saturated brine, dried over anhydrous sodium sulfate. The solvents were evaporated to give crude solid, which was purified by silica gel column chromatography using petroleum ether/25-50% ethyl acetate as eluent to afford desired products. Yield: 293 mg (65%). ^{1}H NMR (400 MHz, $CDCl_3$, ppm) δ 8.86-8.84 (m, 1H), 8.28-8.26 (m, 1H), 8.04-8.02 (m, 1H), 7.94-7.90 (m, 1H), 7.72-7.67 (m, 2H), 7.65-7.61 (m, 1H), 7.26 (d, J = 2.4 Hz, 1H), 7.21-7.19 (m, 1H), 6.98-6.95 (m, 1H), 6.88 (d, J = 8.4 Hz, 1H), 6.80 (d, J = 2.8 Hz, 1H), 6.72 (d, J = 8.8 Hz, 1H), 6.65-6.62 (m, 1H), 3.84 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$, ppm) δ 169.37, 167.85, 163.47, 153.22, 152.46, 150.34, 147.29, 137.39, 135.26, 131.01, 130.98, 130.00, 129.28, 129.13, 128.98, 127.73, 126.72, 126.13, 125.25, 124.19, 117.66, 112.18, 111.17, 110.66, 101.11, 82.58, 55.73. MS(ESI) for $C_{27}H_{18}NO_6^+$ ($[M+H]^+$): calcd: 452.1, found: 452.1.

2. UV-visible Absorbance and Fluorescence Emission

UV-visible spectra were recorded on ThermoFisher Evolution 300 UV-vis spectrometer. Fluorescence spectra were recorded using a HITACHI F-4600 spectrometer. The PMT voltage was 700 V, excitation slit and emission slit were 2.5nm. The path length was 1 cm with cell volume of 3.0 mL. The stock solution of FP and FM were prepared in DMSO (1 mM). The solutions of Na^+, K^+, Ca^{2+}, Mg^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+}, Ba^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+}, Sn^{4+} and Cu^{2+} were prepared from their chloride salts; the solutions of Ag^+, Pb^{2+} and Al^{3+} were prepared from their nitrate salts; the solutions of $Mn^{2+}$and Ni^{2+} were prepared from their sulphate salts.

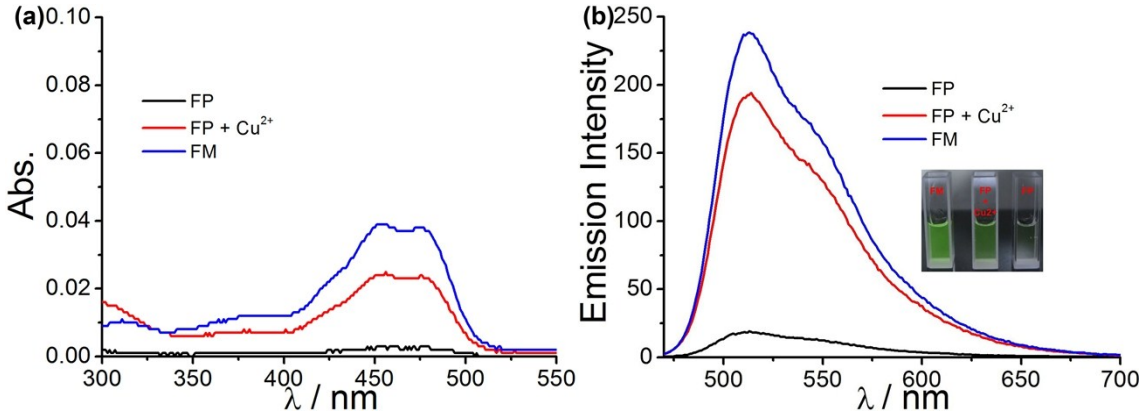


Fig. S1 (a) Absorption spectra of FM (1 μM) (blue line), FP (1 μM) (black line) before and (red line) after reaction with Cu^{2+} (20 μM) in HEPES buffer (10 mM, pH 7.0, 25 °C). (b) Fluorescence spectra of FM (1 μM) (blue line), FP (1 μM) (black line) before and (red line) after reaction with Cu^{2+} (20 μM) in HEPES buffer (10 mM, pH 7.0, 25 °C, λ_{ex} = 460 nm). The fluorescence colour changes of FP before and after the reaction and FM in HEPES buffer under illumination with a 254 nm UV lamp are shown in the inset.

3. pH Effect on the Fluorescence Properties

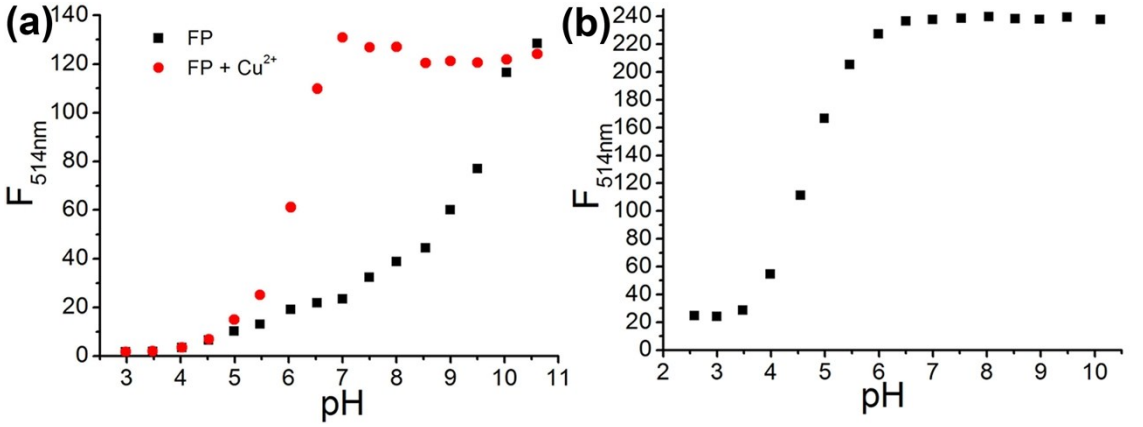


Fig. S2 (a) Effect of pH on fluorescence intensity at 514 nm for FP in the absence (black square) and presence (red cycle) of Cu^{2+} in HEPES buffer (10 mM, 25 °C, λ_{ex} = 460 nm). (b) Effect of pH on fluorescence intensity at 514 nm for FM in HEPES buffer (10 mM, 25 °C, λ_{ex} = 460 nm).

4. Solubility

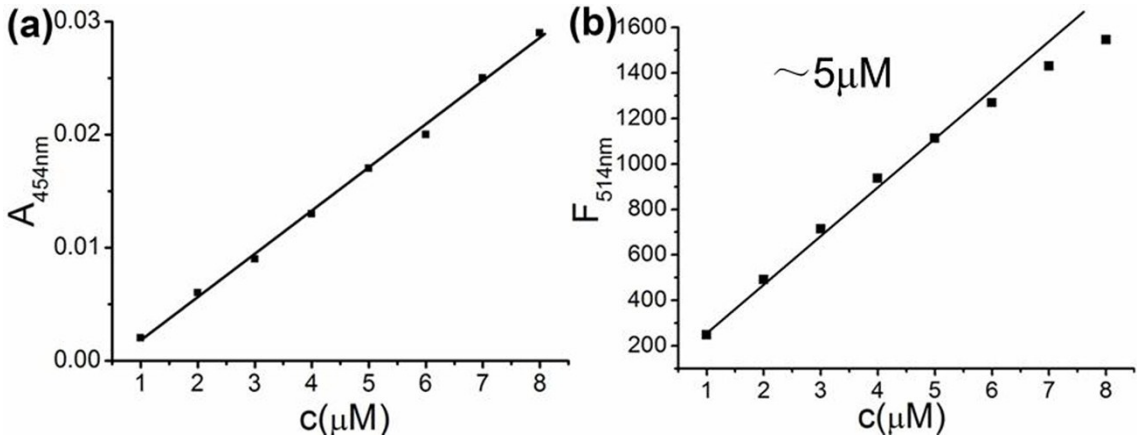


Fig. S3 (a) Plot of absorbance at 454 nm against FP concentration in HEPES buffer (10 mM, pH 7.0, 25 °C). (b) Plot of fluorescence intensity at 514 nm against FM concentration in HEPES buffer (10 mM, pH 7.0, 25 °C, λ_{ex} = 460 nm).

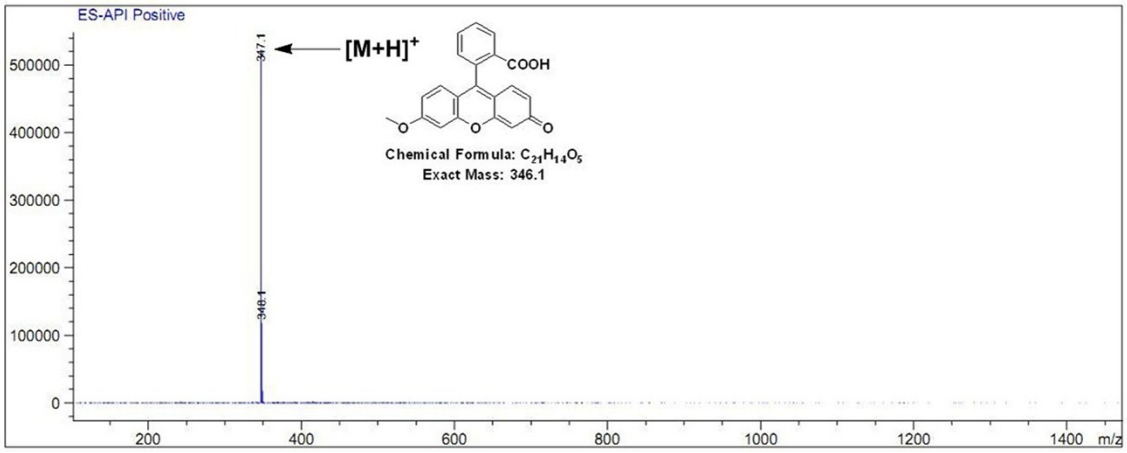


Fig. S4 ESI-MS spectrum of FP (1 μM) after addition of Cu^{2+} (20 μM) in HEPES buffer (10 mM, pH 7.0, 25 °C).

5. Determination of the detection limit (LOD)

The detection limit was calculated according to the method described in reference.[S2] Fluorescence titrations of FP (1 μM) in the presence of Cu^{2+} (0 - 1 μM) were measured by three times. A linear regression curve was fitted according to the fluorescence intensity at 514 nm as a function of the concentrations of Cu^{2+}, and k was obtained. The emission spectrum of FP (1 μM) in HEPES buffer (10 mM, pH 7.0, 25 °C, λ_{ex} = 460 nm.) was collected for 30 times to determine the background noise σ. The detection limit (3σ/k) was then determined to be 55 nM.

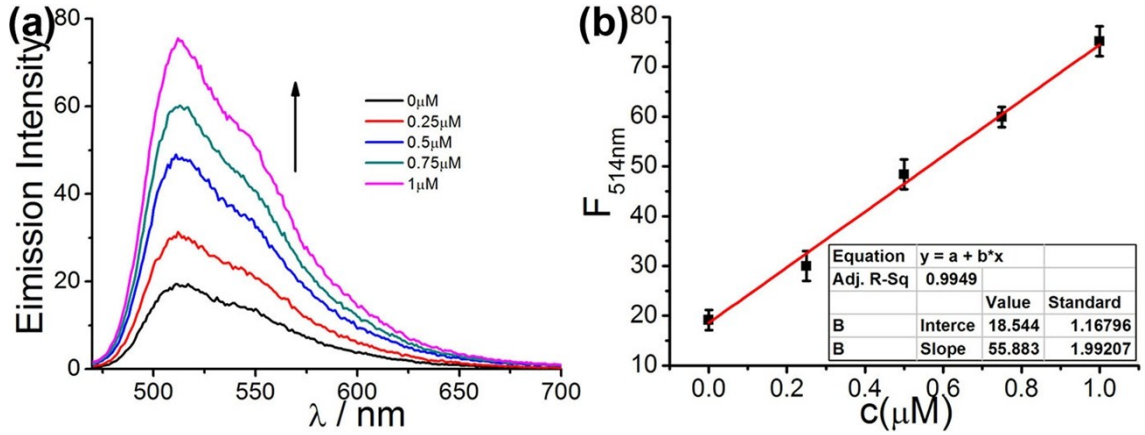


Fig. S5 (a) Fluorescence spectra of FP (1 μM) upon addition of Cu^{2+} (0 - 1 μM). (b) The fluorescence intensities of FP at 514 nm as a function of the concentrations of Cu^{2+} in the range of 0 - 1 μM upon excitation at 460 nm and the calculation of the detection limit of FP for Cu^{2+}.

6. Determination of quantum yields

Fluorescence quantum yield was determined in the reference of fluorescein (Φ = 0.85) in 0.1 M aqueous NaOH.[S3] The quantum yields of FP, FM and the reaction solution of FP with Cu^{2+} are calculated according to following equation.

$\Phi_x = \Phi_s(A_s S_x)/(A_x S_s)$

Φ_s is the fluorescence quantum yield of fluorescein, A_x and A_s are the absorbance of FP, FM, the reaction solution of FP with Cu^{2+} and the standard. S_x and S_s are integrated fluorescence emission corresponding to FP, FM, the reaction solution of FP with Cu^{2+} and the standard.

7. Determination of kinetic rate constant

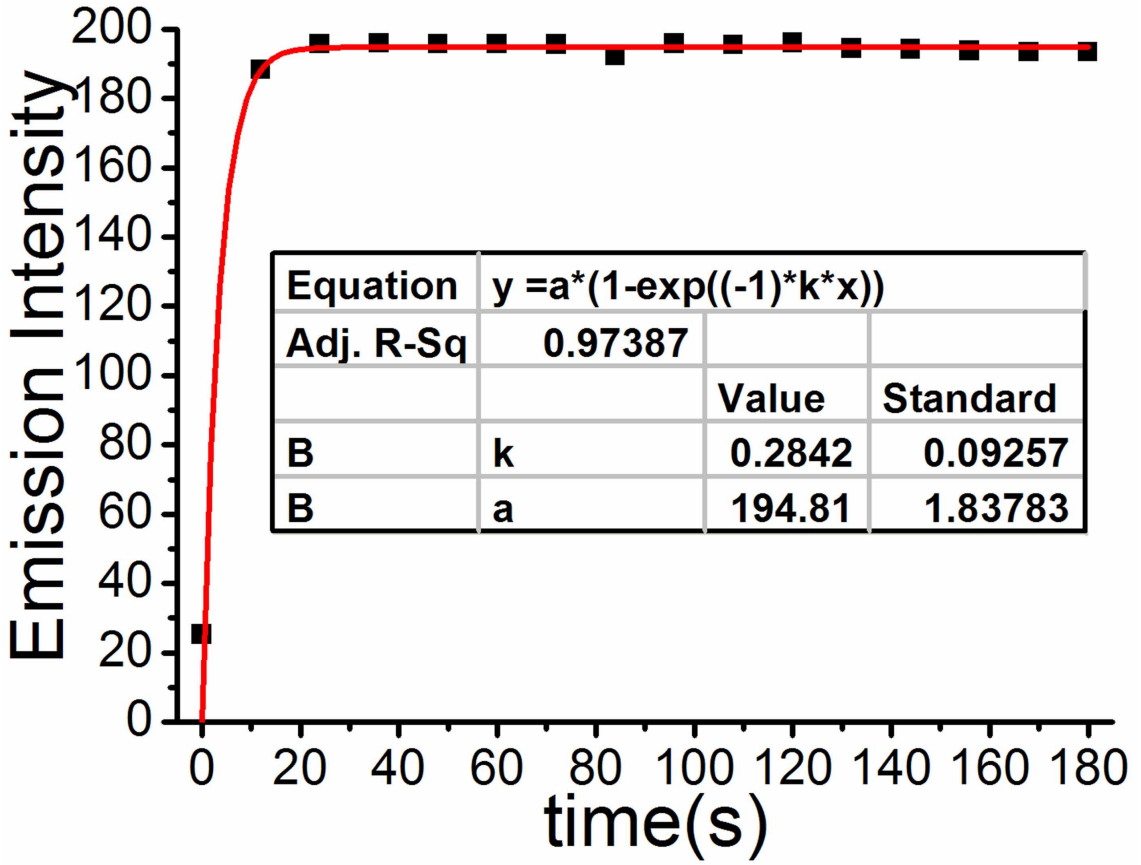


Fig. S6 Kinetic plot of fluorescence emission intensity at 514 nm of the pseudo-first order reaction of 1 μM FP to 100 μM Cu^{2+}, using excitation wavelength at 460 nm. The slope of the plot corresponds to the observed reaction rate of **0.284 s^{-1}**.

8. Comparison of various reaction-based probes for Cu^{2+}

Table S1 Performances comparison of various reaction-based probes for Cu^{2+}

Linear range	LOD	Testing media	Temperature	Reaction time	Ref.
0.5 - 10 μM	0.2 μM	H_2O-CH_3CN (10 mM HEPES, 4:1, v/v, pH 7.4)	-	5 min	S4
0.1 - 0.9 μM	35 nM	H_2O-DMSO (10 mM Tris-HCl, 99:1, v/v, pH 7.0)	25°C	5 min	S5
0.5 - 10 μM	-	H_2O-CH_3CN (10 mM HEPES, 7:3, v/v, pH 7.4)	25°C	-	S6
0 - 0.1 μM	20 nM	H_2O-DMSO (10 mM HEPES, 99:1, v/v, pH 8.0)	50°C	15 min	S7
	0.3 μM	H_2O-CH_3CH_2OH (10 mM Tris-HCl, 9:1, v/v, pH 7.4)	-	-	S8
0.08 - 30 μM	13 nM	H_2O-CH_3CN (HEPES, 4:1, v/v, pH 7.0)	-	-	S9
	20 nM	H_2O-CH_3CN (HEPES, 1:4, v/v, pH 7.0)	-	5 min	S10
1 - 20 μM	33 nM	H_2O-CH_3CN (25 mM Tris-HCl, 4:6, v/v, pH 6.0)	50°C	2 h	S11
-	87 nM	H_2O-CH_3CN (10 mM acetate, 5:5, v/v, pH 5.0)	-	5 min	S12
-	30 nM	H_2O-CH_3CN (1:10, v/v)	60°C	1.5 h	S13
-	0.25 μM	CH_3CN	-	15 min	S14

0.025 - 0.25 μM	3.5 nM	CH_3CN	25°C	2 min	
-	0.30 μM	H_2O-CH_3CN (5 mM Tris-HCl, 0.1 M KCl, 8:2, v/v, pH 7.2)	50°C	3 h	S15
-	37 nM	CH_3CN		20 min	
-	0.64 μM	H_2O-CH_3CN (10 mM Tris-HCl, 5:5, v/v, pH 7.0)	rt	6 h	S16
0.25 - 5 μM	11.1 nM	H_2O (HEPES, pH 7.4)	rt	2 min	S17
0 - 1 μM	55 nM	H_2O (10 mM HEPES, pH 7.0)	25°C	1 min	This Work

Reference

(S1) T. O. Zaikova, A. V. Rukavishnikov, G. Bruce Birrell, O. H. Griffith and J. F. W. Keana, *Bioconjugate Chem.*, 2001, **12**, 307.

(S2) Y. Chen, C. Zhu, Z. Yang, J. Chen, Y. He, Y. Jiao, W. He, L. Qiu, J. Cen and Z. Guo, *Angew. Chem. Int. Ed.*, 2013, **52**, 1688.

(S3) T. Wang, Jr. E. F. Douglass, K. J. Fitzgerald and D. A. Spiegel, *J. Am. Chem. Soc.*, 2013, **135**, 12429.

(S4) R. An, D. Zhang, Y. Chen and Y. Cui, *Sens Actuators B*, 2016, **222**, 48.

(S5) Z. Zhou, N. Li, A. Tong, *Anal. Chim. Acta*, 2011, **702**, 81.

(S6) H. Wen, Q. Huang, X.-F. Yang and H. Li, *Chem. Commun.*, 2013, **49**, 4956.

(S7) N. Li, Y. Xiang and A. Tong, *Chem. Commun.*, 2010, **46**, 3363.

(S8) L. Zhou, X. Zhang, Q. Wang, Y. Lv, G. Mao, A. Luo, Y. Wu, Y. Wu, J. Zhang and W. Tan, *J. Am. Chem. Soc.*, 2014, **136**, 9838.

(S9) L. Yuan, W. Lin, B. Chen and Y. Xie, *Org. Lett.*, 2012, **14**, 432.

(S10) M. Kumar, N. Kumar, V. Bhalla, P. R. Sharma and T. Kaur, *Org. Lett.*, 2012, **14**, 406.

(S11) X. Q. Chen, J. Jia, H. M. Ma, S. J. Wang and X. C. Wang, *Anal. Chim. Acta*, 2009, **632**, 9.

(S12) M. H. Kim, H. H. Jang, S. Yi, S.-K. Chang and M. S. Han, *Chem. Commun.*, 2009, 4838.

(S13) Q. Y. Wu and E. V. Anslyn, *J. Am. Chem. Soc.*, 2004, **126**, 14682.

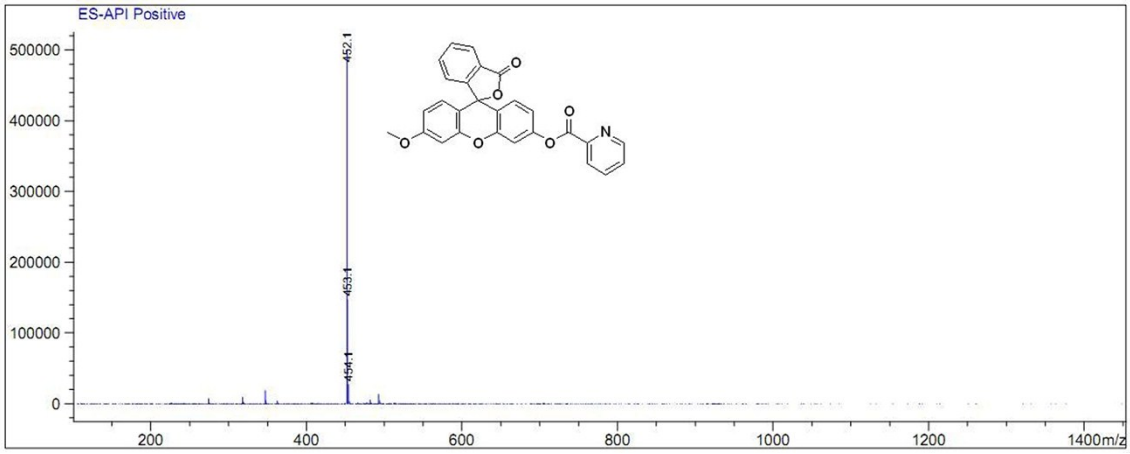
(S14) Y. Xiang and A. Tong, *Luminescence*, 2008, **23**, 28.

(S15) A. F. Li, H. He, Y. B. Ruan, Z. C. Wen, J. S. Zhao, Q. J. Jiang and Y. B. Jiang, *Org. Biomol. Chem.*, 2009, **7**, 193.

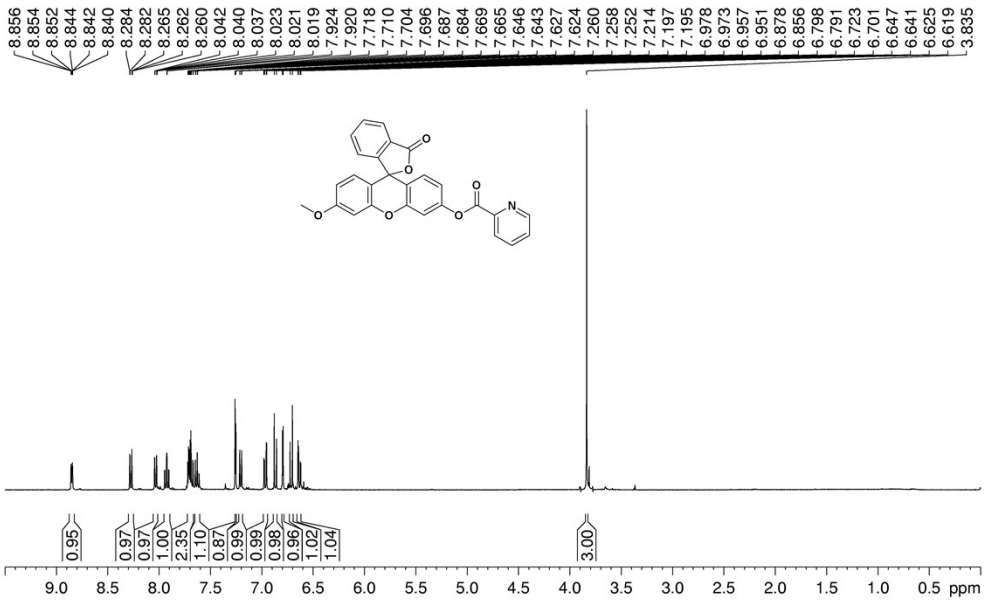
(S16) L. Mei, Y. Xiang, N. Li and A. Tong, *Talanta*, 2007, **72**, 1717

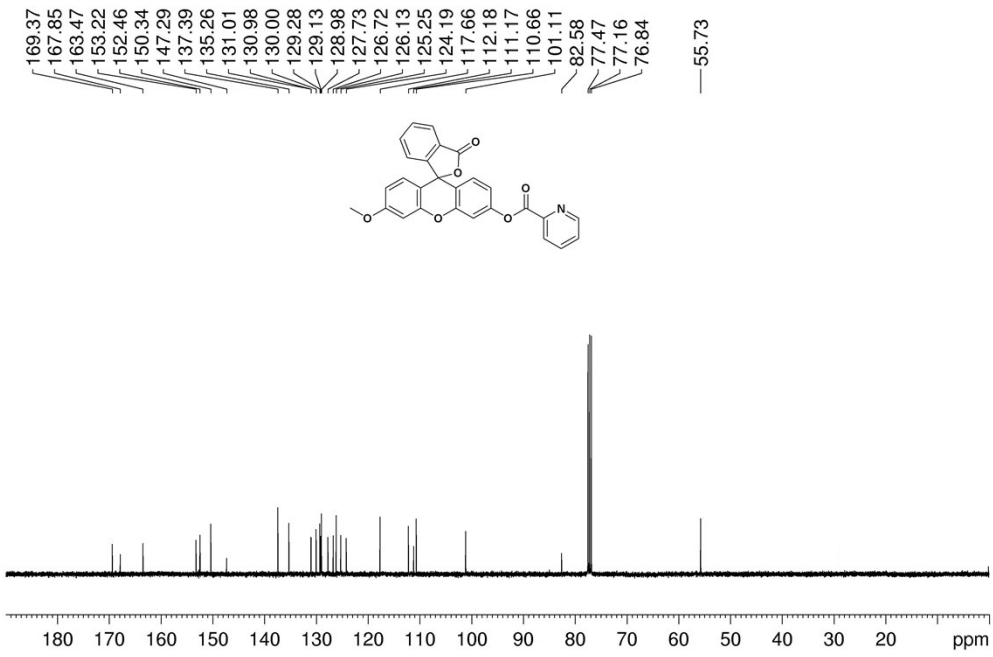
(S17) B. Wang, X. Cui, Z. Zhang, X. Chai, H. Ding, Q. Wu, Z. Guo and T. Wang, *Org. Biomol. Chem.*, 2016, **14**, 6720.

9. ESI-MS Spectra



10. NMR Data


8.856
8.854
8.852
8.844
8.842
8.840
8.284
8.282
8.265
8.262
8.260
8.042
8.040
8.037
8.023
8.021
8.019
7.924
7.920
7.718
7.710
7.704
7.696
7.687
7.684
7.669
7.665
7.646
7.643
7.627
7.624
7.260
7.258
7.252
7.214
7.197
7.195
6.978
6.973
6.957
6.951
6.878
6.856
6.798
6.791
6.723
6.701
6.647
6.641
6.625
6.619
3.835
0.95
0.97
0.97
1.00
2.35
1.10
0.87
0.99
0.99
0.98
0.96
1.02
1.04
3.00
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm


169.37
167.85
163.47
153.22
152.46
150.34
147.29
137.39
135.26
131.01
130.98
130.00
129.28
129.13
128.98
127.73
126.72
126.13
125.25
124.19
117.66
112.18
111.17
110.66
101.11
82.58
77.47
77.16
76.84
55.73
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm