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Supporting Information

Photostable Reaction-Based A-A-A Type Two-Photon Fluorescent Probe for Rapidly Detecting and Imaging Sulfur Dioxide

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Experimental Procedures

1. Instrumental and reagents.

All Reagents were purchased from commercial sources and used without further purification. The zebrafish were acquired from Shanghai FishBio Co., Ltd.

^{1}H NMR and ^{13}C NMR spectra were recorded on a BRUKER ASCEndtm400 MHz instrument. HRMS (high resolution mass spectrometry) spectra were obtained with an Agilent 6200 instrument. Fluorescence and UV absorption spectra were obtained with a PerkinElmer LS-55 fluorescence spectrophotometer and Hitachi U-3900 spectrophotometer, respectively. The absolute fluorescence quantum yields were obtained with an FLS1000 fluorescence spectrophotometer (Edinburgh Instruments Ltd., United Kingdom).The two-photon fluorescence emission spectrums and images were carried out on a confocal laser scanning microscope(TCS SP8, Leica, Germany) equipped with a Ti:Sapphire laser (Chameleon Ultra II, Coherent).

2. Measurement of two-photon absorption cross-sections.

Two-photon absorption cross-sections (TPA, σ) were calculated using the two-photon excited fluorescence method.[1] The two-photon fluorescence emission spectrums at different excitation were collected from confocal laser scanning microscopy with a multiphoton femtosecond laser and all other parameters remain the same during the data collection. The two-photon absorption cross-sections of the samples in PBS buffer (10 mM, pH 7.4) were calculated using fluorescein in pH = 11 aqueous solution as a reference according to eqn.:[2]

$$\sigma_S = \sigma_R \times (S_S/ S_R) \times (\Phi_R/ \Phi_S) \times (C_R/ C_S)$$

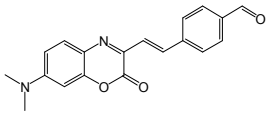
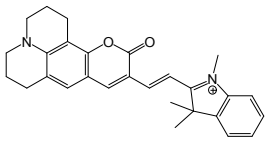
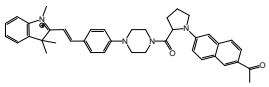
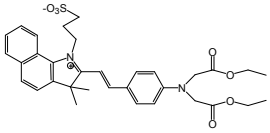
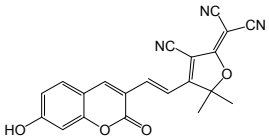
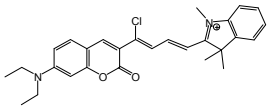
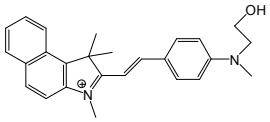
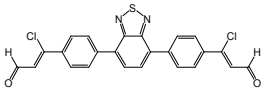
In the equation, the subscripts S and R stand for the sample and reference molecules. C is the concentration, Φ is the quantum yield, S is two-photon excited fluorescence emission integration at different laser wavelengths, and σ_R is the two-photon cross-section of the reference molecule.

3. Zebrafish culture

Zebrafish Culture: 4-day-old larval zebrafishes were used for bio-imaging in vivo. The zebrafish embryos were incubated in E3 embryo media (15 mM NaCl, 0.5 mM KCl, 1 mM $MgSO_4$, 1 mM

$CaCl_2$, 0.15 mM KH_2PO_4 , 0.05 mM Na_2HPO_4, 0.7 mM $NaHCO_3$, 10^{-5}% methylene blue, pH 7.5) at 28 °C for four days. Then the 4-day-old larval zebrafishes were used for subsequent imaging experiments.

Table S1. Comparison of some fluorescent probes for selective detection SO_2 derivatives.

Probes	OPM/ TPM	Detection limit	Response time	TP stability/ σ	References
	TPM	1.86 μM	4.5 min	-/ 3.9 GM	*J. Mater. Chem. B*, 2016, **4**, 7888-7894
	OPM	530 nM	6 min	-	*Talanta*, 2018, **189**, 429–436.
	TPM	50 nM	3 min	-/ 100 GM	*Chem. Commun.*, 2016, **52**, 10289-10292
	TPM	200 nM	5 min	-	*Sensors and Actuators B*, 2018, 255, 1228–1237
	OPM	270 pM	90 s	-	*Chem. Commun.*, 2014, **50**, 183-185.
	TPM	7.12 μM	10 s	-	*Analyst*, 2019, **144**, 4371–4379.
	TPM	560 nM	5 min	-/ 77 GM	*Sensors and Actuators B*, 2016, **233**, 1–6.
	TPM	190 nM	30 s	Photostable/ 91 GM	This work

"-" indicates that there is not provided or not investigated in the manuscript.

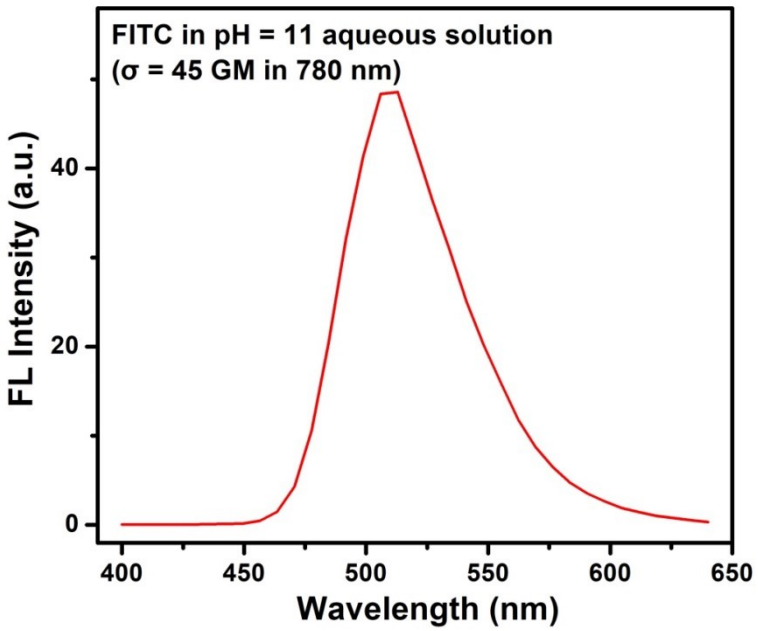


Figure S1. Two-photon fluorescence emission spectrum of FITC in pH 11 aqueous solution at a two-photon laser excitation of 780 nm.

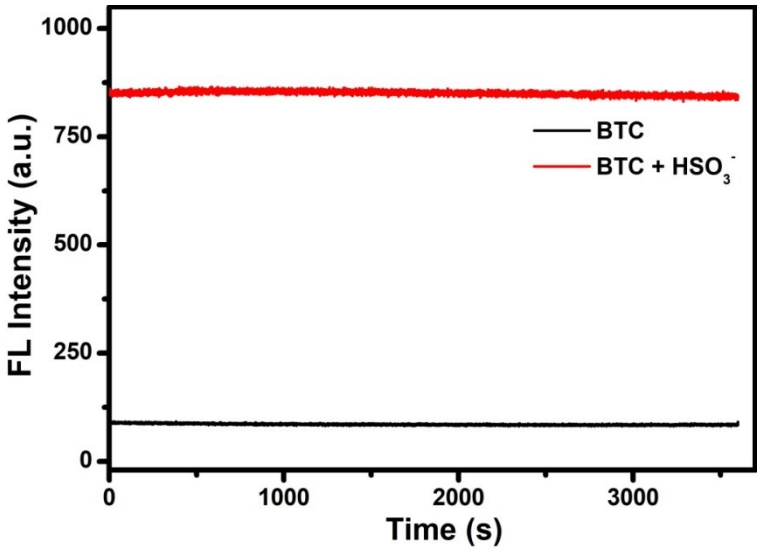


Figure S2. Photostability experiment of BTC (black line) and BTC-HSO_3 (BTC reacted with 200 μM HSO_3^-, red line) in 10 mM, pH 7.4 PBS buffer containing 5% DMSO. Voltage of xenon lamp: 700V, slit: 15 × 15, BTC:10 μM.

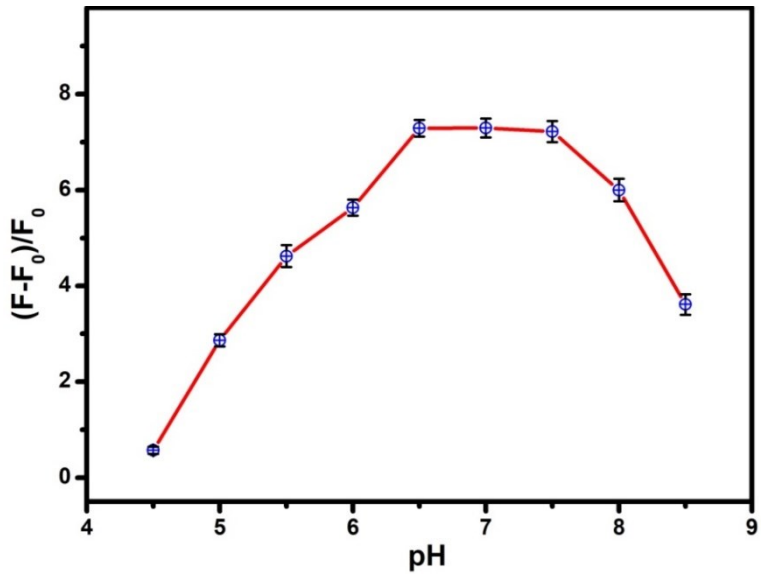


Figure S3. The plot of the variation of fluorescence intensity (F-F_0/F_0) of 10μM BTC in the presence of 200μM HSO_3^- against different pH values in phosphate buffer. F and F_0 are the fluorescence intensity of BTC in the presence and absence of HSO_3^-, respectively.

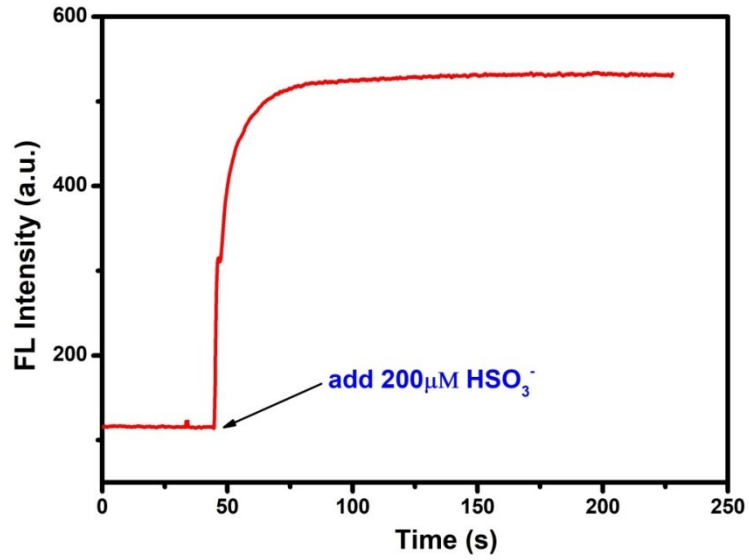


Figure S4. Time-dependent fluorescence intensity at 520 nm of 10 μM BTC probe in the presence of 200 μM HSO_3^- in 10 mM, pH 7.4 phosphate buffer containing 5% DMSO.

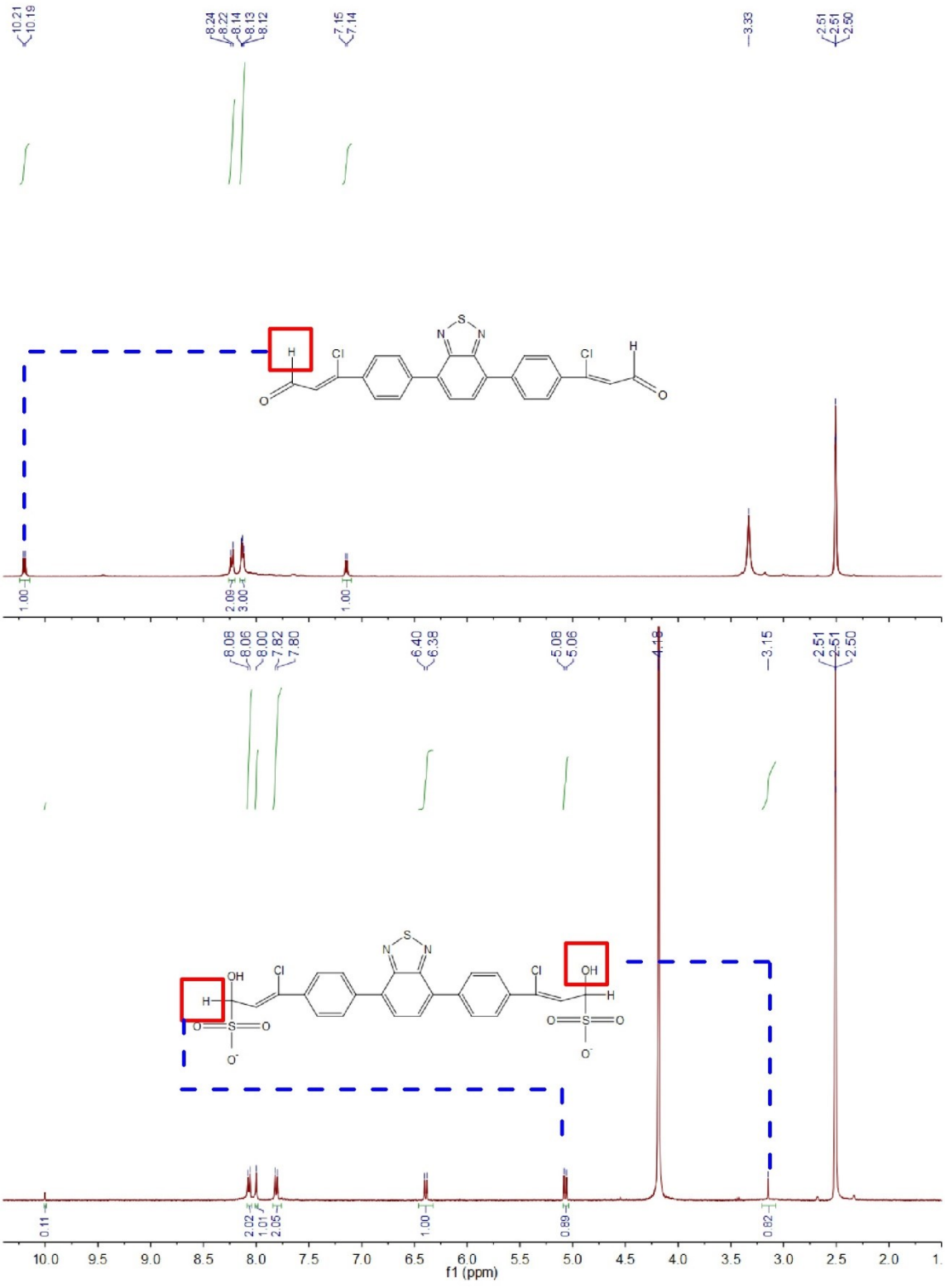


Figure S5. ^{1}H-NMR comparison of the probe BTC with the product BTC-HSO_3 upon addition of HSO_3^- in DMSO-*d*6 and D_2O (*V*/*V*=7:3).

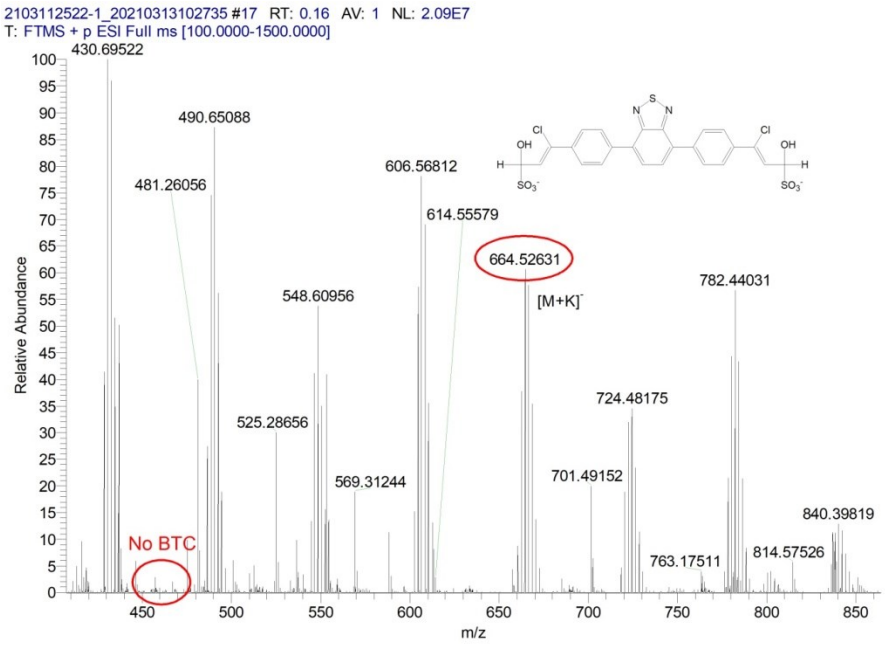


Figure S6. HR-MS of the reaction mixture of BTC and HSO_3^-.

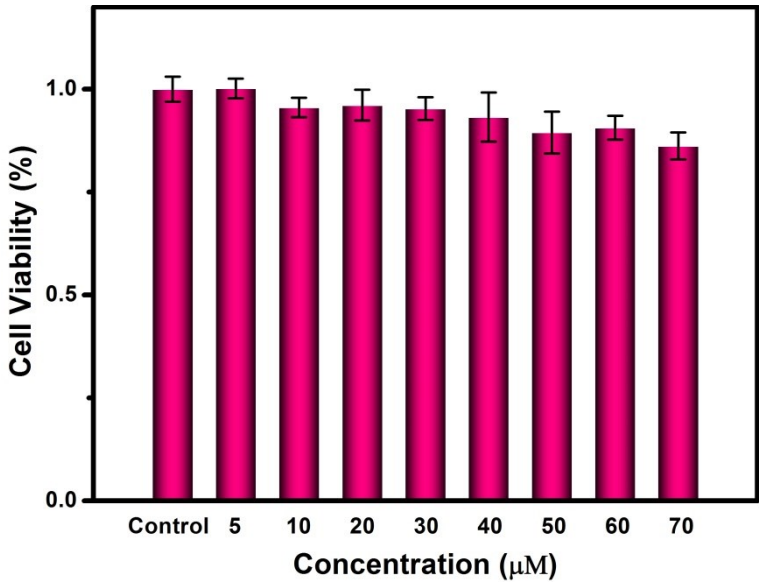


Figure S7. The viability of HeLa cells treated by BTC probe with various concentrations for 24 h.

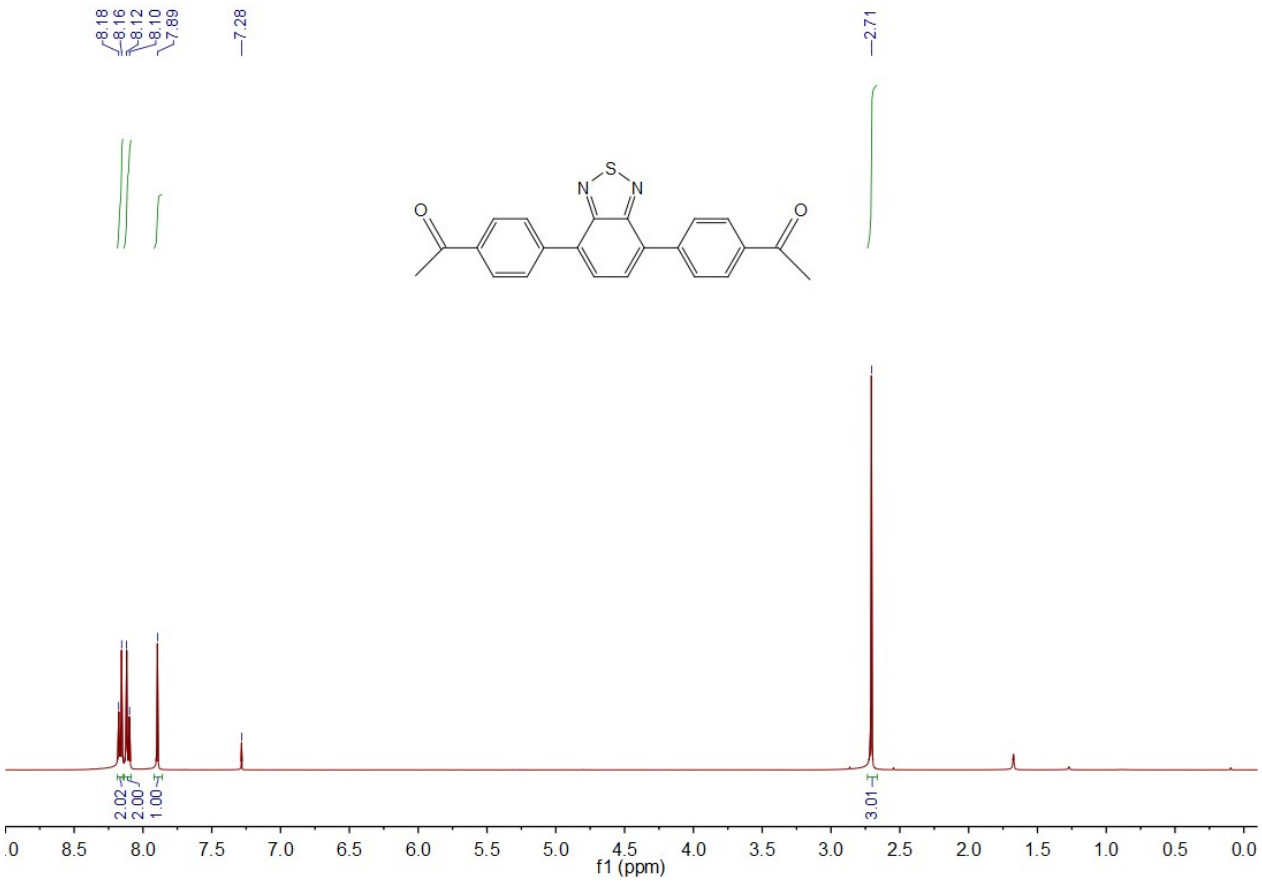


Figure S8. [1]H NMR of Product 1.

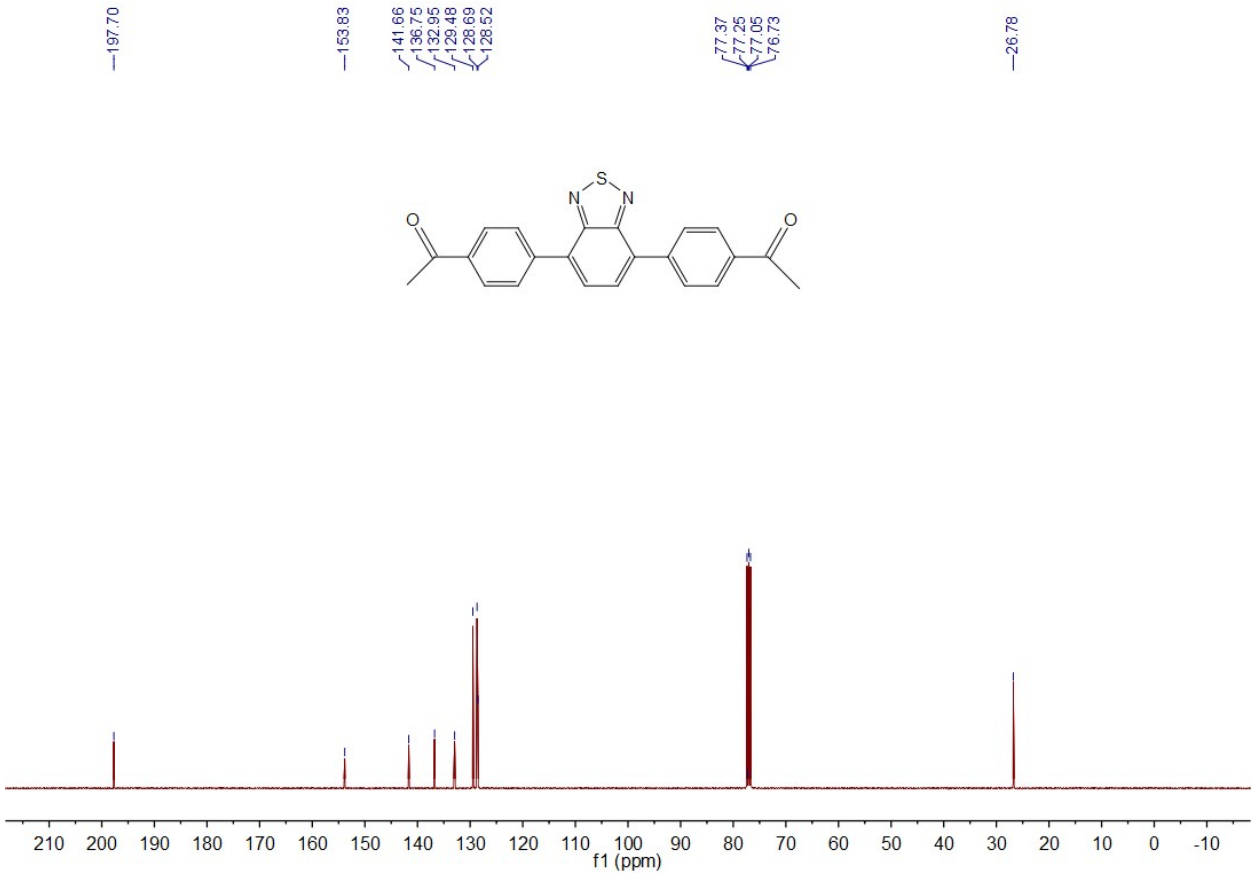


Figure S9. [1]C NMR of Product 1.

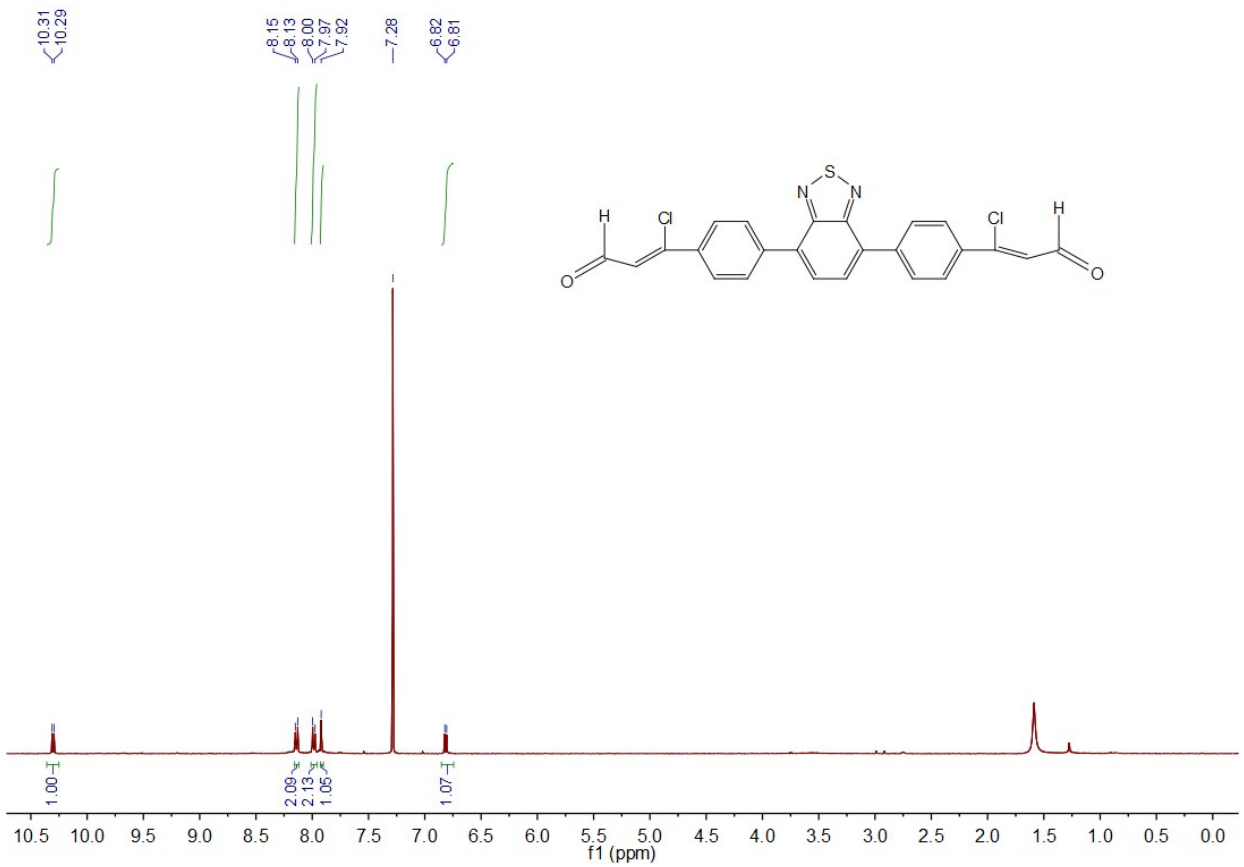


Figure S10. [1]H NMR of BTC.

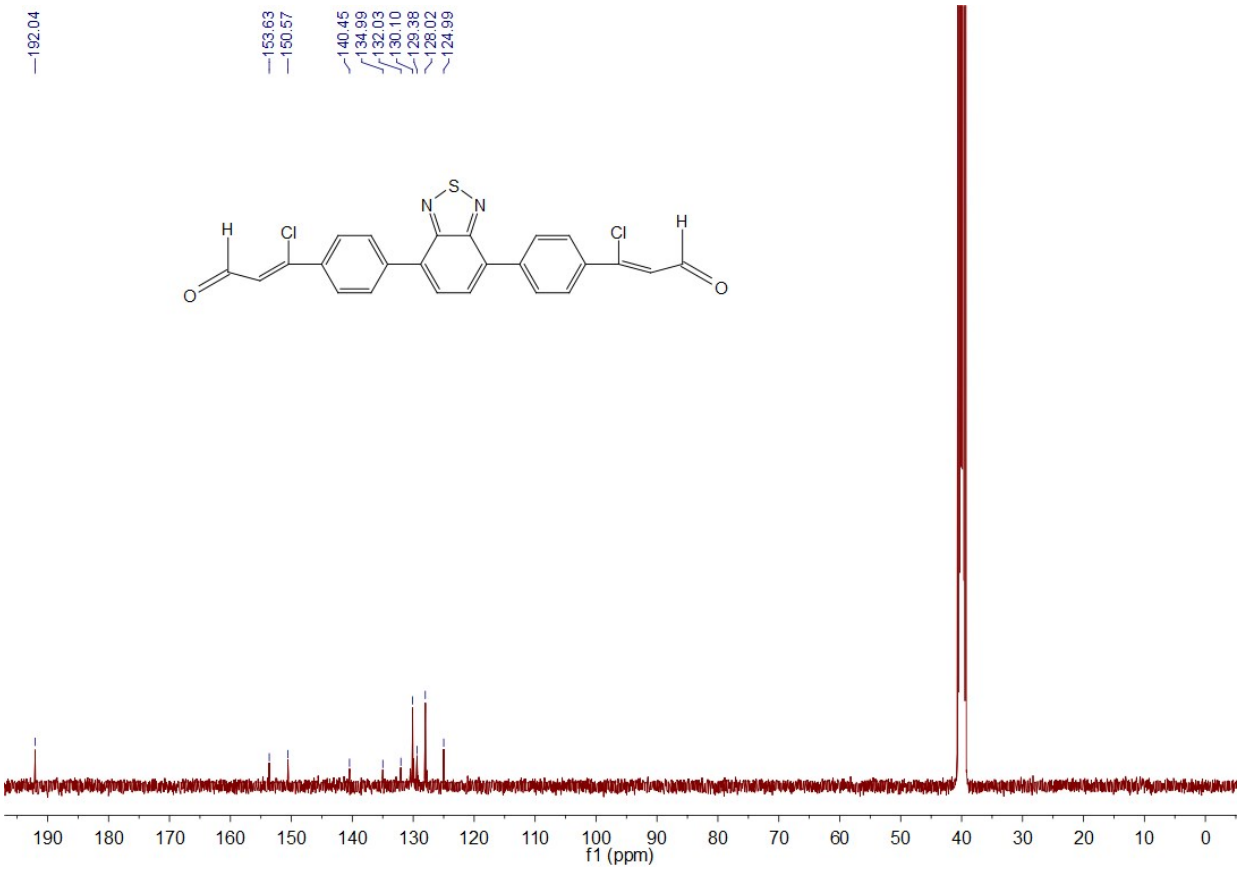


Figure S11. [1]CNMR of BTC.

Supplementary References

(1) N. S. Makarov, M. Drobizhev, A. Rebane, *Opt. Express*, 2008, **16**, 4029-4047.

(2) C. Xu, W. W. Webb, *J. Opt. Soc. Am. B*, 1996, **13**, 481-491.