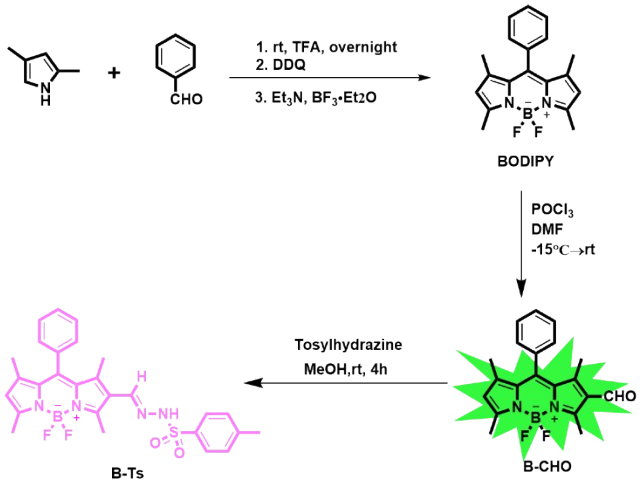
Supporting information for

An ultrafast responsive BODIPY-based fluorescent probe for the detection of endogenous hypochlorite in live cells

Liang Qiao, [a] Hai-Liang Nie, [a] Yu-Long Wu, [a] Fang-Yun Xin, [a] Cong-Cong Gao, [a] Jing Jing, * [a] and Xiao-Ling Zhang*[a]

Key laboratory of Cluster Science of Ministry of Education
Beijing Key Laboratory of Photoelectronic / Electrophotonic Conversion Materials
School of Chemistry and Chemical Engineering
Beijing Institute of Technology
5 South Zhongguancun Street, Beijing 100081, P. R. China.
E-mail: zhangxl@bit.edu.cn, hellojane@bit.edu.cn

Synthesis and characterizations of BODIPY ..... - 2 -
Synthesis and characterizations of B-CHO ..... - 2 -
Synthesis and characterizations of the product of B-Ts with hypochlorite ..... - 3 -
Normalized UV-vis spectra of B-CHO and B-Ts in ethanol solution ..... - 3 -
Determination of extinction coefficient of B-CHO and B-Ts ..... - 3 -
Determination of quantum yields of B-CHO and B-Ts ..... - 4 -
Selection of solvent ..... - 5 -
Selection of optimum amount of H_2O ..... - 5 -
Determination of the concentration of sodium hypochlorite ..... - 6 -
Preparations of various analytes[4,5] ..... - 7 -
Comparison of the recently reported ClO^- fluorescent probes ..... - 9 -
^1H NMR, ^{13}C NMR and ESI-MS spectra ..... - 12 -



Scheme. S1 The syntheses of BODIPY, B-CHO and B-Ts

Synthesis and characterizations of BODIPY

BODIPY was synthesized using the established procedure.[1] 2,4-Dimethylpyrrole (213 mg, 2.2mmol) and benzaldehyde (106 mg, 1mmol) were dissolved in CH_2Cl_2 with a catalytic amount of TFA (1-2 drops). The mixture was stirred for 16 h at r.t. Then a solution of 2,3- dichloro-5,6-dicyanobenzoquinone (DDQ) (227 mg, 1mmol) in ethyl acetate was added, and the mixture was stirred for 15 min. Finally, $BF_3 \cdot OEt_2$ (2 mL, excess) and triethylamine (2 mL, excess) were added, and the mixture was stirred for 3 h at r.t. The crude mixture was diluted with CH_2Cl_2 and washed with H_2O. The organic extracts were dried over $MgSO_4$, filtered and evaporated under reduced pressure. Flash chromatography (petroleum ether:CH_2Cl_2=1:1) afforded 235 mg of BODIPY as a red solid (72% yield). ^{1}H NMR (400 MHz, Chloroform-*d*): δ 7.52 – 7.45 (m, 3H), 7.28 (dd, *J* = 4.4, 3.1 Hz, 2H), 5.98 (s, 2H), 2.56 (s, 6H), 1.37 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 155.42, 143.16, 141.72, 134.99, 131.42, 129.13, 128.93, 127.93, 121.21, 14.60, 14.35.

Synthesis and characterizations of B-CHO

Formylation of BODIPY was conducted by Vilsmeier-Haack reaction.[2] A mixture of DMF (6 mL) and $POCl_3$ (6 mL) was stirred in an ice bath for 5 min under argon. After being warmed to room temperature, it was stirred for additional 30 min. To this reaction mixture was added BODIPY (158 mg, 0.5mmol) in dichloroethane (60 mL), the temperature was raised to 50℃, and the mixture was stirred for an additional 2h. The reaction mixture was cooled to room temperature and slowly poured into saturated aqueous $NaHCO_3$ (200 mL) under ice-cold conditions. After being warmed to room temperature, the reaction mixture was further stirred for 30 min and washed with water (2 ×150

mL). The organic layers were combined, dried over anhydrous MgSO4, and evaporated in vacuo. The crude product was further purified using column chromatography (silica gel, EtOAc/hexane=1:4, v/v) to give B−CHO (157mg, 89%) as a reddish-brown powder. ^{1}H NMR (400 MHz, Chloroform-d): δ 10.01 (s, 1H), 7.53 (q, J = 3.6 Hz, 3H), 7.28 (dd, J = 6.5, 2.9 Hz, 2H), 6.15 (s, 1H), 2.83 (s, 3H), 2.62 (s, 3H), 1.65 (s, 3H), 1.42 (s, 3H). ^{13}C NMR (101 MHz, CDCl3): δ 185.91, 161.61, 156.48, 147.28, 143.54, 142.91, 134.14, 129.48, 127.67, 126.29, 123.99, 15.10, 14.84, 13.02, 11.56.

Synthesis and characterizations of the product of B-Ts with hypochlorite

Compound B-Ts (52.0 mg, 0.1mmol) was dissolved in 20.0 mL Ethanol/Water (1/1, v/v) then 10 equivalents of ClO^- (1.0mmol) was added. Reaction mixture was extracted three times with DCM (20 mL portions) and dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. The resultant residue was purified by silica gel column chromatography (PE /ethyl acetate = 5/1, v/v) to afford a red solid.

Product: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.01 (s, 1H), 7.53 (tt, *J* = 3.8, 2.1 Hz, 3H), 7.31 – 7.27 (m, 2H), 6.15 (s, 1H), 2.83 (s, 3H), 2.62 (s, 3H), 1.65 (s, 3H), 1.42 (s, 3H). HR ESI-MS calcd for $C_{20}H_{20}BF_2N_2O^+$ (B-CHO·H^+) [M+H^+] =353.1631, found 353.1634.

B-CHO: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.01 (s, 1H), 7.53 (q, *J* = 3.6 Hz, 3H), 7.28 (dd, *J* = 6.5, 2.9 Hz, 2H), 6.15 (s, 1H), 2.83 (s, 3H), 2.62 (s, 3H), 1.65 (s, 3H), 1.42 (s, 3H).

Normalized UV-vis spectra of B-CHO and B-Ts in ethanol solution

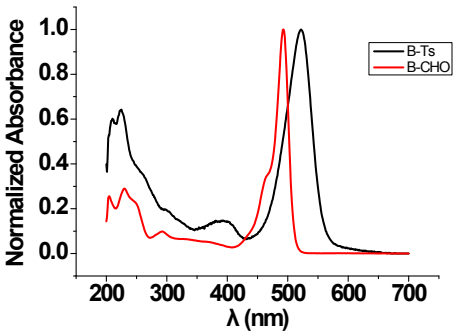


Fig. S1. Normalized UV-vis absorbance spectra of B-CHO and B-Ts

Determination of extinction coefficient of B-CHO and B-Ts

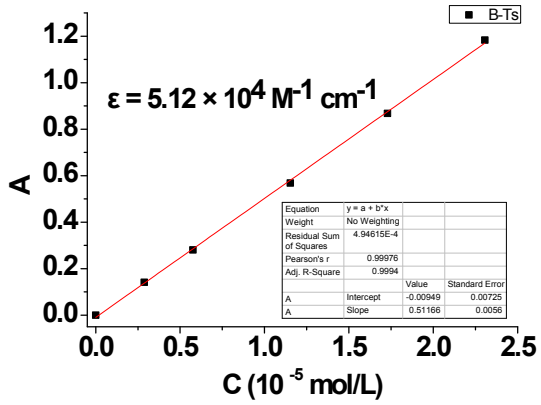


Fig. S2 Absorbance vs concentration of B-Ts diluted ethanol solution

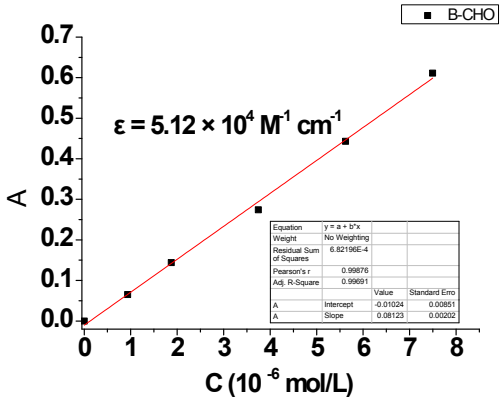


Fig. S3 Absorbance vs concentration of B-CHO diluted ethanol solution

Determination of quantum yields of B-CHO and B-Ts

The quantum yield of B-CHO and B-Ts was measured in ethanol with fluorescein in 0.1 M NaOH solution as the standard (Φ=0.925).[3] The absorbance of the solutions had better be in the range of 0-0.1 to minimize the re-absorption effect. The excitation wavelength was set at 470nm, and the emission range was 480-650nm with the slit widths set at 2.5 nm. The quantum yield was calculated using the following equation:

$$\Phi=\Phi_S\times(F/F_S)\times(A_S/A)\times(n^2/n_S^2)=\Phi_S\times(G/G_S)\times(n^2/n_S^2)$$

where Φ is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, G is the gradient of the curve of integrated intensity against absorbance at λ=470nm and n is the refractive index of the solvents used. Subscript S refers to the standard.

For 0.1 M NaOH, we used the wavelength-dependent refractive index of water. Also for ethanol, a wavelength-dependent refractive index was employed.[4-6] Therefore, $\Phi_{B\text{-}CHO}=0.614$, $\Phi_{B\text{-}Ts}=0.0272$.

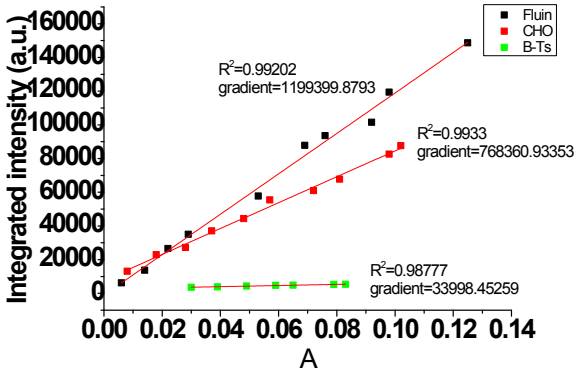


Fig.S4 The integrated fluorescence intensity vs absorbance for B-CHO, B-Ts and fluorescein, respectively

Selection of solvent

First, the optimum solvent was selected. Upon addition of hypochlorite to ethanol solution of B-Ts, an instant colour change from pink to green was observed and the fluorescence spectra showed an increase in fluorescence intensity. While in other solvents, such as DMSO and acetonitrile, the changes of color were not that obvious. Thus, ethanol was chosen for further investigation (data not shown).

Selection of optimum amount of H_2O

Subsequently, the optimum amount of water was screened. First, the water content was set at 95%, however, the response time was too long (about 1h, see Fig. S6) and colour change of solution was not observable by naked eye. Besides, the shape of the peaks and the hydrophilicity/hydrophobicity were also taken into consideration. Altogether, when the percentage of water was 50%, the absorption and fluorescence emission spectra were optimum, as shown in Fig. S7. Besides, the colour change of the probe system was more obvious. Therefore, the percentage of water was set to 50.

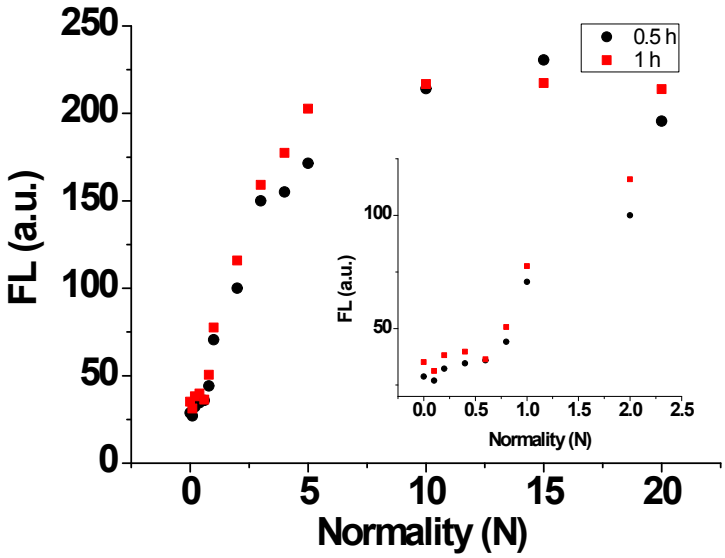


Fig. S5 Fluorescence titration curve when different amounts of hypochlorite were added into the B-Ts solution and were left to react for 0.5 and 1h, respectively. Inset: the titration curve corresponding to 0.5-7.5μM of hypochlorite for clarification.

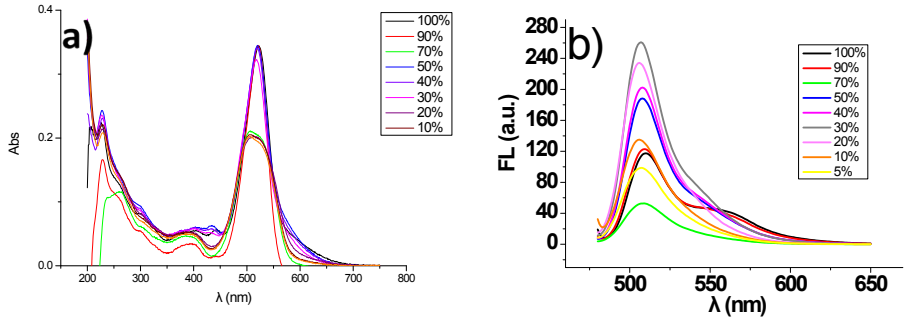


Fig. S6 UV-vis absorbance (a) and fluorescence emission spectra (b) of B-Ts solution with different water content.

Determination of the concentration of sodium hypochlorite

The concentration of the hypochlorite was quite vital in this experiment. One practice to determine the exact concentration of a hypochlorite solution is iodiometry. Under acidic conditions, hypochlorite oxidized iodide ion to iodine, which was measured by titration with sodium thiosulfate using flour as indicator. In this experiment, the concentration was determined by measuring the absorbance of hypochlorite solution at 294nm at pH=12. The molar extinction coefficient is 350 M^{-1} cm^{-1}.[3]

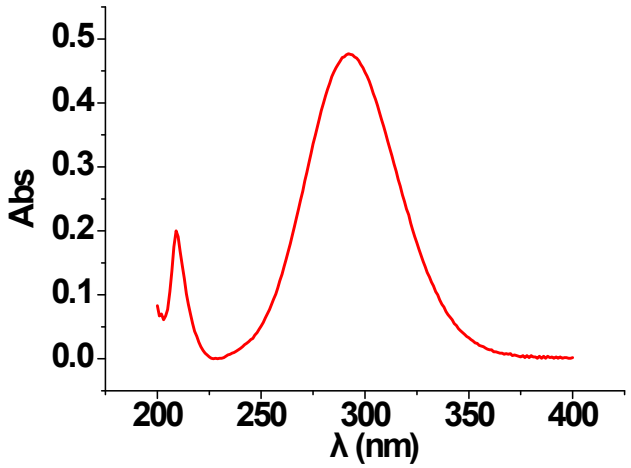


Fig. S7 UV-vis absorbance spectrum of NaClO at pH=12

Preparations of various analytes[4,5]

Preparation of H_2O_2

The H_2O_2 solution was obtained from H_2O_2 stock solution. The accurate concentration of the stock H_2O_2 stock solution was determined by measuring the absorbance at 240nm where ε_{240}= 43.6 $M^{-1}cm^{-1}$. [6]

Preparation of ClO^-

The sodium hypochlorite solution was obtained from sodium hypochlorite stock solution. The accurate concentration of the stock NaClO stock solution was determined by measuring the absorbance at 292nm under basic conditions (pH=12) where ε_{292}= 350 $M^{-1}cm^{-1}$.

Preparation of ROO·

ROO· was generated from 2,2'-azobis(2-amidinopropane) dihydrochloride, which was added into the testing solutions directly.

Preparation of TBHP

Tert-Butyl hydroperoxide solution (10mM) was added into the testing solutions directly.

Preparation of ·OH

Hydroxyl radical was generated in situ by Fenton reactions. 10 equivalents of ferrous sulfate were added to H_2O_2 to prepare the radical solution. The concentration of •OH was equal to the H_2O_2 concentration.

Preparation of t-BuO·

Tert-butoxy radical was generated in situ by Fenton reactions. 10 equivalents of ferrous sulfate were added to TBHP to prepare the radical solution. The concentration of t-BuO• was equal to the TBHP concentration.

Preparation of $^1O_2$

Singlet oxygen was generated from the reaction of H_2O_2 with sodium hypochlorite.

Preparation of NO•

Nitric oxide was generated from SNP (sodium nitroferricyanide(III) dihydrate).

Preparation of $ONOO^-$

Peroxynitrite solution was synthesized according to literature report.1 Briefly, hydrogen peroxide (0.7 M) was acidified with hydrochloric acid (0.6 M). Then this mixture, sodium

nitrite (0.6 M), sodium hydroxide (1.5 M) aqueous solution was mixed within 1–2 s under vigorous stirring. The excess hydrogen peroxide was removed by passing the solution through a short column of manganese dioxide. The resulting solution was split into small aliquots and stored at lower than −18 °C. The aliquots were thawed immediately before use, and the concentration of peroxynitrite was determined by measuring the absorption of the solution at 302 nm. The extinction coefficient of peroxynitrite solution in 0.1 M NaOH is 1670 M−1 cm−1 at 302 nm.

Preparation of GSH

The source of GSH was reduced glutathione.

Preparation of NO_2^-

The source of $NO_2^-$was sodium nitrite anhydrous.

Preparation of NO_3^-

The source of NO_3^- was sodium nitrate anhydrous.

Preparation of Fe^{2+}

The source of Fe^{2+} was ferrous sulfate tetrahydrate.

Preparation of Fe^{3+}

The source of Fe^{3+} was ferric chloride hexahydrate.

Preparation of Zn^{2+}

The source of Zn^{2+} was anhydrous zinc chloride.

Preparation of Mg^{2+}

The source of Mg^{2+} was magnesium chloride hexahydrate.

Preparation of Ca^{2+}

The source of Ca^{2+} was anhydrous calcium chloride.

Preparation of S^{2-}

The source of S^{2-} was sodium sulphide nonahydrate.

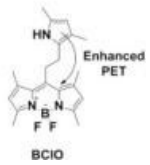
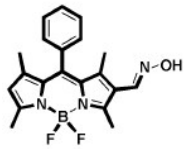
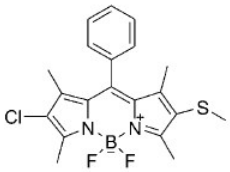
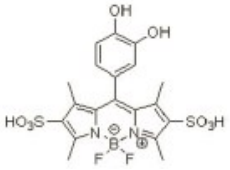
Preparation of Vc

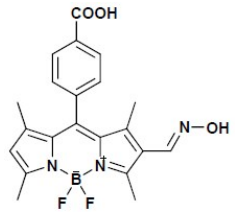
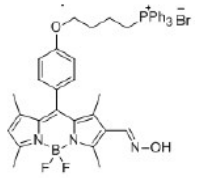
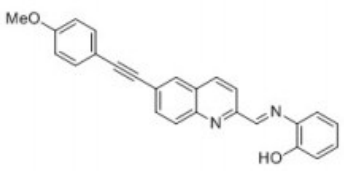
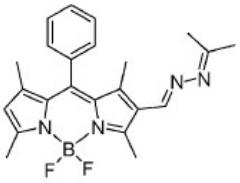
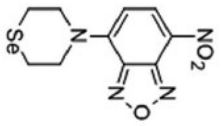
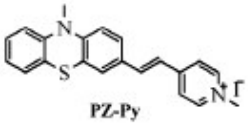
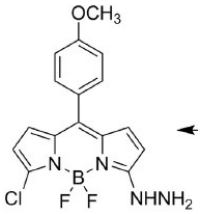
The source of Vc was anhydrous ascorbic acid.

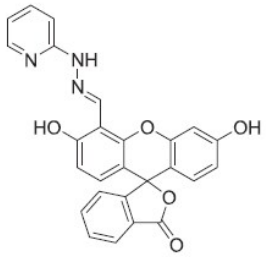
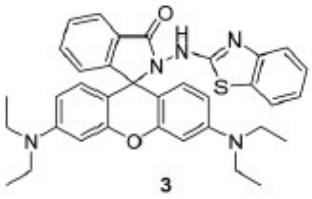
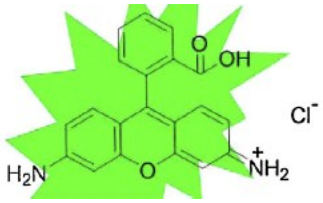
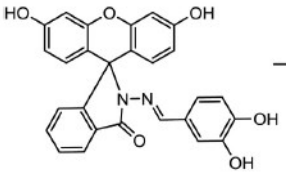
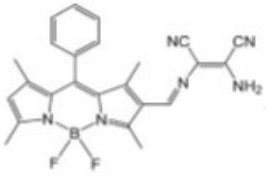
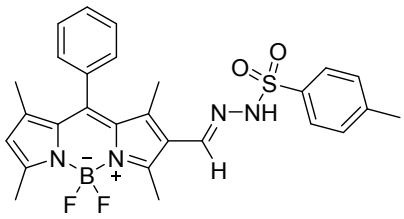
Preparation of citrate

The source of citrate was citric acid dehydrate.

Comparison of the recently reported ClO^- fluorescent probes

Probe & Reference	Testing system	LOD	Response time
 J. Am. Chem. Soc. 2014,136, 12820−12823	PBS (0.01 M) solution (ethanol/water = 1:9 v/v, pH 7.4)	0.56 nM	within 1 s
 *Chem. Commun.,* 2013,**49**, 7836--7838	pH 7.2 buffer – DMF (v/v, 4 : 1)	0.5 μM	within seconds
 *Chem. Commun.,* 2015, 51, 6781--6784	PBS (pH 7.4, 10 mM, containing 0.1% DMSO)	0.12 nM for RMClO-1 and 0.84 nM for RMClO-2	within 5s
 Chem. Asian J. 2011, 6, 1358 – 1361	HEPES buffer (10 mm, pH 7.4, 0.2%DMSO)	N.A	1h
 Analyst, 2014, 139, 2986 – 2989	phosphate buffer (10 mM, pH 7.8),	0.3 μ M	60min

 Anal. Methods, 2013, 5, 5589 – 5596	0.1 M pH 7.4 PBS buffer	17.7 nM	<1s
 Analyst, 2013, 138, 6091 – 6096	PBS buffer (pH 7.4, 10 mM) and EtOH (1/9,v/v)	0.52μM	within seconds
 Dalton Trans., 2015, 44, 6613 – 6619	pH 7.4 PBS–DMSO (1 : 9, v/v)	0.31 μM	within 10 minutes
 *New J. Chem.,* 2015, 39, 6892-6898	PBS buffer–MeOH (v/v = 70/30, 70 mM PBS, and pH 7.4),	0.205 μM	within 3 min,
 RSC Adv., 2015, 5, 79519 – 79524	acetonitrile–water solution (1 : 1 v/v, 50 mM PBS buffer solution at pH 7.4)	3.3nM	<10 s
 *Chem. Commun.,* 2015, 51, 1442--1445	PBS (pH 7.3, 10 mM, containing 0.05% DMSO)	17.9 nM	within just a few seconds
	PBS (10 mM, pH 7.2, 10% CH3CN)	93nM	within 1 min

Dyes and Pigments 126 (2016) 218-223			
 Dyes and Pigments 120 (2015) 22-29	PBS buffer (10 mM, pH 7.4)	7.3nM	within 5 s
 RSC Adv., 2015, 5, 99664 – 99668	MeCN–PBS (v/v =3 : 7, pH =7.4)	1.06nM	MeCN–PBS (v/v =3 : 7, pH =7.4),
 Sensors and Actuators B 224 (2016) 307 – 314	HEPES (10 mM, pH 7.4)	4.1 nM	5s Turn-off type
 Dyes and Pigments 107 (2014) 127-132	H2O/DMSO (v/v =20/1, Tris-HCl buffer, 0.05 M, pH 7.4)	40 nM	within seconds
 Talanta, 2016, 161: 847-853	PBS buffer (0.01 M, pH 7.4, containing 20% EtOH, v/v)	19.8 nM	<10 s
 This work	PBS buffer (pH 7.4, 10 mM) and EtOH (1:1, v/v)	7.5nM	0.2s

^{1}H NMR, ^{13}C NMR and ESI-MS spectra

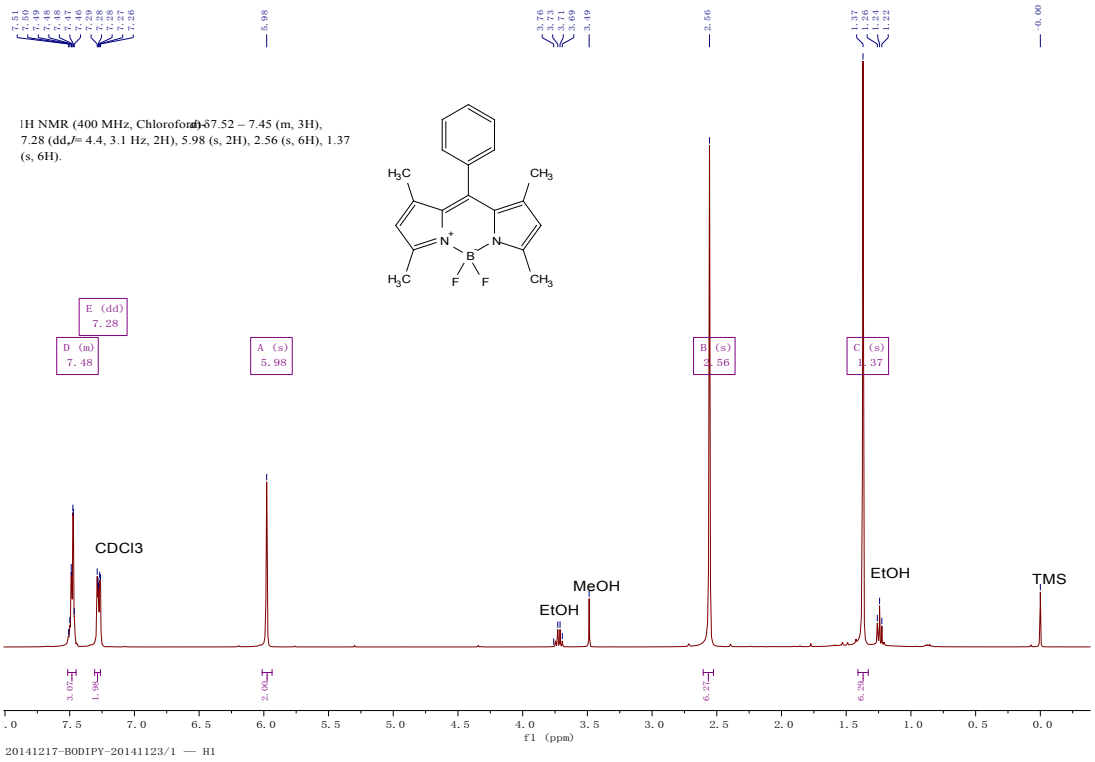
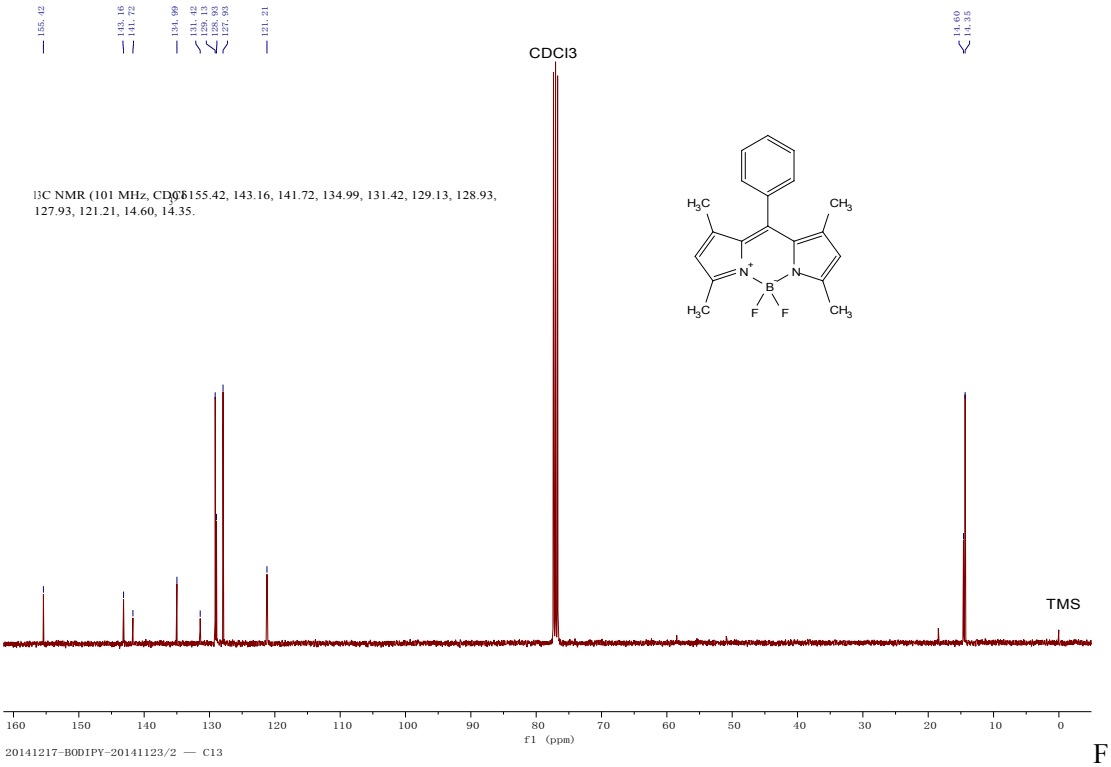


Fig. S8 ^{1}H NMR spectrum of BODIPY



F

ig. S9 ^{13}C NMR spectrum of BODIPY

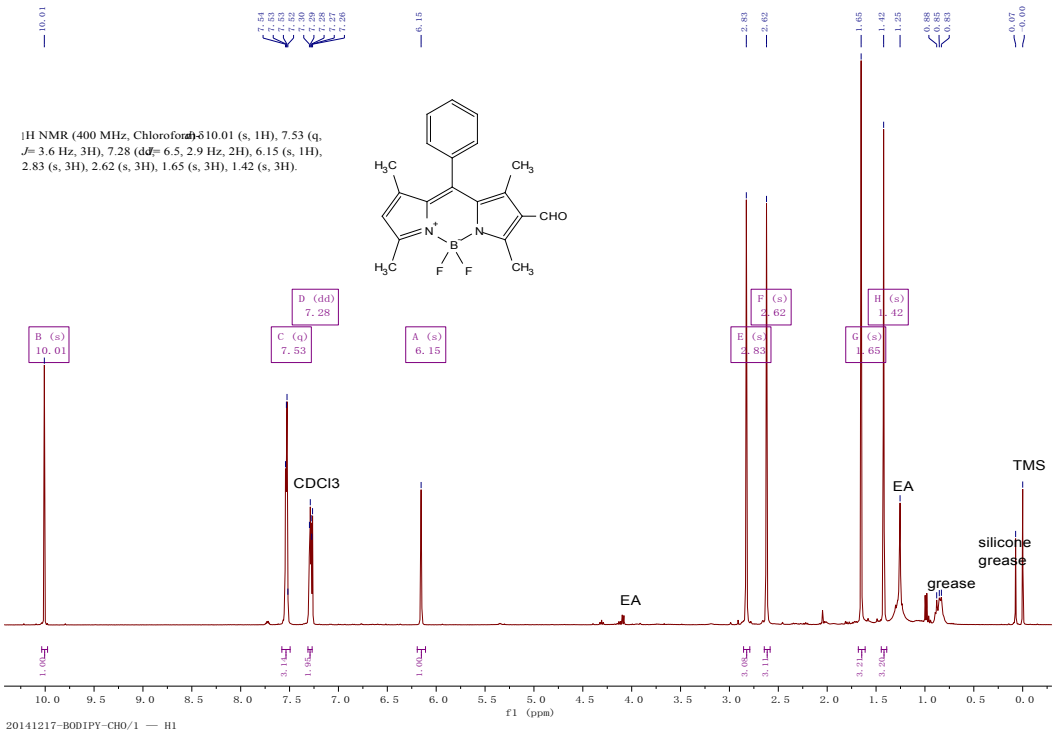


Fig. S10 ^{1}H NMR spectrum of B-CHO

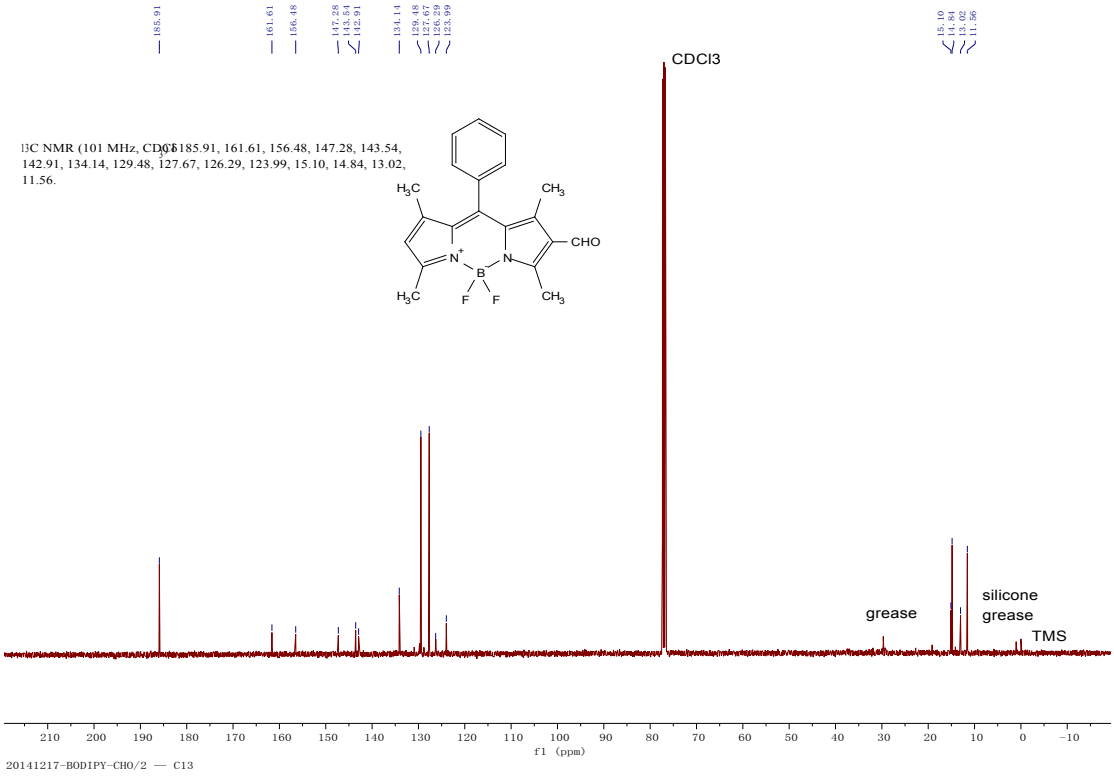


Fig. S11 ^{13}C NMR spectrum of B-CHO

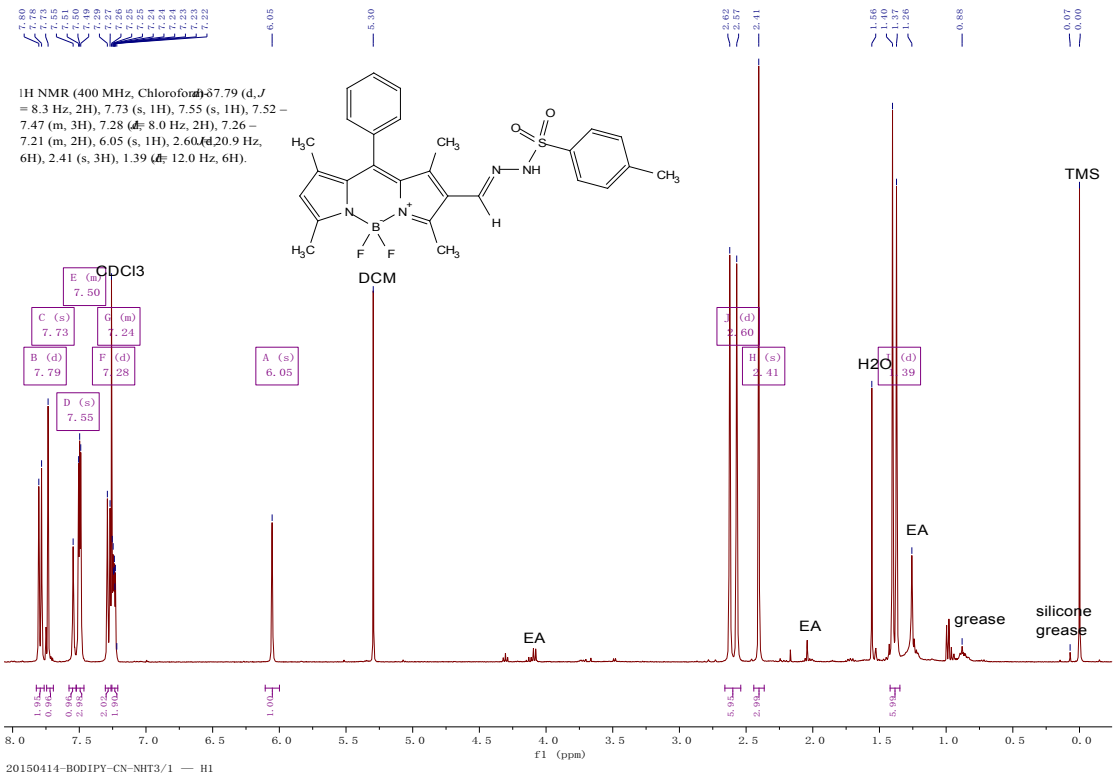


Fig.S12 [1]H NMR spectrum of B-Ts

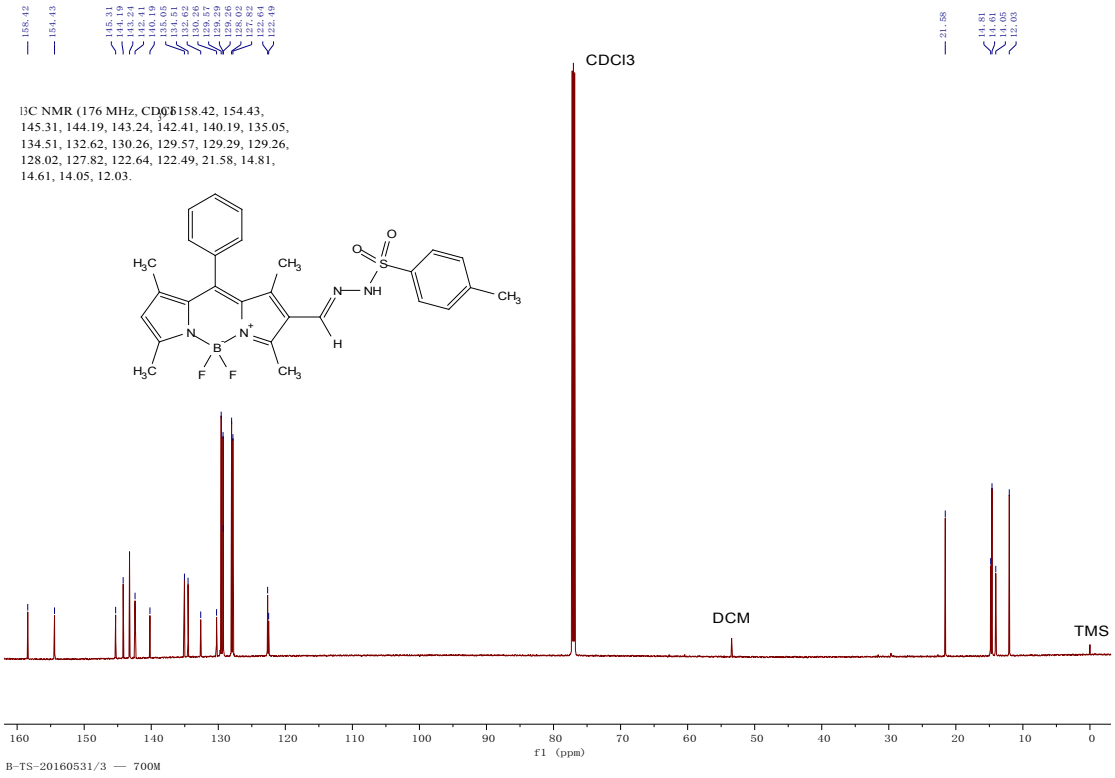


Fig. S13 [13]C NMR spectrum of B-Ts

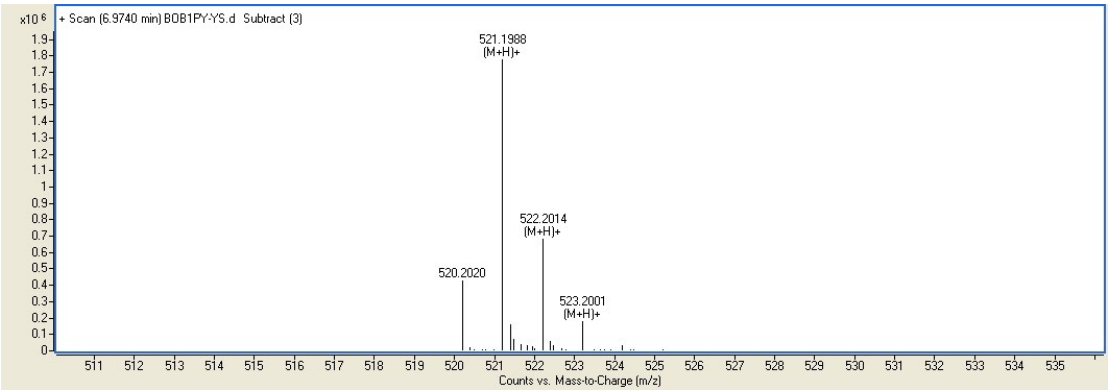


Fig. S14 High resolution ESI mass spectrum of B-Ts

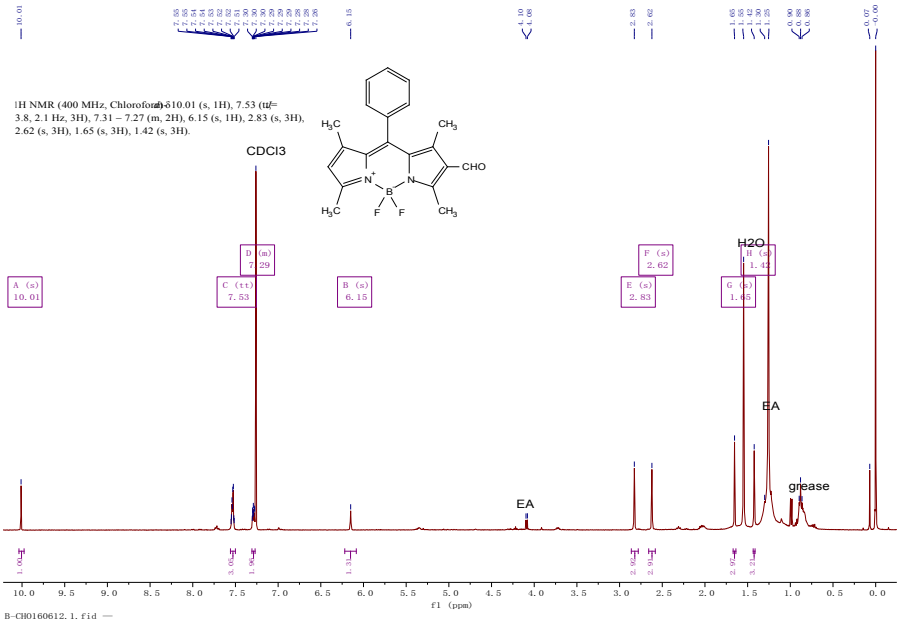


Fig. S15 ^{1}H NMR spectrum of the product of reaction of B-Ts with hypochlorite

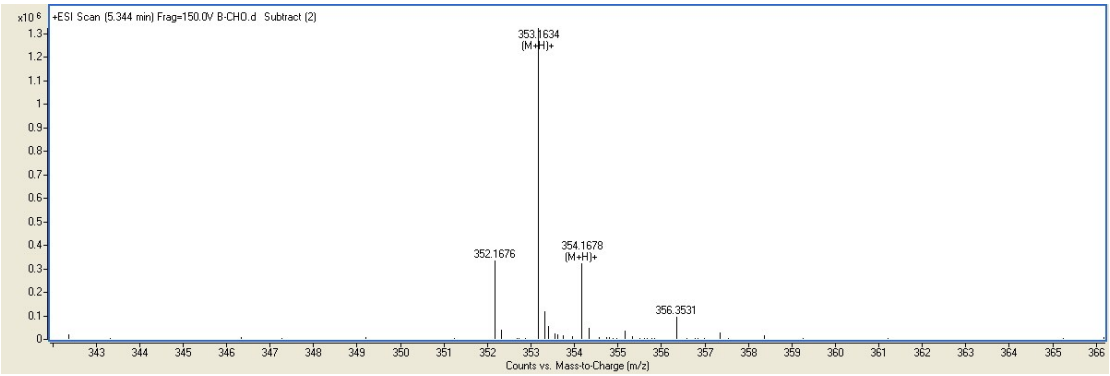


Fig. S16 High resolution mass spectrum of the reaction product

(1) Vazquez-Romero, A.; Kielland, N.; Arevalo, M. J.; Preciado, S.; Mellanby, R. J.; Feng, Y.; Lavilla, R.; Vendrell, M. *J Am Chem Soc* **2013**, *135*, 16018.

(2) Jiao, L.; Yu, C.; Li, J.; Wang, Z.; Wu, M.; Hao, E. *The Journal of organic chemistry* **2009**, *74*, 7525.

(3) Yan, Y.; Wang, S.; Liu, Z.; Wang, H.; Huang, D. *Analytical chemistry* **2010**, *82*, 9775.

(4) Ma, Z.; Sun, W.; Chen, L.; Li, J.; Liu, Z.; Bai, H.; Zhu, M.; Du, L.; Shi, X.; Li, M. *Chemical communications* **2013**, *49*, 6295.

(5) Hu, J. J.; Wong, N.-K.; Gu, Q.; Bai, X.; Ye, S.; Yang, D. *Organic Letters* **2014**, *16*, 3544.

(6) Pirmohamed, T.; Dowding, J. M.; Singh, S.; Wasserman, B.; Heckert, E.; Karakoti, A. S.; King, J. E.; Seal, S.; Self, W. T. *Chemical communications* **2010**, *46*, 2736.