Electronic Supplementary Material (ESI) for Analytical Methods.

Supporting Information

A new turn-on fluorescent probe towards hypochlorite in living cells

Jing Guo,[a] Zhixiang Zhang,[c] Ziyu Kuai,[b] Rui Wang,[a] Qingbiao Yang,[*a] Yaming Shan[*b] and Yaoxian Li[a]

[a] *College of Chemistry, Jilin University, Changchun 130021, P. R. China. Fax: +86-431-88499576; Tel: +86-431-88499576*
E-mail: yangqb@jlu.edu.cn

[b] *National Engineering Laboratory for AIDS Vaccine, College of Life Science, Jilin University, Changchun 130021, P. R. China. Fax: +86-431-85167790; Tel: +86-431-85167790*
E-mail: shanym@jlu.edu.cn

[c] *Institute of Theoretical Chemistry, Jilin University, Changchun 130021, P. R. China.*

Table of contents

Preparation of ROS/RNS

Preparation of H_2O_2

The stock H_2O_2 solution was purchased form J&K Chemical Co. (30 wt %). The concentration of H_2O_2 was measured by iodometric titration.

Preparation of •OH

Hydroxyl radical was generated by Fenton reaction[1]. To prepare •OH solution, ferrous chloride was added in the presence of 10 equiv. of H_2O_2.

Preparation of $^1O_2$

Singlet oxygen was generated from the H_2O_2/MoO_4^{2-} system in alkaline media[2].

Preparation of t-BuOO•

t-BuOO· was generated from TBHP. The concentration of TBHP was determined by the titration of $S_2O_3^{2-}$.

Preparation of $O_2^{\bullet-}$

Superoxide radical was generated from potassium superoxide, the radical could stably existed in 18-crown-6 ether and Me_2SO solutions[3].

Preparation of NO

Nitric oxide was generated from SNP (Sodium Nitroferricyanide (III) Dihydrate)[4].

Preparation of HOCl

The stock NaClO solution was purchased form J&K Chemical Co. (10 wt% Calculated as free chlorine). The concentration of OCl^- was measured by the iodometric titration.

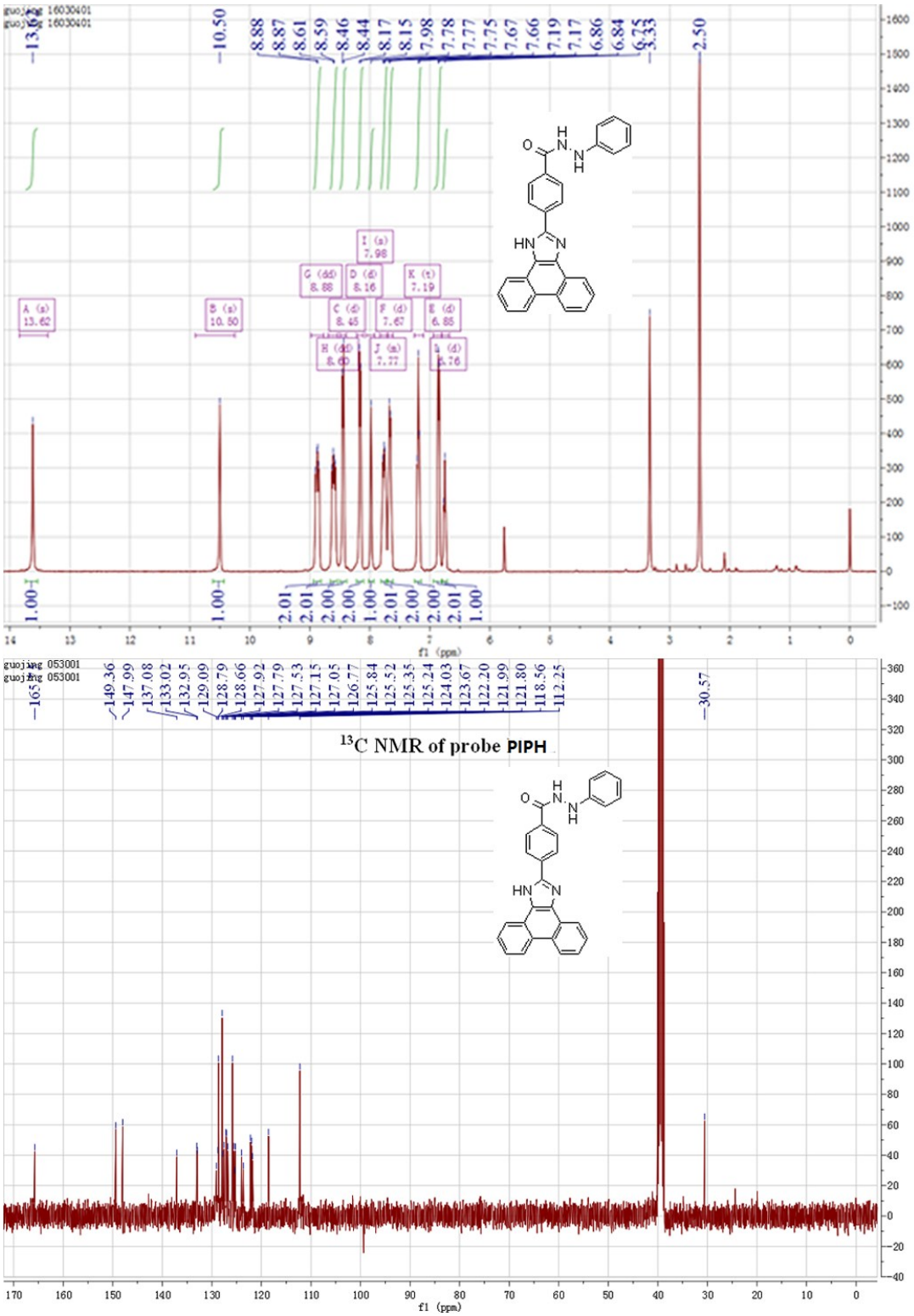

Figure S1 ^{1}H NMR spectrum of probe PIPH in d_6-DMSO; ^{13}C NMR spectrum of the probe in d_6-DMSO.

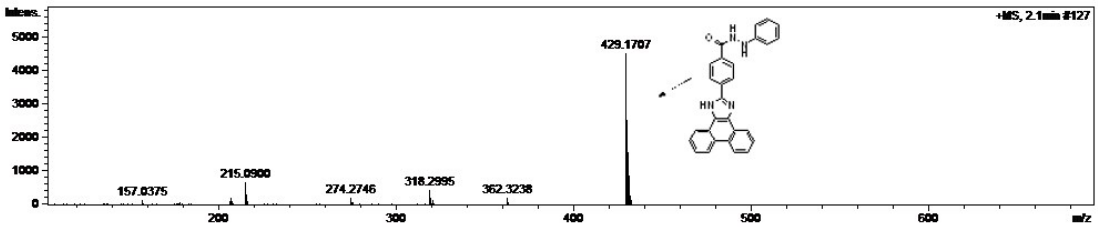

Figure S2 High-resolution mass spectra of PIPH.

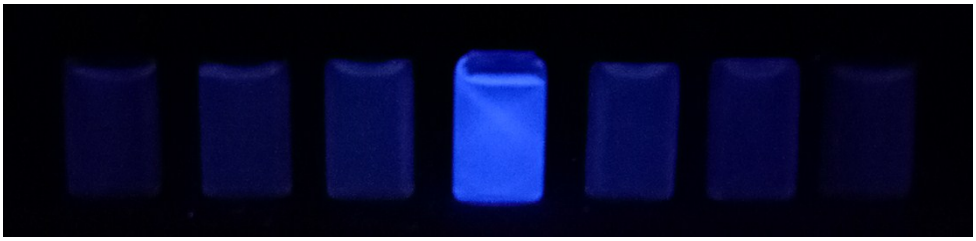

Figure S3 Fluorescence intensity of the PIPH (5 μM) treated with OCl^- (20.0 equiv.) (the 4thvial) and other other ROS/RNS (200.0 equiv.) (including •OH, H_2O_2, $^1O_2$, OCl^-, $O_2^{\bullet-}$, t-BuOO• and NO) under illumination with a 365 nm UV lamp.

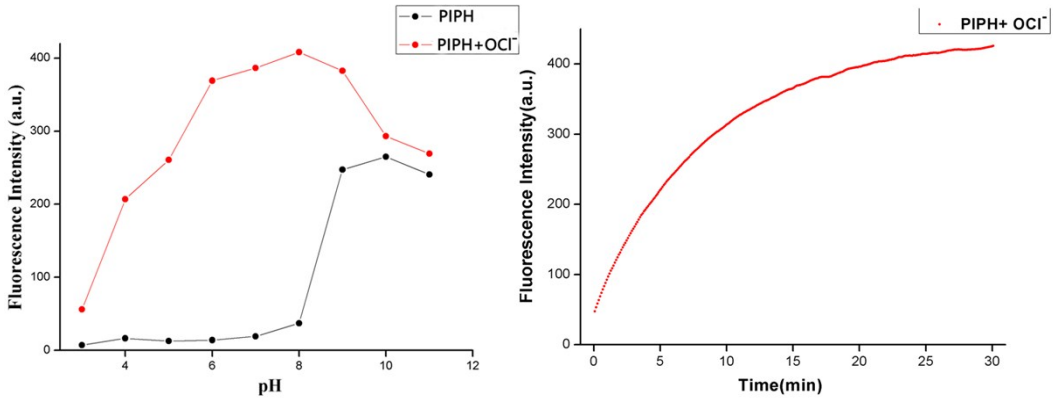

Figure S4 0.2 M NaOH aqueous solution was added to 200 mL mixed-acid system (0.06 M HAc - 0.06 M HCl - 0.06 H_3BO_3) dropwise to prepare different pH level of the aqueous solution. The maximum intensity of probe PIPH in DMF-water (1:1, v/v) with or without OCl^- was measured (left); Time-dependent fluorescence intensity (415 nm) changes of probe (5 μM) in the presence of OCl^- (20 equiv.) (λex = 330 nm) .

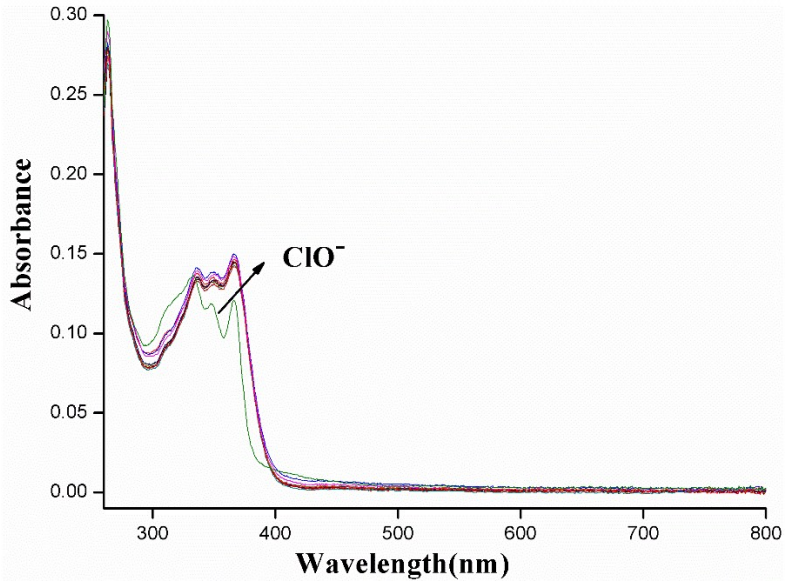

Figure S5 Absorption spectra of PIPH (5 μM) in the presence of OCl^- (20.0 equiv.), and other anions (200.0 equiv.) (including AcO^-, NO_3^-, F^-, SCN^-, N_3^-, H2PO4-, HPO_4^{2-}, HCO_3^-, Cl^-)

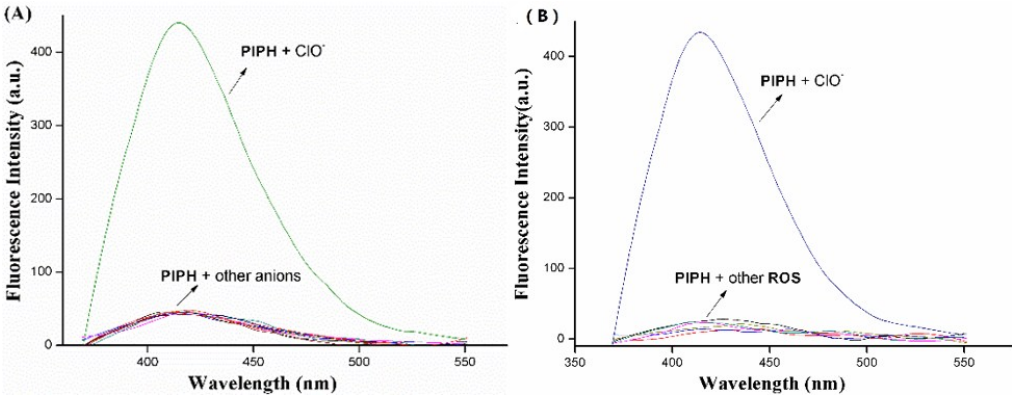

Figure S6 a) Fluorescence spectra of PIPH (5 μM) in the presence of OCl^- (20.0 equiv.) and other anions (200.0 equiv.) (including AcO^-, NO_3^-, F^-, SCN^-, N_3^-, $H_2PO_4^-$, HPO_4^{2-}, HCO_3^-, Cl^-) **b)** Fluorescence spectra of the sensor (5 μM) towards OCl^- (20.0 equiv.) and other ROS/RNS (200.0 equiv.) (including •OH, H_2O_2, $^1O_2$, $O_2^{\bullet-}$, t-BuOO• and NO)

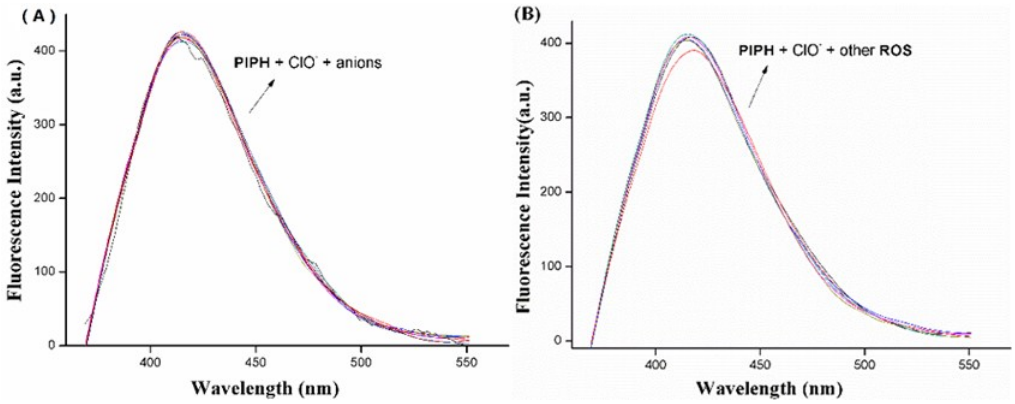

Figure S7 **a)** Fluorescence spectra of the probe (5 μM) in presence of Cys (20.0 equiv.) and other anions (AcO^-, NO_3^-, F^-, SCN^-, N_3^-, $H_2PO_4^-$, HPO_4^{2-}, HCO_3^-, Cl^-) **b)** Fluorescence spectra of the sensor (5 μM) towards OCl^- (20.0 equiv.) in addition of other ROS/RNS (•OH, H_2O_2, $^1O_2$, $O_2^{\bullet-}$, t-BuOO• and NO)

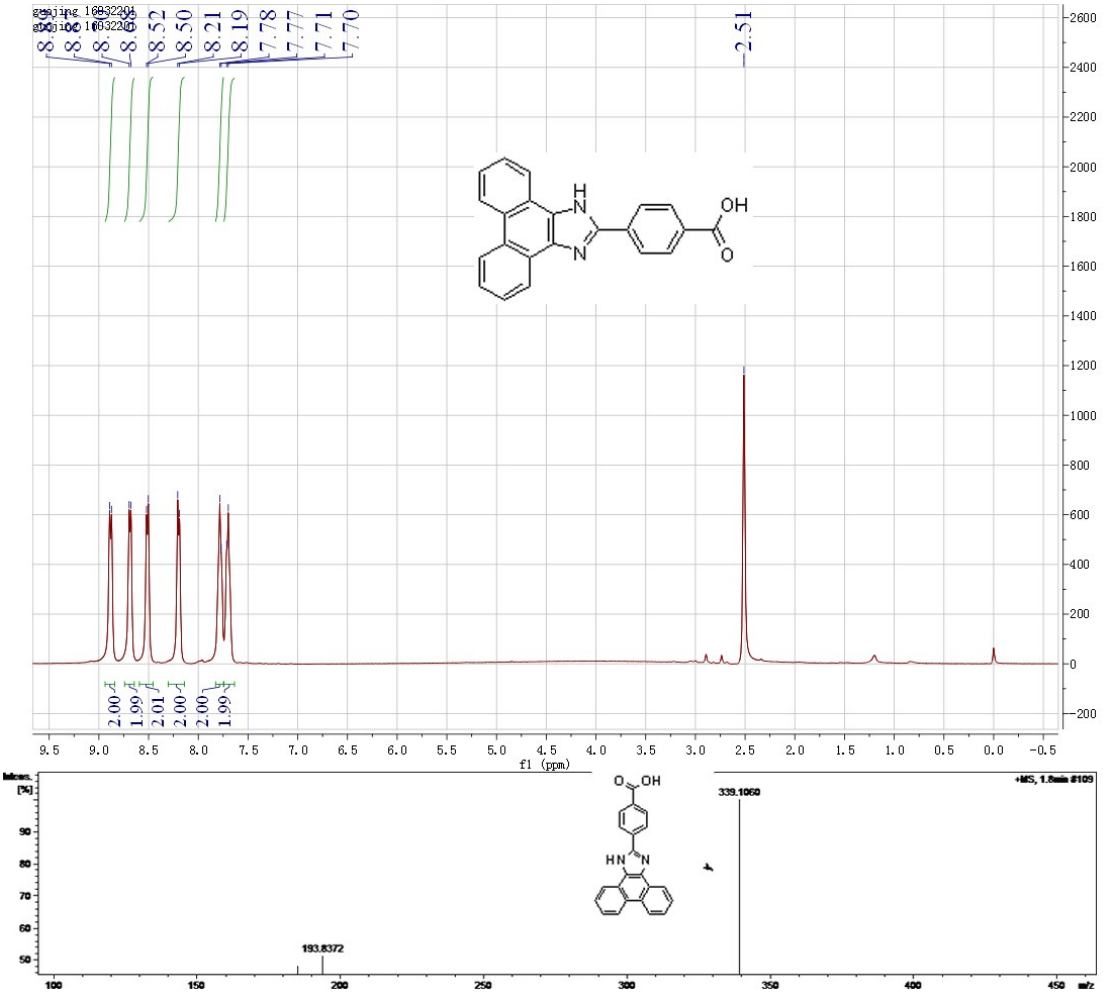

Figure S8 ^1H NMR spectrum of probe PIPH treated with OCl^- in d_6-DMSO. High-resolution mass spectra of the product.

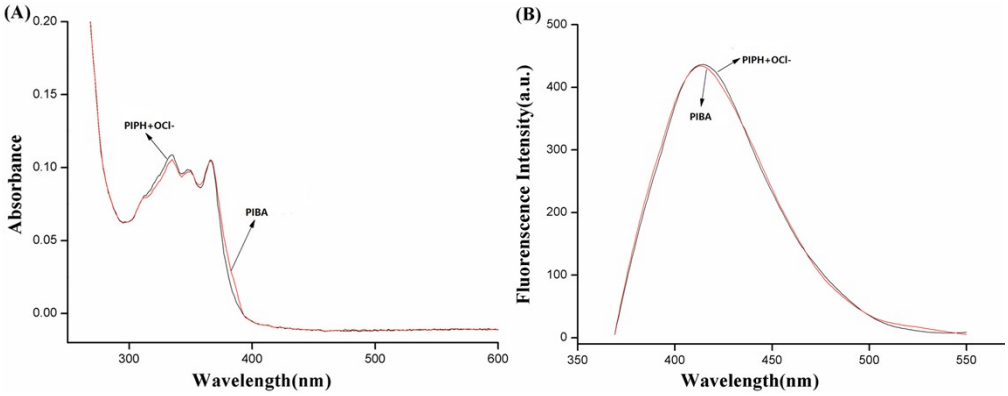

Figure S9 UV-Vis absorption and fluorescence emission spectra of PIPH treated with OCl^- (20.0 equiv.) and PIBA**.**

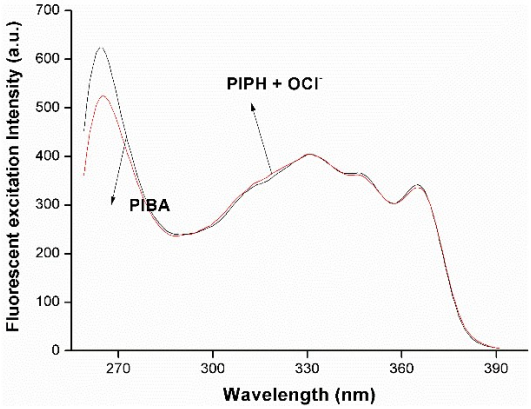

Figure S10 Fluorescence excitation spectra of PIPH treated with OCl^- (20.0 equiv.) and PIBA (λem=415 nm, slits: 2.5nm/2.5nm).

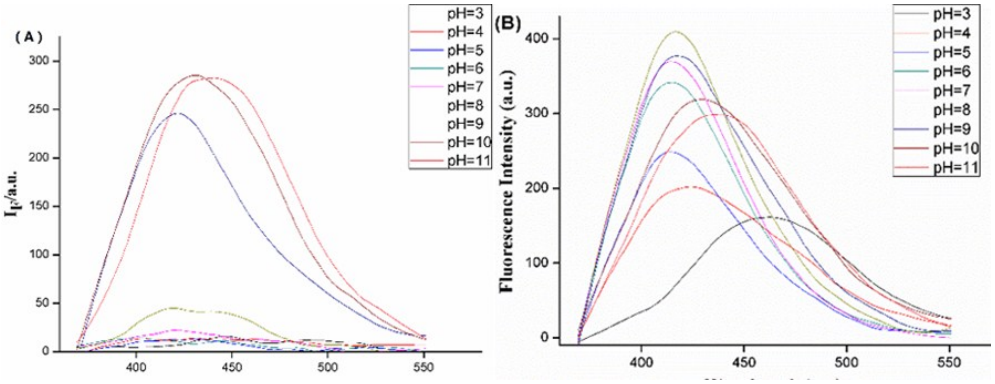

Figure S11 Fluorescence intensity of PIPH at different pH level without **(a)** or with OCl^- (20.0 equiv.) **(b)**

Table 1 Electron transition configurations, excitation energies, and oscillator strengths (*f*) for main absorption band of **PIPH** and **PIBA**. The experimental data is shown for comparison.

	State	Major contrib. [a]	Energy(nm/eV)	*f*	Exp(nm)
PIPH	$S_0 \rightarrow S_1$	H→L (98.0%)	380/3.27	1.1925	370
	$S_0 \rightarrow S_2$	H-1→L (97.2%)	333/3.72	0.0025	310
	$S_1 \rightarrow S_0$	L→H (98.3%)	554/2.24	0.7244	-
PIBA	$S_0 \rightarrow S_1$	H→L (98.3%)	393/3.16	1.1264	405
	$S_1 \rightarrow S_0$	L→H (98.8%)	452/2.74	1.3320	415

[a] 'H' means HOMO and 'L' means LUMO

Notes and references

1 (a) H. H. Fenton, Chem. News, 1876, 33, 190-190; (b) K. Setsukinai, Y. Urano,K. Kakinuma, H. J. Majima, T. Nagano, J. Biol. Chem., 2003, 278, 3170-3175.

2 (a) J. M. Aubry, J. Org. Chem., 1989, 54, 726-728; (b) N. Umezawa, K. Tanaka, Y. Urano, K. Kikuchi, T. Higuchi, T. Nagano, Angew. Chem. Int. Ed., 1999, 38, 2899-2901.

3 H. Gampp, S. J. Lippard, Inorg. Chem., 1983, 22 (2), pp 357-358.

4 (a) Z. Sun, F. Liu, Y. Chen, P. K. H. Tam, D. Yang, Org. Lett., 2008, 10, 2171-2174; (b) Y. Yang, H. J. Cho, J. Lee, I. Shin, J. Tae, Org. Lett., 2009, 11, 859-861.