Electronic Supplementary Material (ESI) for Photochemical & Photobiological Sciences.

Styrylisoxazole based Fluorescent Probes for the Detection of Hydrogen Sulfide

Jagadish Katla and Sriram Kanvah*

[#]Department of Chemistry, Indian Institute of Technology Gandhinagar, Palaj, Gandhinagar 382355

[#]*e-mail:kanvah@gatech.edu

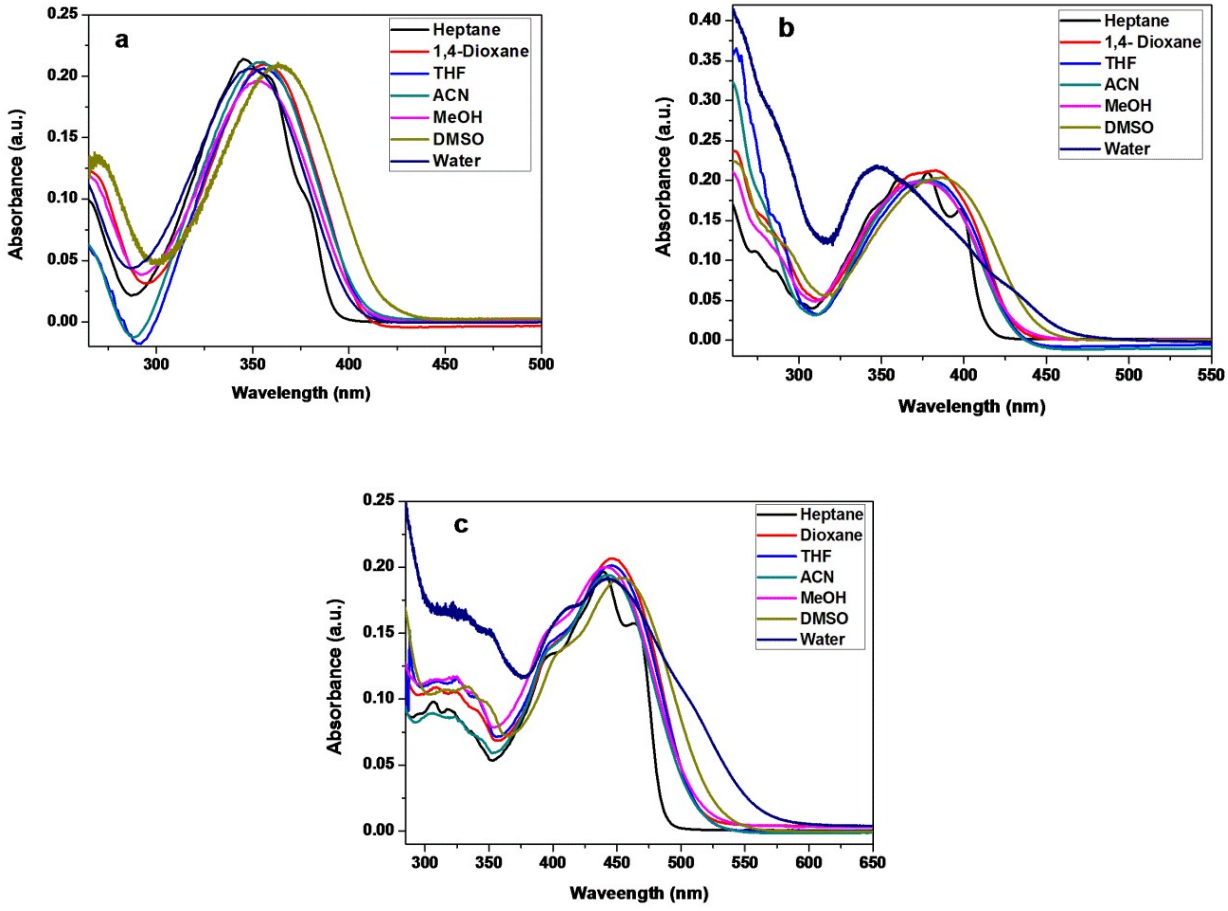

Fig. S1 Absorption of styrenes a) **1**, b) **2** and c) **4** in organic solvents of different polarity .

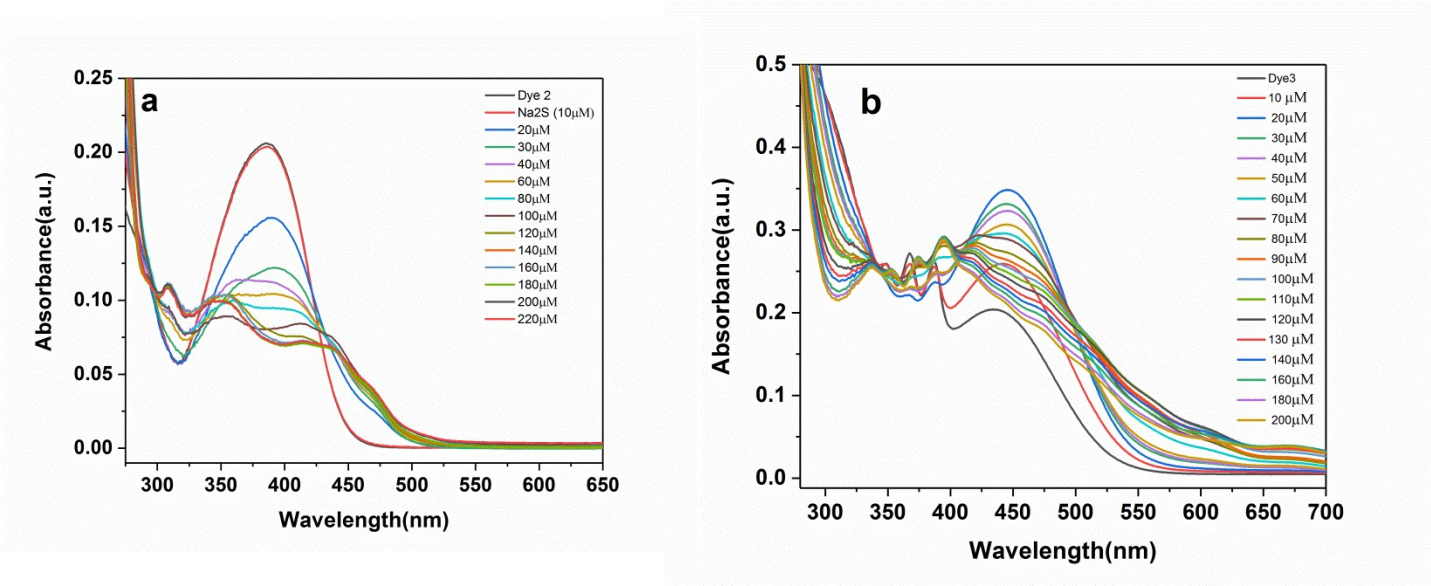

Fig. S2 Absorption of a) **2** and b) **3** with addition of Na_2S

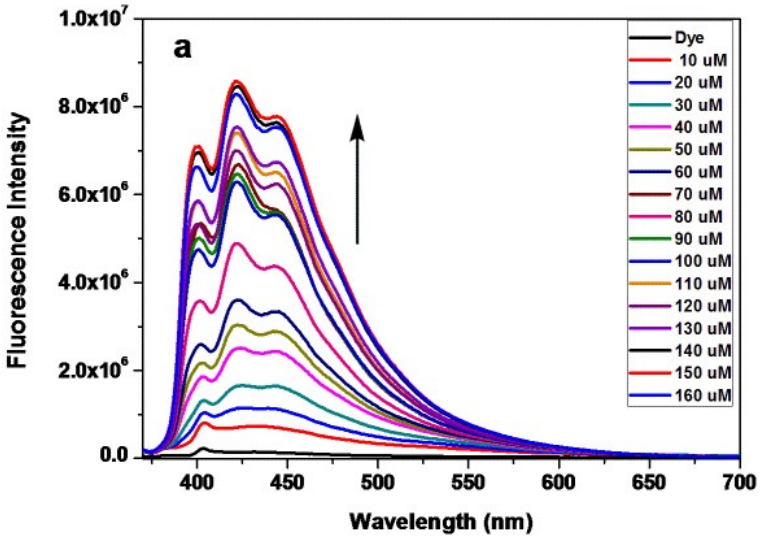

Fig. S3 Fluorescence response (λ_{ex} 350 nm) of probe a) **3** and b) **2** (10 μM) with increasing concentration (0 to 160 μM) of Na_2S in DMSO + H_2O (9:1) binary mixture.

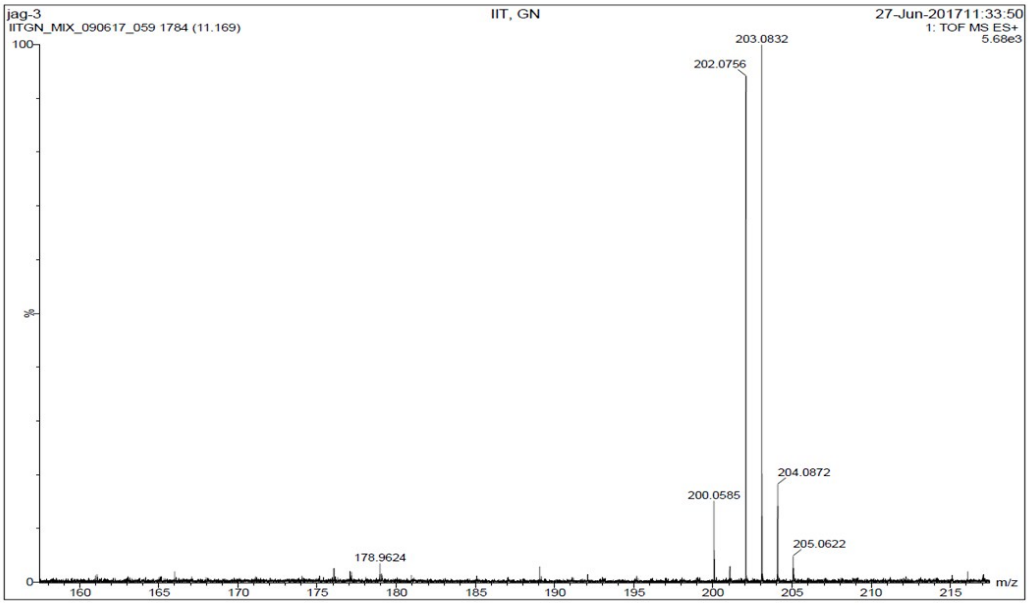

Fig. S4 HRMS data of **1** upon addition of Na_2S

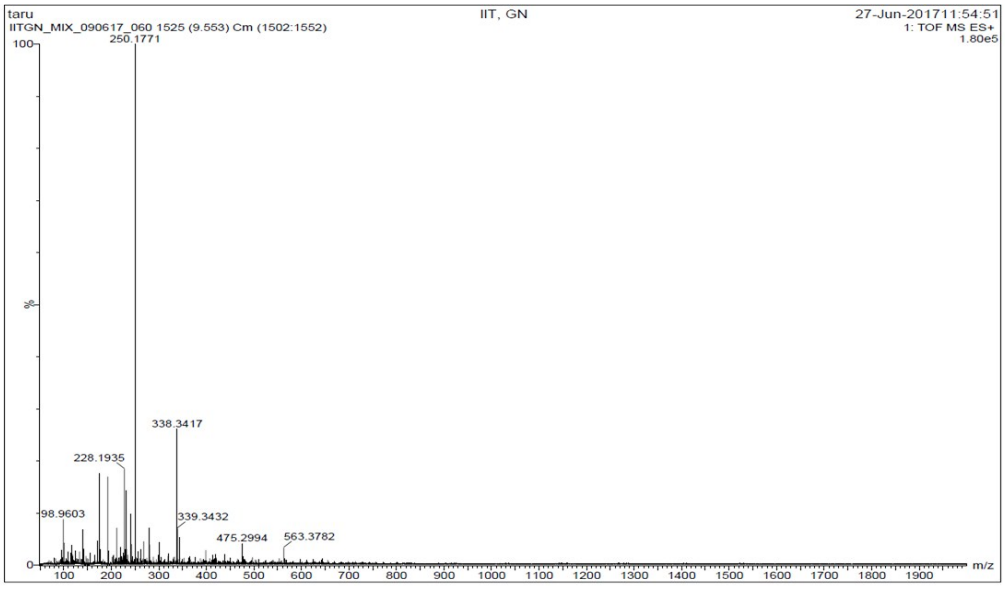

Fig. S5 HRMS data of **2** upon addition of Na_2S

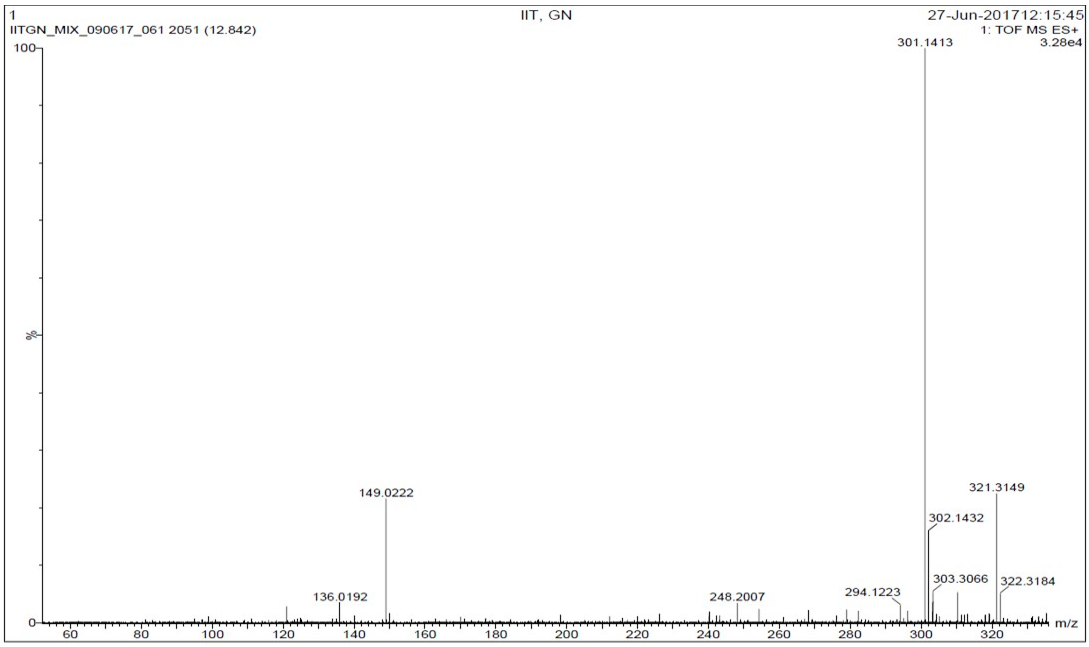

Fig. S6 HRMS data of **3** upon addition of Na_2S

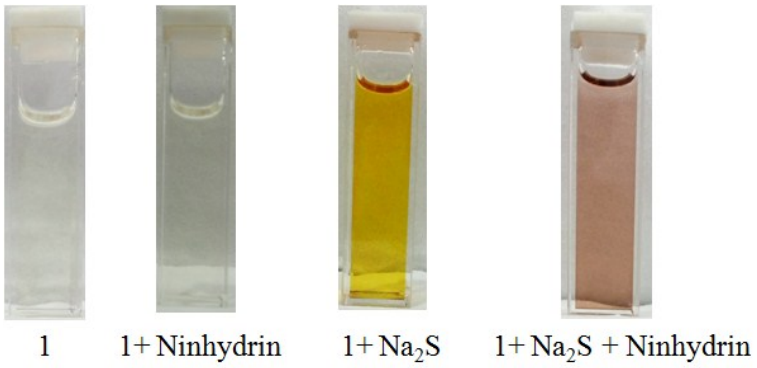

Fig. S7 Confirmation of Amine formation by Ninhydrin test

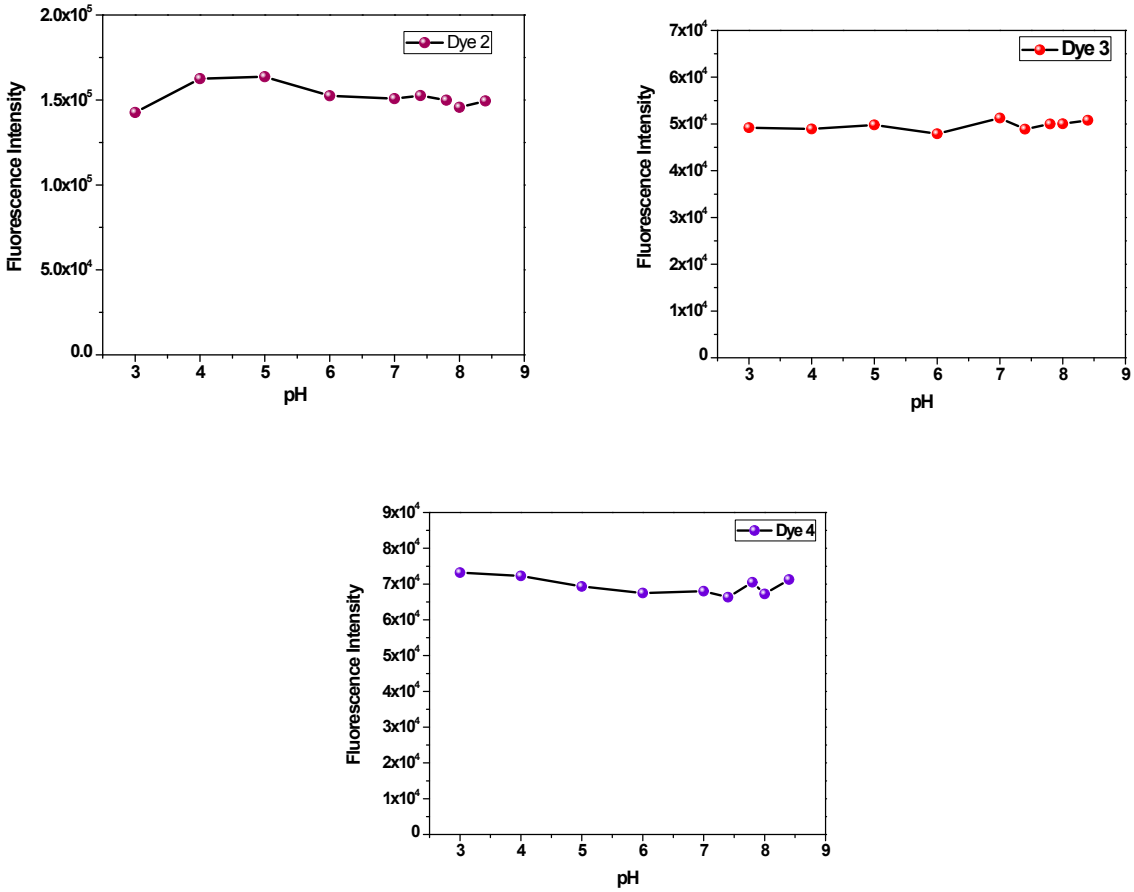

Fig.S8 The effect of pH value on the fluorescence intensity of probe (10.0 μM) in 40 mM HEPES. pH values: 3.0- 8.4

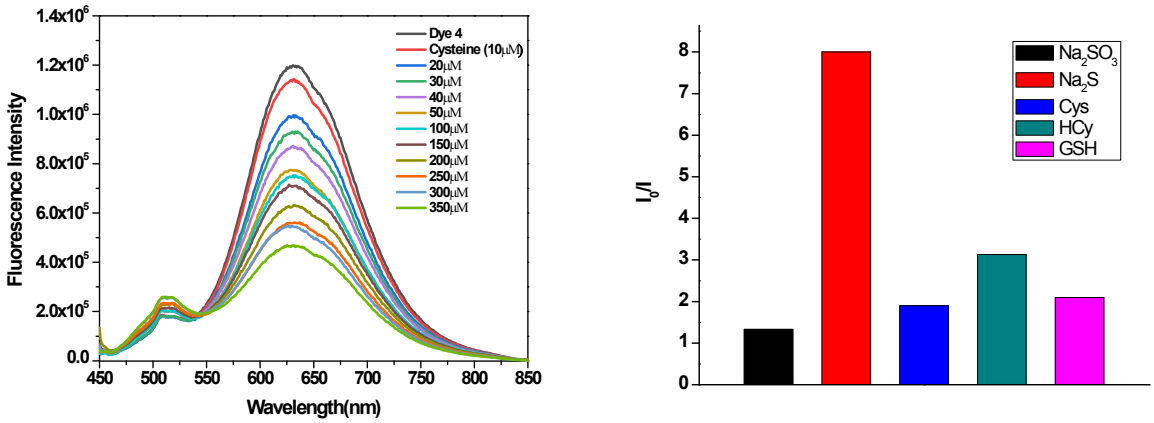

Fig. S9 a) Emission response at λ_{ex}= 450 nm of probe **4** (10 μM) with increasing concentration (0 to 350 μM) of Na_2S in DMSO + H_2O (9:1) binary mixture b) Comparative fluorescence intensity (I_0/I) ratios of (**4**) in DMSO-Water (9:1) solution (10 μM) upon addition of other sulfur analytes (measured at 200 μM of Cys, Hcy and GSH, Na_2S and SO_3^{2-} at λex/λem=450nm/ 631nm. Each spectrum was recorded after 3 min.

Synthetic procedures

Synthesis of pyrene-1- carboxaldehyde: In a three-necked round bottomed flask equipped with a reflux condenser, N_2 inlet and outlet adapters, dry DMF (3.2 mL, 41.3 mmol) in anhydrous 1,2-dichlorobenzene (30 mL) was added. To the reaction mixture phosphorous oxychloride (2.3mL, 24.7 mmol) was added with vigorous stirring at 0°C. The resultant mixture was allowed to stir for 45 minutes at 0°C and at room temperature for 1 h. Pyrene (5.0g, 20.4 mmol) in anhydrous 1,2-dichlorobenzene (20 mL) solution was gradually added to the above reaction mixture using a dropping funnel at room temperature. The contents of the reactions were stirred at room temperature overnight and then refluxed for 2 h. After confirmation of the product using TLC, the reaction mixture was cooled and poured into ice-cooled water with subsequent addition of toluene (100mL). The mixture was neutralized with Na_2CO_3 (5g , final pH 7) at 90°C, stirred for 1h at room temperature and then subjected to liquid separation. The organic phase was washed with water, dried with anhydrous sodium sulfate. The aqueous phase was discarded and the solvent was concentrated under reduced pressure to obtain crude crystals. The product was further crystallized from isopropyl alcohol (55mL).

Synthesis of 3,5-dimethylisoxazole (**5**): A mixture of 2,4-pentanedione (2.5 g, 25 mmol) and hydroxylamine hydrochloride (1.9g, 26.8mmol) in water (10 mL) and ethanol (5 mL) was refluxed for 90min. The mixture was cooled to room temperature, poured on to ice and extracted with dichloromethane. The combined organic phases were dried over anhydrous sodium sulfate, and evaporated. The resulting dark mixture was fractionated (140°C) to obtain (**1)** in 76% yield as a colorless liquid.

3,5-dimethyl-4-nitroisoxazole (**6**): Nitration mixture was prepared by taking 2:1 ratio of concentrated H_2SO_4 (1.3 mL) and concentrated HNO_3 (0.65 mL) at 0°C. 3,5-dimethylisoxazole (**1**) (0.6g, 6.2 mmol) was placed in a 100-ml round flask and cooled using ice/NaCl bath. The nitration mixture prepared above was added dropwise to 3,5-dimethylisoxazole while maintaining the temperature at ~0°C. The reactants were allowed to stand without stirring at room temperature for 3-4 days. The reaction mixture was then heated in a water bath for 10 min and diluted with cold water. The contents were extracted three times with diethyl ether, the organic phases were dried over anhydrous sodium sulfate. Evaporation of the solvent afforded the desired product (**2**) in 96% yield as a white solid.

Characterization Spectral Data

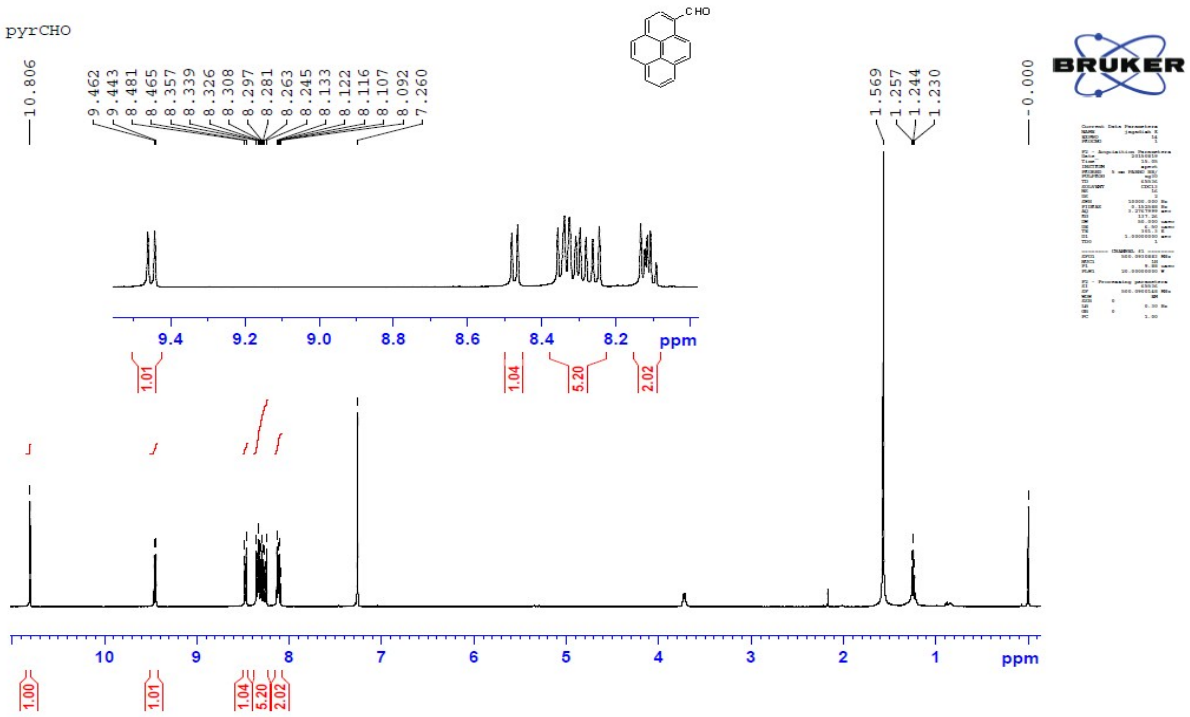

Fig. S10 ^{1}H NMR of pyrene-1-carbaldehyde

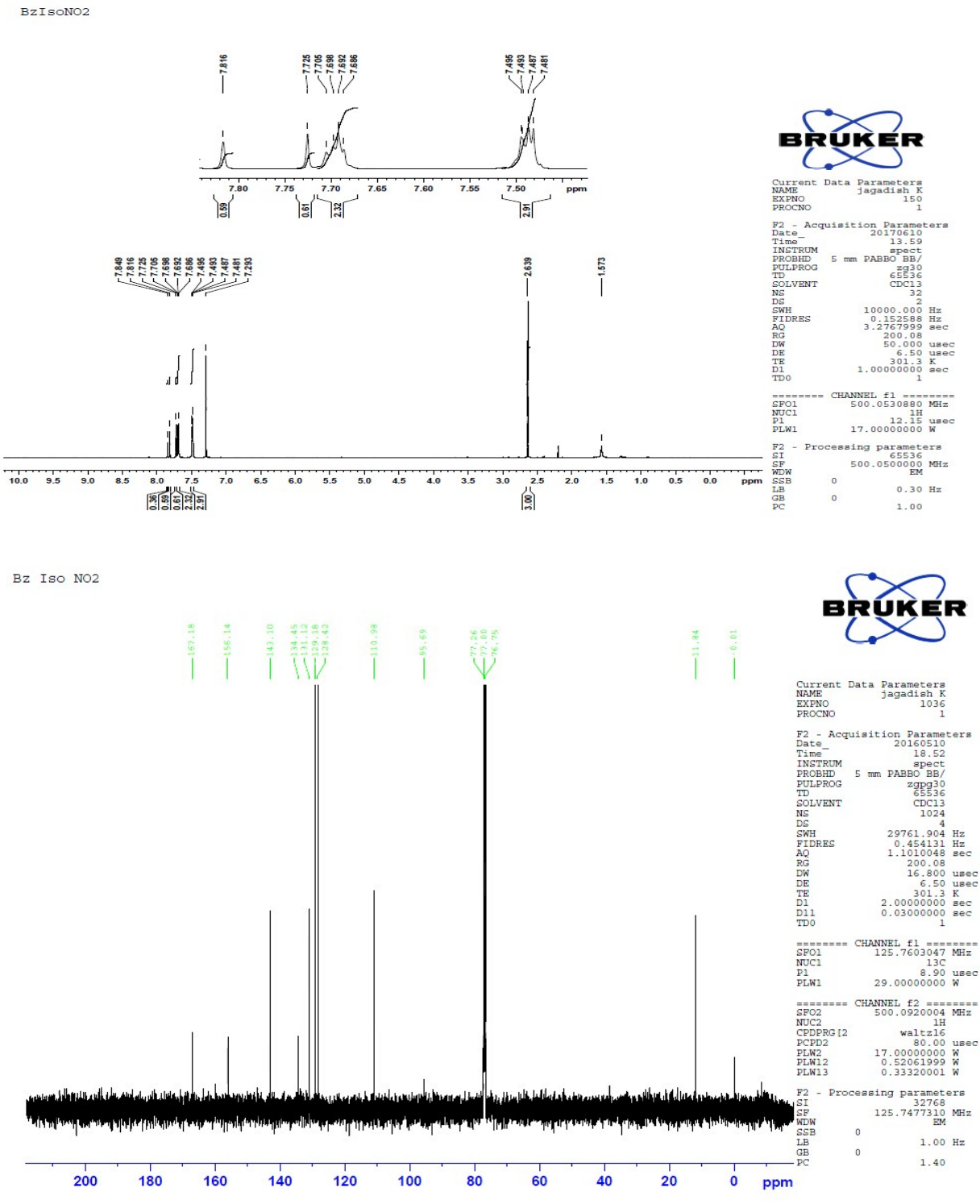

Fig. S11 [1]H NMR and 13C NMR of (E)-3-methyl-4-nitro-5-styrylisoxazole

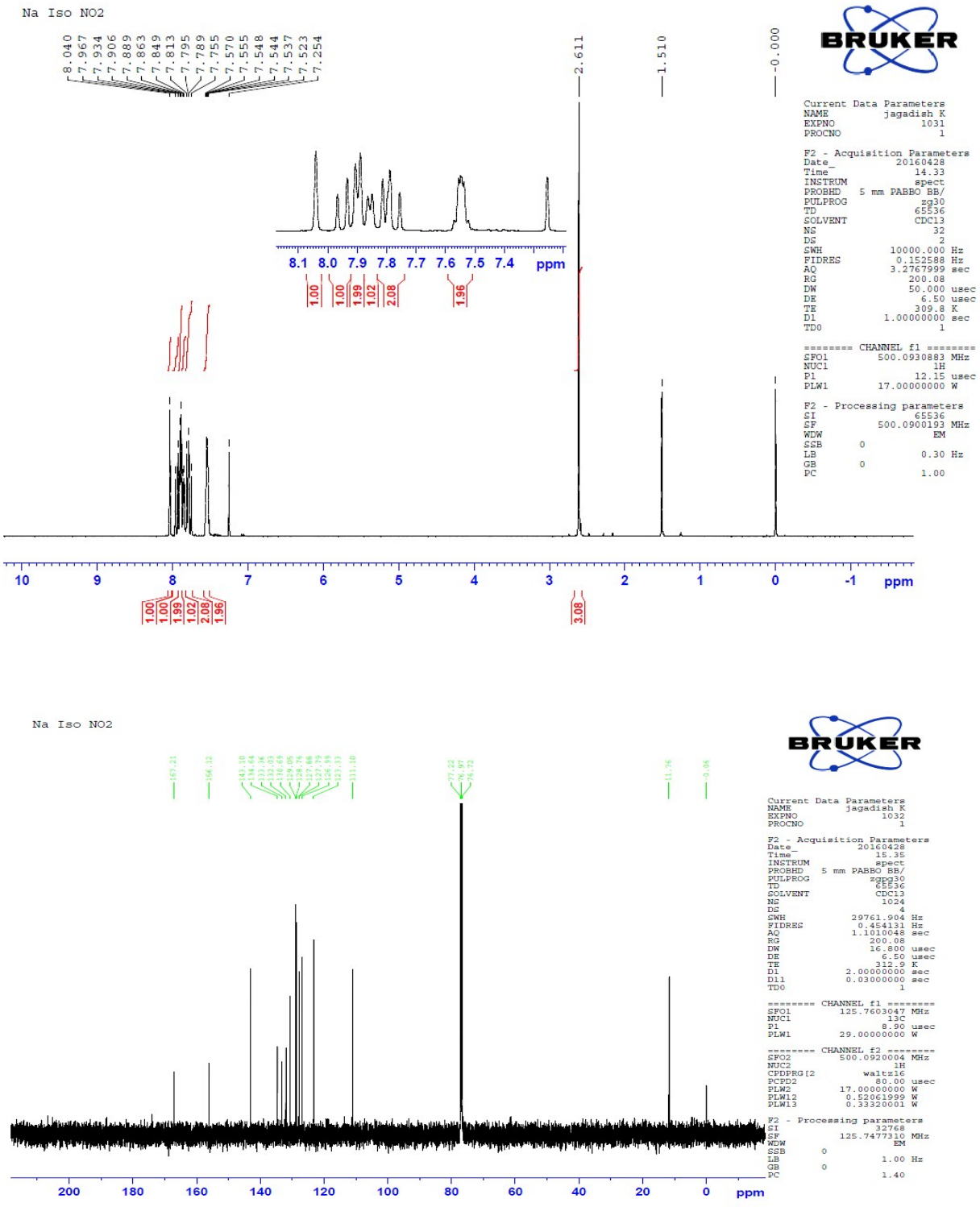

Fig. S12 ^{1}H NMR and 13C NMR of (E)-3-methyl-5-(2-(naphthalen-2-yl)vinyl)-4-nitroisoxazole

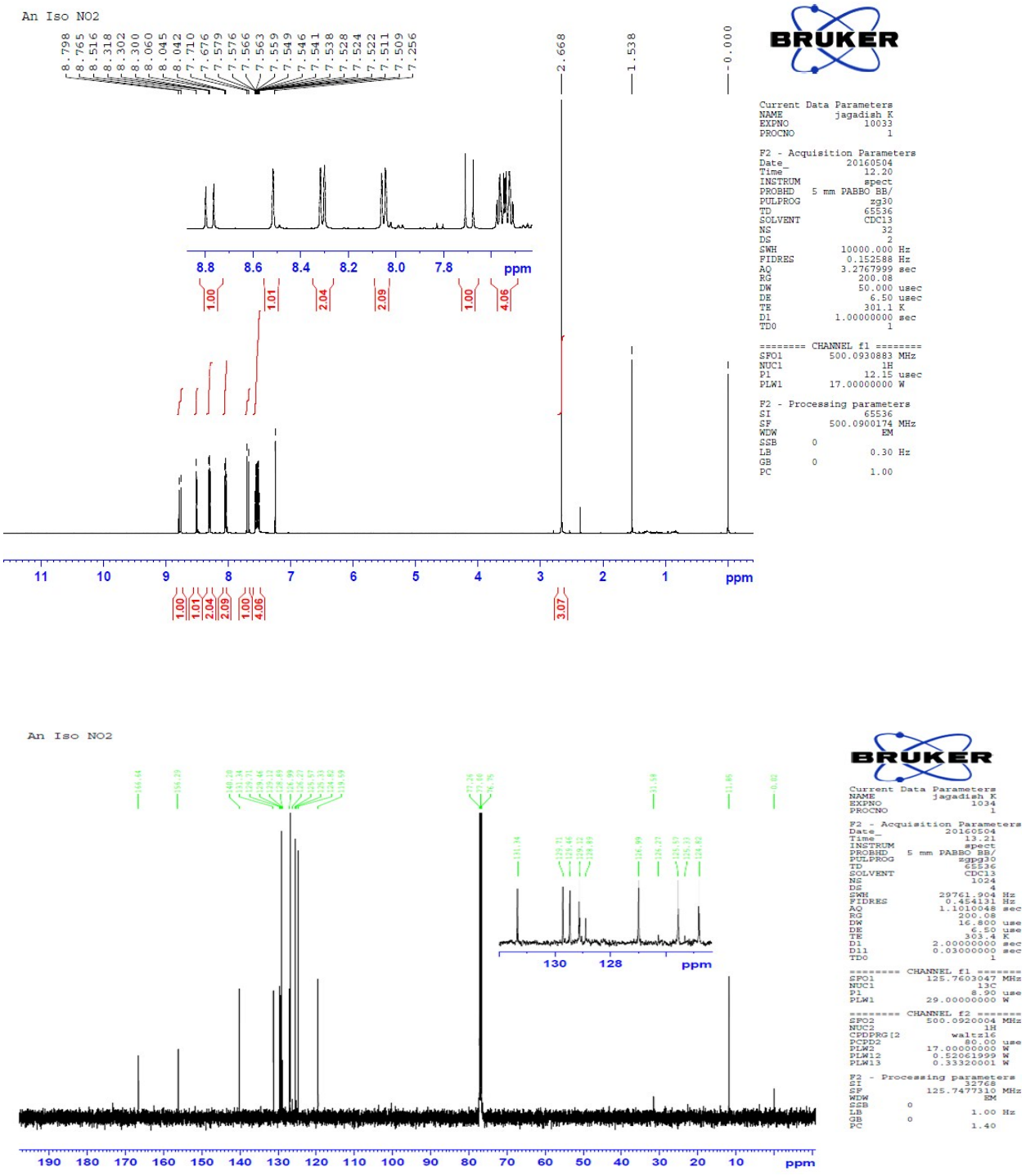

Fig. S13 ^{1}H NMR and 13C NMR of (E)-5-(2-(anthracen-9-yl)vinyl)-3-methyl-4-nitroisoxazole

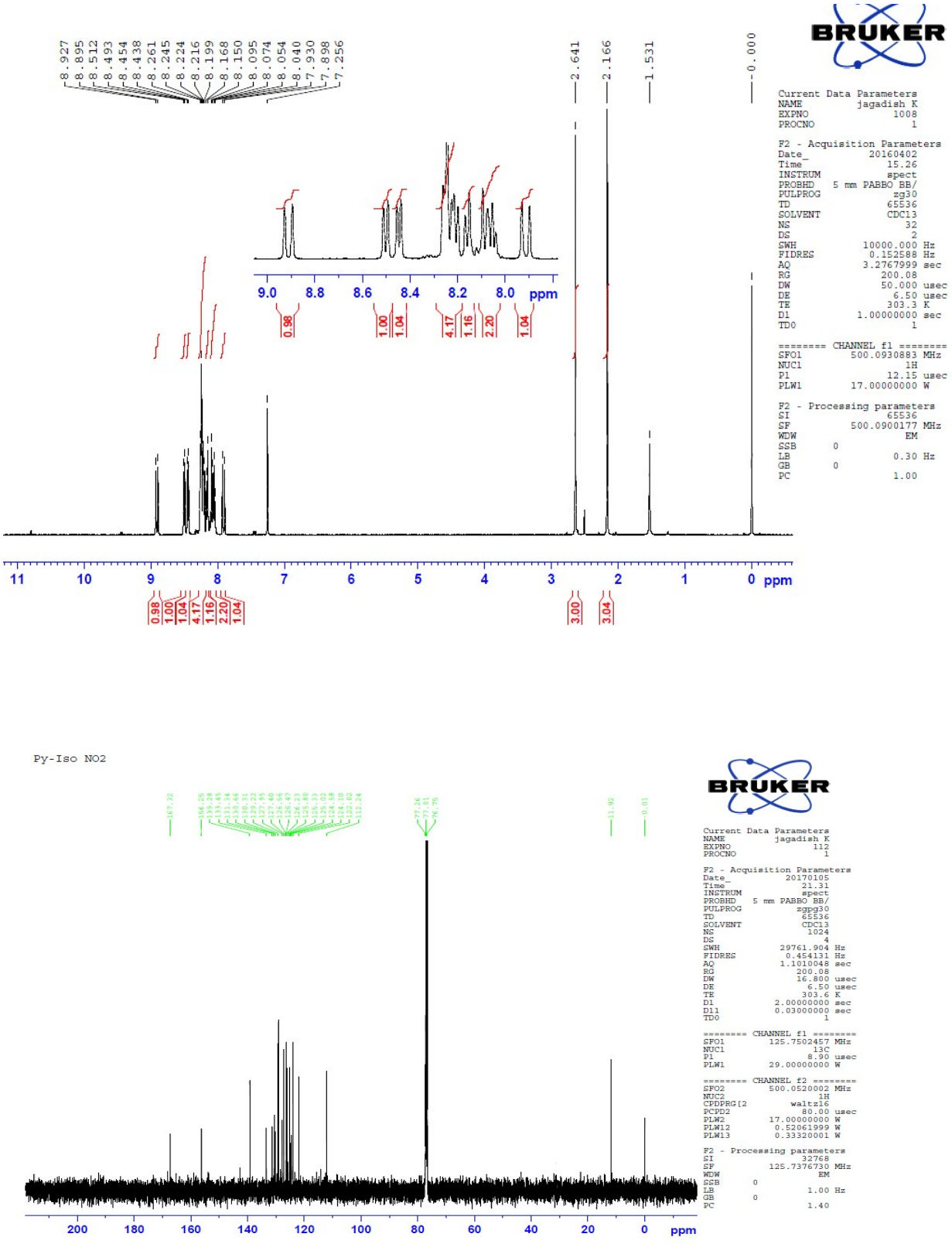

Fig. S14 ^{1}H NMR and 13C NMR of (E)-3-methyl-4-nitro-5-(2-(pyren-1-yl)vinyl)isoxazole