Contents:

1.1. Synthesis

To a solution of 7-chloro-4-nitrobenzo-2,1,3-thiadiazole (NBD-S-Cl) (0.5 mmol) in DCM (5 mL), thiomorpholine or its *S*-oxide (0.5 mmol) and Et_3N (0.6 mmol) were added. The mixture was stirred at a room temperature for 20 h under argon atmosphere. Next, the solvent was evaporated under reduced pressure, and the residue was dissolved in DCM (30 mL). The organic phase was washed with a citric acid solution (20% aq.), distilled water and the saturated $NaHCO_3$ solution. Finally, the organic phase was dried over anhydrous Na_2SO_4, and the solvent was removed in vacuo. The residue was further purified on silica column chromatography using DCM:MeOH (9.5:0.5) as eluent to yield the solid product.

4-Thiomorpholino-7-nitrobenzothiadiazole (NBD-S-TM) was obtained in 85% yield as an orange powder. ^{1}H NMR (DMSO-d_6:$CDCl_3$, 4:1, 600 MHz) δ (ppm): 8.56 (d, J = 9.0 Hz, 1H), 6.88 (d, J= 9.0 Hz, 1H), 4.42-4.43 (m, 4H), 2.84-2.86 (m, 4H); ^{13}C NMR (151 MHz): δ (ppm): 149.6; 147.8; 147.4; 132.5; 128.6; 106.6; 52.7; 27.0

HRMS (ESI): m/z 283.0330 ($[M^+H]^+$), Calcd for $(M + H)^+ = 283.3420$; HRMS (ESI-Na) m/z 305.0150 ($[M^+Na]^+$), Calcd for $(M + Na + H)^+ = 305.3312$.

***4-Thiomorpholino-7-nitrobenzenothiadiazole S*-oxide** (NBD-S-TSO) was obtained in 50% yield as a dark orange powder.^{1}H NMR (DMSO-d_6:$CDCl_3$, 4:1, 600 MHz) δ (ppm): 8.59 (d, J = 9.0 Hz, 1H), 7.00 (d, J= 9.0 Hz, 1H), 4.81 (d, J = 15 Hz 2H), 4.33 (t, J = 12.6 Hz, 2H), 3.11-3.16 (m, 2H), 2.90-2.92 (m, 2H); ^{13}C NMR (151 MHz): δ (ppm): 149.5; 147.5; 132.5; 129.1; 128.9; 106.8; 45.3; 41.4

HRMS (ESI): m/z 299.0277 ($[M^+H]^+$), Calcd for $(M + H)^+ = 299.3414$; HRMS (ESI-Na) m/z 321.0091 ($[M^+Na]^+$), Calcd for $(M + Na + H)^+ = 321.3306$.

1.2. Determination of quantum yield.

Fluorescence quantum yields were determined by the comparative method using the procedure described previously [S1]. Fluorescence quantum yields were determined using solutions of Quinine sulfate (ΦF = 0.79 in 0.1 M NaOH) as a standard [S2]. The quantum yield was calculated using the following equation:

$$\Phi^F_x = \Phi^F_s(A_sF_x/A_xF_s)(n_x/n_s)^2$$

- Φ^F is the fluorescence quantum yield;
- A is the absorbance at the excitation wavelength;
- F is the area under the corrected emission curve;

- n is the refractive index of the solvents used;
- subscripts s and x refer to the standard and unknown samples, respectively.

1.3. Determination of detection limit.

The limit of detection (LOD) is the lowest signal and was calculated from the titration curve of the NBD-S-T fluorescence in the presence of HOCl and the mean blank. The fluorescence intensity of NBD-S-TM was measured and the standard deviation of the blank measurements was obtained and determined as σ using the following equation:

$$\sigma = \sqrt{\frac{\sum(\bar{x} - Xi)^2}{n - 1}}$$

- σ is the standard deviation of the blank;
- x is the blank mean;
- Xi is the values of the blank measures;
- n is the blank number tested (n = 11).

Detection limit LOD is calculated using the following equation

Detection limit = 3.3σ/s

- s is the slope of fluorescence intensity versus HOCl concentrations.

1.4. Stability in the presence of H_2S

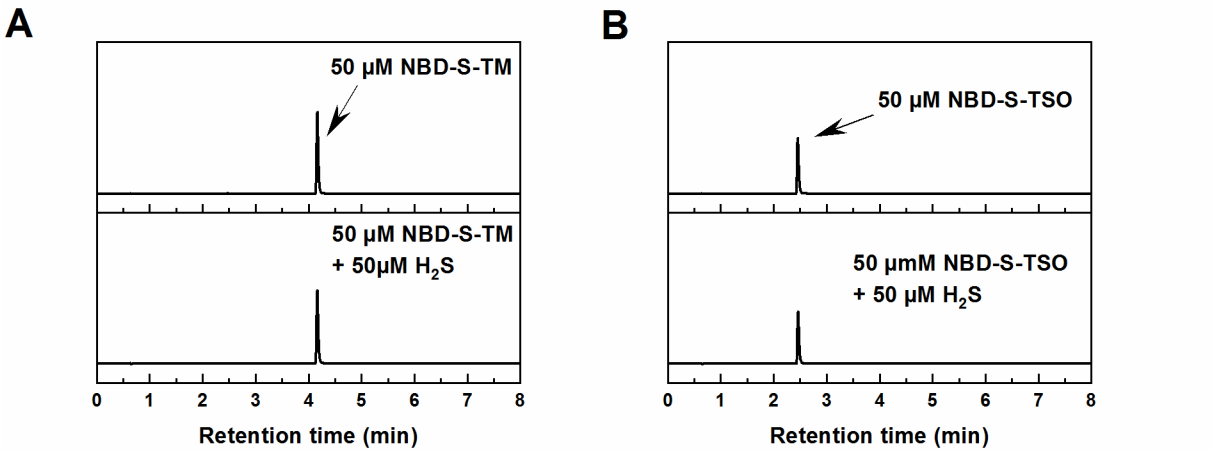


Figure S1. (**A**) The NBD-S-TM probe and (**B**) NBD-S-TSO standard stability in the presence of H_2S. All experiments were carried out in an aqueous solution containing phosphate buffer (50 mM, pH 7.4) and MeCN (10%) at room temperature. The traces were collected using an absorption detector set at 500 nm.

1.5. ^{1}H NMR, ^{13}C NMR and ESI MS spectra

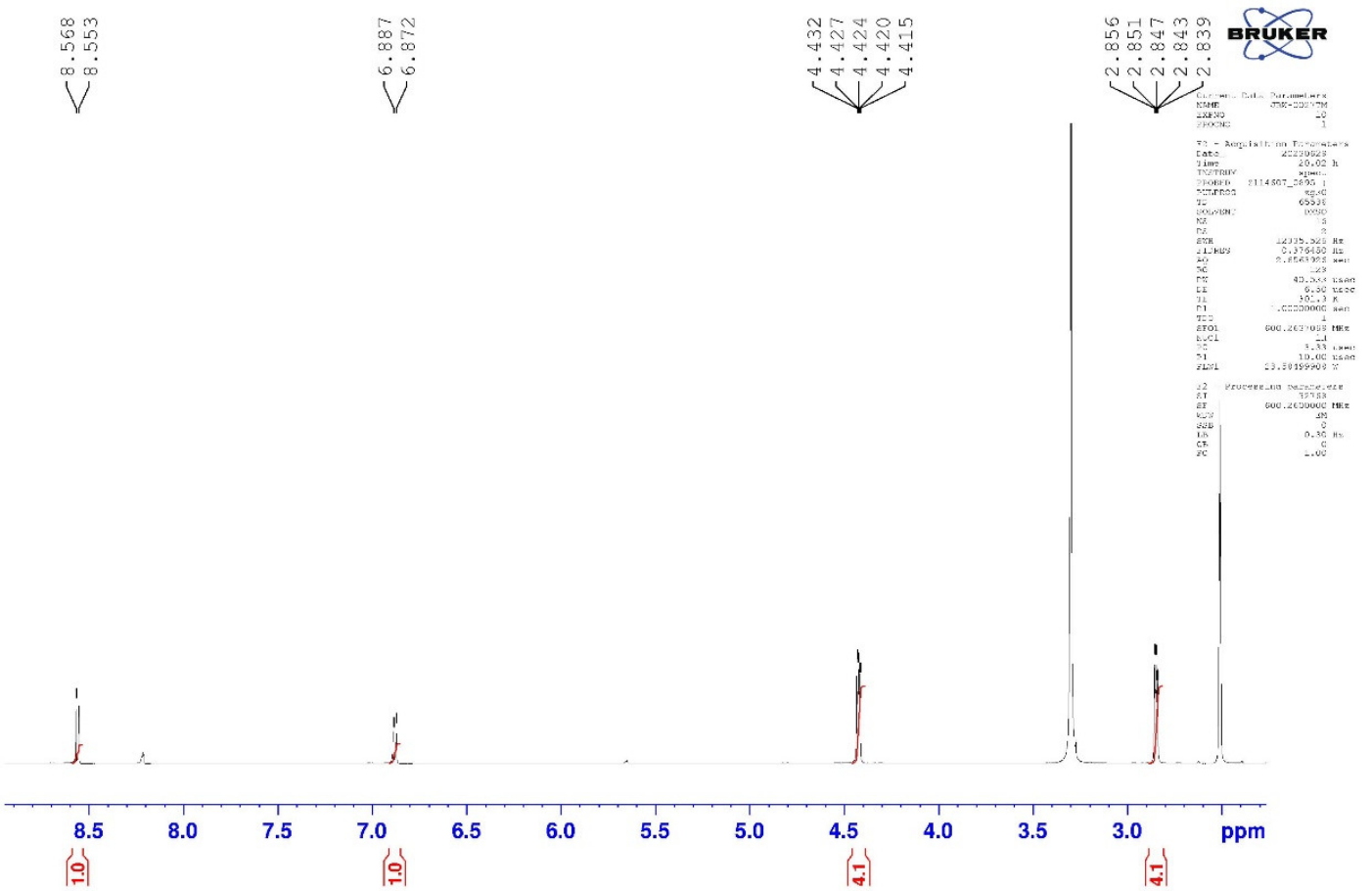


Figure S2. ^{1}H NMR spectra of NBD-S-TM.

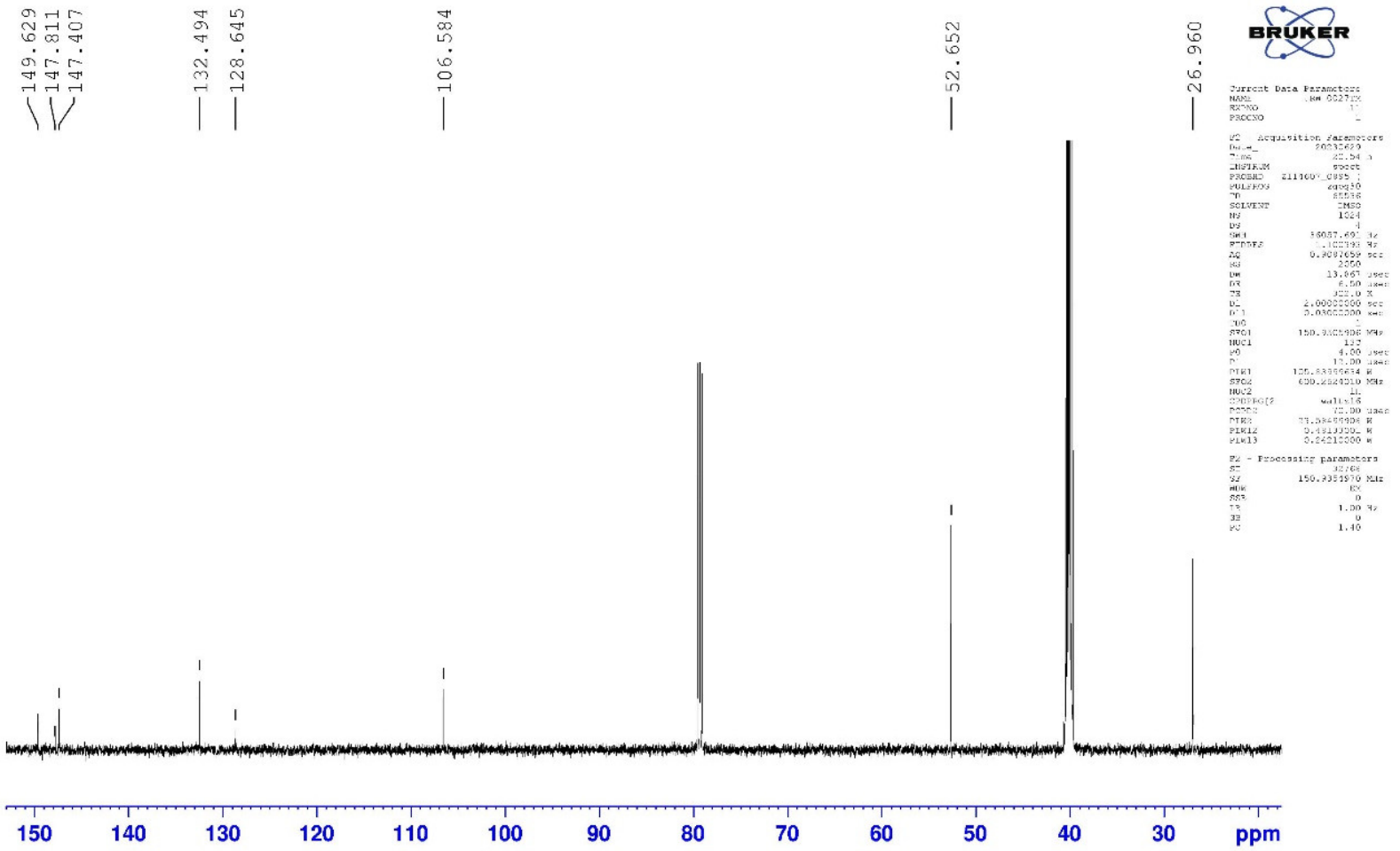
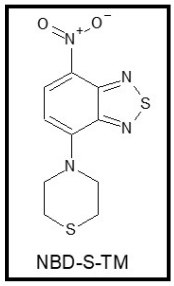


Figure S3. ^{13}C NMR spectra of NBD-S-TM.

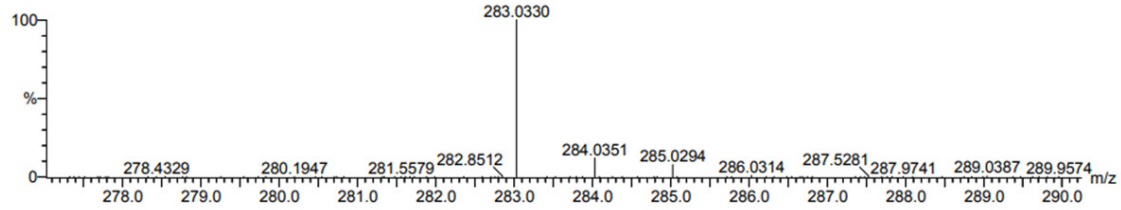
Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9

Monoisotopic Mass, Even Electron Ions
75 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-15 H: 0-30 N: 0-4 O: 0-3 S: 1-2
211217_NBD_S_TM_posB 17 (0.197) Cm (15:20)



TOF MS ES+
1.40e+006



Minimum:				-1.5				
Maximum:		5.0	5.0	80.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
283.0330	283.0323	0.7	2.5	7.5	400.1	n/a	n/a	C10 H11 N4 O2 S2

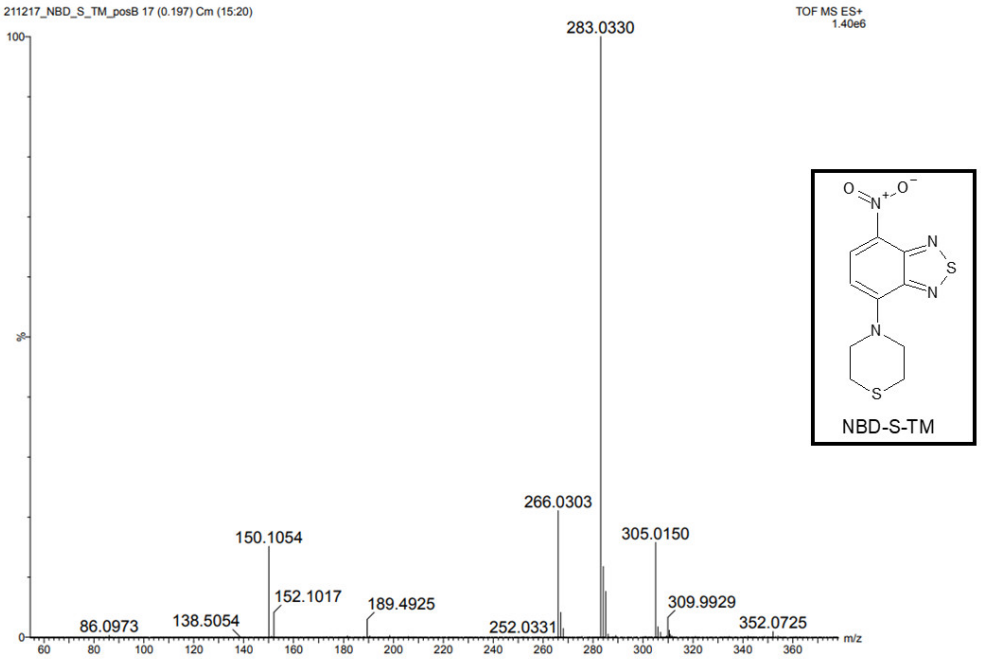


Figure S4. ESI MS spectra of NBD-S-TM.

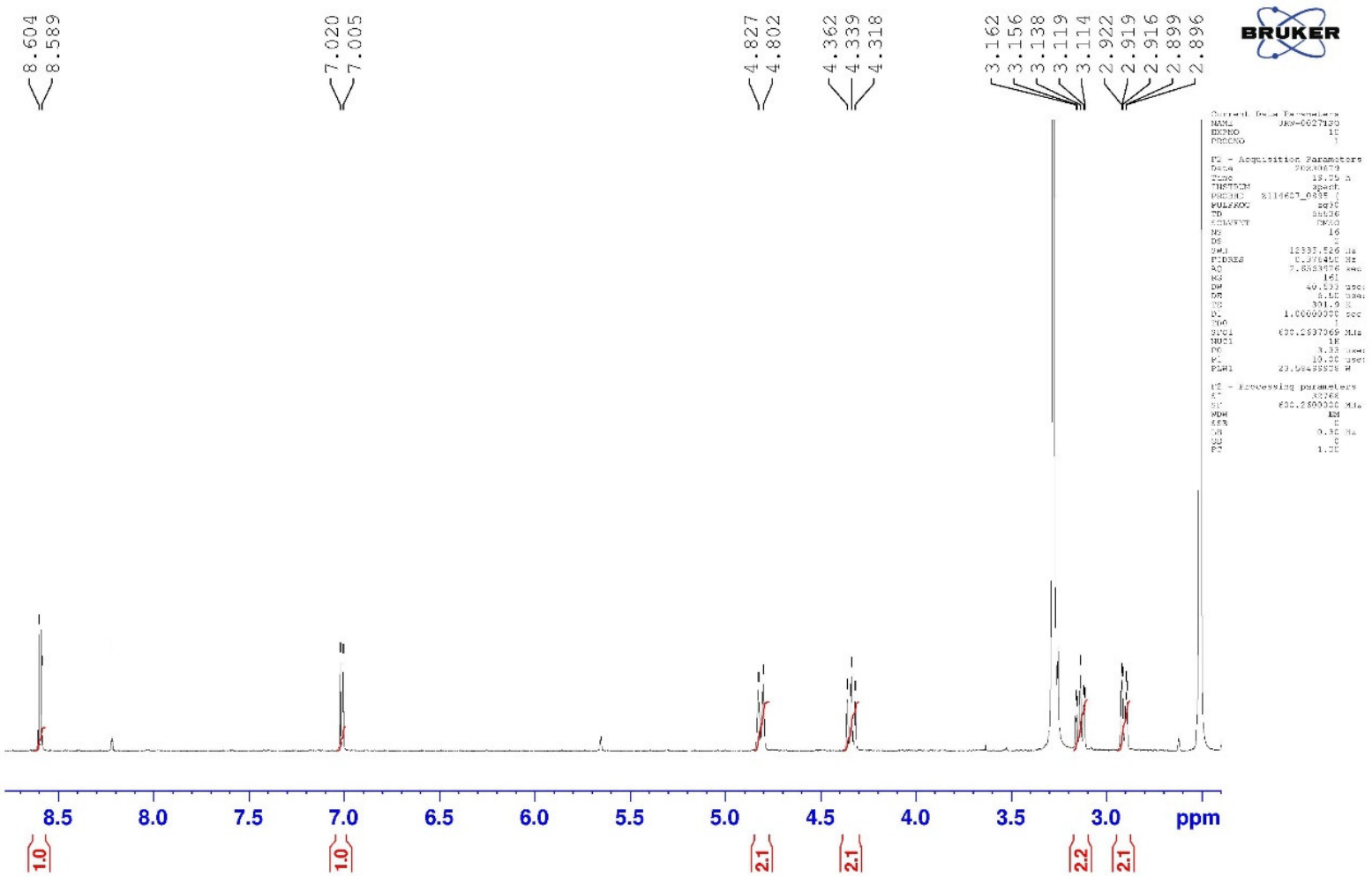


Figure S5. ^{1}H NMR spectra of NBD-S-TSO.

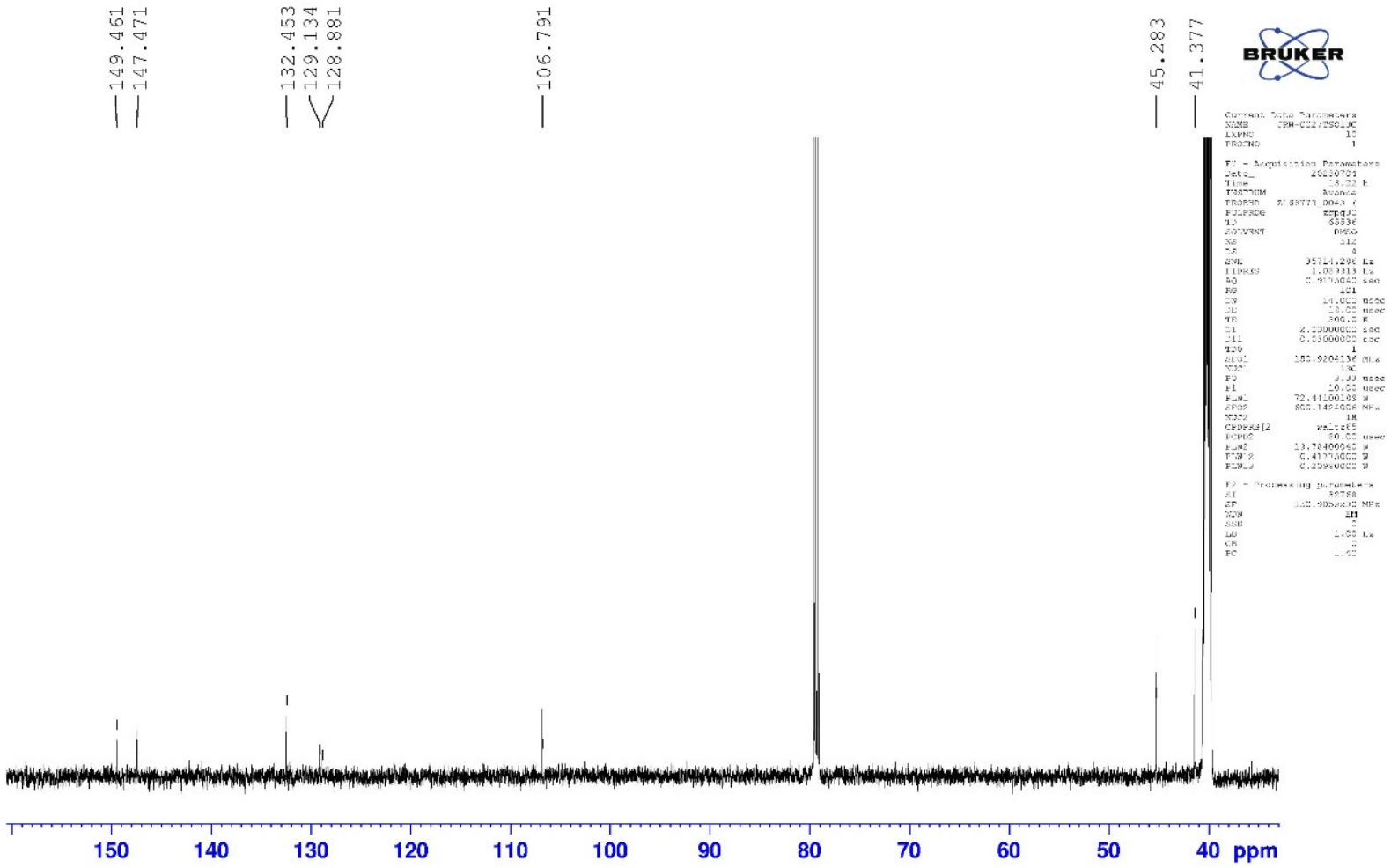


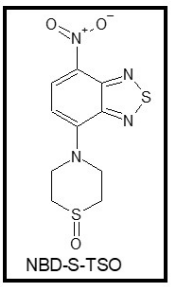
Figure S6. ^{13}C NMR spectra of NBD-S-TSO.

Elemental Composition Report **Page 1**

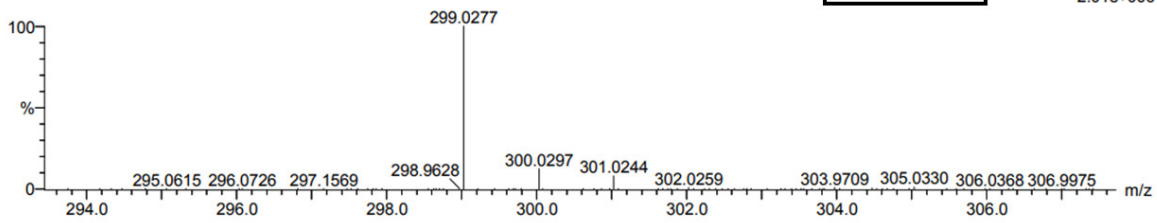
Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 80.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 9

Monoisotopic Mass, Even Electron Ions
61 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-15 H: 0-30 N: 0-4 O: 0-3 S: 1-2

211217_NBD_S_TSO_posB 17 (0.197) Cm (15:20-(4:8+47:52))



TOF MS ES+
2.01e+006



Minimum:				-1.5				
Maximum:		5.0	5.0	80.0				
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
299.0277	299.0273	0.4	1.3	7.5	593.4	n/a	n/a	C10 H11 N4 O3 S2

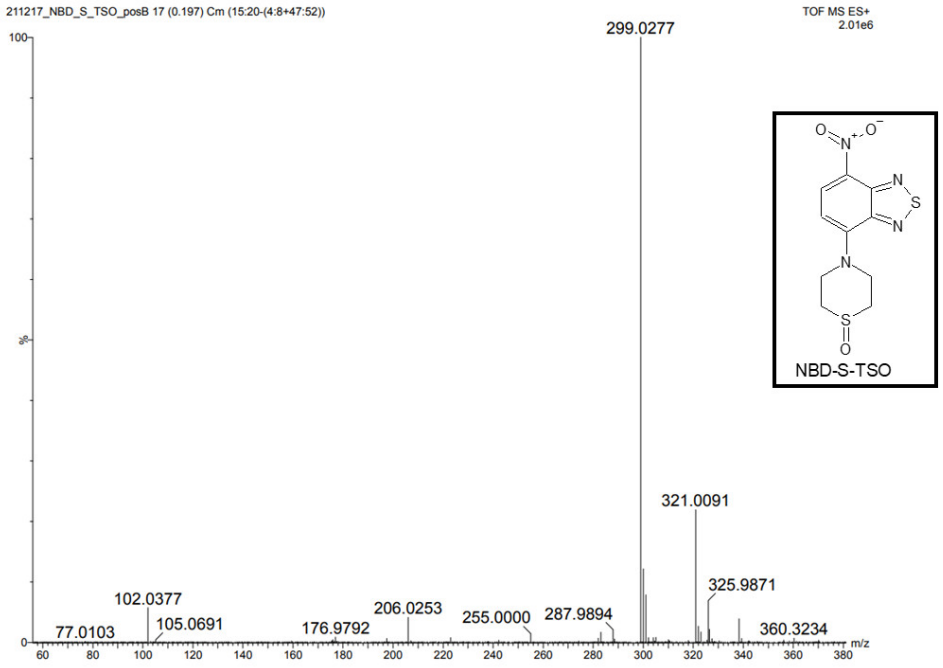


Figure S7. ESI MS spectra of NBD-S-TSO.

1.6. Comparison of fluorescent probes based on HOCl response

Table S1. Comparison of fluorescent probes based on HOCl response.

Probe	LOD	Response time	Mechanism	References
BCy-S	35.2 nM	less than 40 s	TICT	[3]
Probe 1	72 nM	30 s	ICT	[4]
FNIR-HOCl	70 nM	less than 20 s	-	[5]
NBD-TM	72 nM	within 1 s	PET	[1]
NBD-Se-TM	258 nM	within 1 s	PET	[6]
HBTN	24.5 nM	less than 20 min	-	[7]
PTA	33.9 nM	45 s	ICT	[8]
NBD-S-TM	60 nM	within 1 s	PET	This work

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