Improved Lipophilic Probe for Visualizing Lipid droplets in Erastin-Induced Ferroptosis

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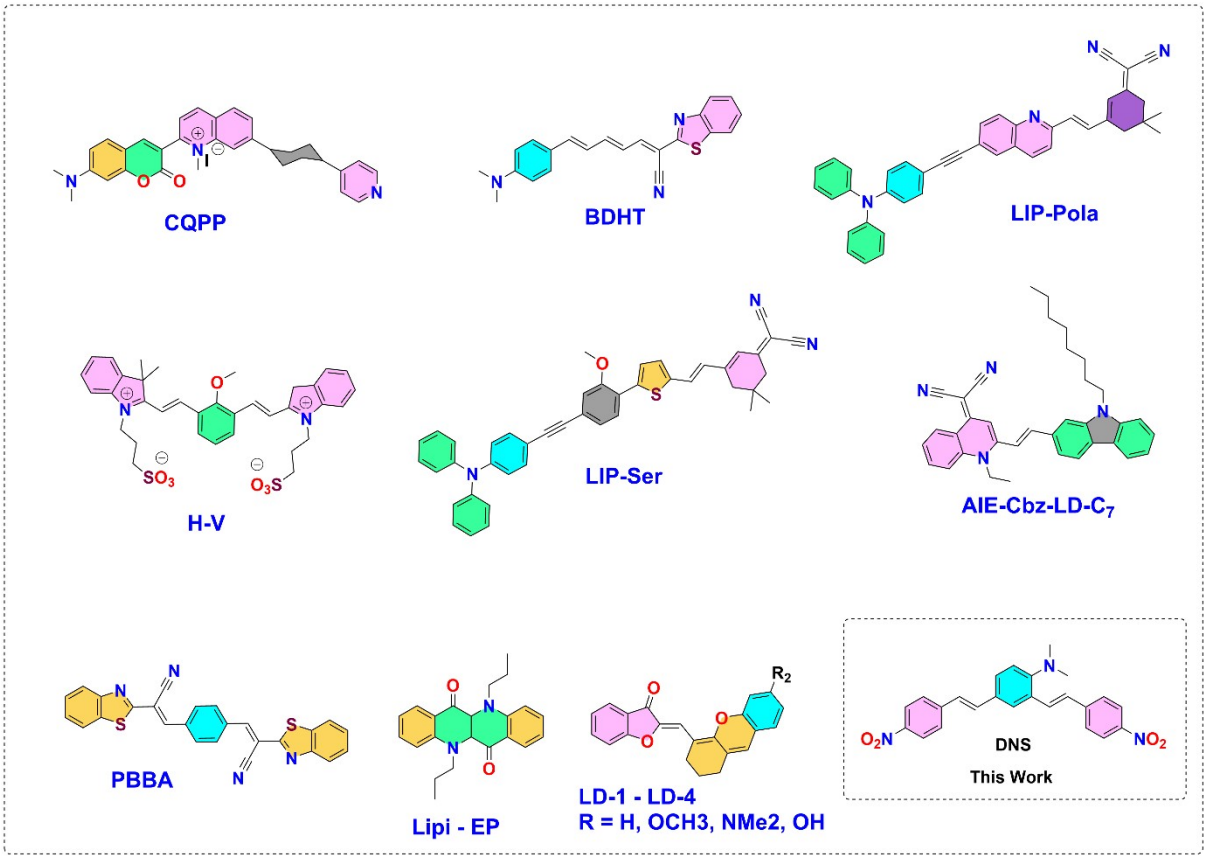
S1: Reported Probes for Ferroptosis in LDs



S2: Absorption and emission spectra

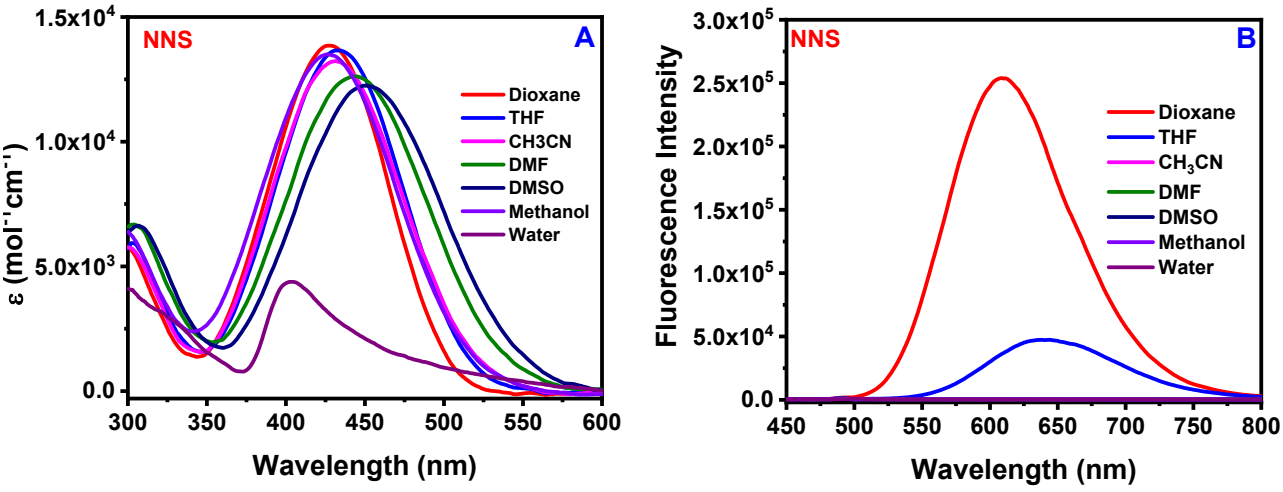


Fig S2: A) Absorption & B) Emission data of NNS in different solvents.

S3: Lifetime data:

Table S3: Lifetime decay of NNS and DNS in different solvents

NNS						
Components	τ_1 (ns)	A1	τ_2 (ns)	A2	Average Lifetime (τ) (ns)	χ^2
Dioxane	0.2	7.5%	1.3	92.4%	1.0	1.53
THF	0.5	100%	-	-	0.5	1.34
DMSO	1.1	17.8%	0.03	82.1%	0.03	1.21
Water	0.03	41.7%	0.24	51.2%	0.06	1.47
DNS						
Components	τ_1 (ns)	A1	τ_2 (ns)	A2	Average Lifetime (τ) (ns)	χ^2
Dioxane	0.2	63.4%	0.5	36.5%	0.2	1.06
THF	0.06	71.7%	0.55	21.2%	0.08	1.25
DMSO	0.02	78.1%	0.48	21.8%	0.02	1.37
Water	--- Lifetime value too little (beyond instrument limit to measure)					

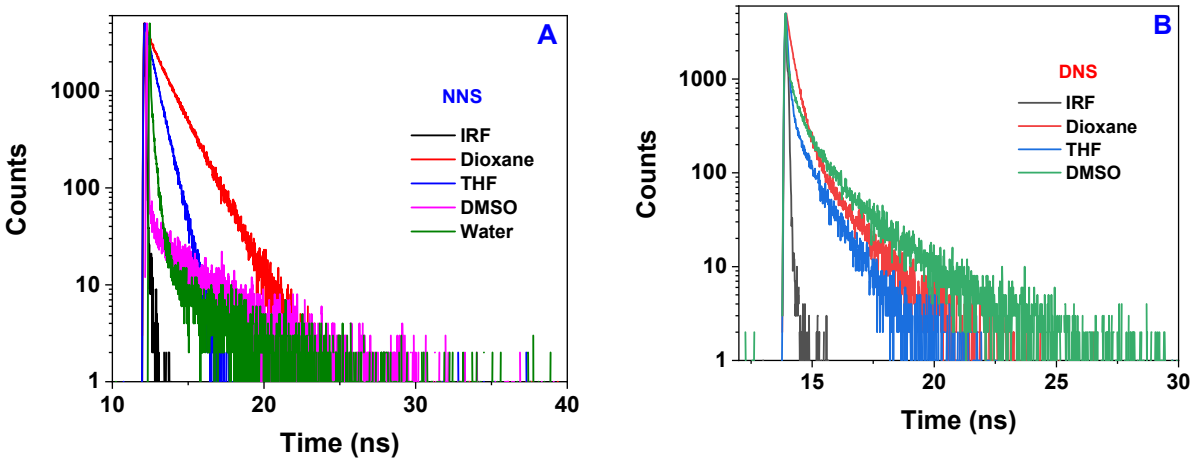


Fig S3: Fluorescence lifetime plot of A) NNS and B) DNS in different solvent environments.

S4: Optimized geometries

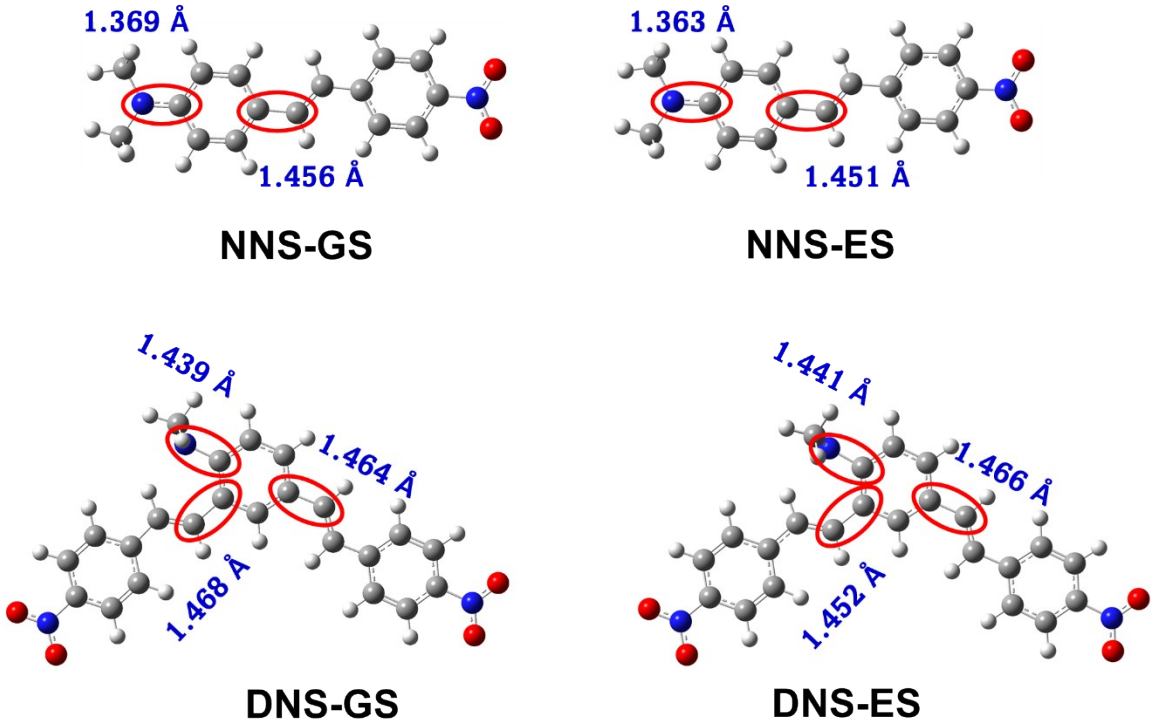


Fig S4: Optimized geometries of the monomer and dimer derivatives at the ground and excited states obtained from the B3LYP/6-311G (d, p) level of theory with selected bond distances.

S5: Energy level diagram

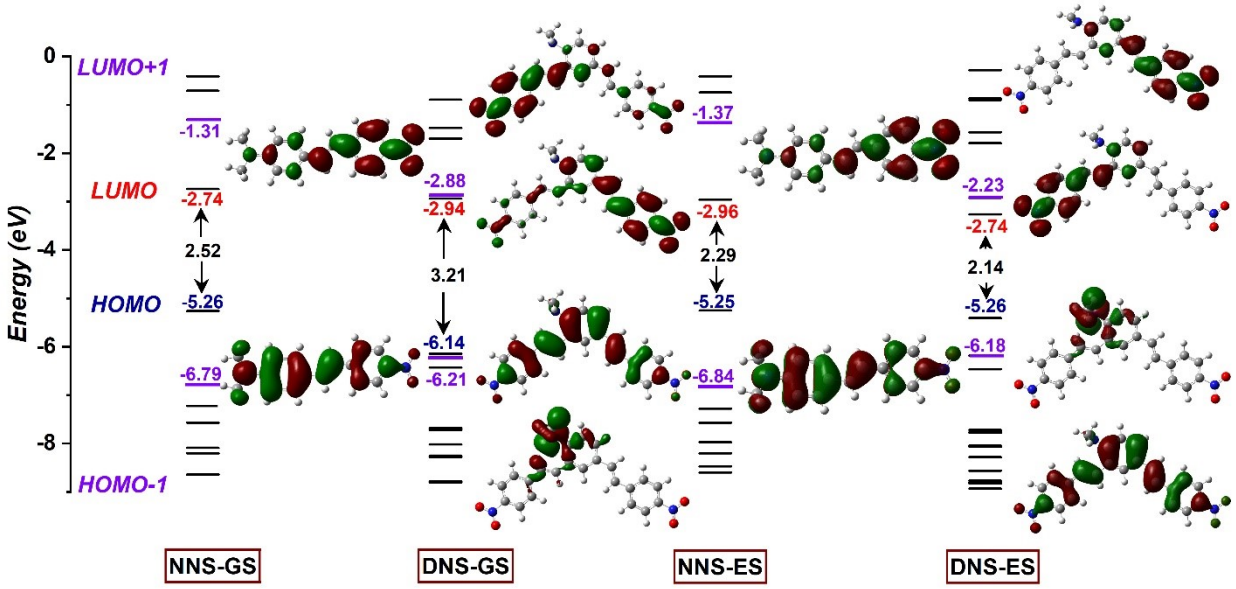
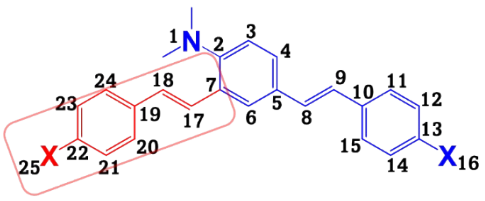


Fig S5: Schematic energy level diagram illustrating the energy levels, HOMO-LUMO gap, and isodensity surface plots of the frontier molecular orbitals for NNS and DNS molecules obtained at the ground and excited states. The green and red surfaces represent positive and negative signs of the MO at isovalue of 0.02 a.u., respectively.

S6: Geometrical parameters



: NIL; X = NO_2 (NNS) : 1; X = NO_2 (NNS)

	NNS-GS	NNS-ES	DNS-GS	DNS-ES
N1-C2	1.370	1.363	1.439	1.441
C2-C3	1.392	1.423	1.396	1.391
C3-C4	1.383	1.377	1.389	1.387
C4-C5	1.408	1.415	1.404	1.407
C5-C6	1.411	1.417	1.401	1.398
C6-C7	1.381	1.376	1.405	1.411
C7-C2	1.421	1.425	1.421	1.420
C5-C8	1.451	1.456	1.464	1.466
C8-C9	1.354	1.355	1.347	1.346
C9-C10	1.455	1.465	1.462	1.462
C10-C11	1.414	1.411	1.411	1.410
C11-C12	1.382	1.385	1.383	1.384
C12-C13	1.397	1.404	1.395	1.395
C13-C14	1.394	1.402	1.392	1.392
C14-C15	1.384	1.388	1.386	1.386
C15-C10	1.413	1.408	1.409	1.408
C13-X16	1.457	1.432	1.465	1.466
C7-C17			1.468	1.453
C17-C18			1.349	1.365
C18-C19			1.463	1.440
C19-C20			1.411	1.422
C20-C21			1.384	1.376
C21-C22			1.395	1.413
C22-C23			1.392	1.411
C23-C24			1.386	1.378
C24-C19			1.409	1.419

C22-X25			1.465	1.407

Fig S6: Geometrical parameters of the NNS and DNS molecules optimized at the ground and excited states.

S7: TDDFT simulated absorption spectral data

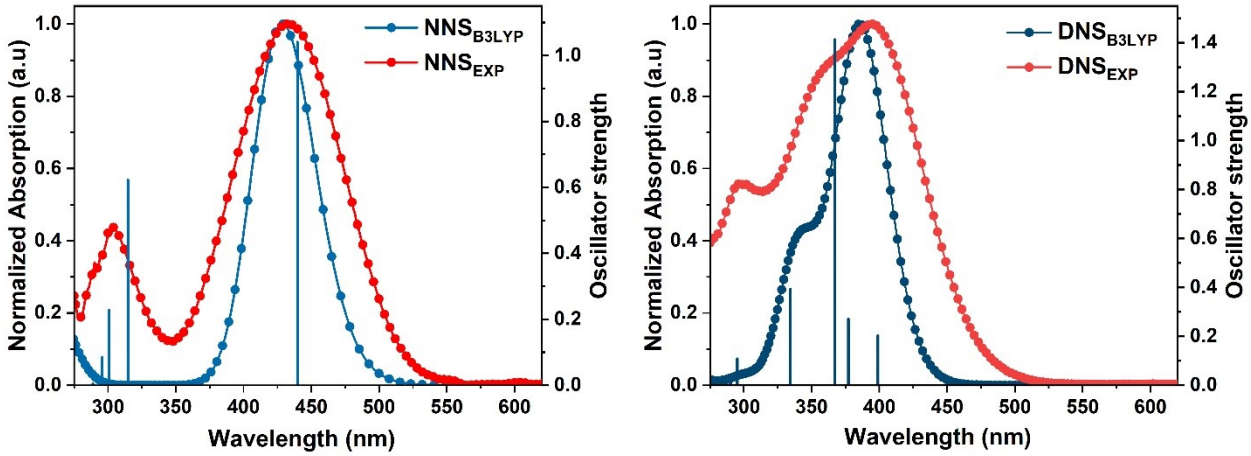


Fig S7: TDDFT simulated absorption spectral data and oscillator strength obtained from B3LYP/6-311G (d, p)/C-PCM (THF) level of theory and compared with the experimental absorption recorded in THF.

Table S7**.** Comparison of absorption properties of the molecules with computed excitation energy, oscillator strength, major transitions involved in frontier molecular Orbitals, and dipole moments.

	λ_{exp}[a] (nm)	λ_{B3LYP}[b] (nm)	f_{B3LYP}[b]	Major transitions	μ_g[c] (Debye)	μ_e[d] (Debye)
NNS	434	428.4	1.61	HOMO->LUMO (91%), H-1-> LUMO, (3%), HOMO->L+1 (4%)	13.9	12.6
DNS	394	385.7	2.13	H-1->LUMO (30%), HOMO->L+1 (56%), HOMO->LUMO (6%), HOMO->L+3 (3%)	9.4	9.1

[Absorption] spectra measured in THF in the concentration of 1×10^{-5} M at ambient temperature; [simulated] absorption maximum and oscillator strength obtained at the TDDFT/ B3LYP/6-311G**/C-PCM(THF) level of theory functional; [c]μ_g: ground state dipole moment; [d]μ_e: transient dipole moment.

S8: Computed total density of states predicted at the ground state.

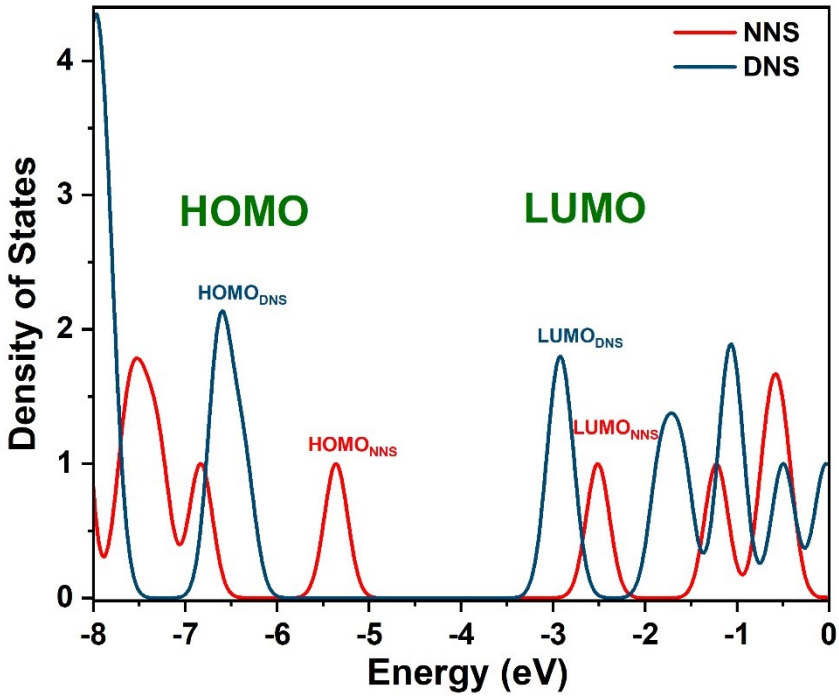


Fig S8: Computed total density of states (TDOS) of the NNS and DNS molecules predicted at the ground state.

S9: Illustration of the effect of *ortho*-substitution

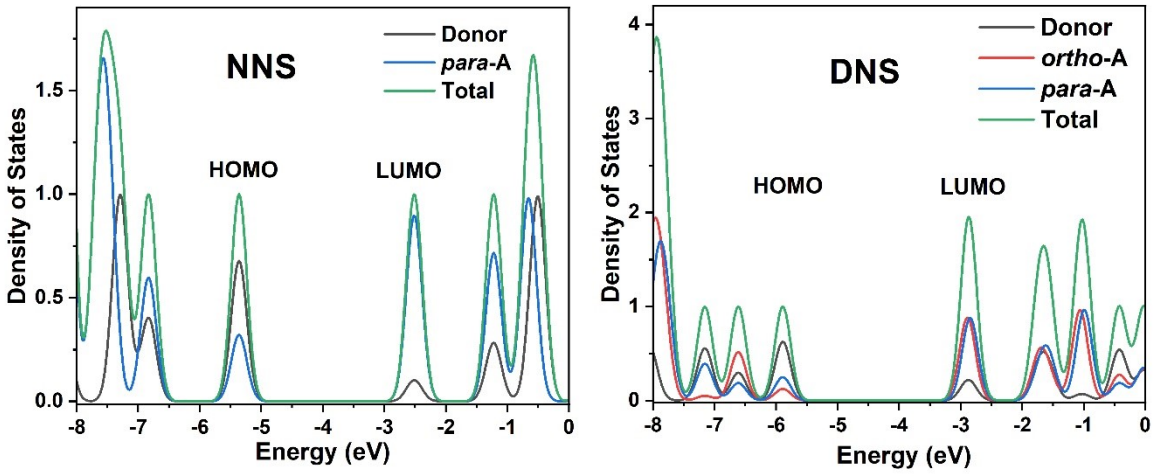


Fig S9: Computed total density of states (TDOS) and projected DOS of the NNS and DNS molecules predicted at the ground state to illustrate the effect of *ortho*-substitution.

S10: Molecular orbital density coefficients

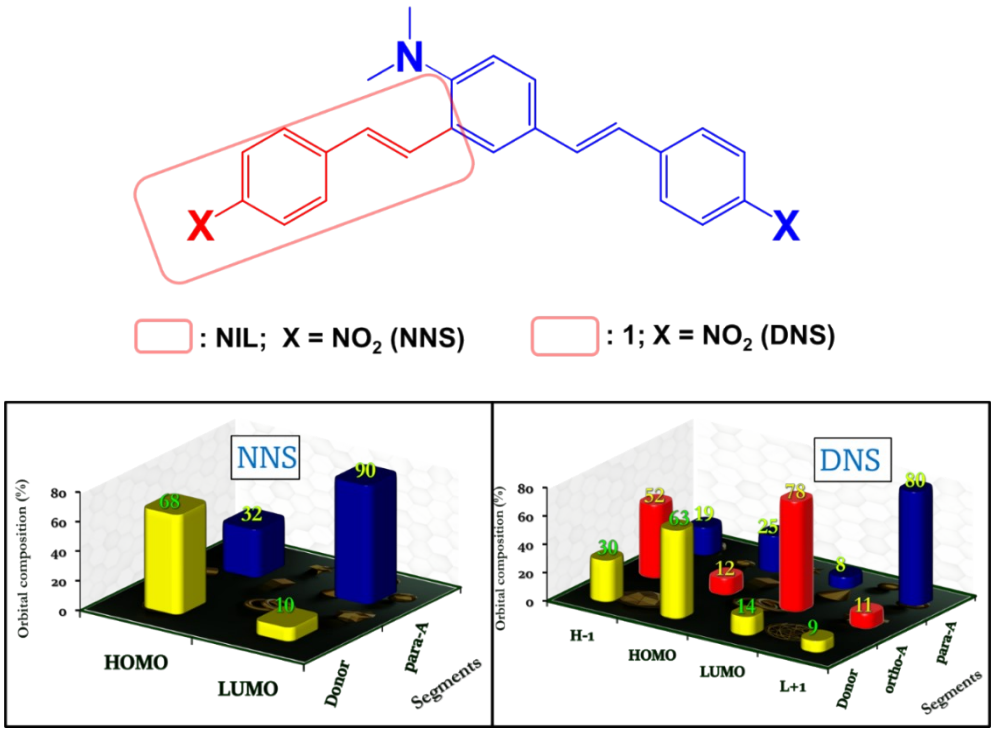


Fig S10: Percentage contribution of the molecular orbital density coefficients of the molecules extracted from the corresponding partitioned segments (D, and *para*-A) for NNS and (D, *ortho*-A, and *para*-A) obtained corresponding to the H-1, HOMO, LUMO, and LUMO+1 energy levels for DNS molecule.

S11: Computed total density of states (TDOS)

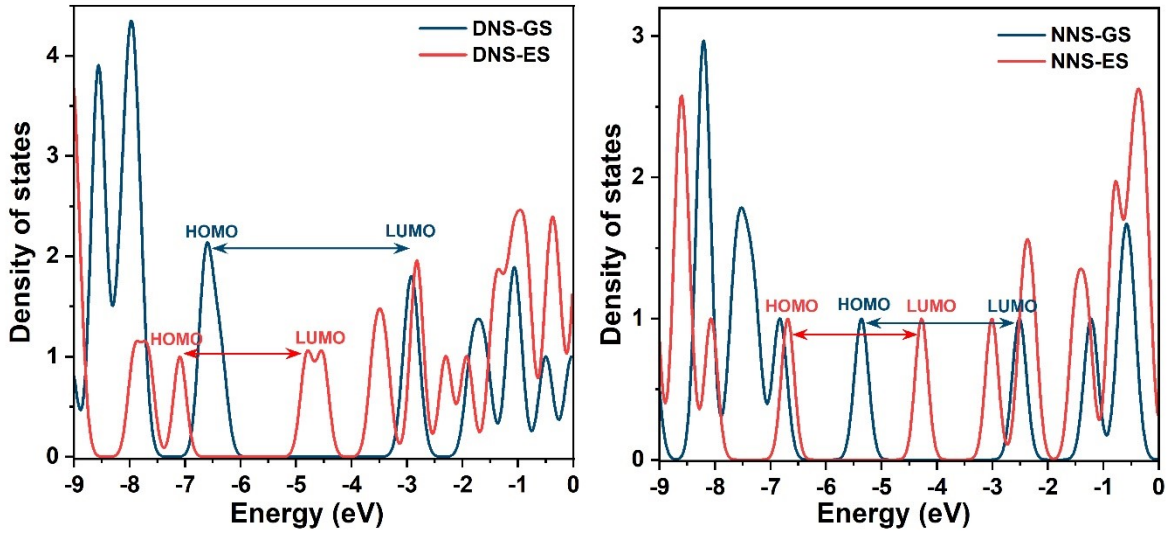


Fig S11: Computed total density of states (TDOS) of the NNS and DNS molecules predicted at the ground and excited states and their respective transformations.

S12: Cytotoxicity assay (MTT Assay)

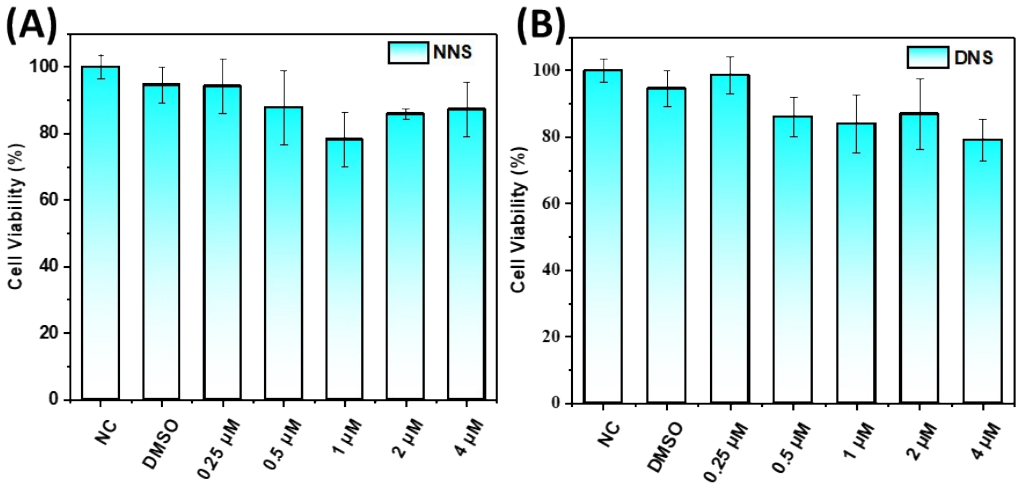


Fig S12: Cytotoxicity assay for NNS (A) and DNS (B) at various concentrations.

S13: Photostability and phototoxicity of the probes:

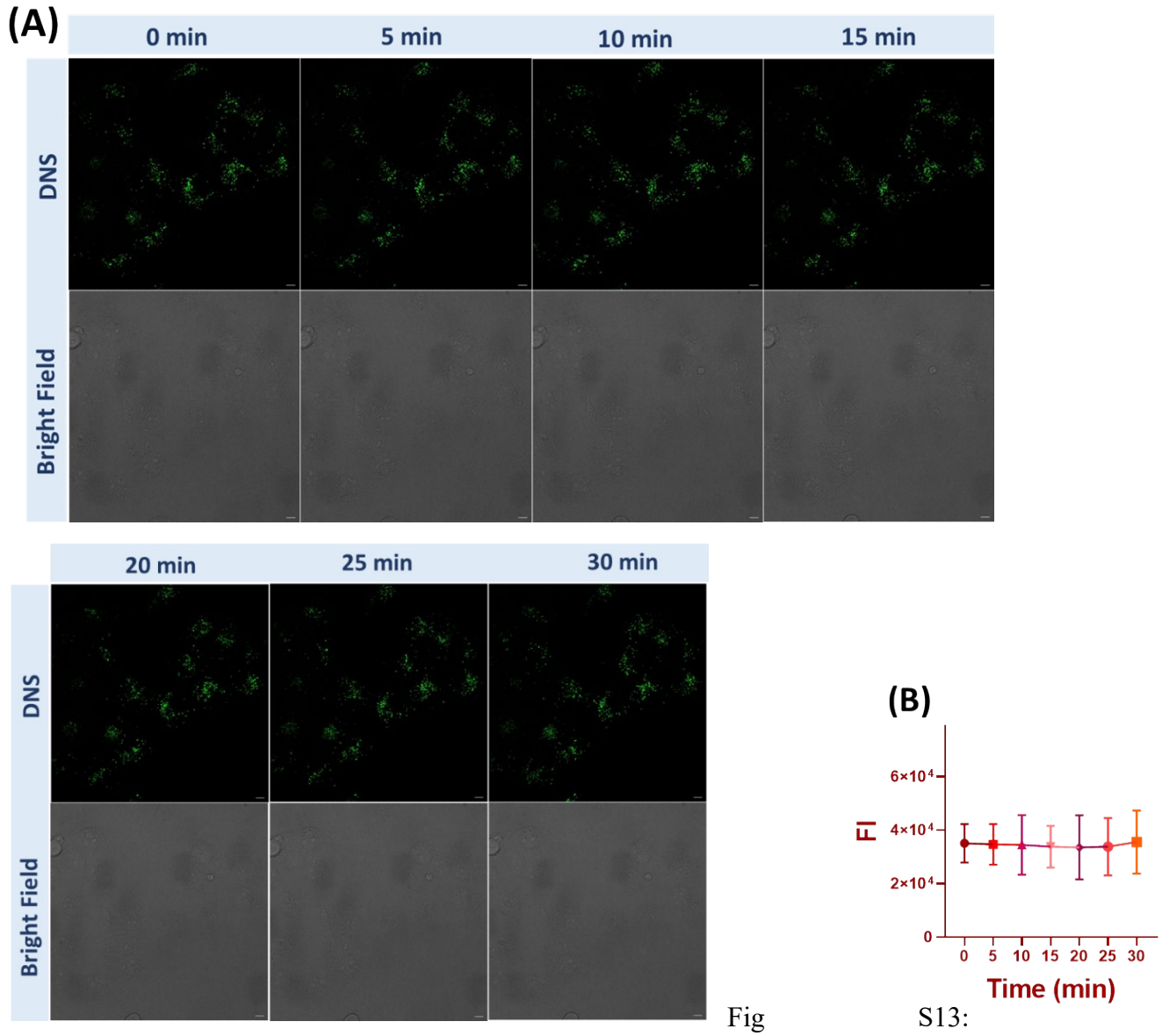


Fig S13: Photostability and Phototoxicity Experiment: A) CLSM images of DNS upon continuous exposure to 405 nm laser for 30-minute duration. B) Fluorescence intensity vs time plot at different time intervals. (Scale Bar 10 µm)

S14: Synthetic Procedures:

1. Synthesis of 4-(dimethylamino)isophthalaldehyde:

4-(dimethylamino)isophthalaldehyde was synthesized using known procedure in the literature[1]

2. Synthesis of NNS ((E)-N, N-dimethyl-4-(4-nitrostyryl)aniline):

Nitrophenylacetic acid (1.2 equivalent) and piperidine (0.1 equivalent) were mixed together and stirred, followed by the addition of 4 dimethylamino benzaldehyde (1 equivalent). The reaction mixture was heated at 140 ºC for 2h to get red-colored precipitate. The precipitate was further filtered and washed with hexane and diethyl ether. Yield: 62%; ^{1}H NMR (500 MHz, DMSO) δ 8.19 (d, *J* = 8.8 Hz, 2H), 7.77 (d, *J* = 8.9 Hz, 2H), 7.52 (d, *J* = 8.8 Hz, 2H), 7.44 (d, *J* = 16.3 Hz, 1H), 7.13 (d, *J* = 16.4 Hz, 1H), 6.75 (d, *J* = 8.8 Hz, 2H), 2.97 (s, 6H). HRMS: Observed Mass:269.1293; Calculated Mass: 269.1290

3. Synthesis of DNS (N, N-dimethyl-3,4-bis((E)-4-nitrostyryl)aniline):

Nitrophenylacetic acid (1.2 equivalent) and piperidine (0.1 equivalent) were mixed together and stirred followed by the addition of 4 dimethylamino isothalaldehyde (1 equivalent).. The reaction mixture was heated at 140 ºC for 2h to get red colored ether to yield red color solid. Yield: 51%; ^{1}H NMR (500 MHz, DMSO) δ 8.25 (dd, *J* = 8.7, 8.0 Hz, 4H), 8.01 (d, *J* = 1.9 Hz, 1H), 7.87 (dd, *J* = 23.3, 8.9 Hz, 4H), 7.60 (d, *J* = 16.5 Hz, 2H), 7.52 (s, 1H), 7.45 – 7.37 (m, 2H), 7.13 (d, *J* = 8.4 Hz, 1H), 2.78 (s, 6H). ^{13}C NMR (126 MHz, DMSO) δ 153.34, 146.62, 146.31, 144.94, 133.49, 131.16, 130.41, 129.70, 128.90, 127.69, 127.38, 126.61, 126.50, 125.06, 124.64, 124.58, 118.91, 44.72, 40.50, 40.42, 40.33, 40.25, 40.16, 39.99, 39.83, 39.66, 39.49. HRMS: Observed Mass: 416.1356; Calculated Mass: 416.1610

S15: Spectral Details – ^{1}H NMR and ^{13}C NMR Spectral copies

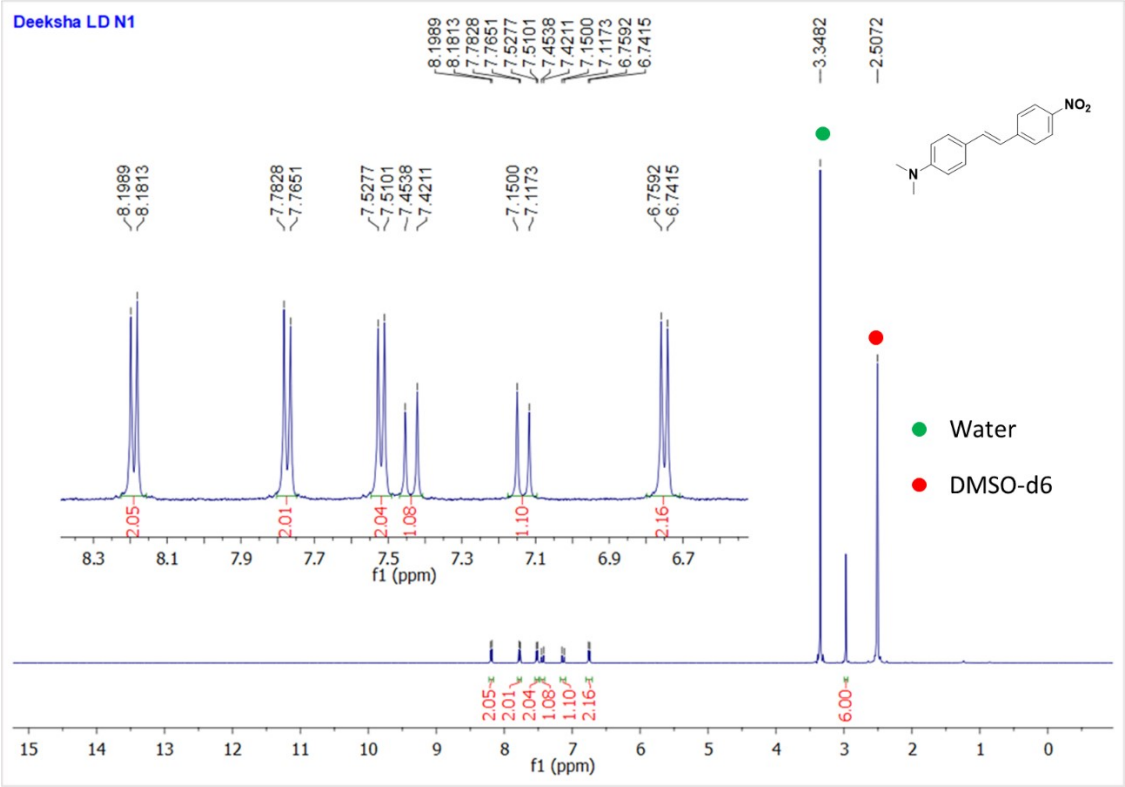


Fig S15a: ^{1}H NMR of NNS

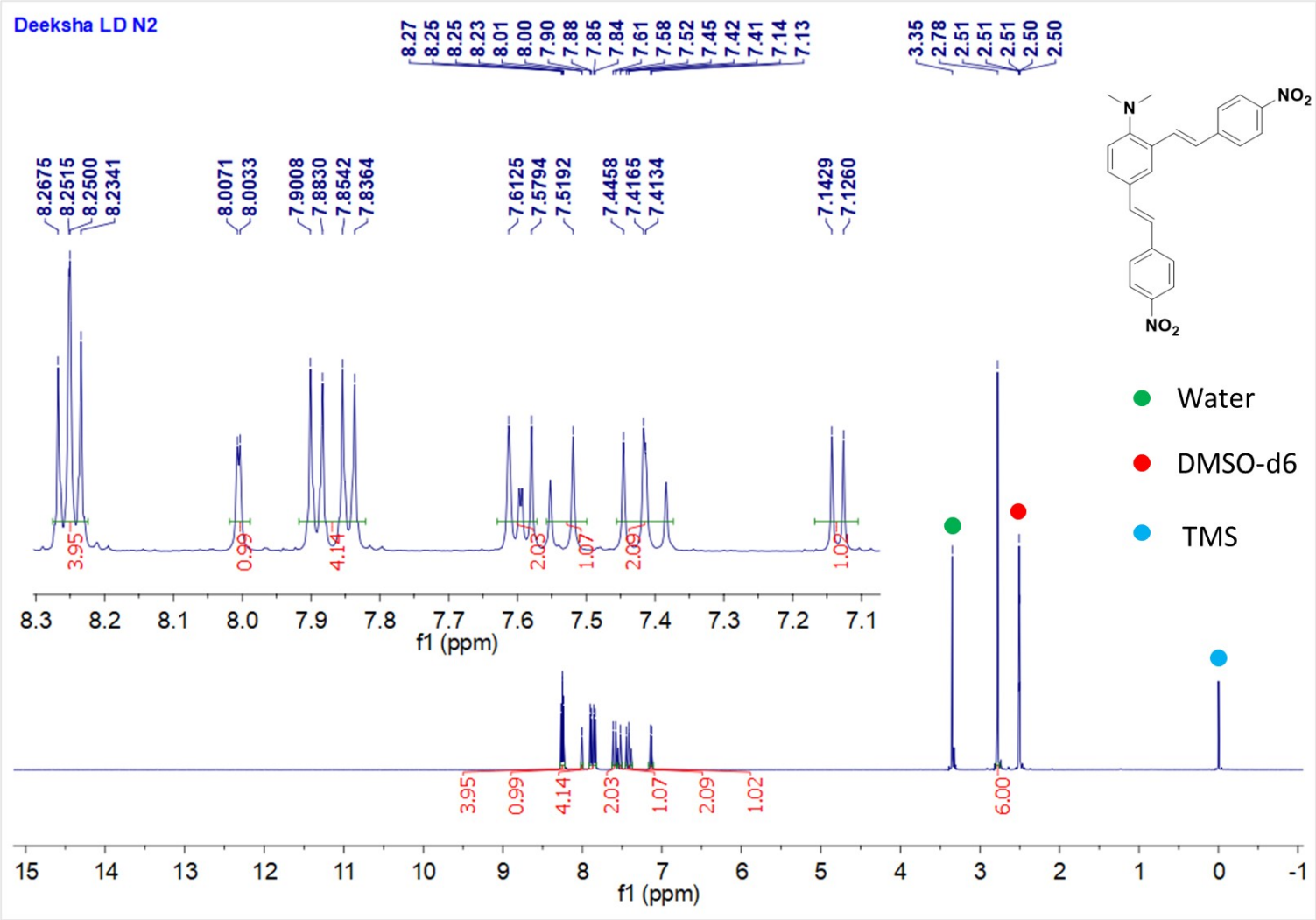


Fig S15b: ^{1}H NMR of DNS

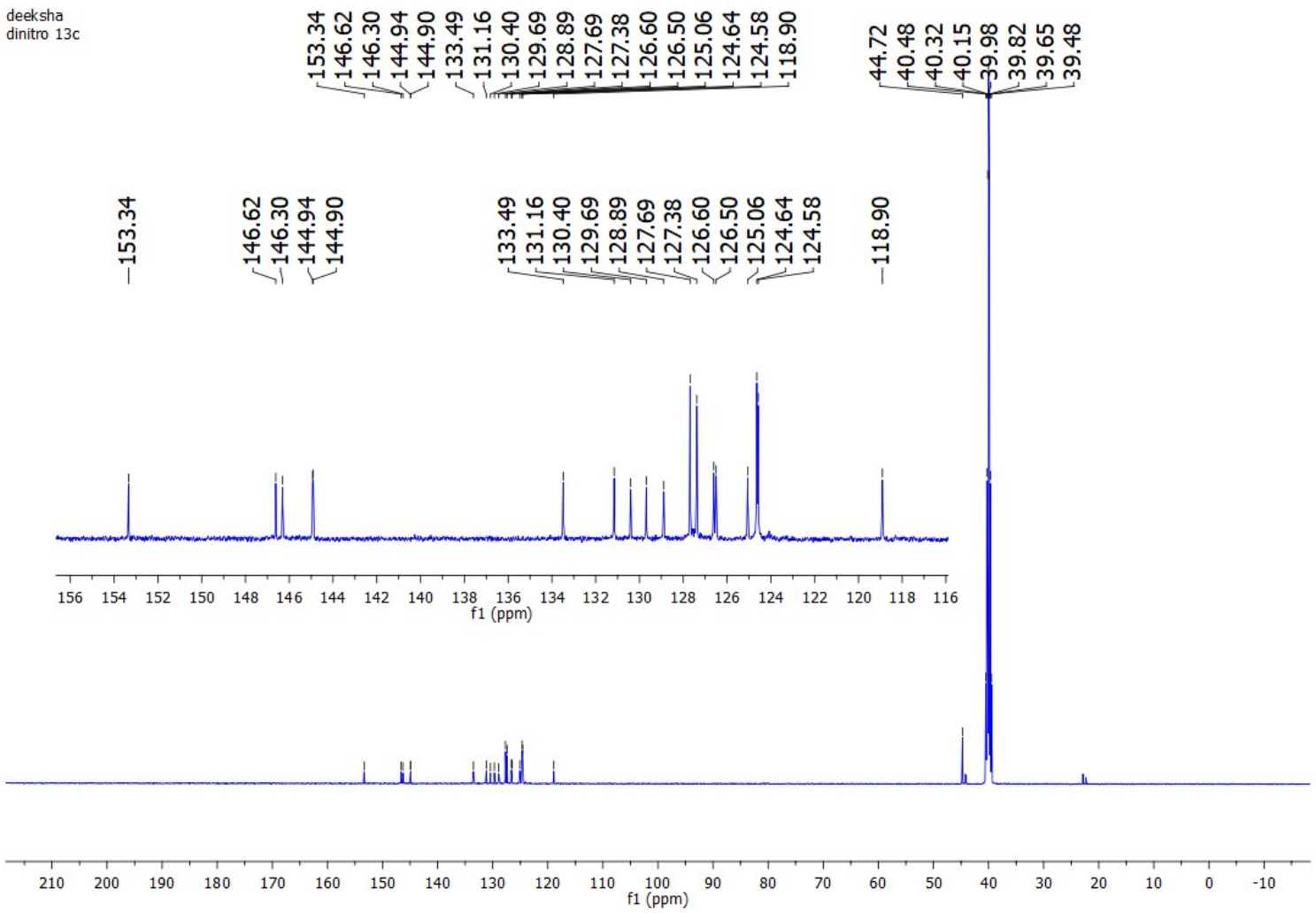


Fig S15c: ^{13}C NMR of DNS

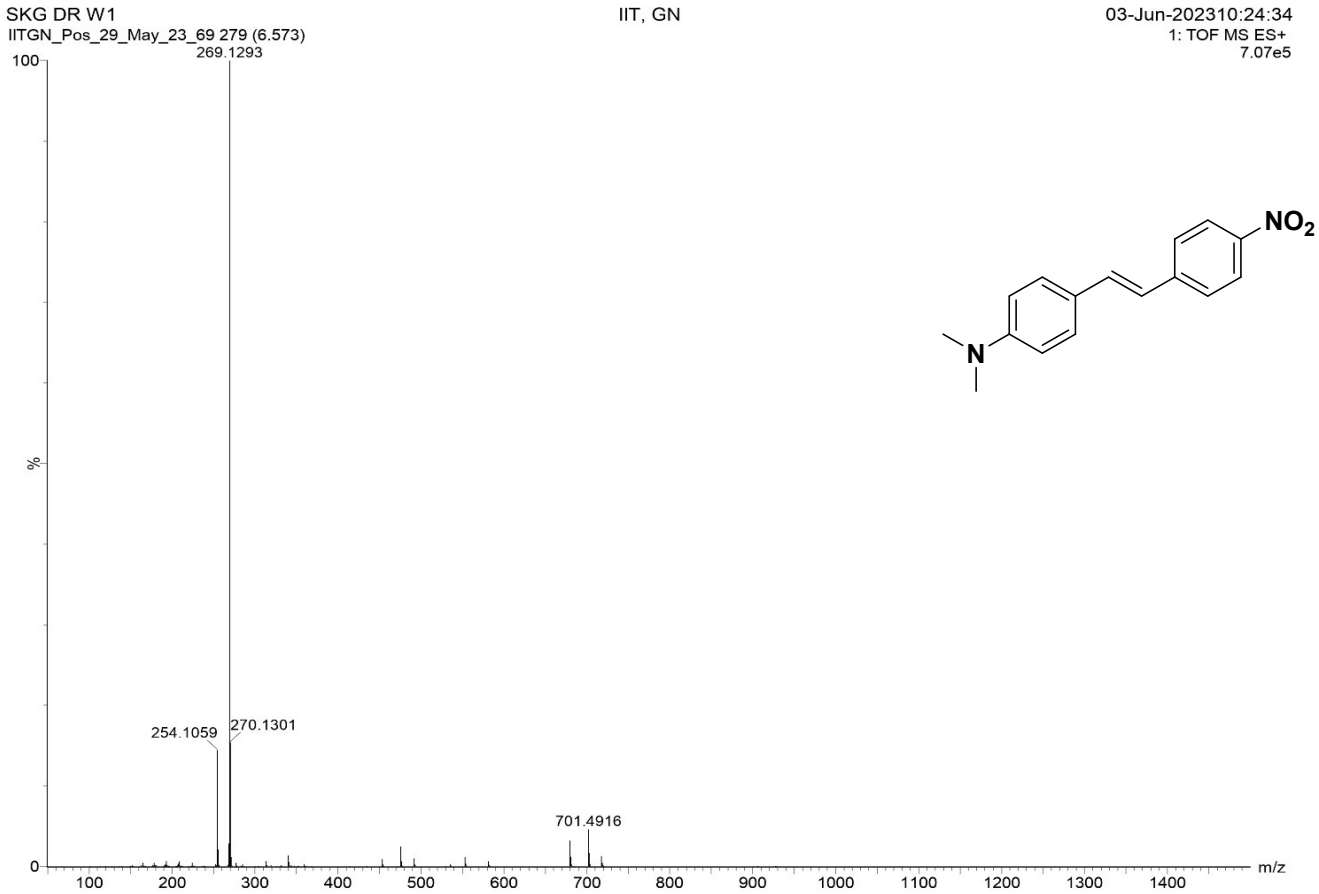


Fig S15d: HRMS of NNS

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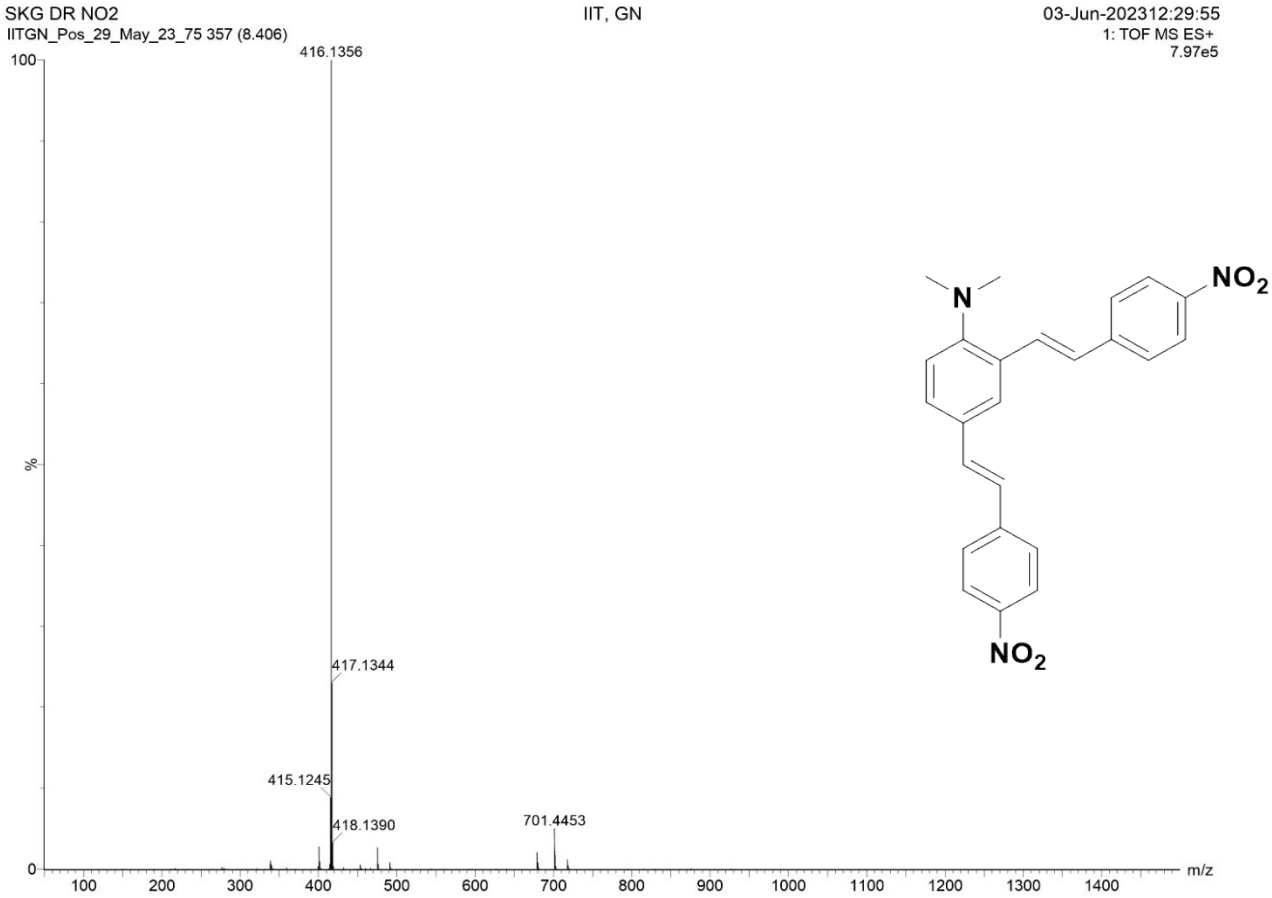

Fig S15e: HRMS of DNS

1. W. Fang, W. Zhao, P. Pei, R. Liu, Y. Zhang, L. Kong and J. Yang, *Journal of Materials Chemistry C*, 2018, **6**, 9269-9276.