Electronic Supplementary Information

A Twisted Intramolecular Charge Transfer Probe for Rapid and Specific Detection of Trace Biological SO_2 Derivatives and Bio-imaging Application

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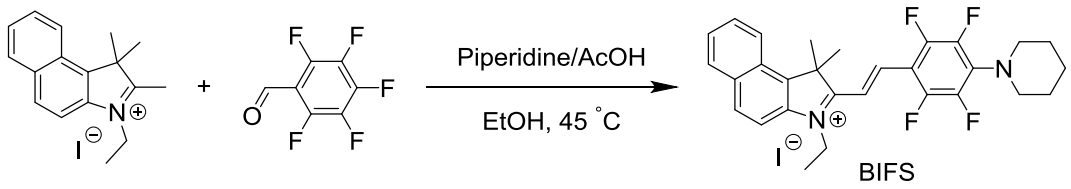
1.1 General

Reagents and Instrumentation. All chemicals and solvents were of analytical grade and were used without further purifications. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV-400 spectrometer with tetramethylsilane (TMS) as the internal standard. The chemical shift was recorded in ppm and the following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. Mass spectra were measured on a HP-1100 LC-MS spectrometer. UV-vis spectra were recorded on Hitachi UV 3310 spectrometer. Fluorescence spectra were recorded on a Hitachi FL-4500 fluorometer. Fluorescent images were acquired on a Nikon A1 confocal laser-scanning microscope with a 60 objective lens. The solvents used for UV-vis and fluorescence measurements are of HPLC grade. SYTO 9 Green Fluorescent Nucleic Acid Stain was purchased from Invitrogen corporation.

1.2 Synthesis and characterization data of probe



Scheme 1 Synthetic route for probe **BIFS**

To a 100 mL round-bottom flask with stirring bar was added 3-ethyl-1,1,2-trimethyl-1*H*-benz[e]indolium iodide (183 mg, 0.5 mmol), pentafluorobenzaldehyde (118 mg, 0.6 mmol), piperidine (two drops) and acetic acid (two drops). Then, 30 mL C_2H_5OH was added as solvent and the reaction mixture was heated at 45 ℃ for 4 h. After the reaction was completed, the solvent was removed under reduced pressure, and the crude product was separated by column chromatography on silica gel with CH_2Cl_2/C_2H_5OH = 30/1 as eluent to afford probe BIFS as a dark red solid (425 mg, 70 %).

^{1}H NMR (400 MHz, d^6-DMSO) δ (ppm) = 8.45 (d, *J* = 8.0 Hz, 1H, Ar-H), 8.33 (d, *J* = 8.0 Hz, 1H, Ar-H), 8.24 (d, *J* = 8.0 Hz, 1H, Ar-H), 8.19 (d, *J* = 8.0 Hz, 1H, Ar-H), 8.07 (d, *J* = 16 Hz, 1H, HC=CH,),7.82 (t, *J* = 8.0 Hz, 1H, Ar-H), 7.75 (t, *J* = 8.0 Hz,

1H, Ar-H), 7.51 (d, *J* = 16 Hz, 1H, -CH=CH-), 4.75 (q, *J* = 8.0 Hz, 2H, -CH_2), 3.41 (s, 4H, -CH_2-), 2.00 (s, 6H, -CH_3), 1.65 (s, 6H, -CH_2-), 1.54 (t, *J* = 8.0 Hz, 3H, -CH_3).

^{13}C NMR (100 MHz, d^6-DMSO) δ (ppm) 182.0, 139.5, 138.6, 137.0, 133.9, 131.8, 130.5, 129.0, 128.0, 127.2, 123.7, 115.6, 113.8, 54.4, 52.2. 52.2, 43.58, 26.6, 25.8, 23.8, 14.16.

^{19}F NMR (376 MHz, d^6-DMSO) δ (ppm) -139.75 (d, *J* = 15 Hz, 2F), -152.17 (d, *J* = 15 Hz, 2F).

1.3 Determination of the detection limit

The fluorescence spectrum of **BIFS** was measured three times and the standard deviation of a blank measurement was achieved. The fluorescence intensity at 467 nm was plotted as a concentration of SO_3^{2-}. The detection limit was calculated by using the equation: detection limit = 3*σ*/*k*: where*σ*is the standard deviation of the blank measurement, *k* is the slope between the fluorescence intensities of **BIFS** versus HSO_3^- concentration.

1.4 Cell culture and fluorescence imaging

A 549 cells (Perking Union Medical College, China) were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (Invitrogen Corp., Carlsbad, CA) and penicillin (100 units/mL)-streptomycin (100 μg/mL) liquid (Invitrogen Corp., Carlsbad, CA) at 37 ^oC in a humidified incubator containing 5% CO_2 in air. The cells were incubated for 2 days before dye loading on an uncoated 35 mm diameter glass-bottomed dish (D110100, Matsunami, Japan). Then, the cells were rinsed with PBS, incubated with DMEM containing 10% FBS, 10 μM probe **BIFS** and SYTO 9 (1 μM) for 30 min at 37 ^oC, washed with PBS twice, and mounted on the microscope stage. Fluorescence images were captured using a Nikon A1 Application. The cells were furthermore incubated with 10 μM $NaHSO_3$ for 10 minutes, and then washed with PBS twice for confocal laser-scanning microscopy measurement. Fluorescence images were captured using a Nikon A1 Application.

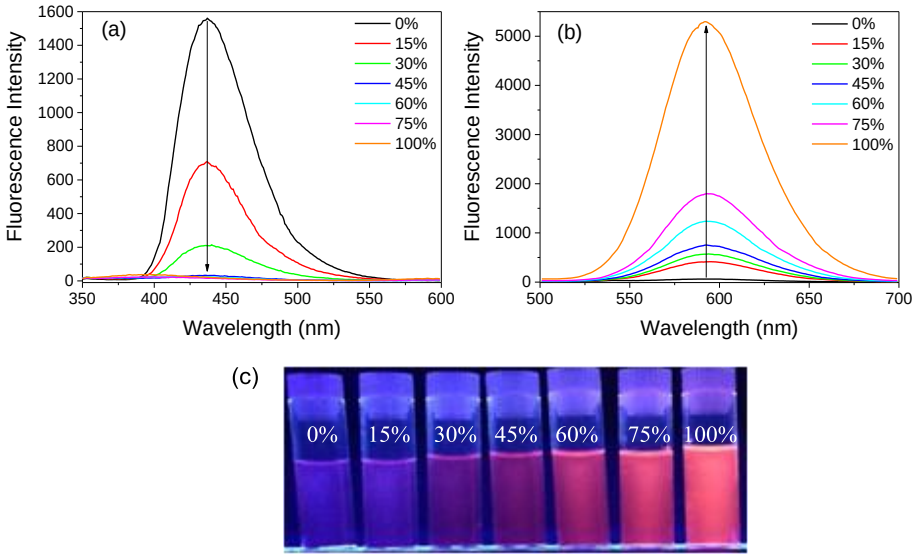


Fig. S1 Fluorescence spectra of **BIFS** in mixture solvents of glycerol/ MeOH. (a) Excitation wavelength: 322 nm, slit: 2.5/2.5 nm. (b) Excitation wavelength: 470 nm, slit: 5/10 nm. (c) Fluorescence photograph of probe **BIFS** (10 μM) in mixture solvents of glycerol/MeOH.

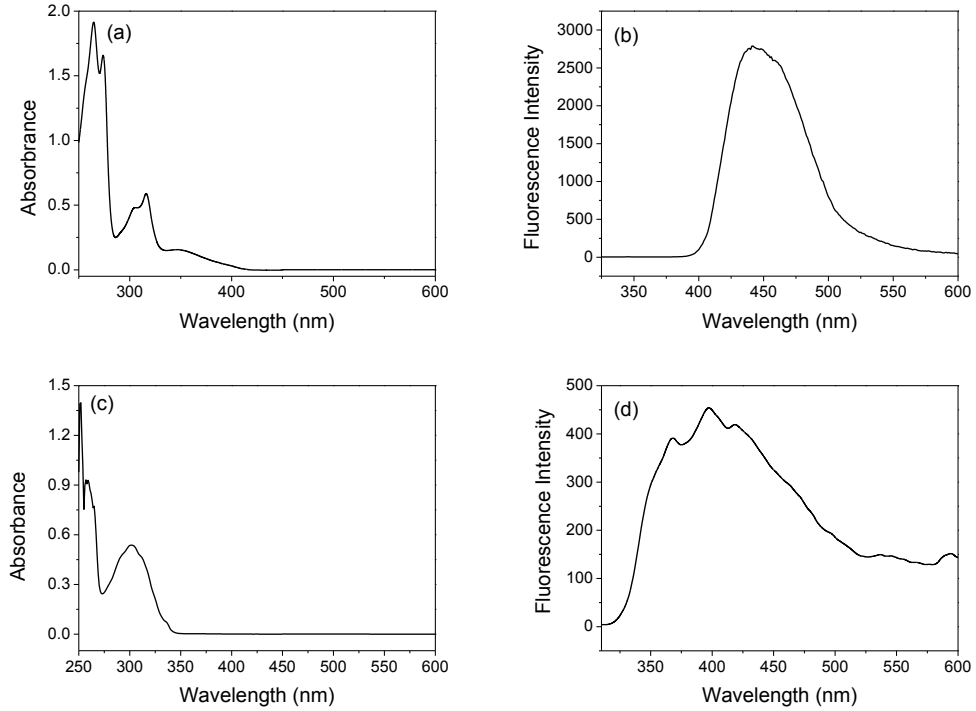


Fig.S2 (a) UV-vis absorption and (b) fluorescence spectra of 3-ethyl-1,1,2-trimethyl-1*H*-benzo[*e*]indolium in EtOH. (c) UV-vis absorption spectra and (d) fluorescence spectra of 1,1,2-trimethyl benzo[*e*]indole in EtOH.

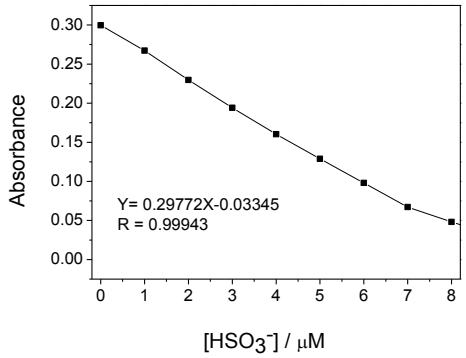


Fig. S3. Absorbance changes of the probe (10 μM) at 497 nm versus concentrations of HSO_3^- in glycerol/PBS solution (v/v = 4/6, pH 7.40).

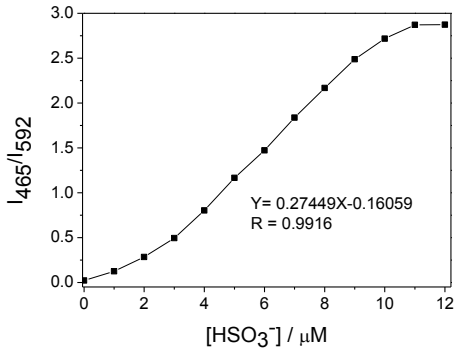


Fig. S4. Fluorescence intensity changes of probe (10 μM) versus concentrations of HSO_3^- in glycerol/PBS solution (v/v = 4/6, pH 7.40).

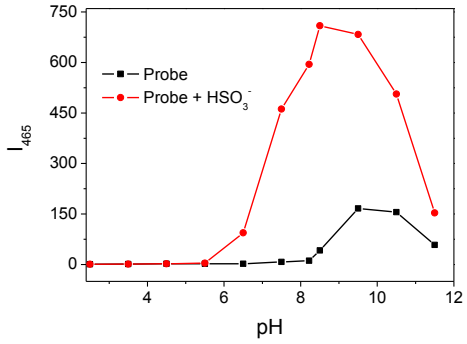


Fig. S5. pH-dependent fluorescence responses of the probe (10 μM) to HSO_3^- (1.0 equiv.) in glycerol/PBS solution (v/v = 4/6, pH 7.40). λ_{ex} = 322 nm. Slit: 2.5 nm.

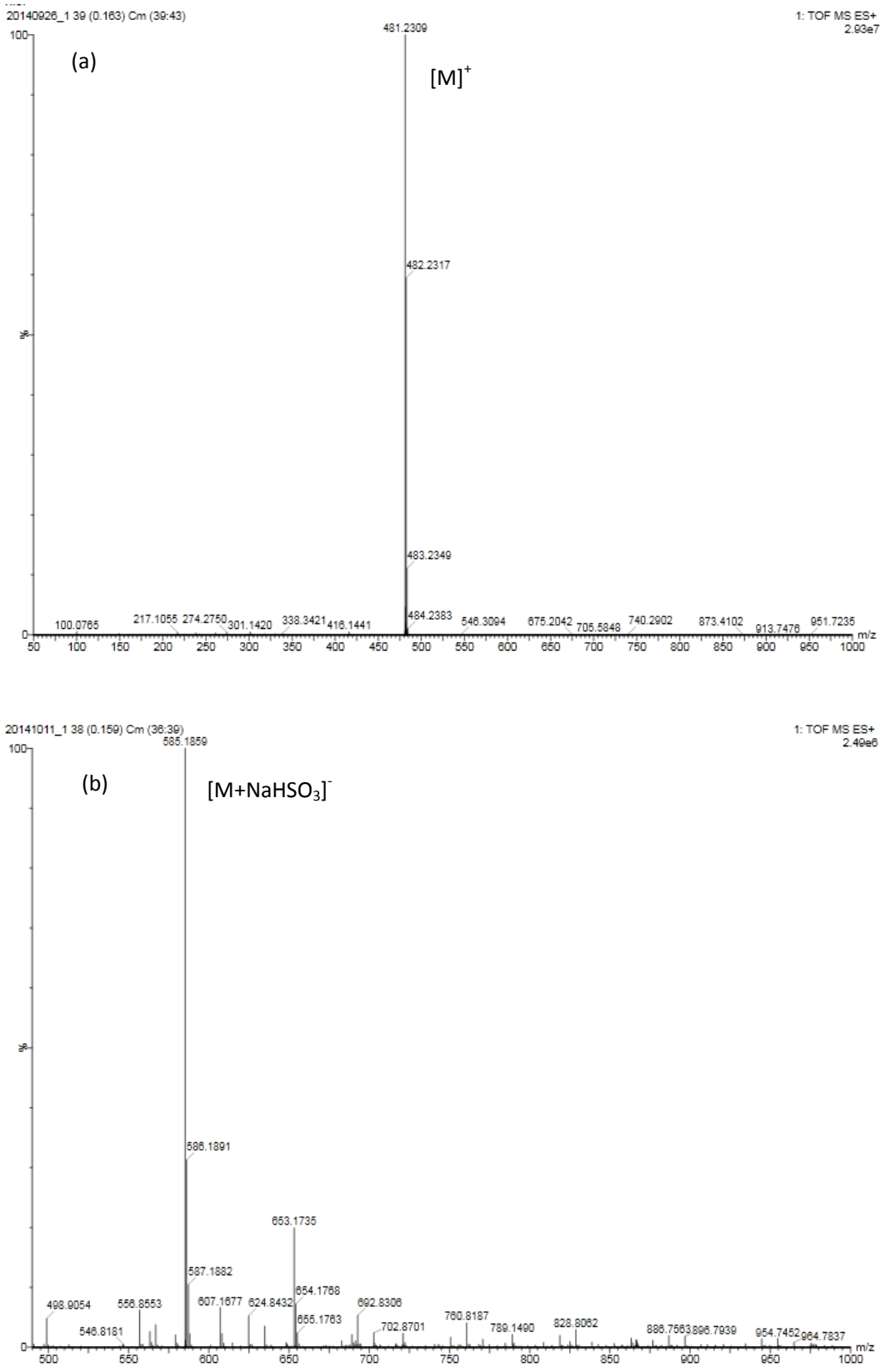


Fig. S6. HR-MS spectra of (a) the probe **BIFS** and (b) **BIFS**-$NaHSO_3$ adduct.

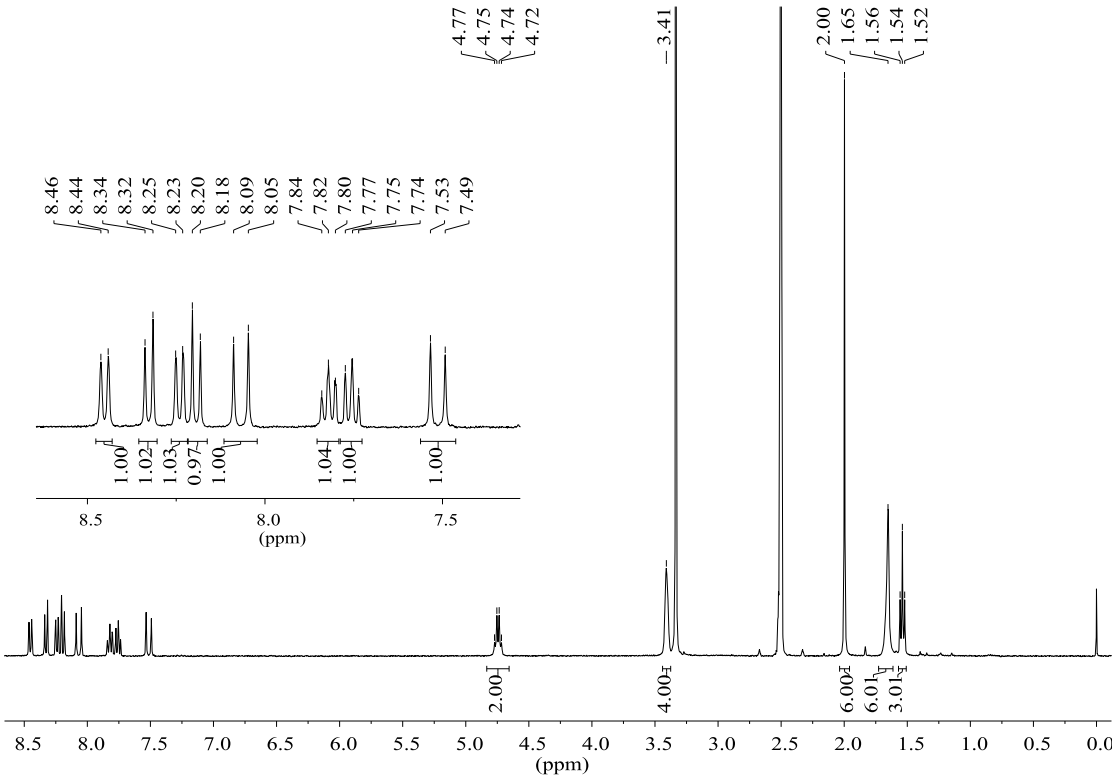


Fig. S7. ^{1}H NMR spectrum of **BIFS** in d^{6}-DMSO (400 M Hz)

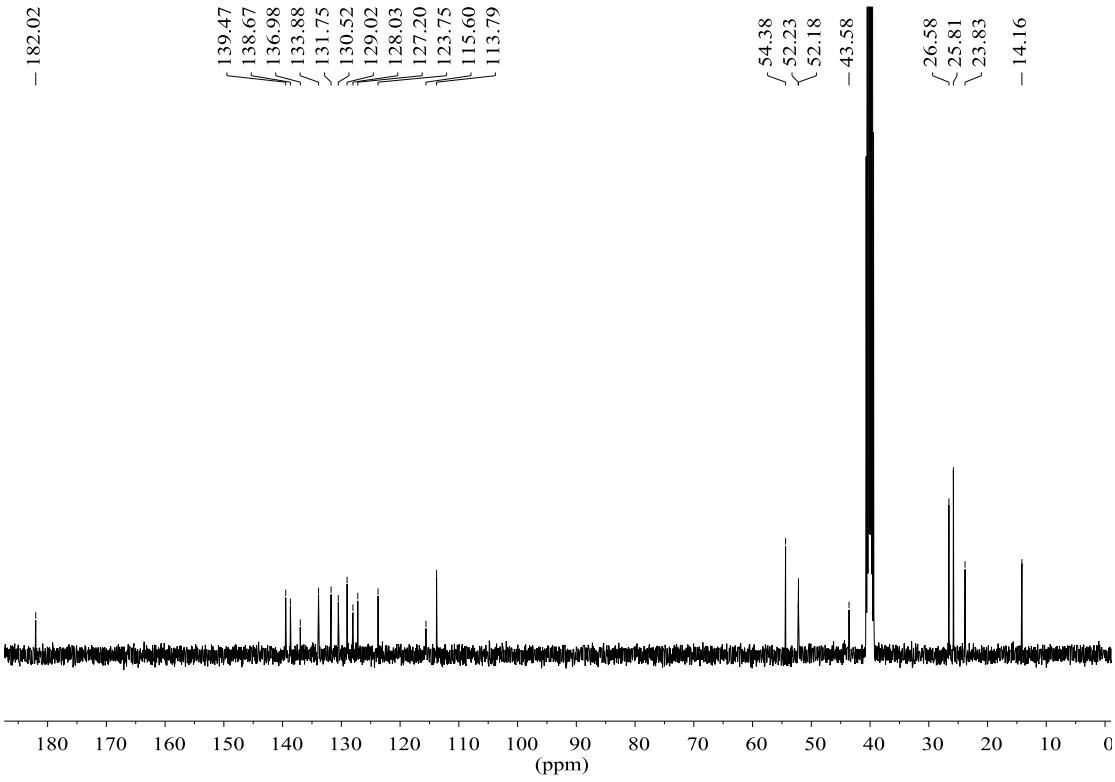


Fig. S8. ^{13}C NMR spectrum of **BIFS** in d^{6}-DMSO (100 M Hz)

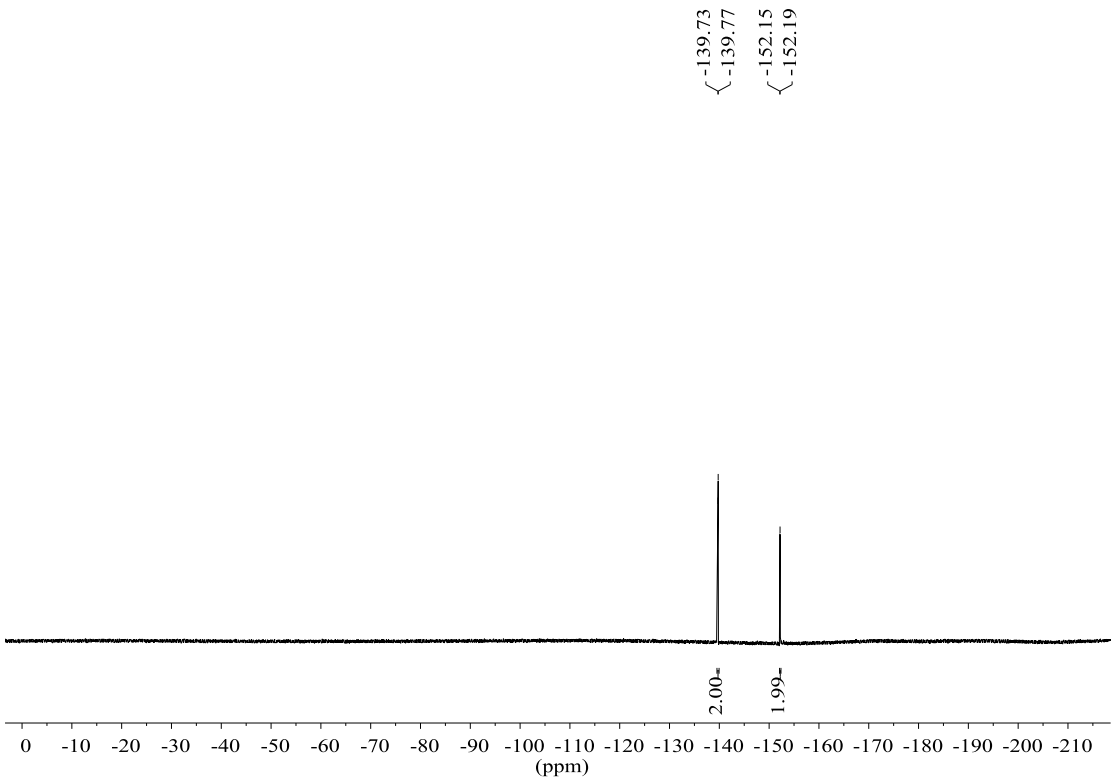


Fig. S9. ^{19}F NMR spectrum of **BIFS** in d^{6}-DMSO (376 M Hz)