Electronic Supplementary Information

A Probe for Ratiometric Near-Infrared Fluorescence and Colorimetric Hydrogen Sulfide Detection and Imaging in Live Cells

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Contents

1. General method for measurements of photophysical properties

Fluorescence spectra were recorded on a Perkin Elmer model LS 55 spectrophotometer. 1 cm cells were used for emission titration. For fluorescence titrations stock solution of ligands **DNPOCy** were prepared (c = 2000 μM) in 10 mM PBS buffer (pH = 7.4). The solutions of guest analytes were prepared in buffer solution in the order of 10^{-3} M. Working solutions were prepared from the stock solutions. Excitation was carried out at 510 nm and 10 nm emission slit widths.

2. Fluorescence study, pH study, HPLC and mass spectra

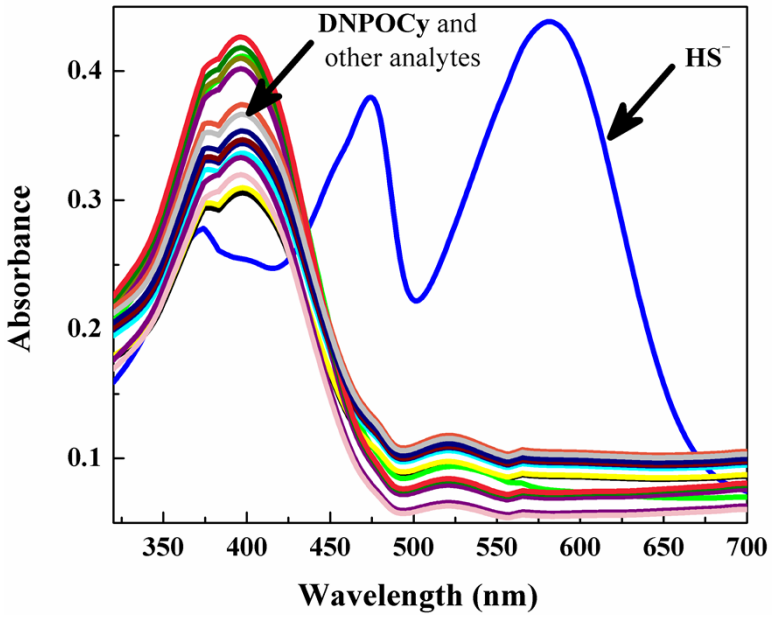

Fig. S1 UV-Vis absorption spectra of **DNPOCy** probe (10.0 μM) in presence of 20.0 μM of NaSH (**HS⁻**), different anions (Cl^-, Br^-, I^-,AcO^-, N_3^-, CN^-, CO_3^{2-}, NO_2^-) at 1 mM, metal ions (K^+,Mg^{2+}, Ca^{2+}, Zn^{2+}) at 1 mM, 5 equiv of reactive oxygen species (H_2O_2, OCl^-), and reducing agents (ascorbic acid, $S_2O_3^{2-}$, SO_3^{2-}). Each spectrum was acquired after 2 min. incubation of the probe with analyte in 10 mM PBS buffer (pH 7.4).

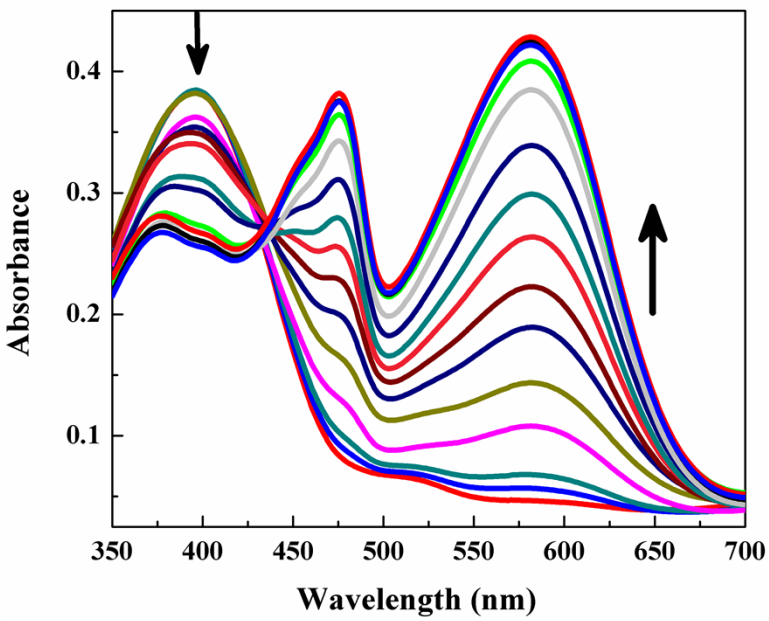

Fig. S2 UV-Vis absorption spectra of **DNPOCy** probe (10.0 μM) upon addition of NaSH (0-30 μM). Each spectrum was acquired after 2 min incubation of the probe with analyte in 10 mM PBS buffer (pH 7.4).

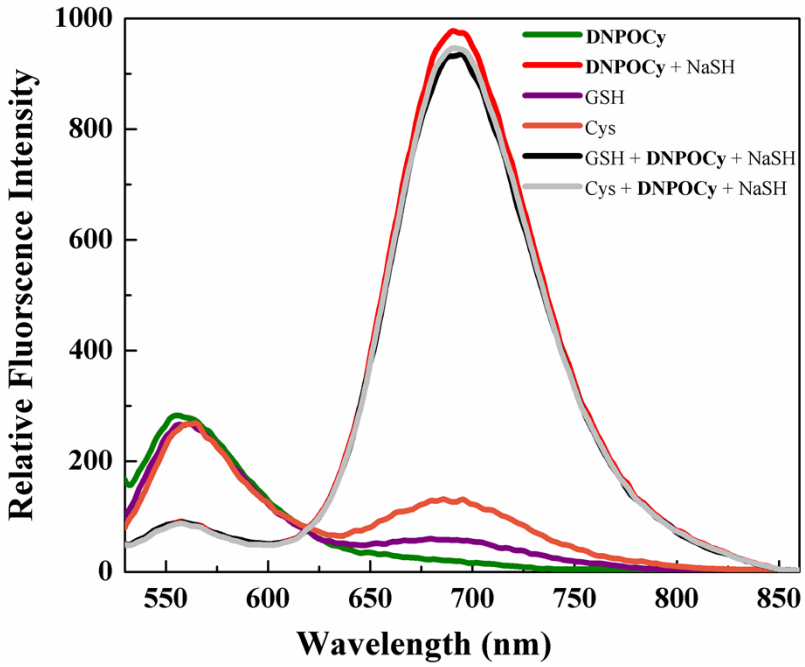

Fig. S3 Fluorescence spectra of **DNPOCy** probe (10.0 μM) upon addition of NaSH (20.0 μM), 1 mM of cysteine (Cys) and glutathione (GSH). Each spectrum was acquired after 2 min incubation of the probe with analyte in 10 mM PBS buffer (pH 7.4).

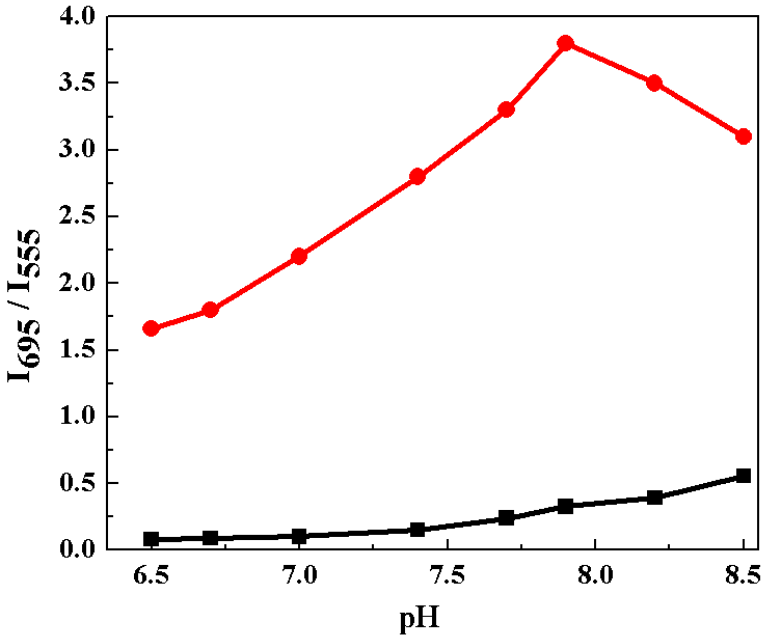

Fig. S4 Effect of pH on the ratiometric emission of **DNPOCy** monitored at 695 and 555 nm. Black trace: **DNPOCy** (10 μM) and red trace: **DNPOCy** (10.0 μM) with 2.0 μM of NaSH in 10 mM PBS buffer.

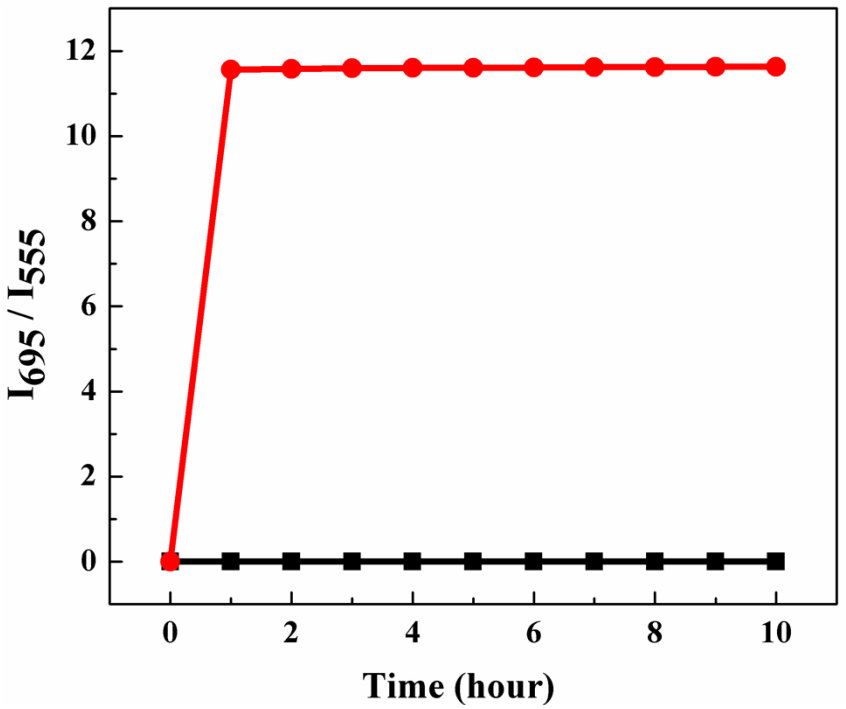

Fig. S5 Time dependent fluorescence response in 10 mM PBS buffer (pH 7.4). Black: only **DNPOCy** probe (10.0 μM). Red: **DNPOCy** probe (10.0 μM) upon addition of NaSH (20.0 μM).

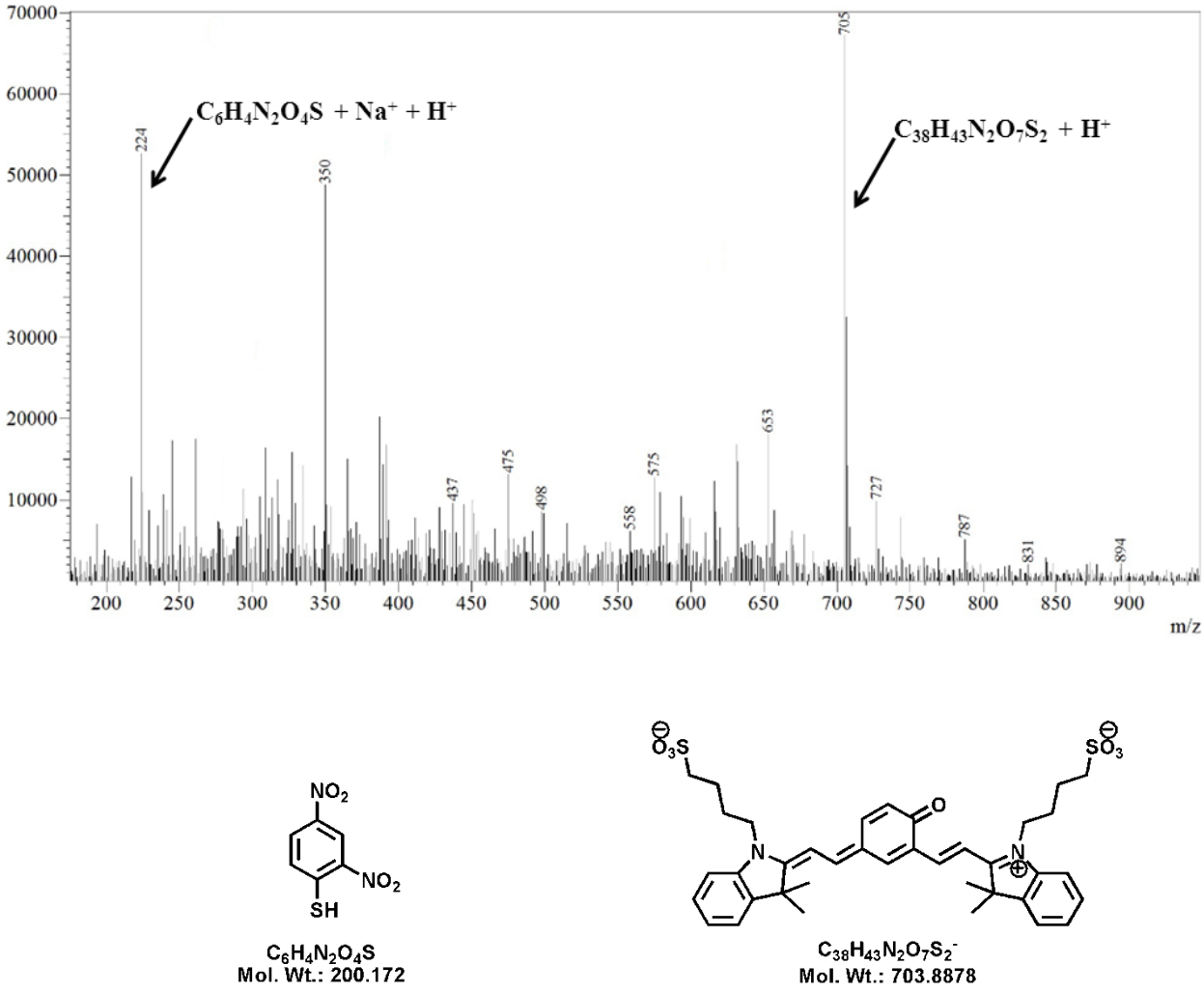

Fig. S6 ESI-mass spectrum (positive ion mode) for the reaction of 10.0 μM **DNPOCy** with NaSH (50.0 μM) in water. Mass peak observed at 705 was attributed to cyanine dye (**Cy-quinone**) (calculated for $[C_{38}H_{43}N_2O_7S_2^- + H]^+$). Peak at m/z = 224.0 corresponding to the side product 2,4-dinitrothiophenol (calculated for $C_6H_4N_2O_4S + Na^+ + H^+$).

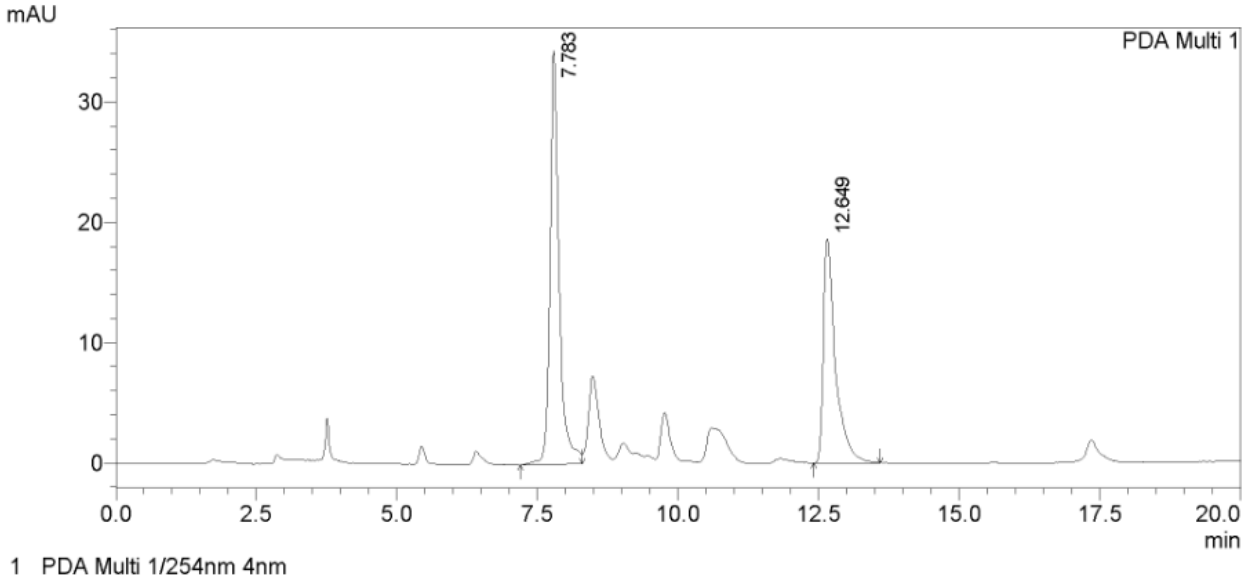

Fig. S7 RP-HPLC (grad. 20-100% ACN in water, 20 min.) of the reaction mixture of **DNPOCy** and NaSH in 10 mM PBS buffer medium (pH = 7.4).

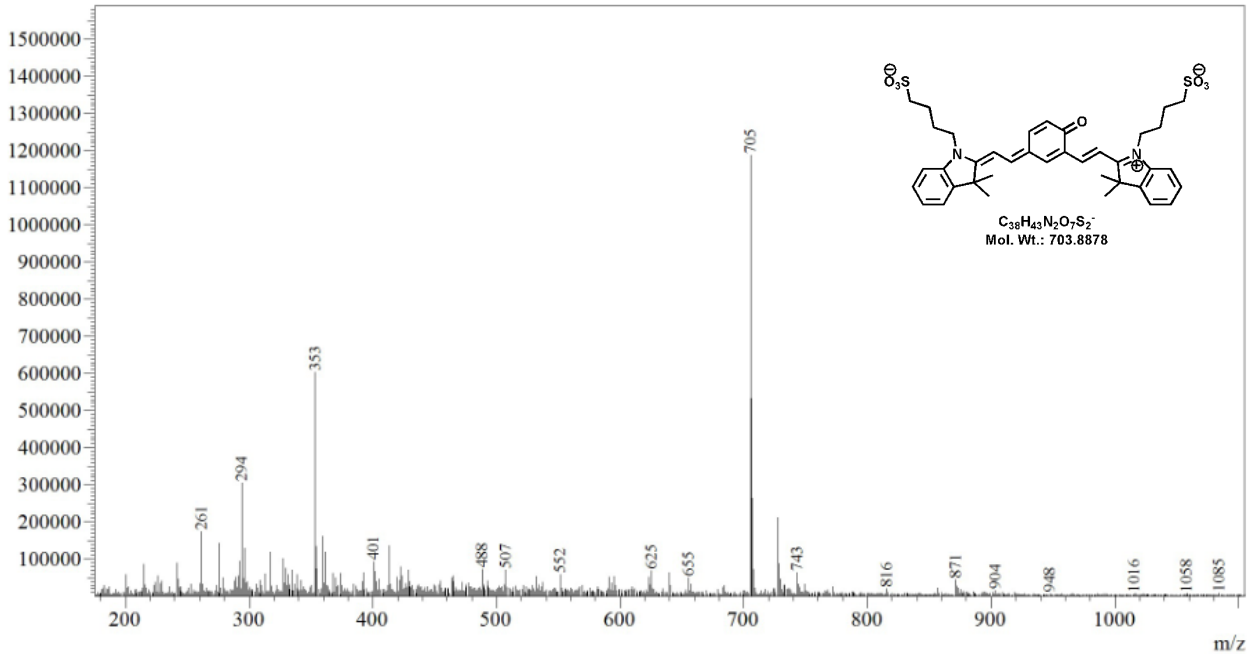

Fig. S8 ESI mass spectra (positive ion mode) of the eluent fraction collected at 7.78 min in RP-HPLC (Figure S7). Mass peak observed at 705 was attributed to cyanine dye (**Cy-quinone**) (calculated for $[C_{38}H_{43}N_2O_7S_2^- + H]^+$).

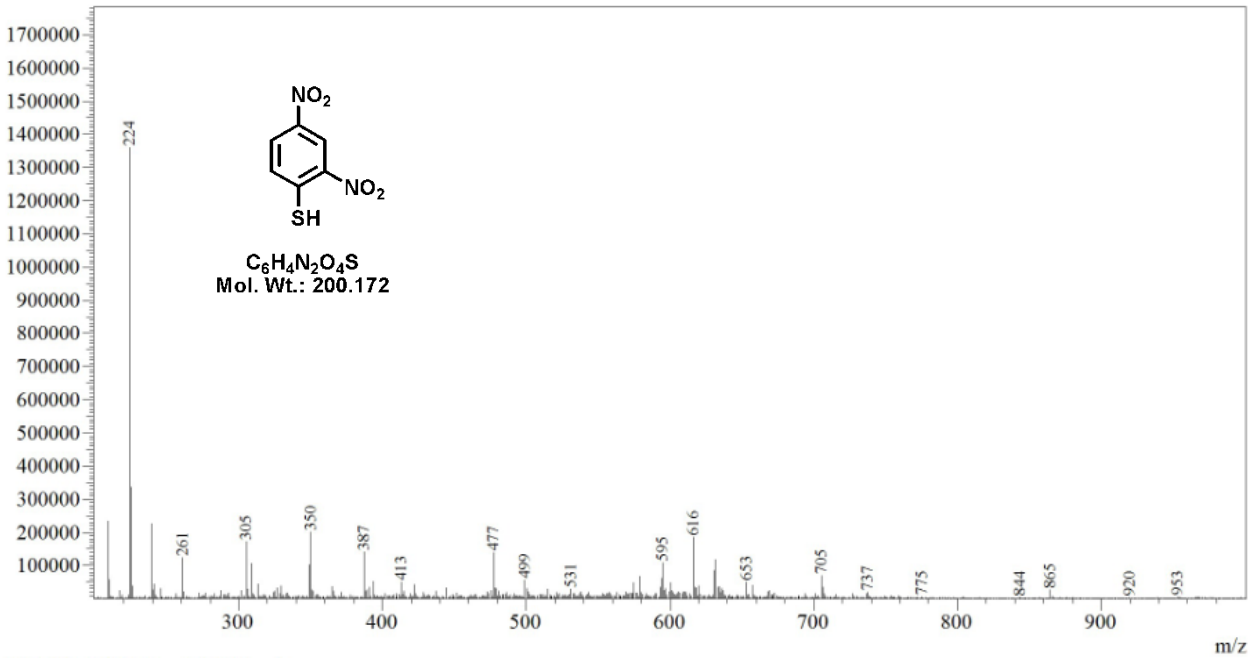

Fig. S9 ESI mass spectra (positive ion mode) of the eluent fraction collected at 12.64 min in RP-HPLC (Figure S7). Peak at m/z = 224.0 corresponding to the side product **2,4-dinitrothiophenol** (calculated for $C_6H_4N_2O_4S + Na^+ + H^+$).

3. Theoretical study

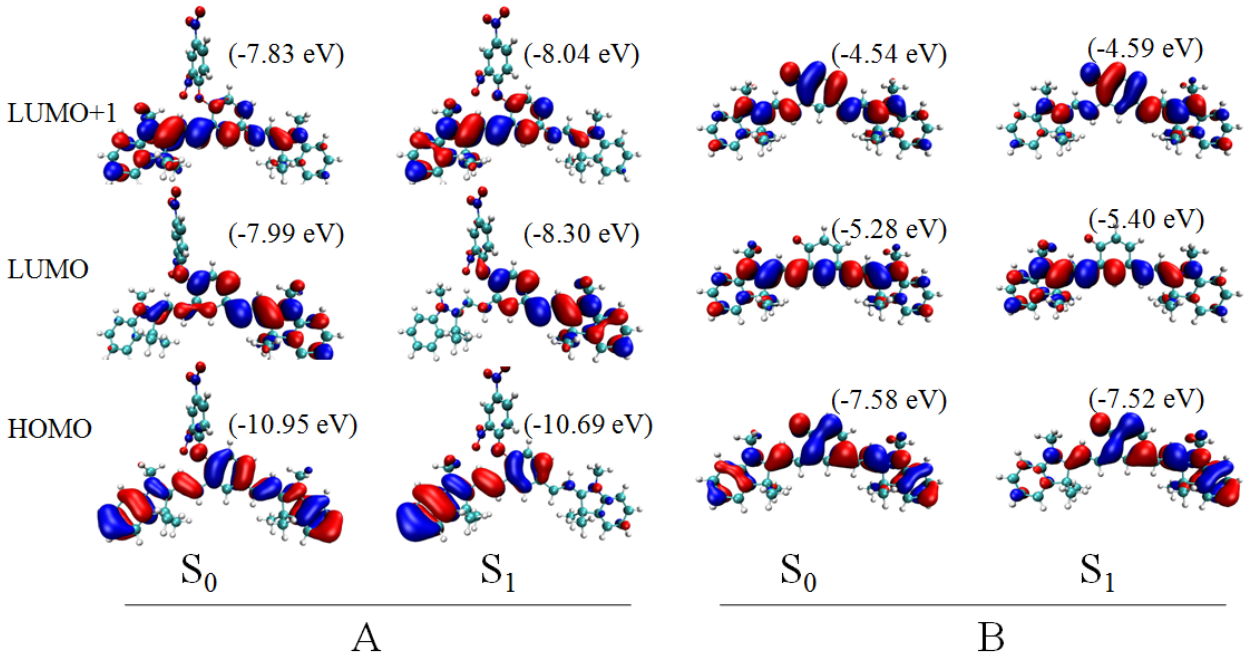

Fig. S10 Frontier molecular orbital of the optimized ground state (S_0) and optimized excited state (S_1) of **DNPOCy** (**A**) and **Cy-quinone** (**B**). The lowest energy excitation is approximately [($c_1\varphi_1 \rightarrow \varphi_2$) + ($c_2\varphi_1 \rightarrow \varphi_3$)]; where c_1 and c_2 are constant gives weightage probability, and φ_1, φ_2 and φ_3 are the ground state molecular orbitals involved in describing excited state) described as 0.4621 (HOMO → LUMO) + 0.5121 (HOMO → LUMO + 1) for **A** and 0.6995 (HOMO → LUMO) + 0.1055 (HOMO → LUMO + 1) for **B**. The emission is approximately describes 0.3277 (LUMO → HOMO - 1) - 0.6098 (LUMO + 1 → HOMO) for **A** and 0.7021 (LUMO → HOMO) for **B**.

Optimized Cartesian coordinate (in Å) of model compound **DNPOCy** (**A**) in ground state (S_0):

Total Energy: SCF Done: E(RB3LYP) = -2064.16330023 Hartree

C	5.53762807	-3.62980890	0.19053002
C	4.64755607	-4.56687992	-0.33264896
C	5.09152708	-5.85389792	-0.60671494
C	6.43247908	-6.16430191	-0.34919294
C	7.31019108	-5.20589690	0.17413804
C	6.87541507	-3.90946189	0.45704902
C	3.56819207	-2.49383291	-0.00575299
C	3.28305107	-3.92397692	-0.48716096
H	4.42431908	-6.60817393	-1.01341792
H	6.79897508	-7.16407291	-0.55926493
H	8.34511308	-5.47180489	0.36308304
H	7.55911107	-3.16960188	0.85842901
C	5.54134306	-1.24065089	0.93656899
H	6.07600907	-1.56408888	1.83235099
H	6.26188406	-0.86969190	0.20179298
H	4.84658006	-0.45226489	1.21197398
C	2.25953008	-4.64277591	0.43440005
H	2.20450108	-5.69631192	0.14567007
H	2.56573008	-4.59626790	1.48358005
H	1.25552407	-4.21991892	0.34723405
C	2.68593306	-1.36134892	0.02329300
H	3.11527406	-0.40795191	0.29503198
N	4.84060307	-2.40422190	0.37758001
C	2.83031406	-3.94741095	-1.96930596
H	2.74254707	-4.98616895	-2.30074294
H	1.85900206	-3.46484196	-2.10677496
H	3.55814006	-3.44810796	-2.61568397
C	1.35140306	-1.41637893	-0.24736499

H	0.90593006	-2.38011793	-0.46946098
C	-8.14416895	0.11119004	0.18738102
C	-7.85002194	-1.24062997	0.00926904
C	-8.88592994	-2.16174397	-0.07447294
C	-10.20261694	-1.69414798	0.01685906
C	-10.47551594	-0.33103697	0.18783604
C	-9.44293595	0.60496503	0.27705902
C	-5.86311595	0.02798104	0.09243601
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H	-8.69057393	-3.22138297	-0.21036992
H	-11.02552794	-2.39875798	-0.04830593
H	-11.50461394	0.00724702	0.25062104
H	-9.66364095	1.65945903	0.40054001
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H	-7.02126896	2.79532403	-0.51362102
H	-7.73424195	2.54999605	1.10168198
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H	-6.39757694	-3.00612698	-1.53923994
H	-6.27977195	-1.39328499	-2.25681996
H	-4.85360794	-2.14393498	-1.51531396
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C	-5.85966093	-2.30728194	1.12009605
H	-6.33599393	-3.28896894	1.04457407
H	-4.77697693	-2.45757693	1.10363705
H	-6.13446993	-1.87270493	2.08564905
C	-3.39548694	-0.22545895	-0.04826199
H	-3.49446694	-1.30447295	-0.09975297
C	-1.66597195	1.61924306	-0.04931903

C	-2.02563195	0.25413206	-0.09566100
C	-0.98412694	-0.68472994	-0.18606899
C	0.37564805	-0.33235293	-0.21837801
C	0.67961505	1.05737407	-0.21127903
C	-0.33995096	2.01304307	-0.11209804
H	-2.42568596	2.38944106	0.03218696
H	-1.23570794	-1.74216094	-0.21192698
H	-0.09005496	3.06695107	-0.08403006
O	1.99569505	1.39023507	-0.30405104
C	2.42574504	2.70110607	-0.39813806
C	2.22523303	3.41158005	-1.58251407
C	3.12907804	3.29947009	0.66175693
C	2.71278003	4.70956405	-1.71287009
H	1.69645703	2.93486304	-2.40126706
C	3.60452404	4.60382109	0.54880390
C	3.39150303	5.28397307	-0.64131010
H	2.57923102	5.27686204	-2.62640110
H	4.13142704	5.07451111	1.36980390
N	3.40362405	2.59146411	1.92385194
N	3.90706402	6.66728107	-0.77242813
O	4.51907002	7.12385209	0.18553986
O	3.67623501	7.24271206	-1.82984414
O	3.50550806	1.36071711	1.88696096
O	3.53161205	3.27480013	2.92827093

Optimized Cartesian coordinate (in Å) of model compound **Cy-quinone** (**B**) in ground state (S_0):
Total Energy: SCF Done: E(RB3LYP) = -1423.81487058 Hartree

C	-7.07731294	-0.19714294	0.00248000
C	-6.49194795	-1.46553194	-0.00096501
C	-7.29689096	-2.59641793	-0.00713902
C	-8.68788696	-2.43369092	-0.01133403
C	-9.25583695	-1.15625992	-0.01010802
C	-8.45613094	-0.00930392	-0.00355901
C	-4.82205694	0.20498405	0.00540702
C	-4.97948595	-1.32179795	0.00217400
H	-6.86345897	-3.59278894	-0.00941403
H	-9.33129197	-3.30794192	-0.01652205
H	-10.33589595	-1.04725791	-0.01509403
H	-8.91106493	0.97517408	-0.00575600
C	-6.27967592	2.21796506	0.02040403
H	-5.90682491	2.66954806	-0.90340396
H	-7.34826692	2.40485807	0.10426003
H	-5.77100392	2.67683204	0.87204804
C	-4.37421395	-1.95279094	-1.27665700
H	-4.60193095	-3.02320994	-1.29551501
H	-4.79664794	-1.50074493	-2.17900900
H	-3.28777294	-1.83587995	-1.31125399
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H	-3.77152392	2.07525404	-0.00884695
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C	8.59653106	0.08372494	-0.00181189
C	4.95787606	0.31106497	0.00015909
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H	10.47277005	-0.96079207	-0.00215389
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H	5.99345908	2.77851395	0.88866812
H	7.50373908	2.50046595	0.00227913
H	6.00039609	2.77624897	-0.89695688
C	4.51684704	-1.85388504	1.28170606
H	4.75095203	-2.92297104	1.29923705
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C	4.51561305	-1.85639401	-1.27641594
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H	3.42846606	-1.74734901	-1.31669694
H	4.93879406	-1.40484801	-2.17868993
C	2.48759407	0.59731999	0.00055308
H	2.34565306	-0.47915401	0.00326806
C	1.26163608	2.80384700	-0.00537391

C	1.29708507	1.35598200	-0.00121692
C	0.05991707	0.67945301	0.00106706
C	-1.17880193	1.32676302	-0.00086194
C	-1.21277192	2.81639102	-0.00525793
C	0.09111109	3.48462401	-0.00708791
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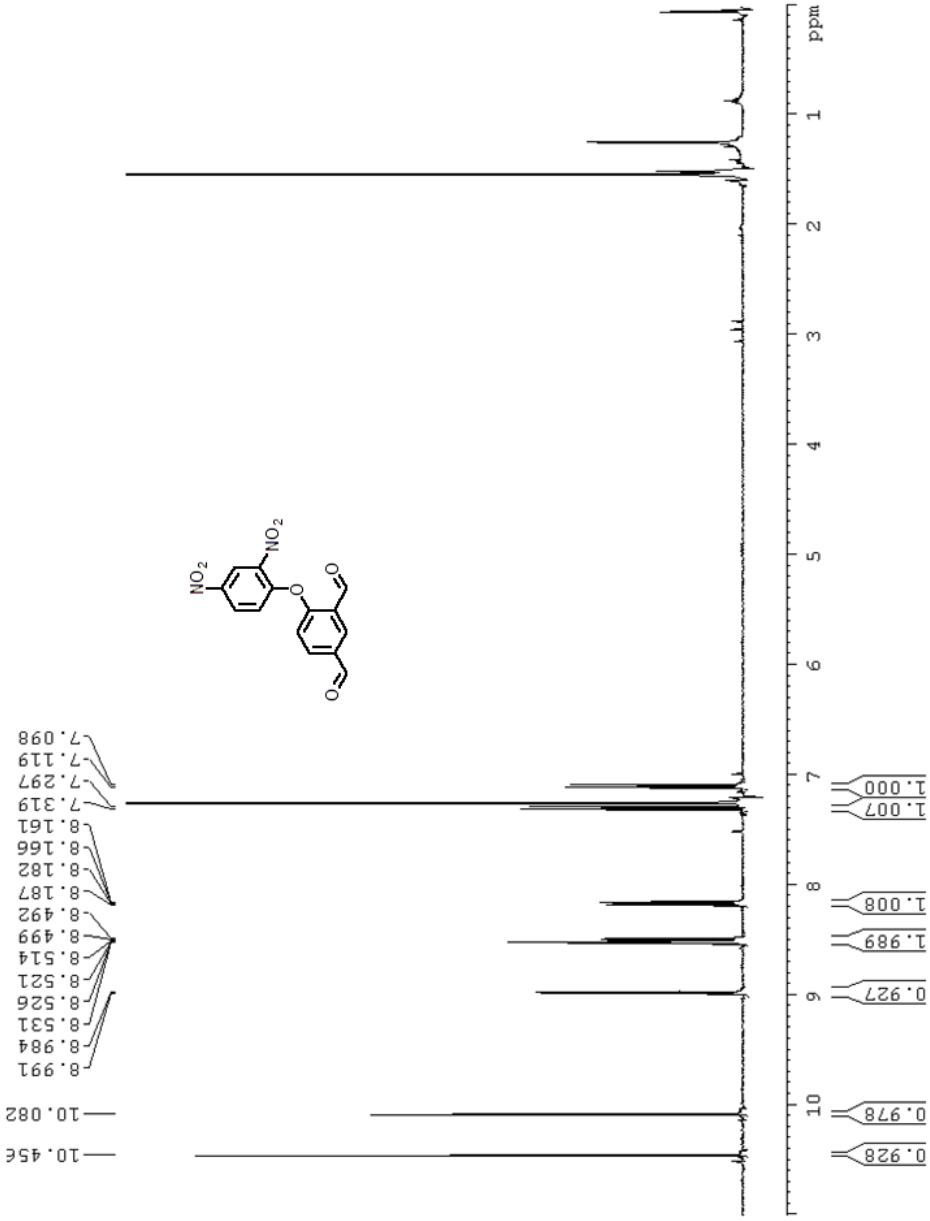
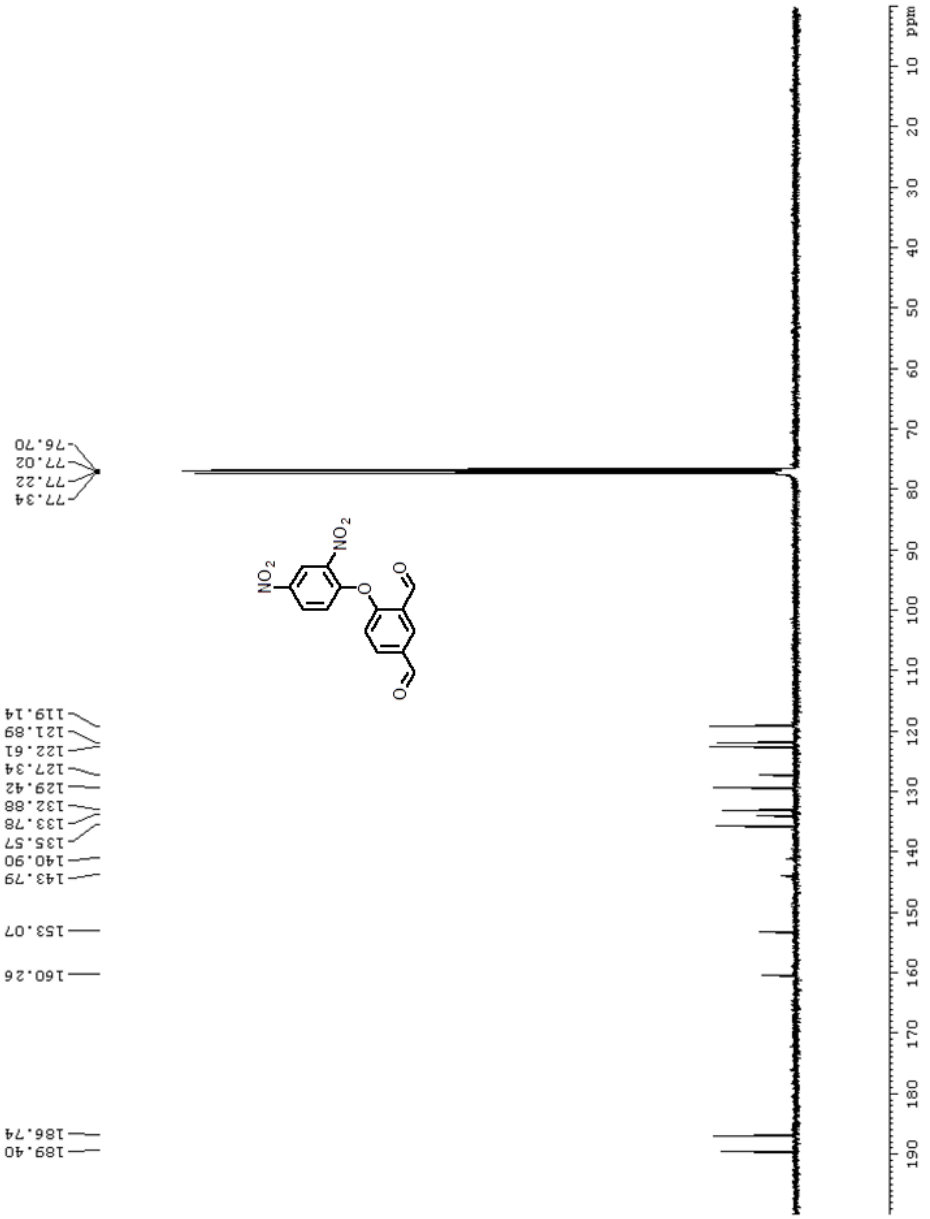
H	0.07272006	-0.41026499	0.00434905
H	0.06245710	4.56972601	-0.01021190
O	-2.26214991	3.47430803	-0.00711293

5. NMR spectra

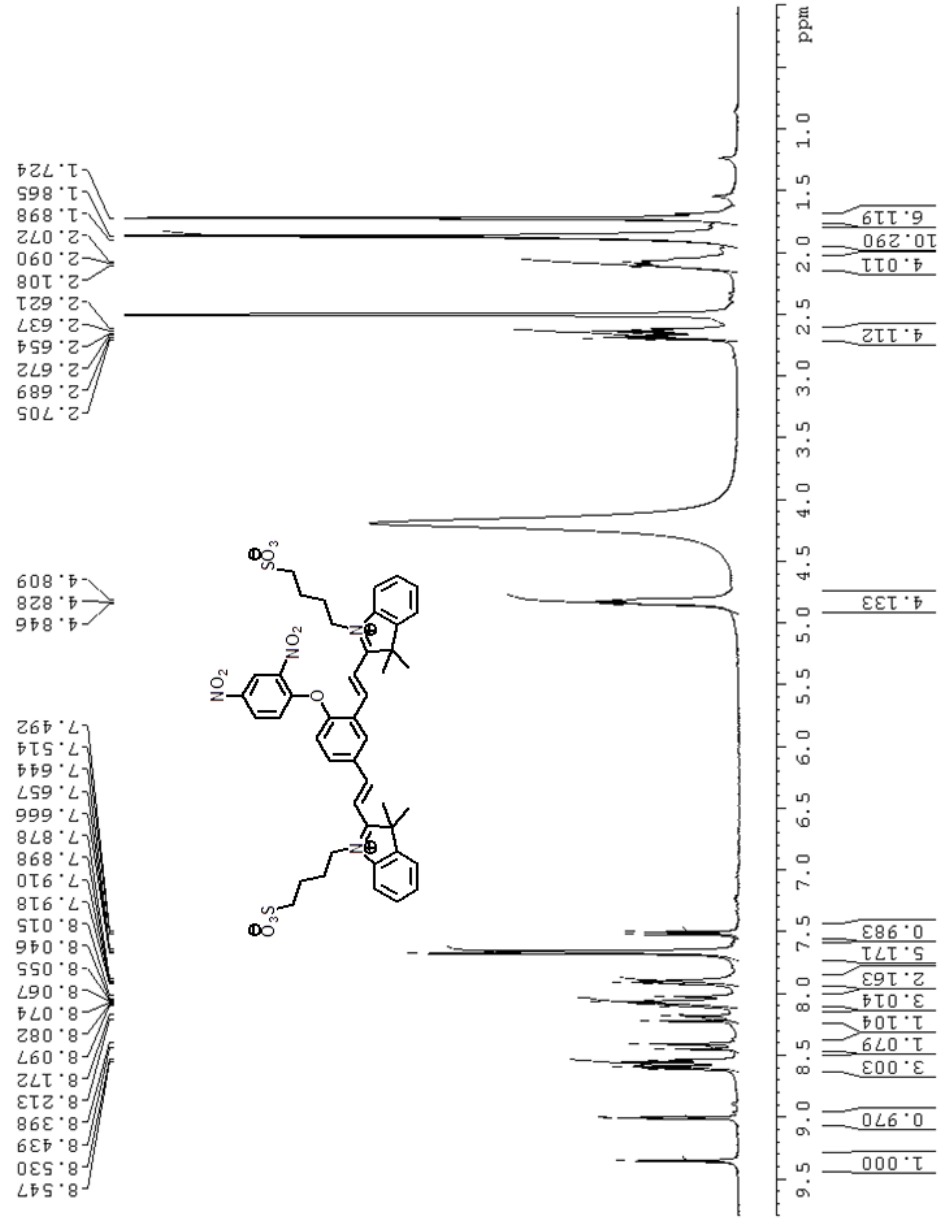
^{1}H NMR spectrum of 2,4-dinitrophenyl 2,4-diformylphenyl ether (1)



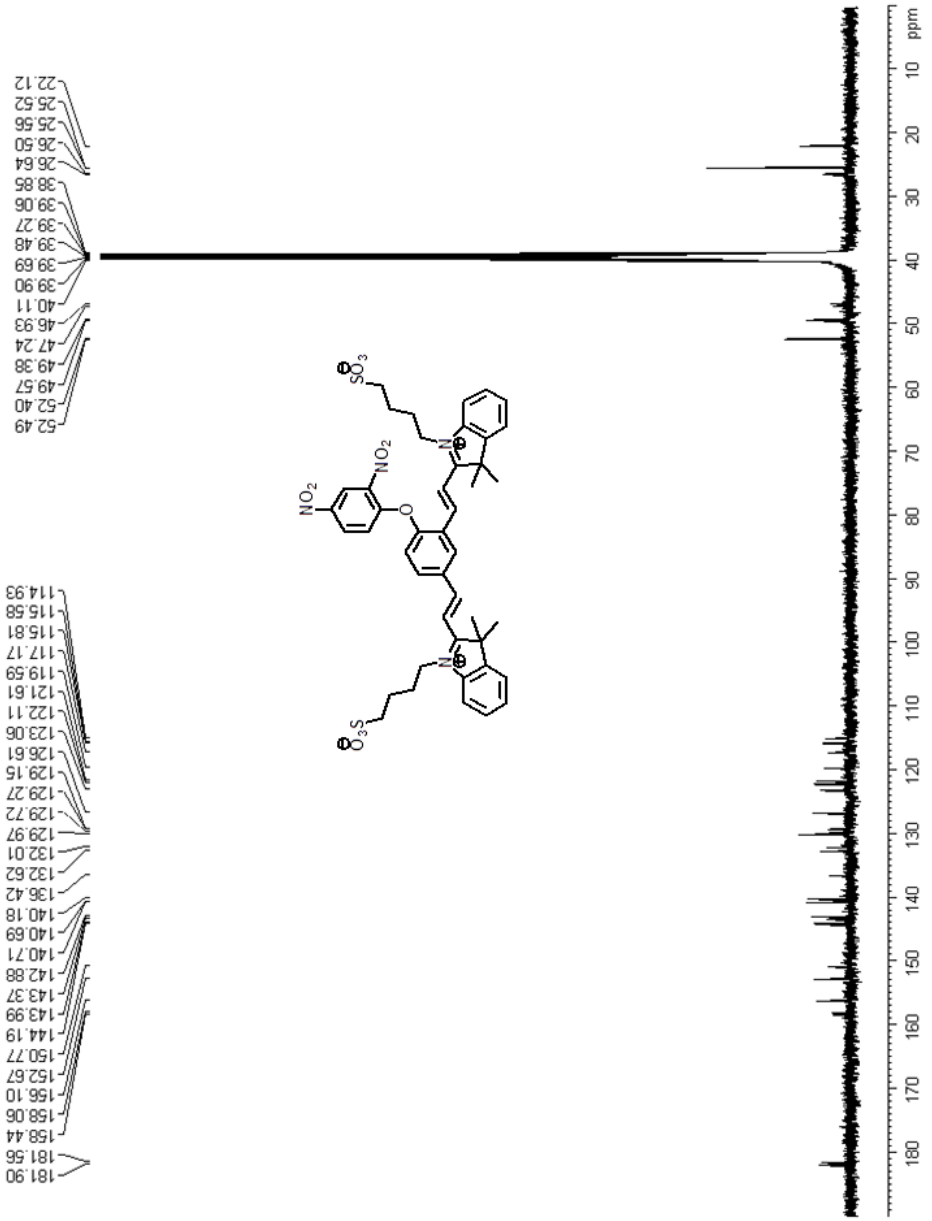
^{13}C NMR spectrum of 2,4-dinitrophenyl 2,4-diformylphenyl ether (1)



^{1}H NMR spectrum of Dinitrophenyl-ether-cyanine (DNPOCy)



^{13}C NMR spectrum of Dinitrophenyl-ether-cyanine (DNPOCy)



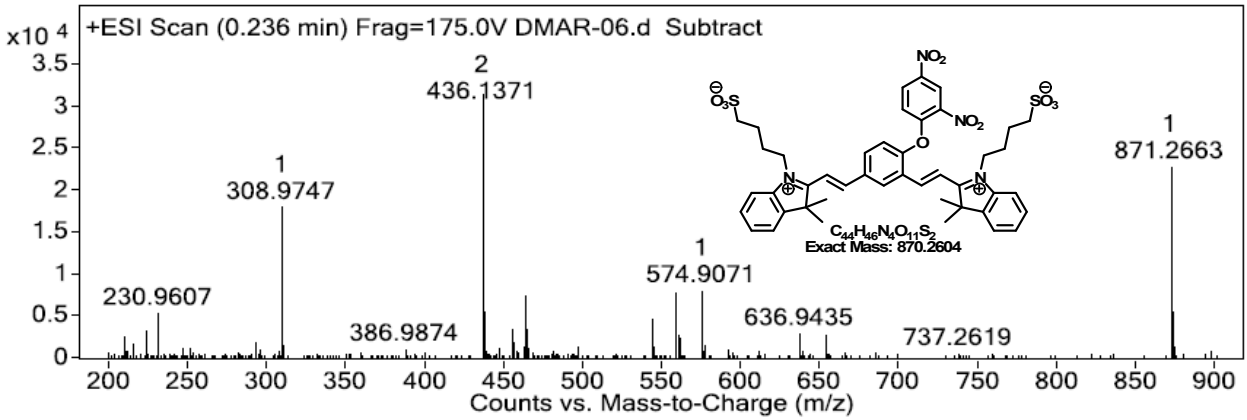


Fig. S11 HRMS spectrum of **DNPOCy**. Observed m/z = 871.2663 $[M + H]^+$ and calculated m/z = 871.2683 for $C_{44}H_{47}N_4O_{11}S_2$.

6. References

(1) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, Jr., J. A.; Peralta, J. E.; Ogliaro, F.; Bearpark, M.; Heyd, J. J.; Brothers, E.; Kudin, K. N.; Staroverov, V. N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J. C.; Iyengar, S. S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, N. J.; Klene, M.; Knox, J. E.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Martin, R. L.; Morokuma, K.; Zakrzewski, V. G.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Dapprich, S.; Daniels, A. D.; Farkas, Ö.; Foresman, J. B.; Ortiz, J. V.; Cioslowski, J.; Fox, D. J. *Gaussian 09, Revision A.1, Gaussian, Inc., Wallingford CT* **2009**.