Electronic Supplementary Material (ESI) for Chemical Communications


# Supporting Information

# A BODIPY aldoxime-based chemodosimeter for highly selective and rapid detection of hypochlorous acid

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## 1. General Methods:

All reagents were purchased from commercial suppliers (Aldrich and Merck) and used without further purification. $^{1}$H NMR and $^{13}$C NMR were measured on a Varian VNMRJ 400 Nuclear Magnetic Resonance Spectrometer. Bruker MALDI-TOF-TOF Mass Spectrometer was used for mass spectrometry analysis. UV absorption spectra were obtained on Shimadzu UV-2550 Spectrophotometer. Fluorescence emission spectra were obtained using Varian Cary Eclipse Fluorescence spectrophotometer. Cell imaging was performed with Olympus IX71 fluorescence microscope. Samples were contained in 10.0 mm path length quartz cuvettes (2.0 mL volume). Upon excitation at 470 nm, the emission spectra were integrated over the range 490 nm to 700 nm. The slit width was 5 nm for both excitation and emission. Melting points were determined using an Electrothermal Melting Point Apparatus 9200. The pH was recorded by HI-8014 instrument (HANNA). All measurements were conducted at least in triplicate.

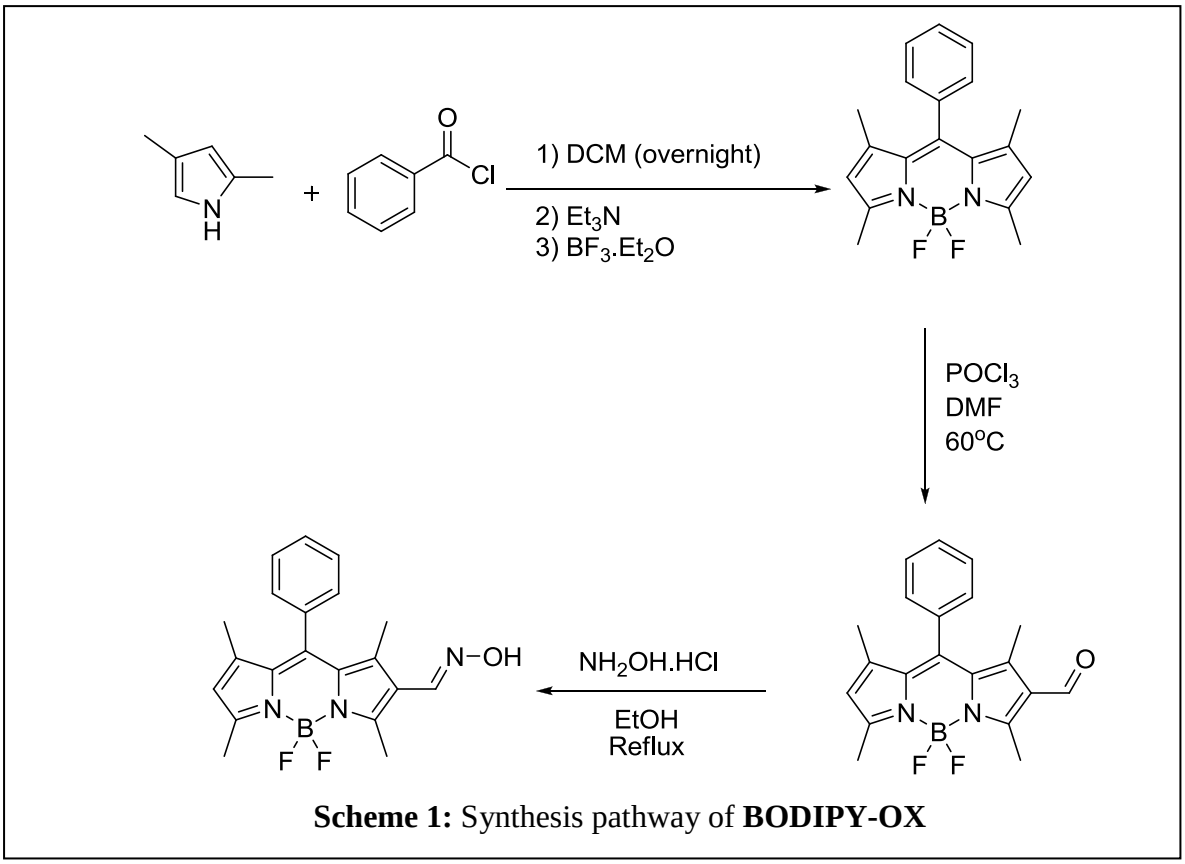
## 2. Determination of Quantum Yields

Fluorescence quantum yields of **BODIPY-OX** and **BODIPY-CNO** were determined by using optically matching solutions of Rhodamine 6G ($\Phi_F$=0.95 in water[1]) as a standard[2]. The quantum yield was calculated according to the equation;

$$\Phi_{F(X)} = \Phi_{F(S)} (A_S F_X / A_X F_S) (n_X / n_S)^2$$

Where $\Phi_F$ is the fluorescence quantum yield, A is the absorbance at the excitation wavelength, F is the area under the corrected emission curve, and n is the refractive index of the solvents used. Subscripts S and X refer to the standard and to the unknown, respectively.

## 3. Synthesis and Characterization of Fluorescent Probe

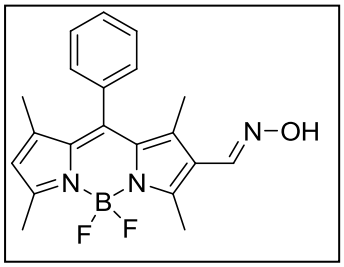



**Scheme 1:** Synthesis pathway of **BODIPY-OX**

**BODIPY** was synthesized according to known procedure.[3]

**BODIPY-AL**: Formylation reaction of **BODIPY** was performed by using known (Vilsmeier Haack reaction) procedure.[4] Mp: 239-241 $^{o}$C. $^{1}$H NMR (400 MHz, $CDCl_3$) δ: 10.00 (s, 1H), 7.54-7,52 (m, 3H), 7.29-7,27 (m, 2H), 6.14 (s, 1H), 2.81 (s, 3H), 2.60 (s, 3H), 1.64 (s, 3H), 1.41 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 185.9, 161.6, 156.4, 147.3, 143.5, 142.9, 134.1, 129.5, 127.7, 126.3, 124.0, 15.1, 14.8, 13.0, 11.6.

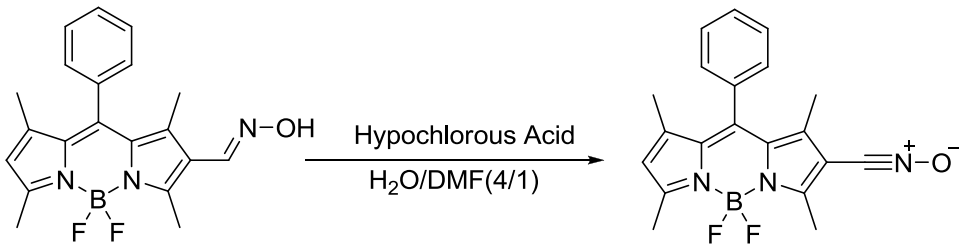
**Synthesis of BODIPY-OX**



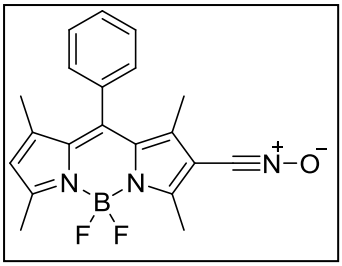

**BODIPY-AL** (150 mg, 0.5 mmol) and hydroxylamine hydrochloride (41.7 mg, 0.6 mmol) in absolute ethanol (10.0 mL) were stirred at reflux temperature for one hour. After the completion of reaction the solvent was removed under reduced pressure and the residue was purified by column chromatography (hexane/ethyl acetate (3/1)). **BODIPY-OX** was obtained as red solid (130.0 mg, 70.0% yield). Mp: 225 $^{o}$C. $^{1}$H NMR (400 MHz, $CDCl_3$) δ: 10.41 (s, 1H), 8.07

(s, 1H), 7.53-7.50 (m, 3H), 7.29-7.27 (m, 2H), 6.05 (s, 1H), 2.71 (s, 3H), 2.56 (s, 3H), 1.46 (s, 3H), 1.38 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 156.8, 153.8, 144.2, 142.1, 141.9, 139.4, 134.3, 131.8, 130.2, 129.0, 128.9, 127.5, 122.3, 121.8, 14.4, 14.2, 13.8, 12.0. MS (MALDI-TOF): m/z: Calcd. for $C_{20}H_{20}BF_2N_3O$: 367.166 $[M]^+$, Found: 368.157$[M+H]^+$.

**Synthesis of BODIPY-CNO from BODIPY-OX**




**Scheme 2:** Reaction of **BODIPY-OX** with hypochlorous acid




Compound **BODIPY-OX** (50.0 mg, 0.14 mmol) was dissolved in 20.0 mL DMF/Water (1/4, buffered by 0,1M phosphate buffer at pH=7.2) then 40 equivalent of HOCl (5.6 mmol) was added. Reaction mixture was stirred at room temperature for 5 min. This mixture was extracted three times with DCM (100 mL portions) and dried over anhydrous $Na_2SO_4$ and the solvent removed under reduced pressure. The resultant residue was purified by silica gel column chromatography (hexane /ethyl acetate (8/1)) to afford **BODIPY-CNO** as red solid (33.2 mg, 65% yield). (decomposes at 150 $^{o}$C). $^1$H NMR (400 MHz, $CDCl_3$) δ: 7.54-7,53 (m, 3H), 7.27-7.25 (m, 2H), 6.13 (s, 1H), 2.62 (s, 3H), 2.61 (s, 3H), 1.42 (s, 6H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ: 156.9, 156.0, 144.2, 143.4, 140.4, 133.5, 131.4, 129.9, 129.7, 127.5, 124.8, 104.3, 13.6, 13.3, 12.8, 12.2. Calcd. for $C_{20}H_{18}BF_2N_3O$: 365.151 $[M]^+$, Found: 365.173$[M]^+$.

## 4. Cell Imaging

MCF10A cells were grown in DMEM/F12 medium supplemented with DHS (Donor Horse Serum), cholera toxin and insulin in an atmosphere of 5 % $CO_2$ at 37 $^oC$. The cells were plated on 24-well plate and allowed to grow for 24h. Before the experiments, the cells were washed with PBS buffer, and then the cells were incubated **BODIPY-OX** (1.0 μM) for 10 min at 37 $^oC$ then washed with PBS three times. After incubating with HOCl (10 μM) for 10 min at 37 $^oC$, cells were rinsed with PBS three times and Hoechst 34580 (2 μM) for 15 min at 37 $^oC$ then washed with PBS three times. Then, the fluorescence images were acquired through an Olympus IX71 fluorescence microscope.

## 5. Preparation of ROS[5]

### Preparation of $H_2O_2$

The stock $H_2O_2$ solution was purchased from Sigma-Aldrich Chemical Co. The concentration of $H_2O_2$ was titrated according to iodometry.

### Preparation of $ROO^{\bullet}$

$ROO^{\bullet}$ was generated from 2,2'-azobis(2-amidinopropane)dihydrochloride. AAPH (2,2'-azobis(2- amidinopropane)dihydrochloride) in deionizer water was added, then stirred at 25 °C for 30 min.

### Preparation of $^{\bullet}OH$

Hydroxyl radical was generated by Fenton reaction[6]. To prepare $^{\bullet}OH$ solution, ferrous chloride was added in the presence of 10 equivalents of $H_2O_2$.

### Preparation of HOCl

Commercial bleach was the source of NaOCl. The concentration of HOCl was determined by titration with $S_2O_3^{2-}$.

## References

1. Brouwer, A. M. *Pure Appl. Chem.* 2011, **83**, 2213-2228.
2. Ajayaghosh, A.; Carol, P.; Sreejith, S. *J. Am. Chem. Soc.* 2005, **127**, 14962-14963.
3. Ekmekci, Z.; M. Yilmaz, D.; Akkaya, E. U. *Org. Lett*. 2008, **10**, 461-464.
4. Isik, M.; Ozdemir, T.; Turan, I. S.; Kolemen, S.; Akkaya, E. U. *Org. Lett.* 2013, **15**, 216-219.
5. Sun, Z. N.; Liu, F. Q.; Chen, Y.; Tam, P. K. H.; Yang, D. *Org. Lett.* 2008, **10**, 2171-2174
6. Fenton, H. H., *Chem. News* 1876, **33**, 190.

## Absorption and Emission Spectra of BODIPY-OX

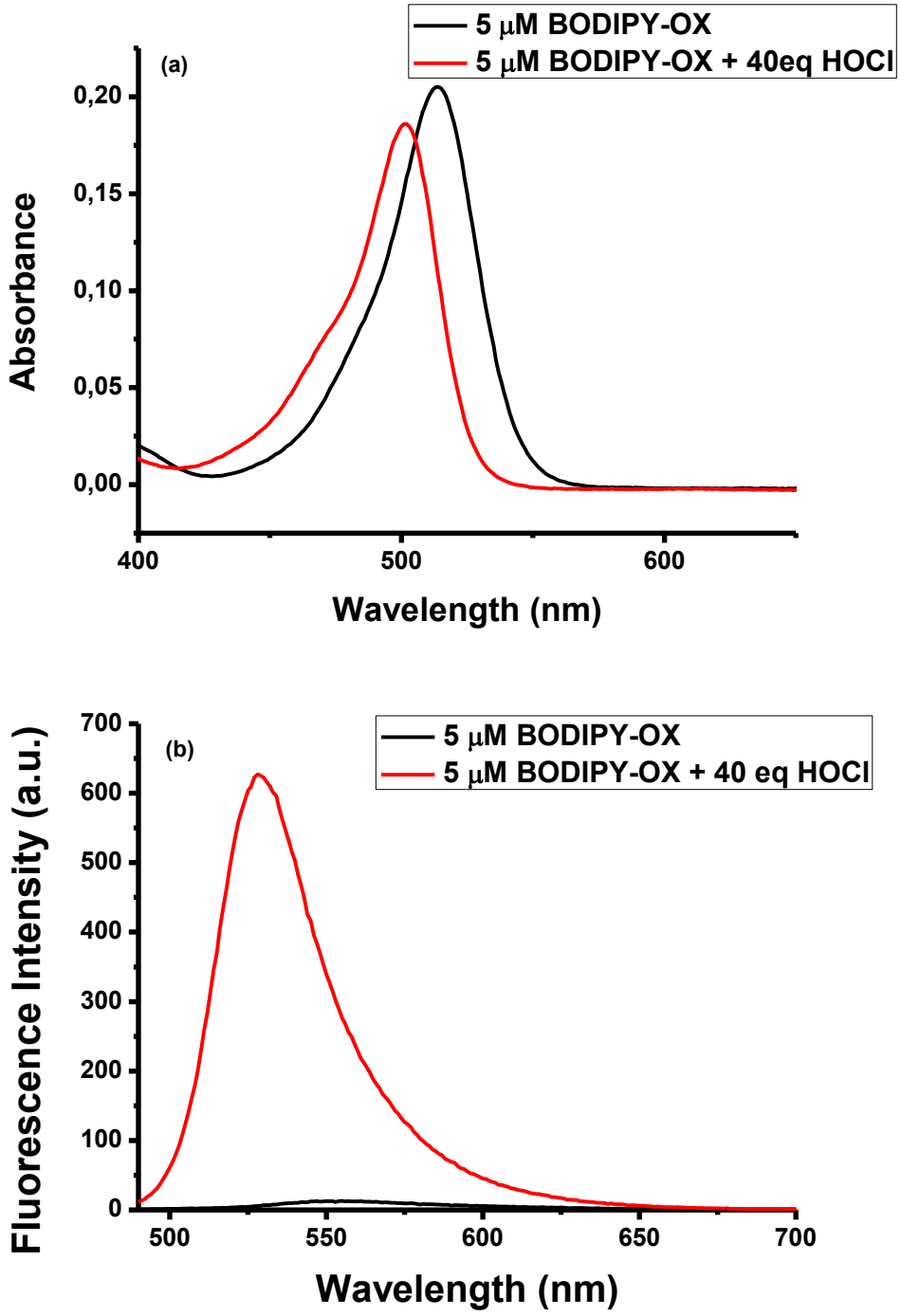



**Figure S1:** Absorption and Emission spectra of **BODIPY-OX** (5.0 μM) and HOCl (200 μM, 40 equiv.) in 0.1M potassium phosphate buffer, pH 7.2/DMF (v/v, 4:1) ($\lambda_{ex}$: 470 nm, at 25 $^{o}$C).

## Fluorescence and Absorbance Titration Spectra of BODIPY-OX with HOCl

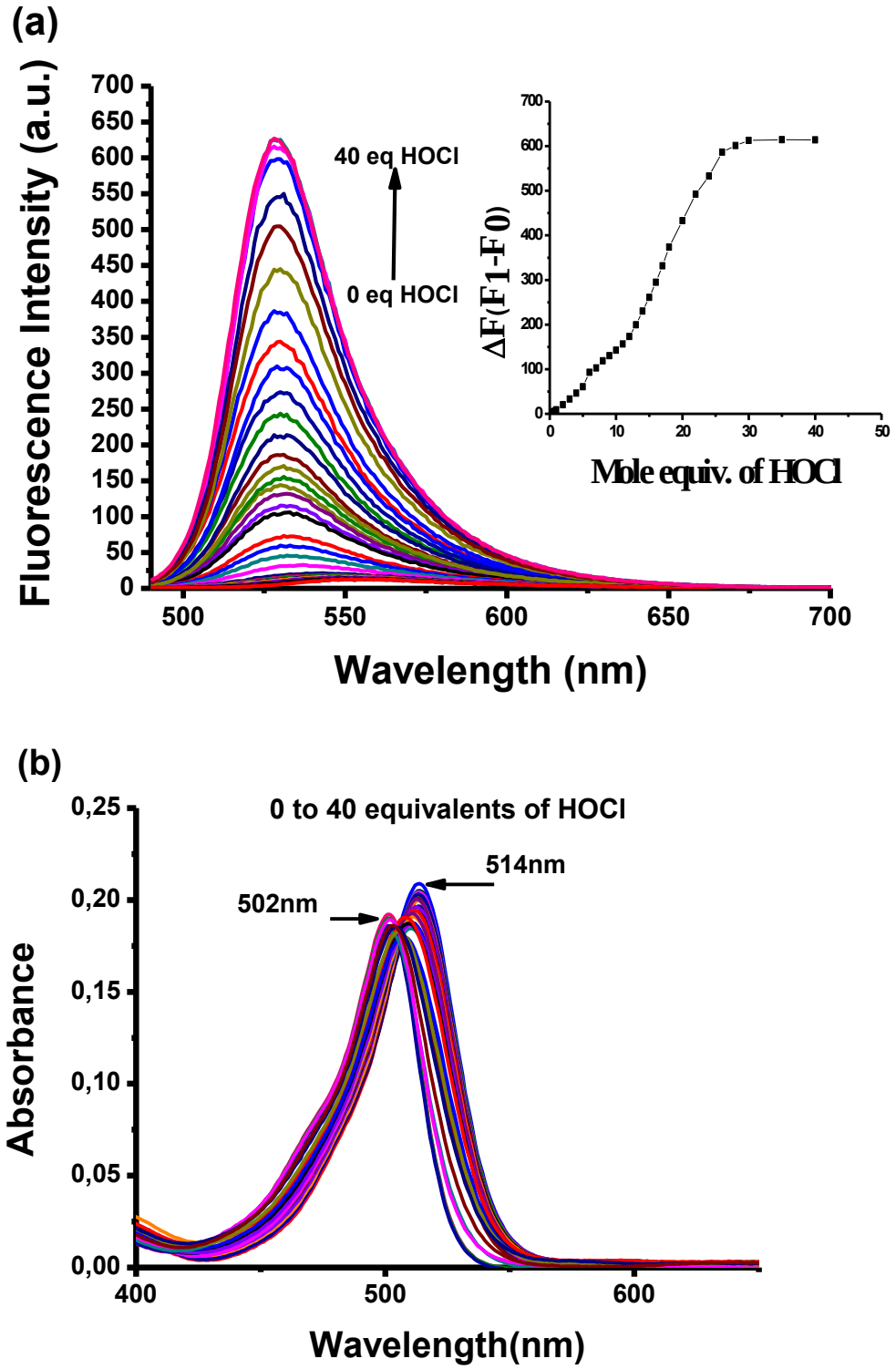



**Figure S2: (a)** Fluorescence and **(b)** absorbance spectra of **BODIPY-OX** (5.0 μM) in 0.1M potassium phosphate buffer, pH 7.2/DMF (v/v, 4:1) in the presence of HOCl (0.5 to 200 μM, 0.1 to 40 equiv.) ($\lambda_{ex}$: 470 nm, at 25 $^{o}$C). Inset: Fluorescence intensity changes of **BODIPY-OX** vs equivalents of HOCl.

## Determination of Effect of Solvent to the Reactivity of BODIPY-OX

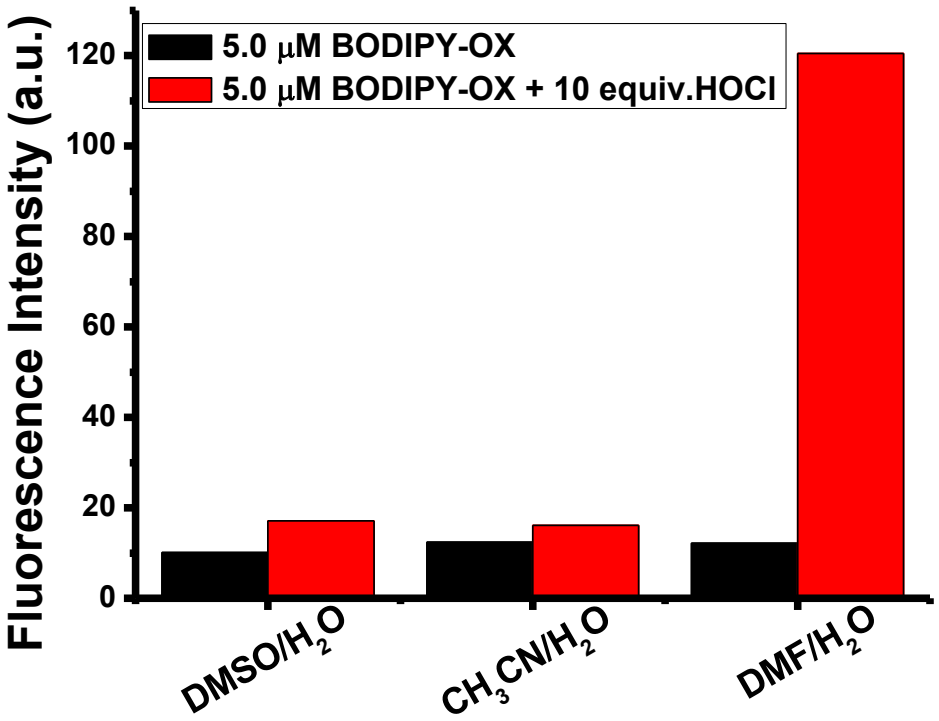



**Figure S3**: Effect of solvent on the reaction of **BODIPY-OX** (5.0μM) with HOCl (50 μM, 10 equiv.) in various organic solvents (($H_2O$/Solvent) v/v, 4:1) ($\lambda_{ex}$: 470 nm, at 25 $^oC$).

## Determination of Effect of DMF/Water Ratio to the Reactivity of BODIPY-OX

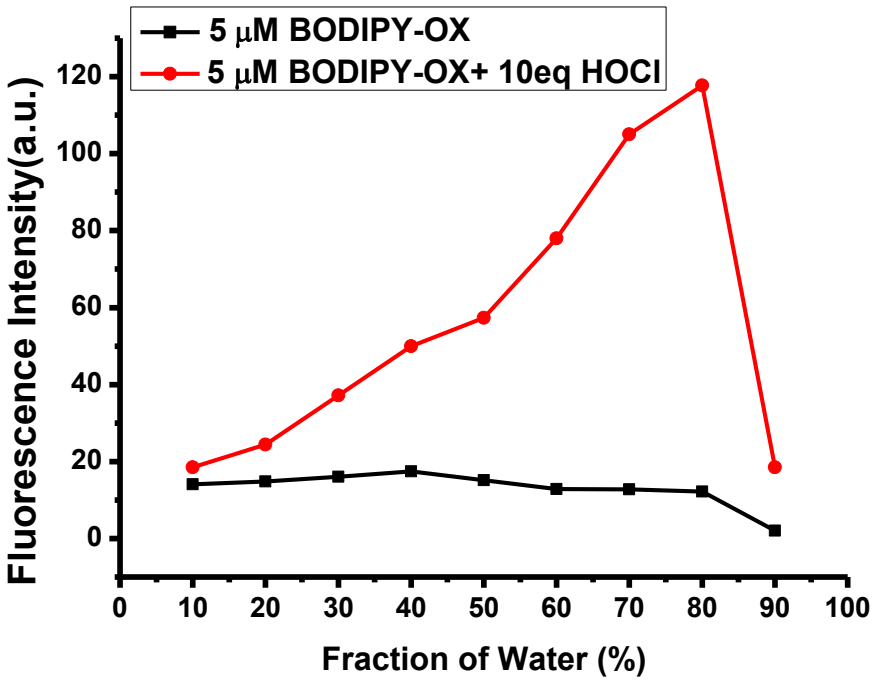



**Figure S4:** Effect of fraction of water on the reaction of **BODIPY-OX** (5.0μM) with HOCl (50 μM, 10 equiv.) in 0.1M potassium phosphate buffer, pH 7.2/DMF (v/v, 4:1) ($\lambda_{ex}$: 470 nm, at 25 $^{o}$C).

## Determination of Effect of pH to the Reactivity of BODIPY-OX

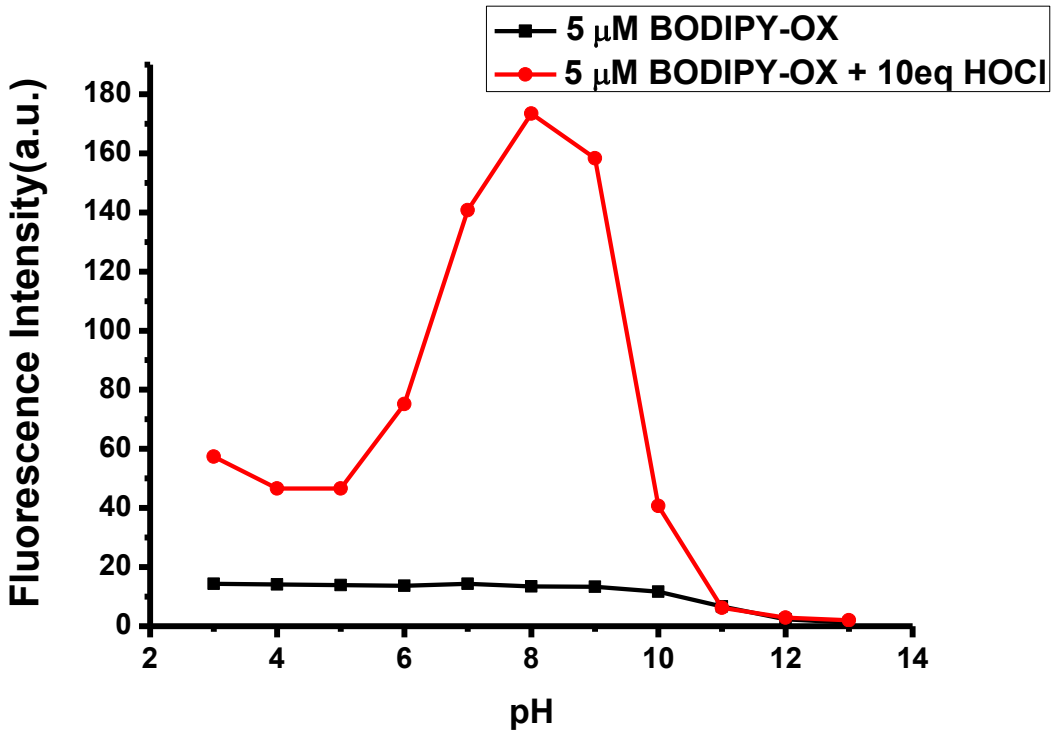



**Figure S5**: Effect of pH on the reaction of **BODIPY-OX** (5.0μM) with HOCl (50 μM, 10 equiv.) in $H_2O$/DMF (v/v, 4:1) ($\lambda_{ex}$: 470 nm, at 25 $^oC$).

# The Fluorescence Responses of BODIPY-OX with HOCl and Other Ions and ROS

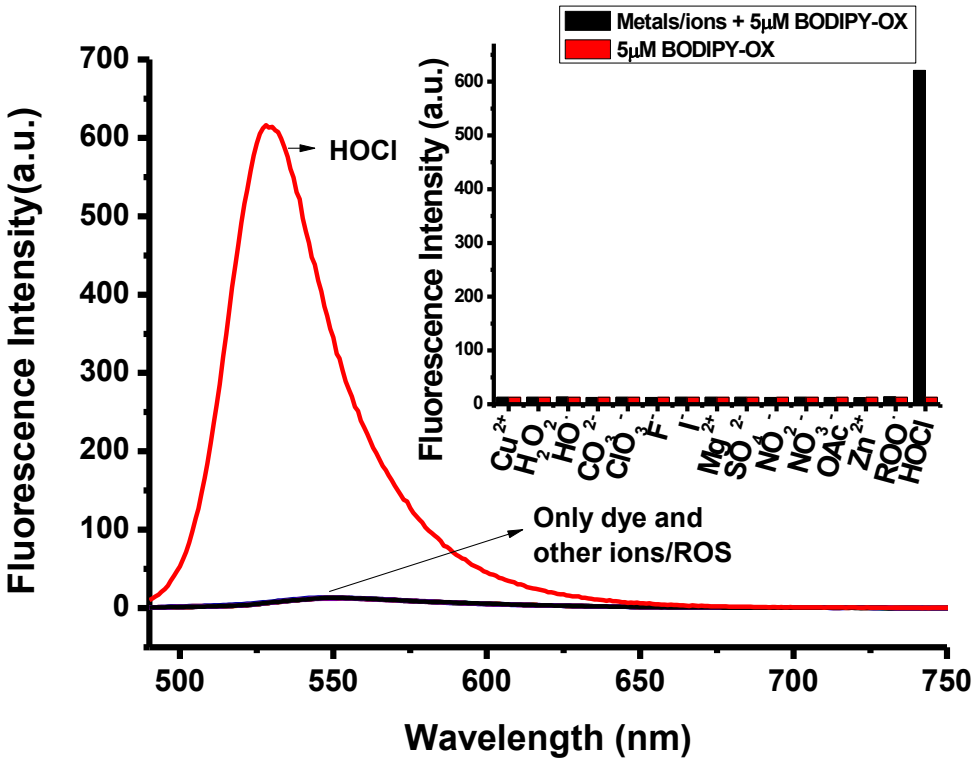



**Figure S6:** Fluorescence intensities of **BODIPY-OX** (5.0 μM), **BODIPY-OX** (5.0 μM) + HOCl (200μM, 40 equiv.), **BODIPY-OX** (5.0 μM) + other ions/ROS (200μM, 40 equiv.) in 0.1M potassium phosphate buffer, pH 7.2/DMF (v/v, 4:1) ($\lambda_{ex}$: 470 nm, at 25 $^{o}$C). Inset: Bar graph notation.

# The Fluorescence Responses of BODIPY-OX in the Presence of HOCl and Other Metal Ions or ROS

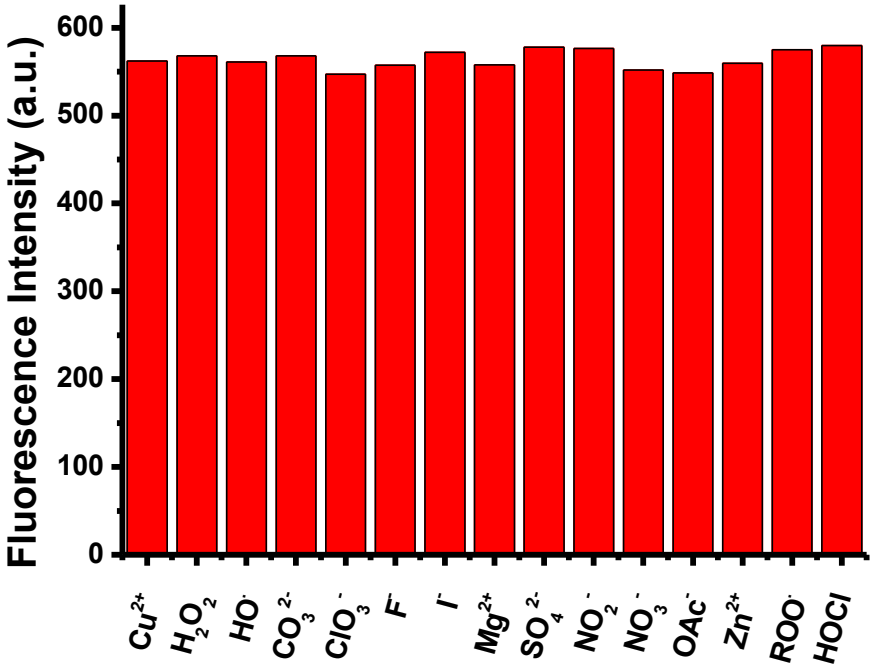
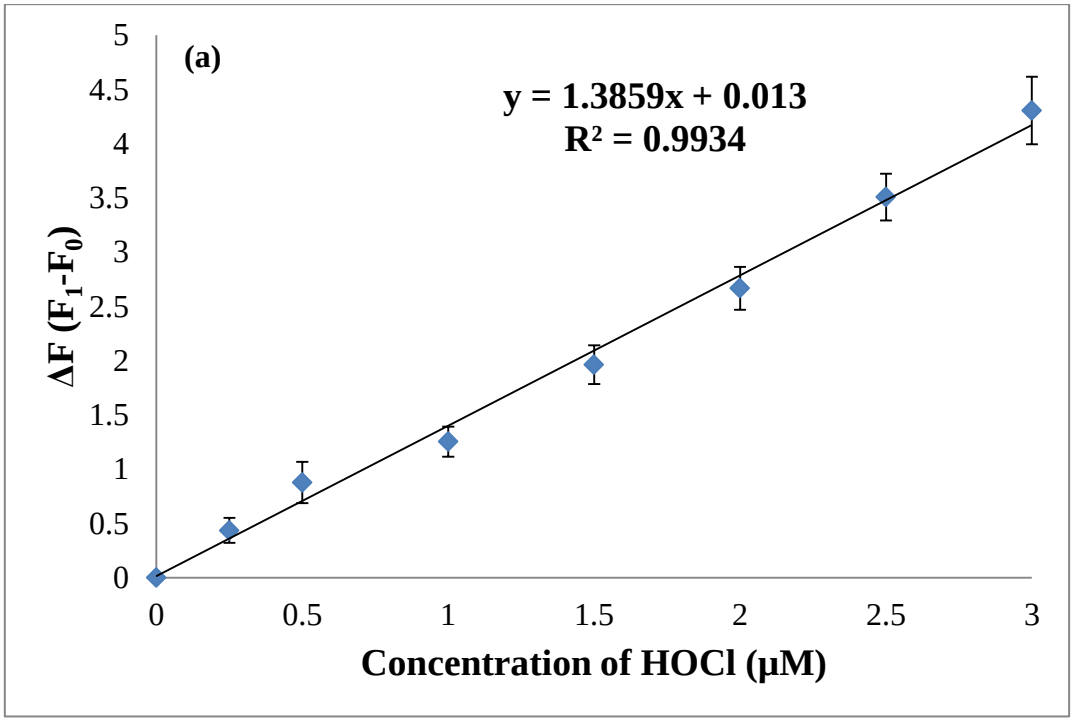



**Figure S7:** Fluorescence intensities of **BODIPY-OX** (5.0 μM) ) in 0.1M potassium phosphate buffer, pH 7.2/DMF (v/v, 4:1) upon addition of HOCl (200 μM, 40 equiv.) in the presence of background species (200 μM, 40 equiv.): $Cu^{2+}$, $H_2O_2$, $HO^{\cdot}$, $CO_3^{2-}$, $ClO_3^{-}$, $F^{-}$, $Mg^{2+}$, $SO_4^{2-}$, $NO^{2-}$, $NO^{3-}$, $OAc^{-}$, $Zn^{2+}$, $ROO^{\cdot}$, HOCl ($\lambda_{ex}$: 470 nm, at 25 $^{o}$C).

## Determination of Detection Limit

The detection limit was calculated based on the fluorescence titration. To determine the detection limit, the emission intensity of **BODIPY-OX** (5.0 μM) without HOCl was measured by 10 times and the standard deviation of blank measurements was determined. Under the present conditions, a good linear relationship between the fluorescence intensity and HOCl concentration could be obtained in the 0 – 3 μM (R = 0.9934). The detection limit is then calculated with the equation: detection limit = 3σbi/m, where σbi is the standard deviation of blank measurements; m is the slope between intensity versus sample concentration. The detection limit was measured to be 0.5 μM.



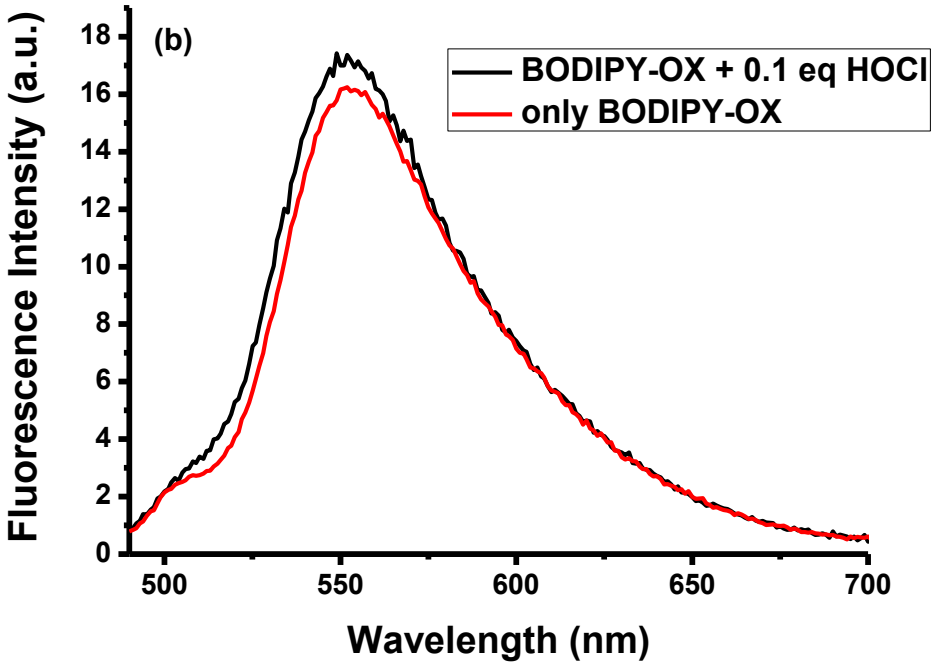



**Figure S8: (a)** Fluorescence changes of **BODIPY-OX** (5.0 μM) upon addition of HOCl (0.25 to 3.0 μM, 0.05 to 0.6 equiv.) **(b)** Fluorescence spectra of **BODIPY-OX** (5.0 μM) in the presence of HOCl (0.5 μM, 0.1 equiv.) in 0.1M potassium phosphate buffer, pH 7.2/DMF (v/v, 4:1) ($\lambda_{ex}$: 470 nm, at 25 $^{o}$C).

## Determination of Stability of BODIPY-OX + HOCl Mixture over Time

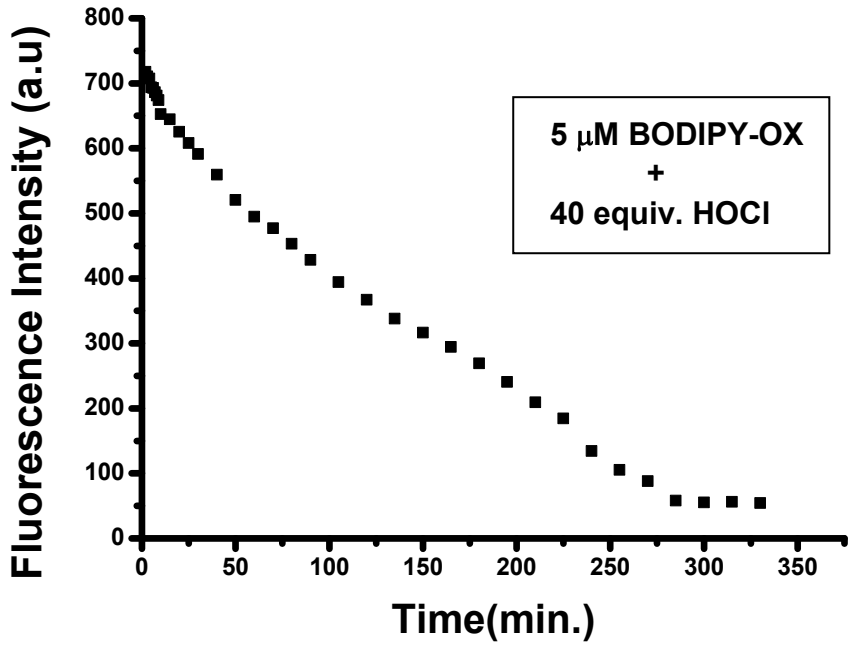



**Figure S9:** Fluorescence intensity change of **BODIPY-OX** (5.0μM) + HOCl (200μM ,40 equiv.) over time in 0.1M potassium phosphate buffer, pH 7.2/DMF (v/v, 4:1)) ($\lambda_{ex}$: 470 nm, at 25 $^{o}$C).

## Reaction – Time Profiles of BODIPY-OX with HOCl

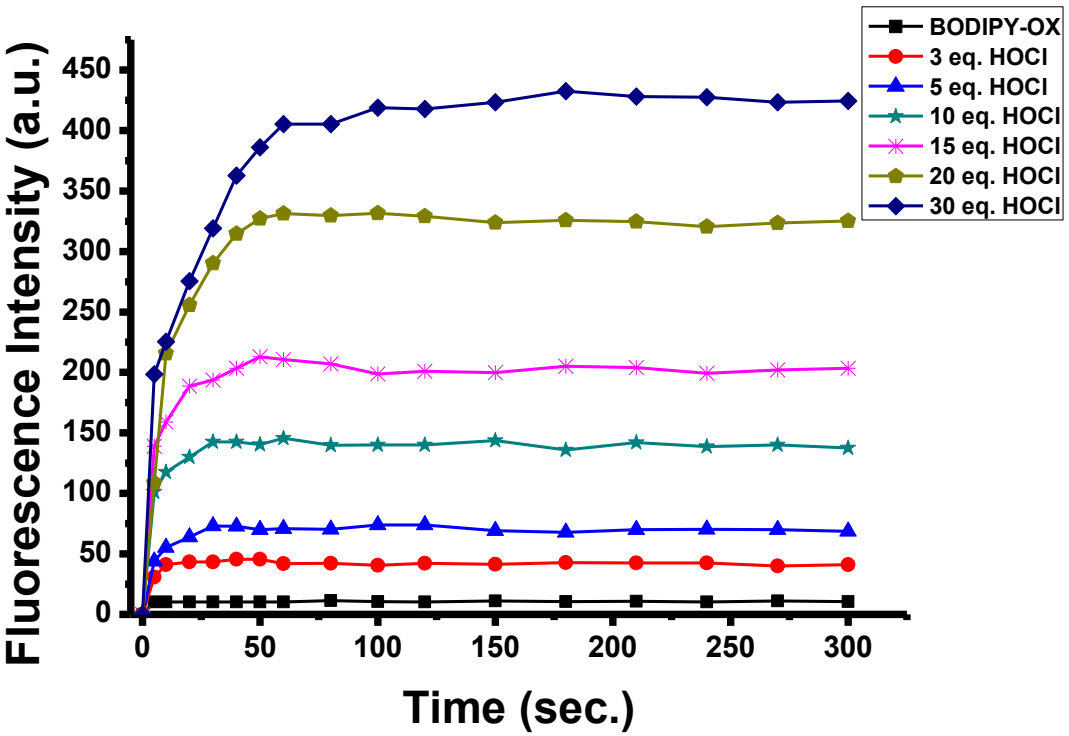
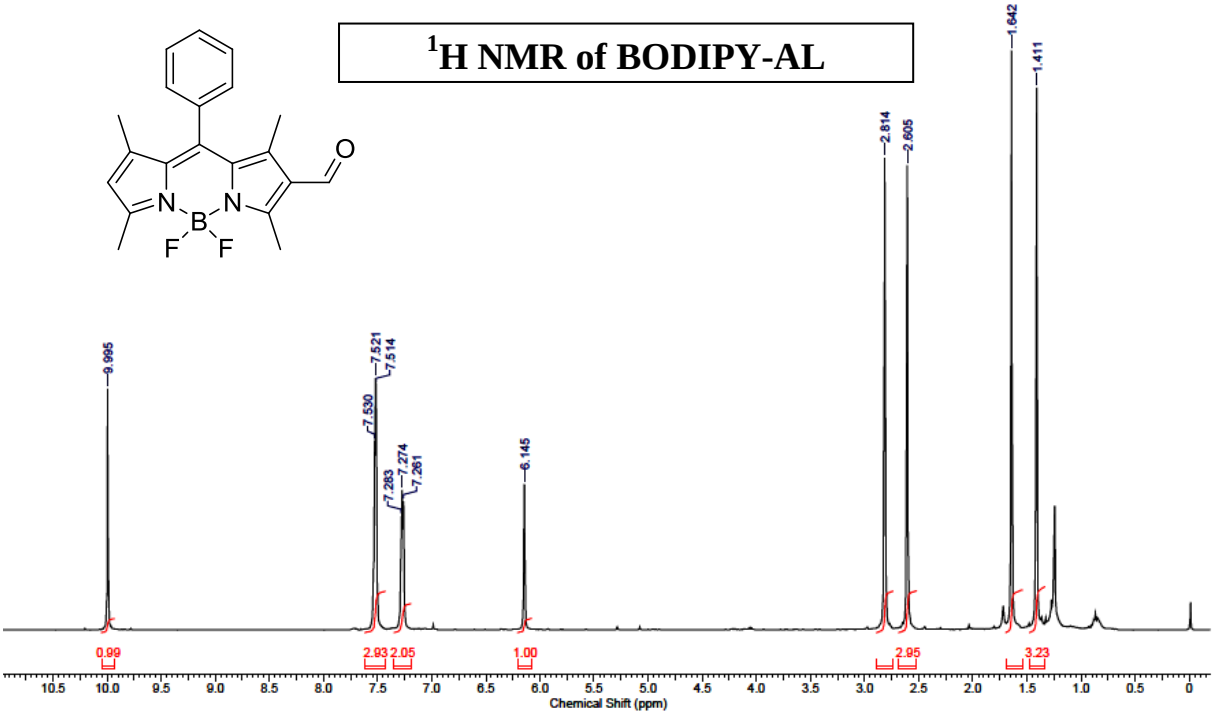
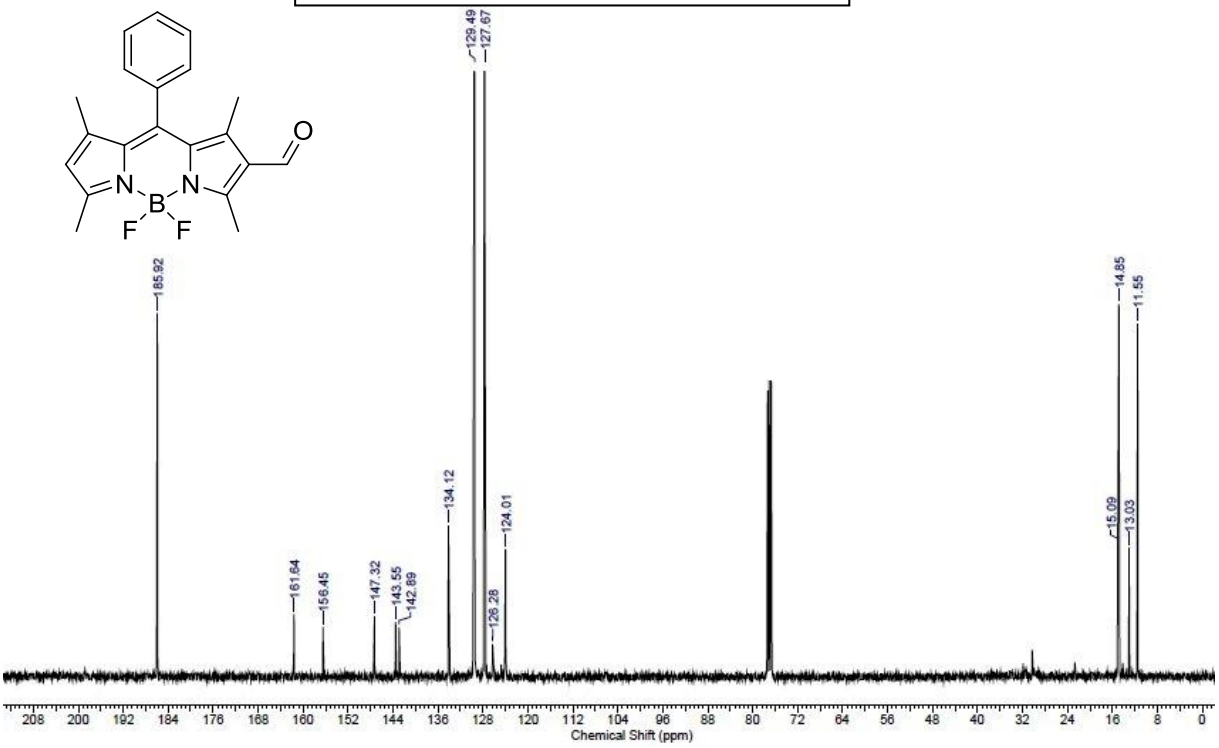



**Figure S10:** Reaction time profiles of BODIPY-OX (5.0μM) in the absence (■) or presence of HOCl [15 (●), 25(▲), 50(☆), 75(*), 100(⬟), 150(◆) μM.]. The fluorescence intensities at 529 nm were continuously monitored at time intervals in 0.1M potassium phosphate buffer, pH 7.2/DMF (v/v, 4:1)) ($\lambda_{ex}$: 470 nm, at 25 $^{o}$C).

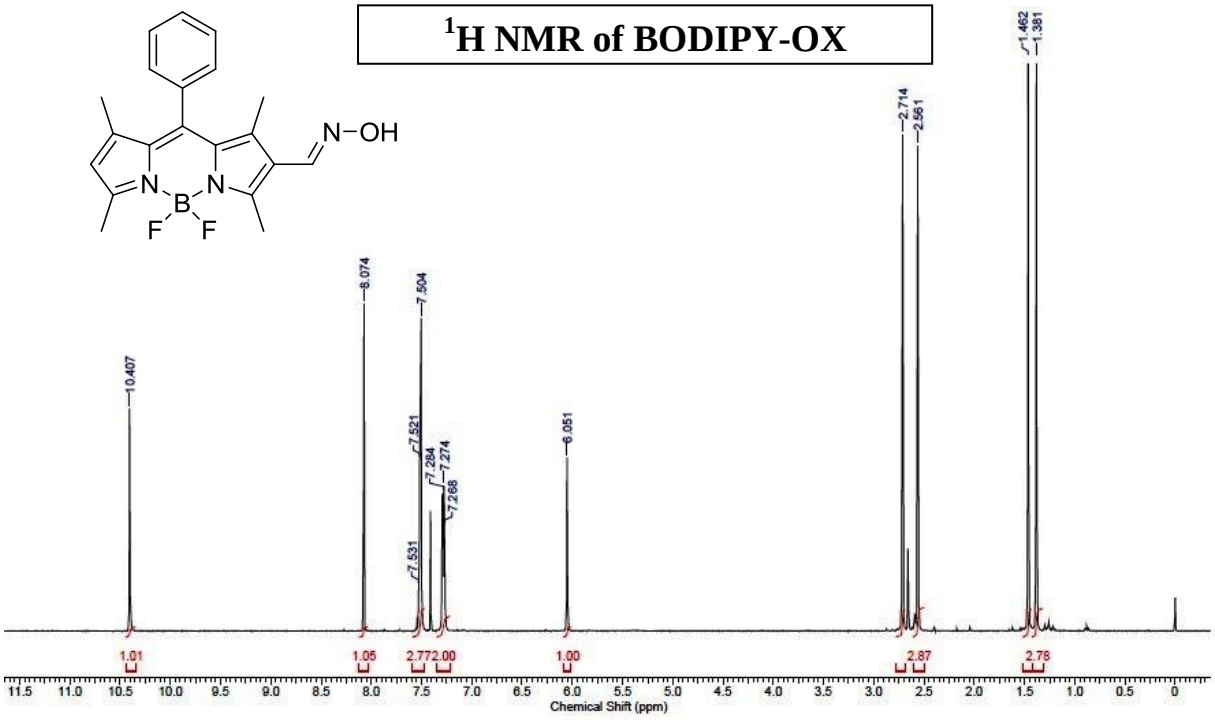
## $^{1}$H NMR of BODIPY-AL



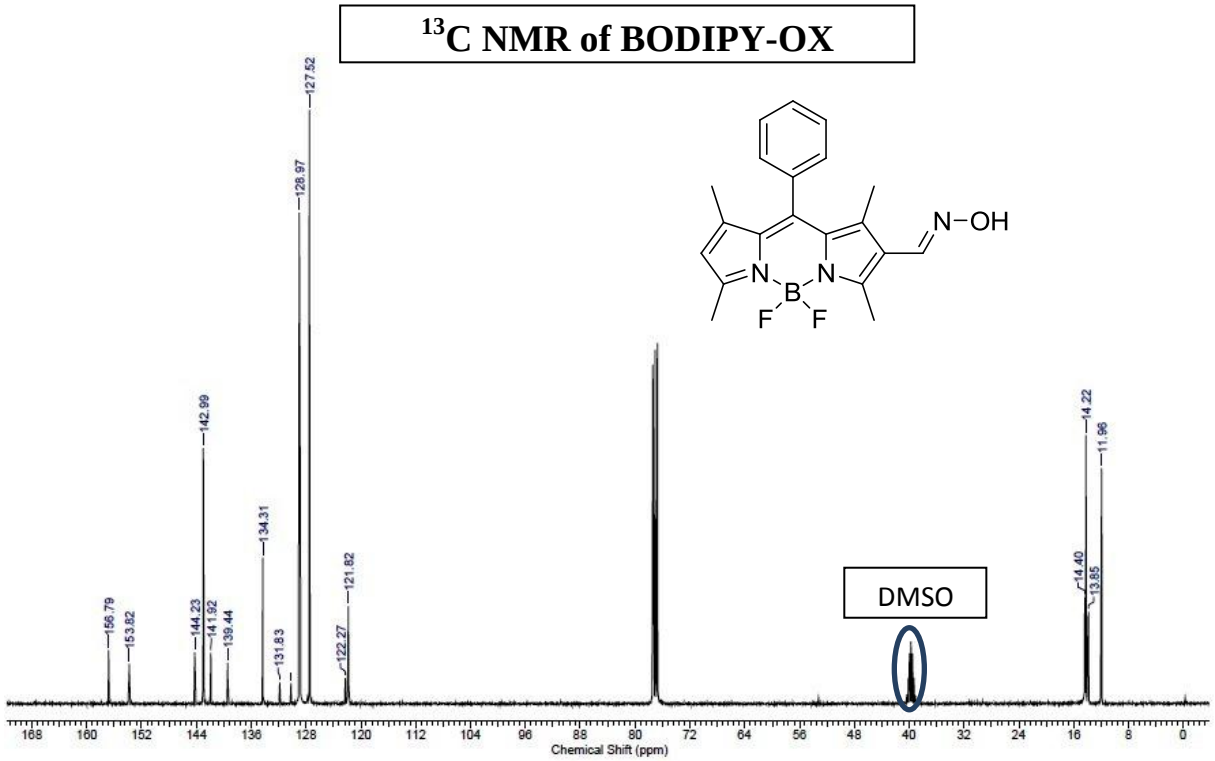

## $^{13}$C NMR of BODIPY-AL



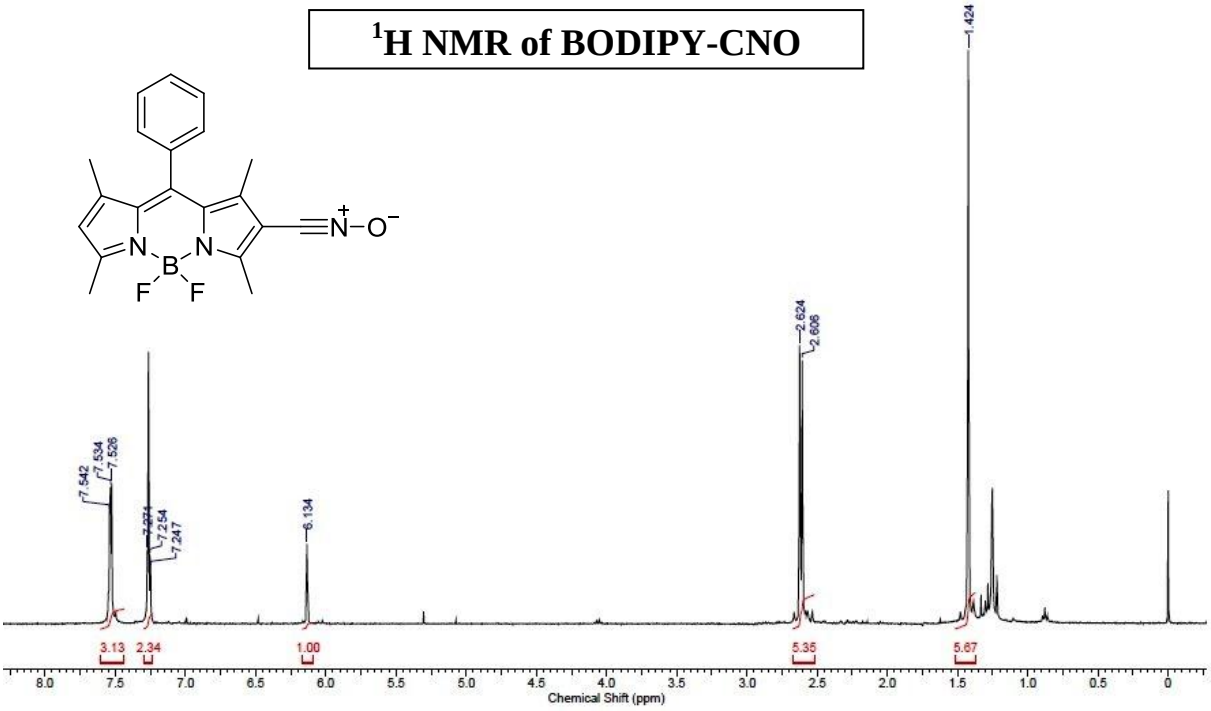
## $^{1}$H NMR of BODIPY-OX



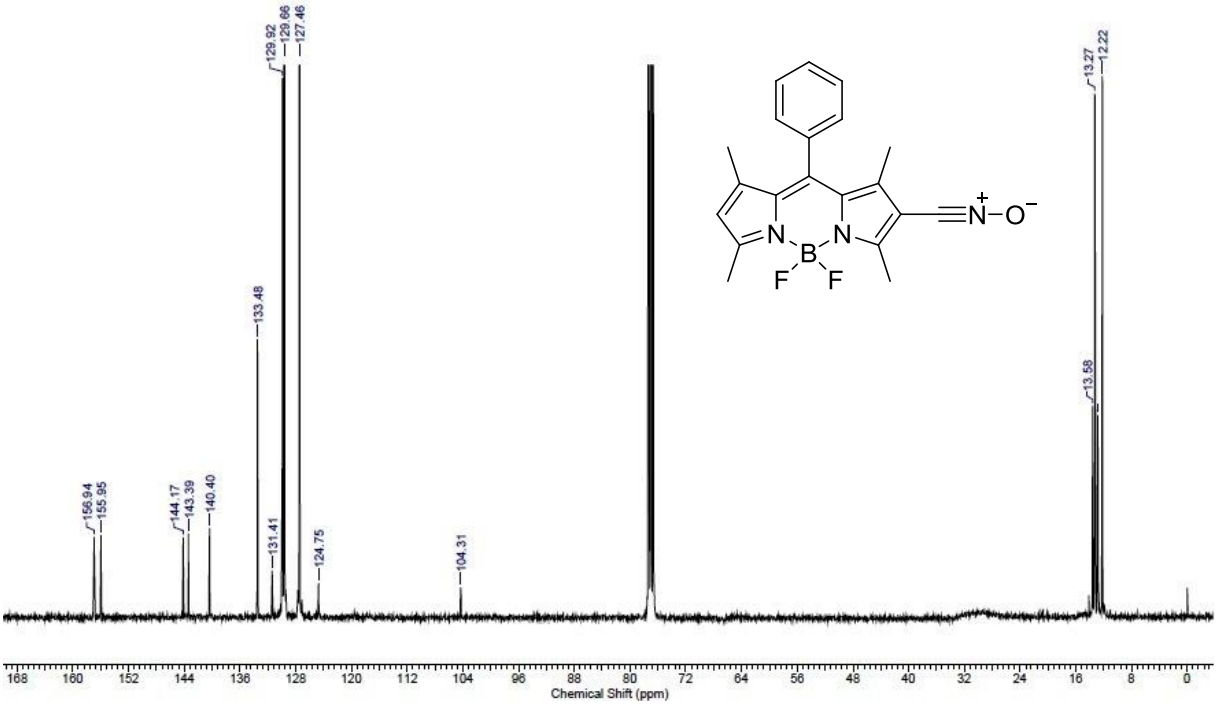

## $^{13}$C NMR of BODIPY-OX



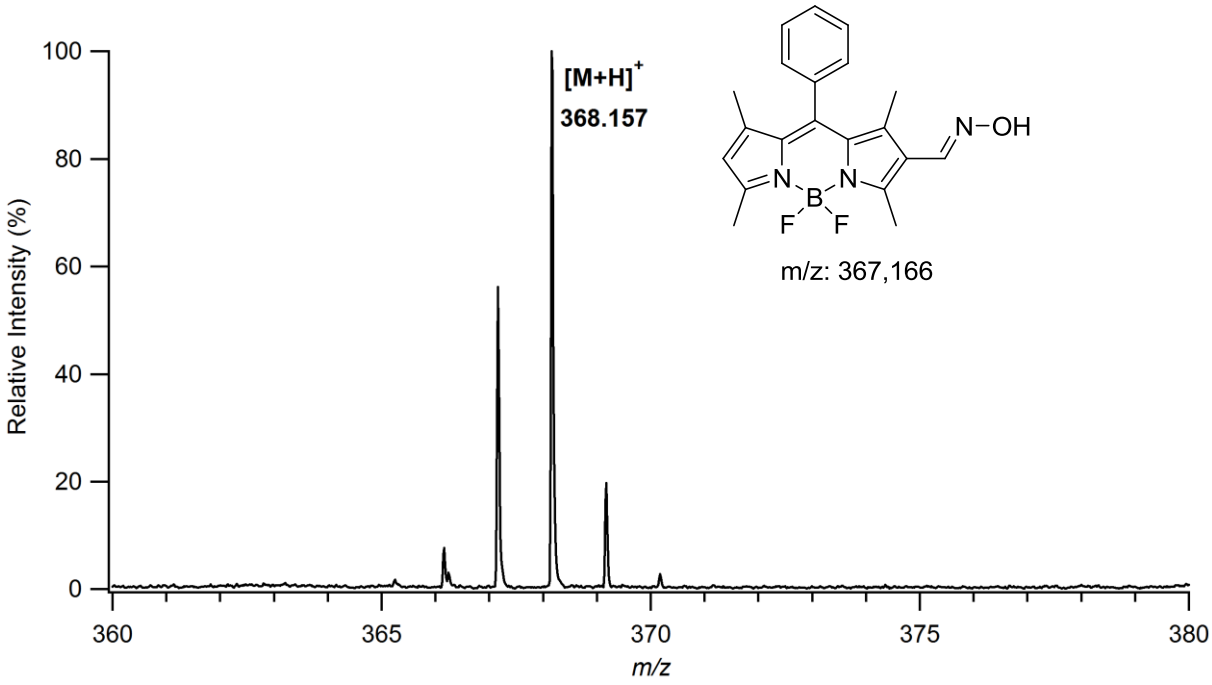
## $^{1}$H NMR of BODIPY-CNO




## $^{13}$C NMR of BODIPY-CNO



**MALDI-TOF TOF MS of BODIPY-OX**



**MALDI-TOF-TOF MS of BODIPY-CNO**