Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
This journal is © The Royal Society of Chemistry 2024

Supporting Information

Multipurpose Mitochondrial NIR Probe for Imaging Ferroptosis and Mitophagy

Deeksha Rajput[a], Nachiket Pradhan[a], Shabnam Mansuri[a], Virupakshi Soppina[c]* and Sriram Kanvah[a]*

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

[b]Department of Biological Engineering, Indian Institute of Technology Gandhinagar, Palaj, Gandhinagar 382355

sriram@iitgn.ac.in

Contents	

Previously reported mitochondrial probes for ferroptosis/mitophagy:

Table S1: Details of Previously reported probes

Probe	**Emission Maxima**	**MMP Independent**	**Ferroptosis/ Mitophagy**	**Viscosity Sensing (Solution/Endogenous in cells)**	**Ref.**
1.	668	Yes	No/Yes	Yes/ Yes	1
2.	675	Yes	Yes/No	Yes/Yes	2
3.	670	No	No/Yes	Yes/Yes	3
4.	704	No	Yes/No	Yes/Yes	4
5.	795	No	Yes/No	Yes/Yes	5
6.	602	No	Yes/No	Yes/Yes	6
7.	553	No	Yes/No	No/No	7
8.	700	Yes	Yes/Yes	Yes/Yes	***This Work***

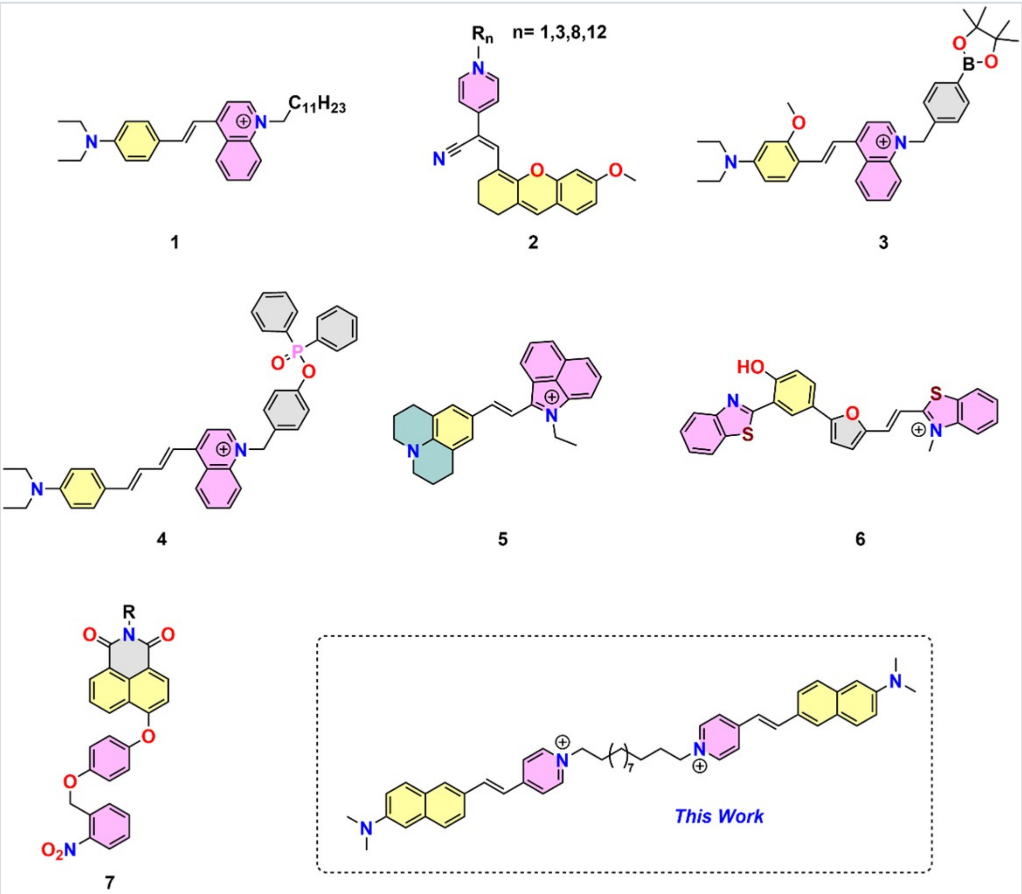


Figure S1: Molecular structures of previously reported probes which detect ferroptosis/mitophagy process but none utilizing this architecture are known.

S2: Absorption and emission spectra:

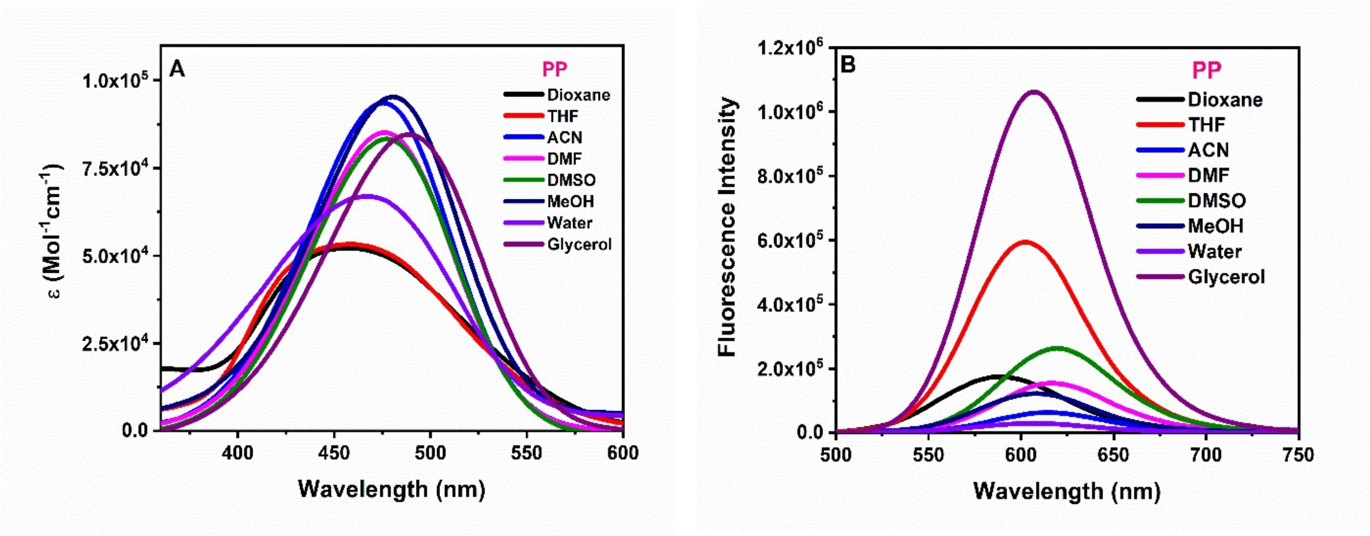


Figure S2: A) Absorption and B) Emission spectra of PP in all solvents. Concentration: 10μM.

S3: Lifetime decay

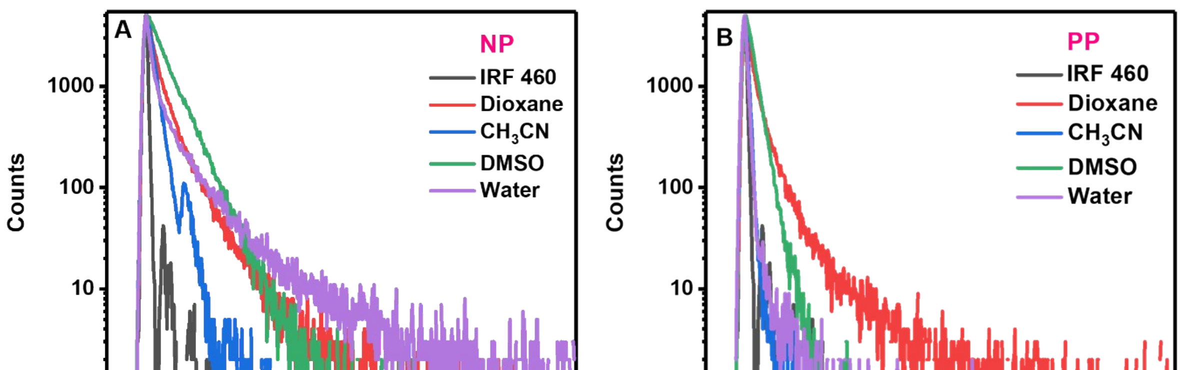


Figure S3: A) & B) Lifetime decay for NP & PP in different solvents.

Table S2: Lifetime decay for the compounds in different solvents.

NP						
Components	τ_1 (ns)	A1	τ_2 (ns)	A2	Average Lifetime (τ) (ns)	χ^2
Dioxane	0.09	47.4%	0.30	52.5%	0.15	1.03
CH_3CN	0.10	100%	-	-	0.10	0.96
DMSO	0.26	100%	-	-	0.26	1.18
Water	0.07	68.0%	0.41	31.9%	0.07	1.04
PP						
Components	τ_1 (ns)	A1	τ_2 (ns)	A2	Average Lifetime (τ) (ns)	χ^2
Dioxane	0.07	63.75%	0.25	36.25%	0.10	1.32
CH_3CN	0.03	100%	-	-	0.03	1.22
DMSO	0.11	100%	-	-	0.11	1.01
Water	0.03	100%	-	-	0.03	1.16

*Most of the decays are very fast and overlap with the instrument response function (IRF). The FWHM of IRF is 70 ps, and the resolution of the experiment is 13 ps. For such data, it is difficult to resolve with our current instrument setup.

S4: Viscosity sensing in solution:

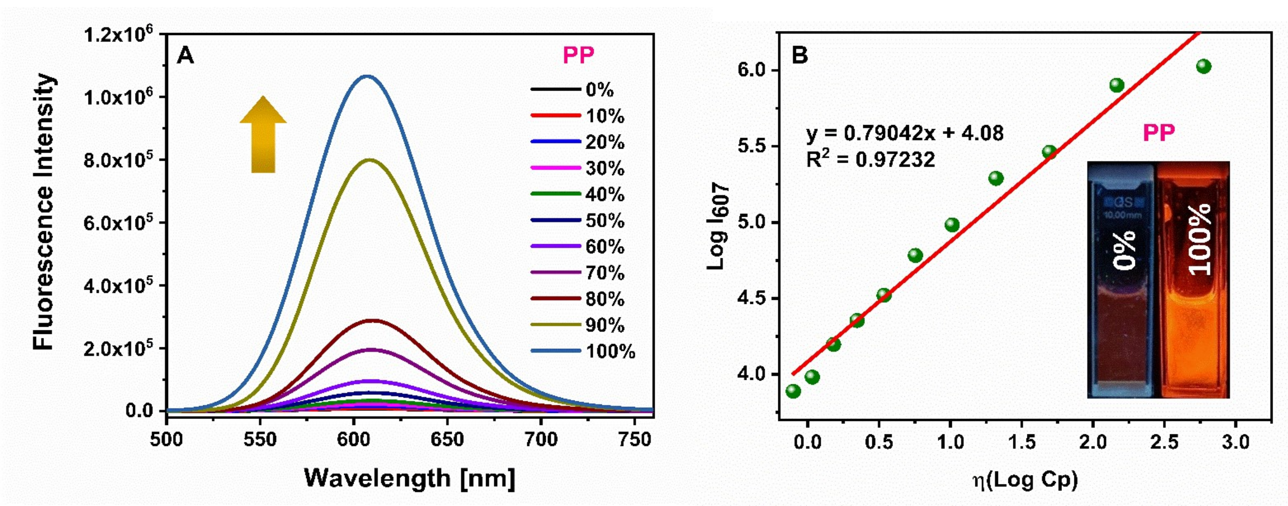


Figure S4: Emission spectra of A) PP in different viscous medium; B) Viscosity vs Log I with linear relationship for PP (Inset – Colour of the fluorophore in 0% glycerol and 100 % glycerol)

S5: Ion selectivity of the probe:

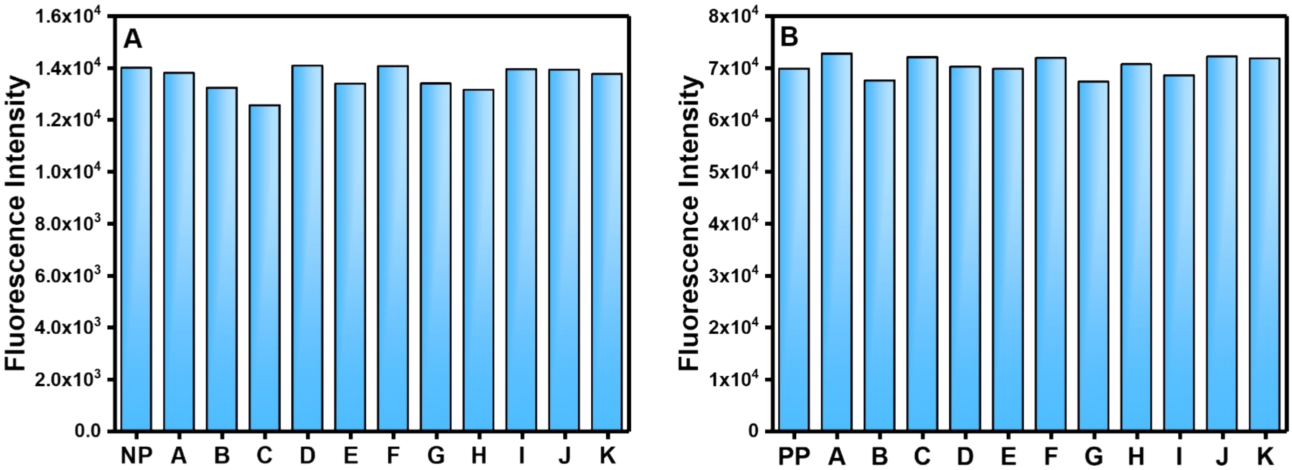


Figure S5: Fluorescence response of A) NP and B) PP to different competitive analytes (A–K: F^-, Cl^-, Br^-, I^-, NO_2^-, NO_3^-, S^{2-}, CH_3COO^-, Ca^{2+}, Mg^{2+}, Fe^{3+}. Concentration of probes and analytes: 10 μM.

S6: Live Cell imaging:

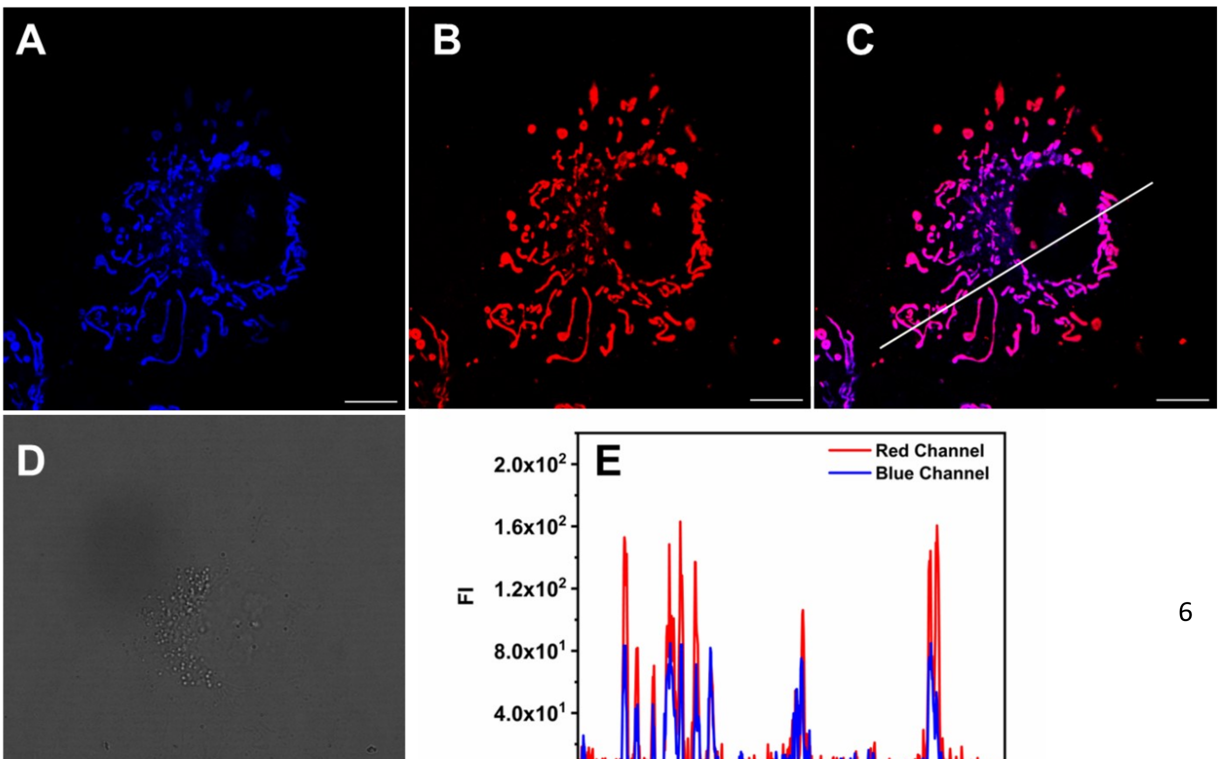


Figure S6: Colocalization of PP in COS-7 cells. CLSM image of A) PP, B) MitoView 405, C) Merged image, D) Bright field image and E) Intensity profile.

S7: Cytotoxicity assay:

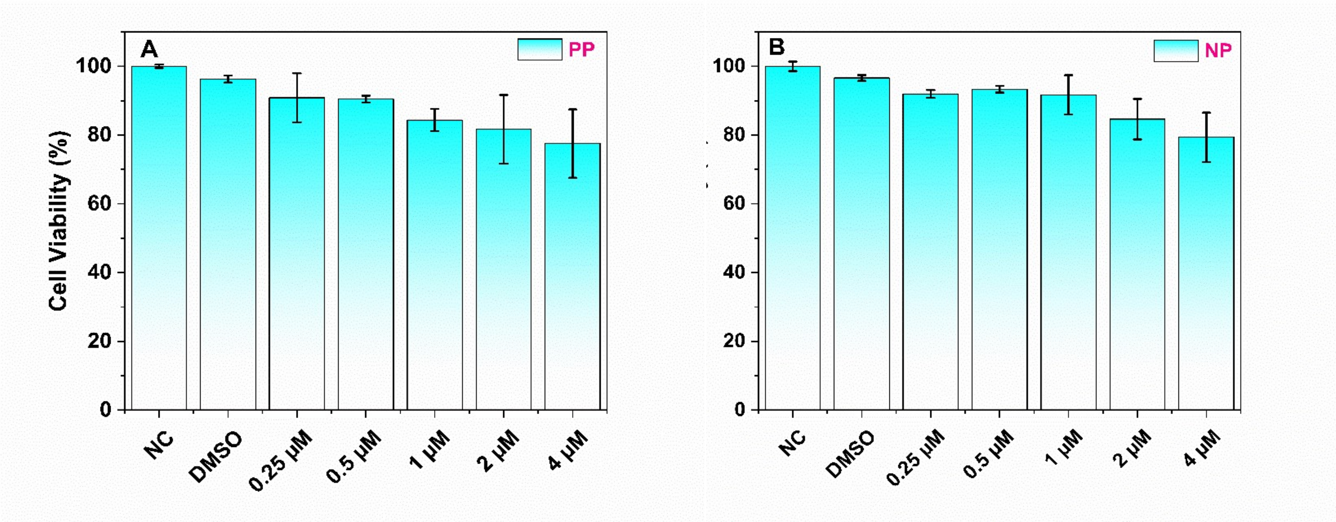


Figure S7: Cytotoxicity assay for PP & NP

S8: Live Cell Imaging with probe NP with Nystatin treatment:

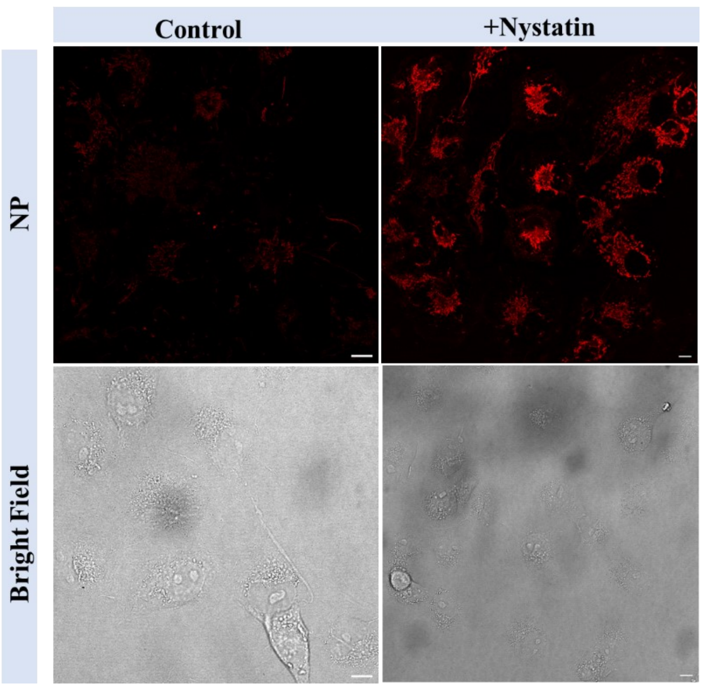


Figure S8: CLSM Images of NP with before (first column) and after Nystatin treatment (second column). (Images with multiple cells)

S9: Live Cell Imaging with probe NP during Ferroptosis:

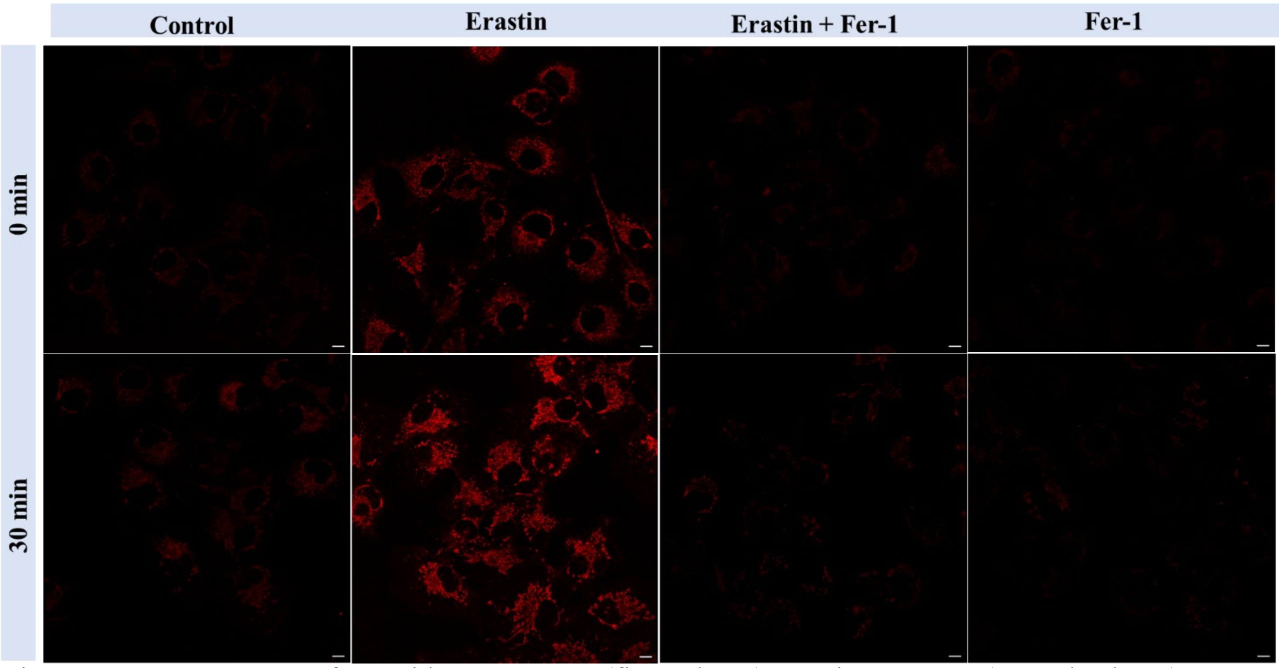


Figure S9: CLSM Images of NP with no treatment (first column), erastin treatment (second column), erastin + ferrostatin-1 treatment (third column), and ferrostatin-1 treatment (fourth column) after 0 min (first row) and 30 min (second row) incubation with NP. (Images with multiple cells)

S10: Synthetic procedures:

General procedures:

Styrene 3 (Heck Coupling):

In a sealed reactor, 1 molar equivalent of N, N-dimethylamino bromonaphthalene derivative **2** was dissolved in dry DMF, followed by the addition of 3 molar equivalents of triethylamine, a pinch of tris (o-tolyl) phosphine ligand and $Pd(OAc)_2$. The contents were stirred for 10 min at room temperature. After the addition of 3 molar equivalents of vinyl pyridine, the reaction was tightly sealed and allowed to heat at 100° C. After completion of the reaction, as monitored by TLC, the reaction mixture is triturated with n-pentane and filtered off to get yellowish orange coloured powder. (64% yield)

General procedure for N-Alkylation: The styryl pyridyl derivatives (**3** or **5**) were dissolved in dry acetonitrile and allowed to stir at room temperature for 15 mins, followed by the addition of 0.51 molar equivalents of 1,12-dibromo dodecane. The reaction mixture was refluxed for 24

h. The solvent was evaporated using a rotatory evaporator and the obtained precipitate was washed with diethyl ether and pentane and filtered to get pure dark red colored solid powder. (80% yield for PP and 70% yield for NP)

S11: Characterization data:

PP: 1,1'-dodecane-1,12-diyl)bis(4-((E)-4-(dimethylamino)styryl)pyridin-1-ium bromide

^{1}H NMR (500 MHz, *DMSO-d6*) *δ* 8.79 (d, *J* = 6.7 Hz, 4H), 8.08 (d, *J* = 6.7 Hz, 4H), 7.94 (d, *J* = 16.0 Hz, 2H), 7.60 (d, *J* = 8.8 Hz, 4H), 7.19 (d, *J* = 16.1 Hz, 2H), 6.79 (d, *J* = 8.9 Hz, 4H), 4.41 (t, *J* = 7.2 Hz, 4H), 3.03 (s, 12H), 1.87 (m, 4H), 1.25 (d, *J* = 17.4 Hz, 16H). ^{13}C NMR (126 MHz, DMSO) δ 153.12, 151.31, 142.87, 141.57, 129.58, 121.86, 121.77, 116.49, 111.34, 58.49, 39.51, 39.42, 39.34, 39.25, 39.17, 39.08, 39.01, 38.91, 38.75, 38.58, 38.41, 29.86, 28.27, 28.21, 27.81, 24.84. HRMS: Observed Mass: 308.2207; Calculated Mass: 308.2247

NP: 1,1'-dodecane-1,12-diyl) bis(4-((E)-2-(6-(dimethylamino) naphthalen-2-yl) vinyl) pyridin-1-ium bromide

^{1}H NMR (500 MHz, *DMSO-d6*) *δ* 8.91 (d, *J* = 6.7 Hz, 4H), 8.21 (d, *J* = 6.8 Hz, 4H), 8.13 (d, *J* = 16.2 Hz, 2H), 8.01 (s, 2H), 7.80 (dd, *J* = 8.8, 5.9 Hz, 4H), 7.73 (d, *J* = 8.8 Hz, 2H), 7.50 (d, *J* = 16.2 Hz, 2H), 7.29 – 7.21 (m, 2H), 6.97 (d, *J* = 2.4 Hz, 2H), 4.47 (t, *J* = 7.2 Hz, 4H), 3.07 (s, 12H), 1.89 (m, 4H), 1.26 (d, *J* = 19.8 Hz, 16H). ^{13}C NMR (126 MHz, DMSO) δ 153.72, 150.06, 144.40, 142.28, 136.43, 130.77, 130.15, 128.86, 127.27, 125.93, 124.40, 123.69, 121.19, 116.99, 105.88, 59.93, 40.59, 40.50, 40.42, 40.33, 40.25, 40.16, 40.09, 40.00, 39.83, 39.66, 39.50, 35.71, 32.69, 30.98, 29.37, 29.30, 28.91, 28.56, 27.97, 27.11, 25.93, 20.31. HRMS: Observed mass: 358.2392; Calculated mass: 358.2404

S12: Spectral copies:

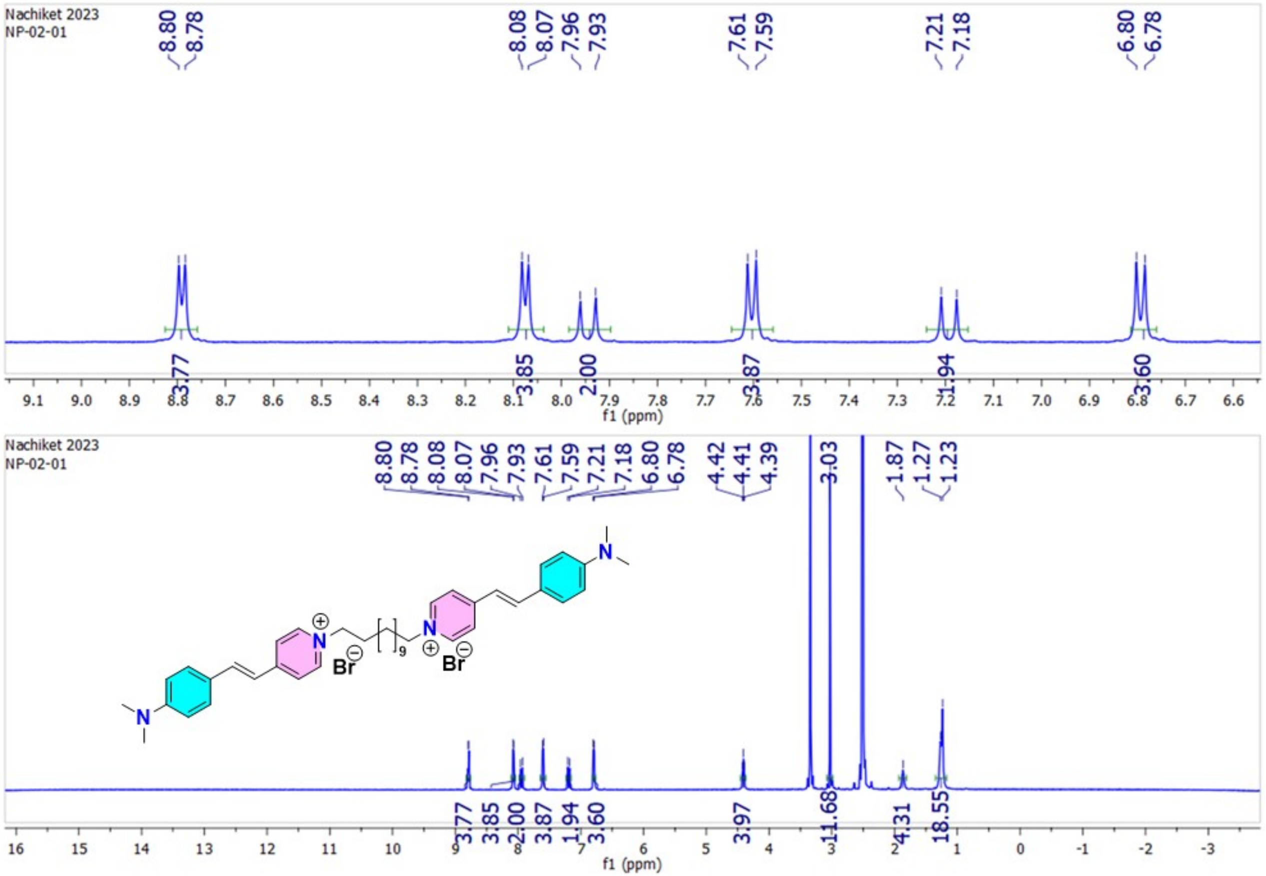


Figure S12a: [1]H NMR of PP

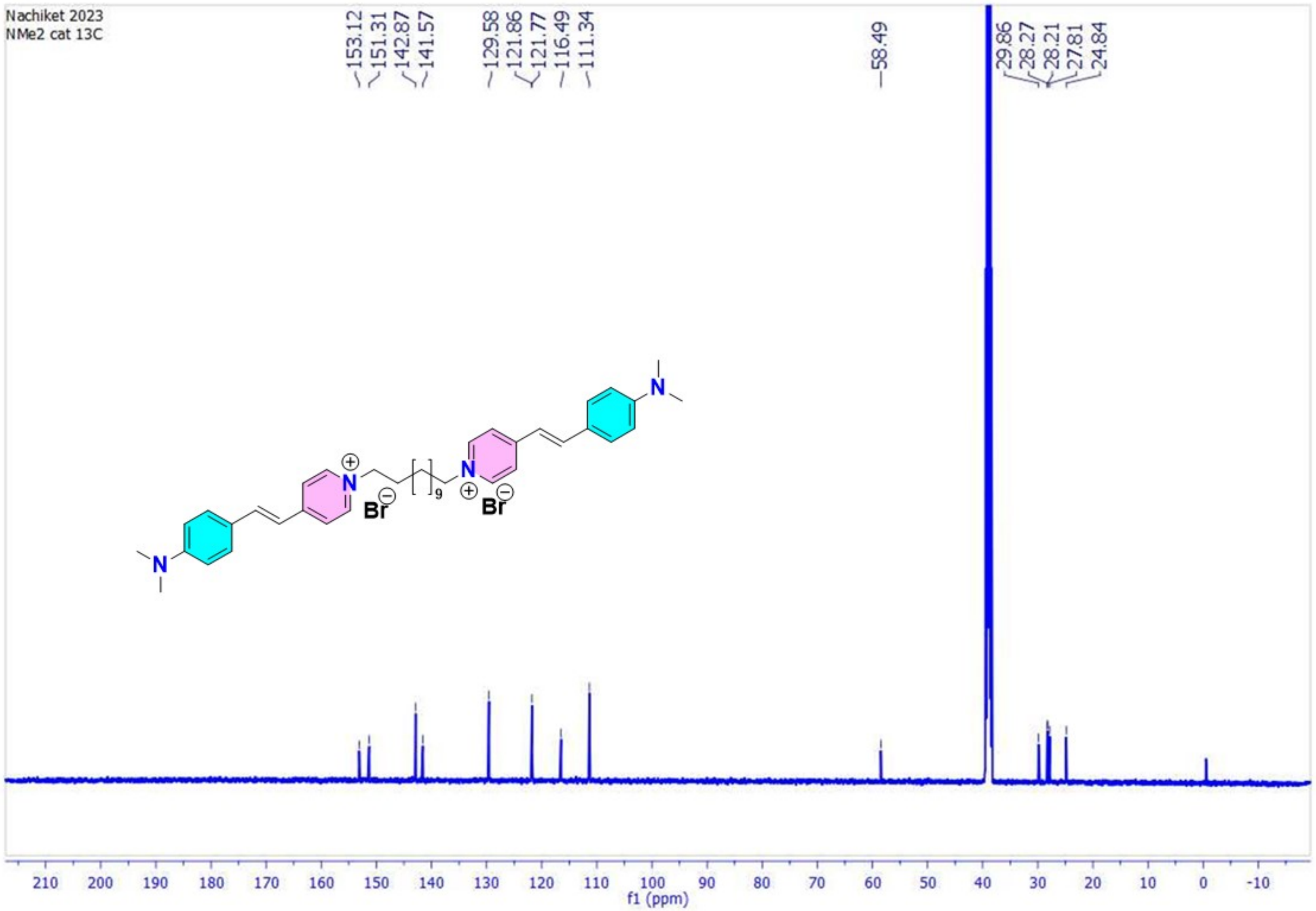


Figure S12b: ^{13}C NMR of PP

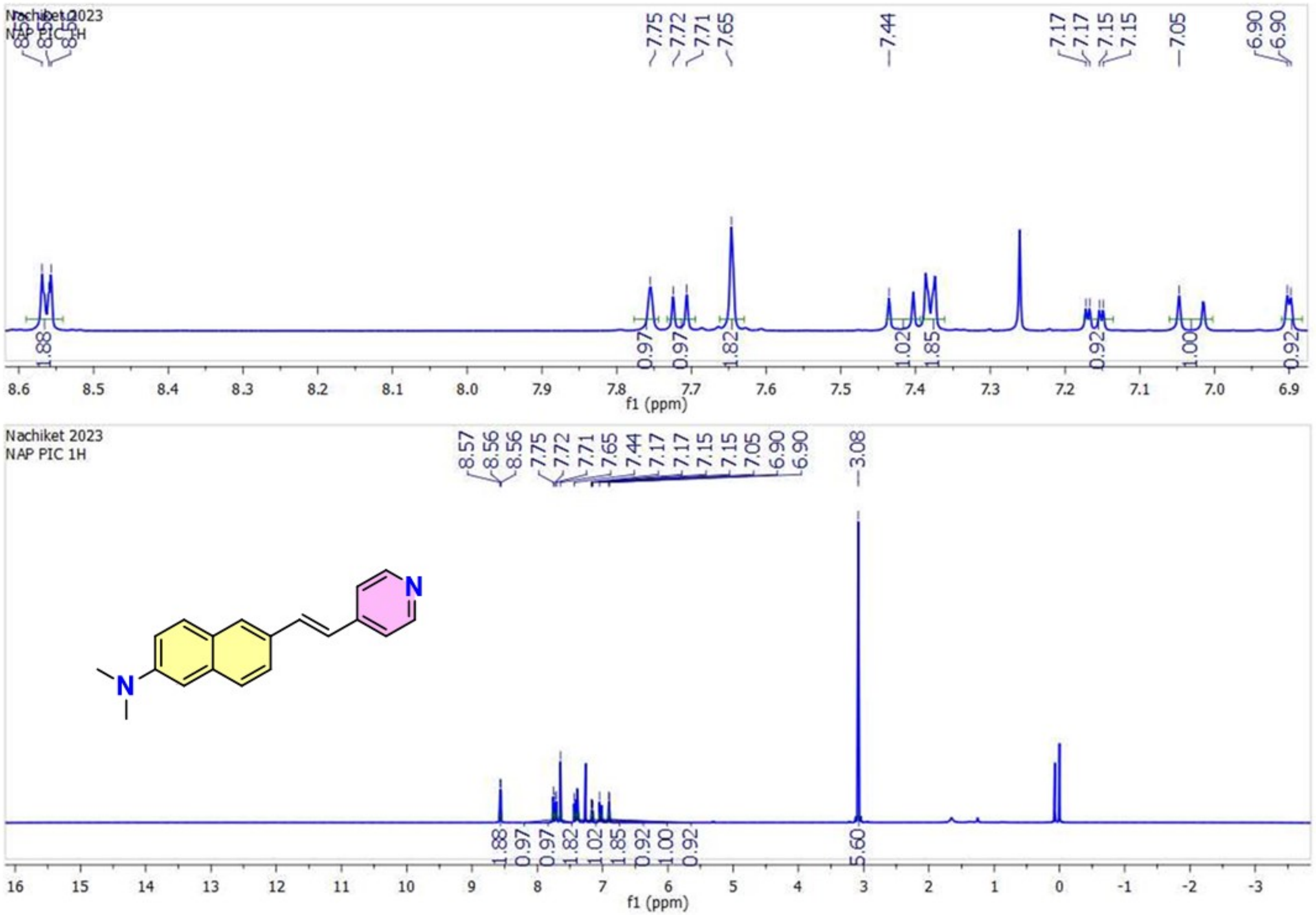


Figure S12c: ^{1}H NMR of Styrene 3

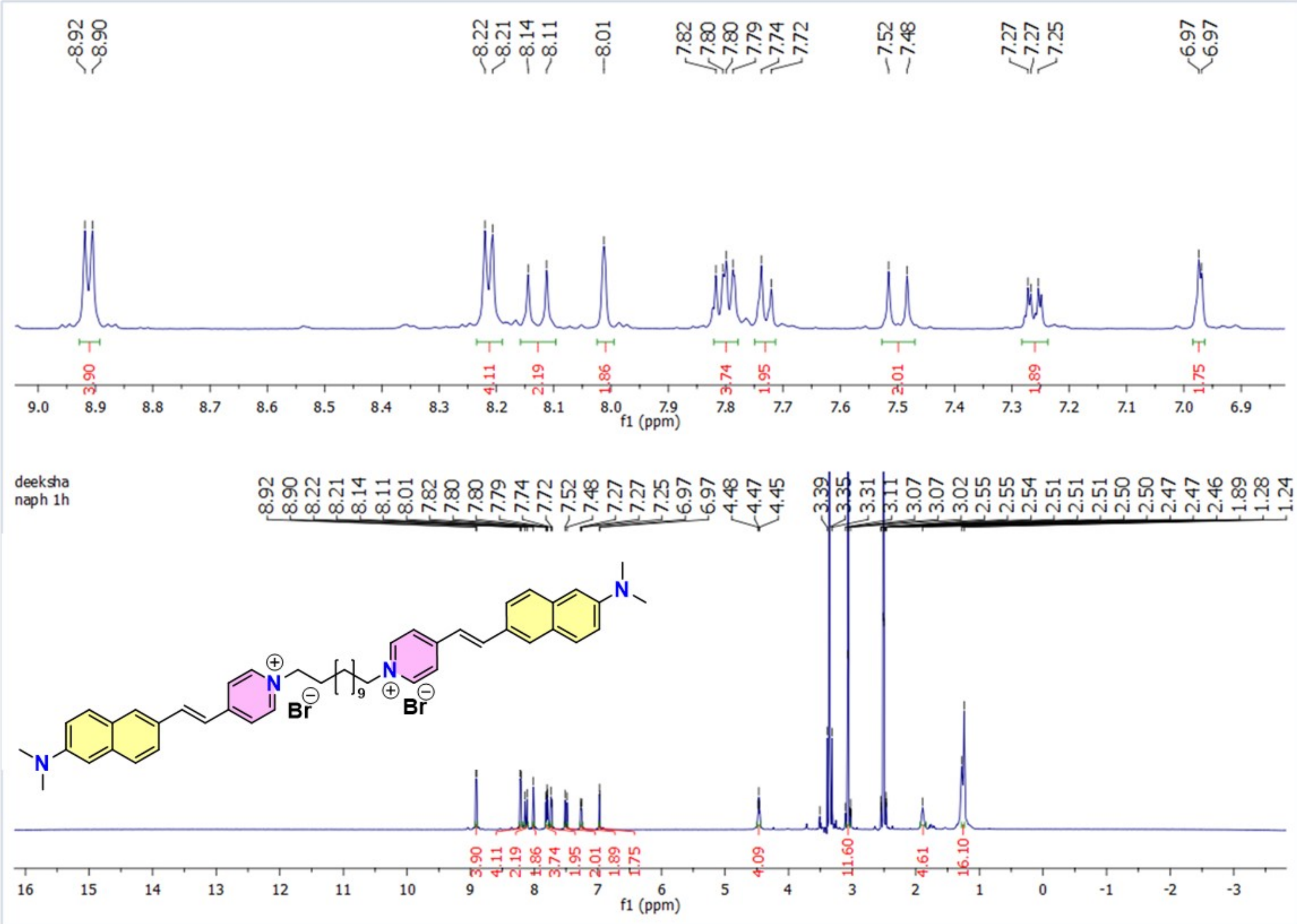


Figure S12d: ^{1}H NMR of NP

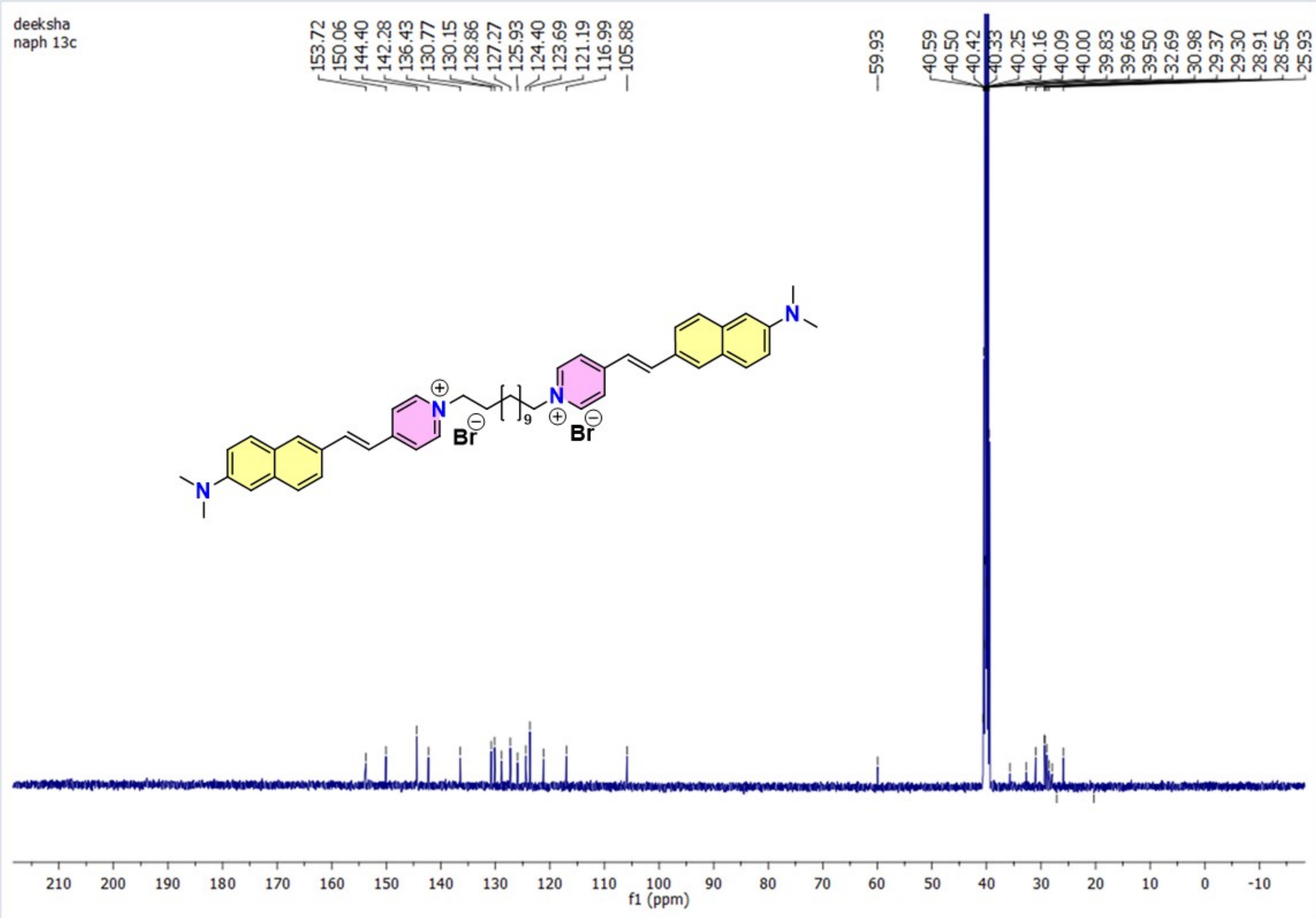
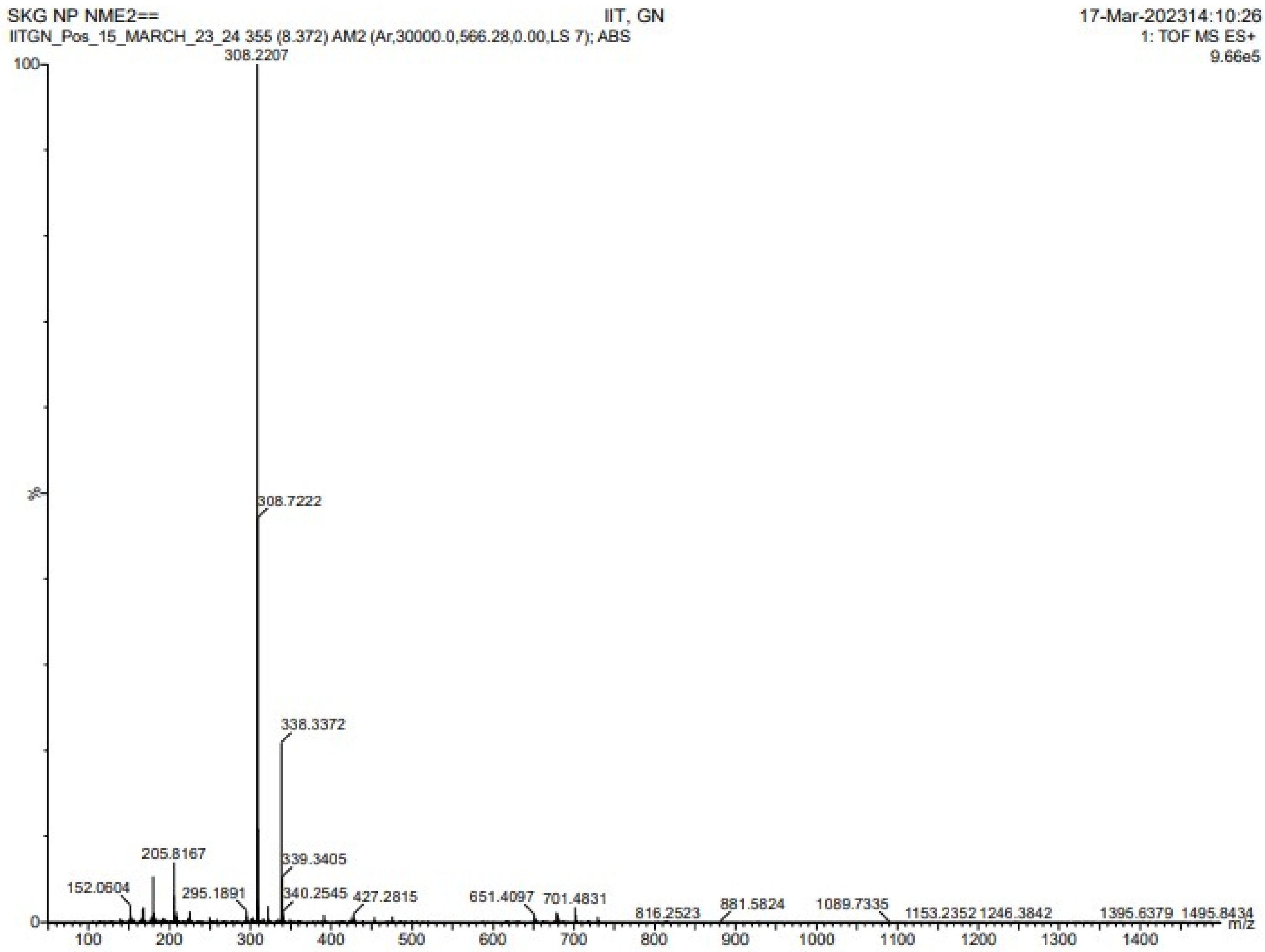


Figure S12e: ^{13}C NMR of NP


SKG NP NME2==
IIT, GN
17-Mar-202314:10:26
IITGN_Pos_15_MARCH_23_24 355 (8.372) AM2 (Ar,30000.0,566.28,0.00,LS 7); ABS
1: TOF MS ES+
9.66e5
308.2207
308.7222
338.3372
205.8167
339.3405
152.0604
295.1891
340.2545
427.2815
651.4097
701.4831
816.2523
881.5824
1089.7335
1153.2352
1246.3842
1395.6379
1495.8434
m/z
%

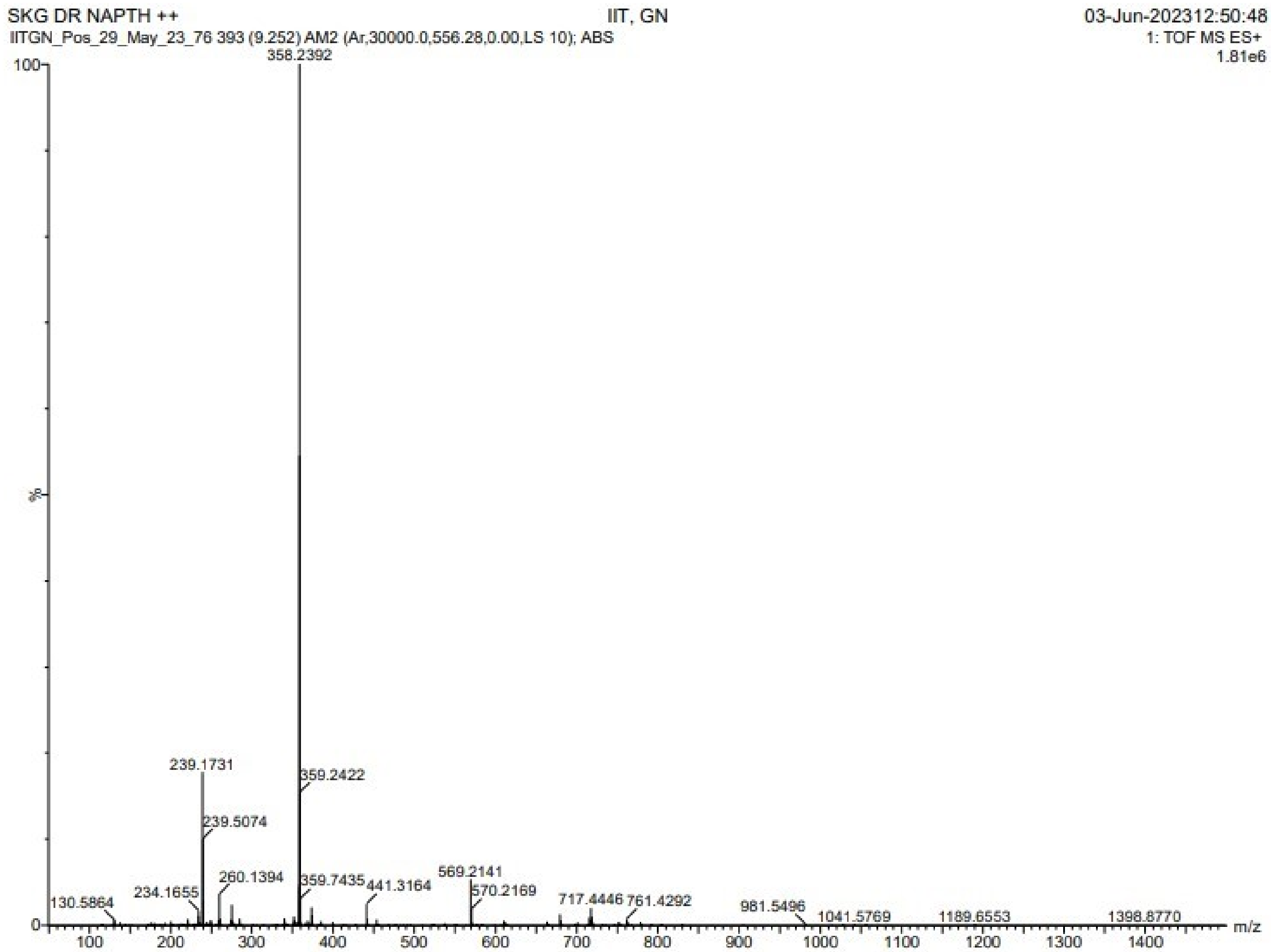


Figure S12g: HRMS of NP

References:

1. J. Hong, X. Guan, Y. Chen, X. Tan, S. Zhang and G. Feng, *Anal. Chem.*, 2023, **95**, 5687-5694.
2. X. Wu, R. Zhang, Y. Li, Y. Gai, T. Feng, J. Kou, F. Kong, L. Li and B. Tang, *Anal. Chem.*, 2023, **95**, 7611-7619.
3. P. Lei, M. Li, C. Dong and S. Shuang, *ACS Biomaterials Science & Engineering*, 2023, **9**, 3581-3589.
4. L. Fan, Q. Yang, Q. Zan, K. Zhao, W. Lu, X. Wang, Y. Wang, S. Shuang and C. Dong, *Anal. Chem.*, 2023, **95**, 5780-5787.
5. J. Yin, Q. Xu, X. Mo, L. Dai, M. Ren, S. Wang and F. Kong, *Dyes and Pigments*, 2022, **200**, 110184.
6. F. Zheng, J. Ding, S. Huang, A. Bi, S. Liu, K. Zhang, F. Chen and W. Zeng, *Dyes and Pigments*, 2023, **217**, 111424.
7. X. Xie, Y. Liu, G. Liu, Y. Zhao, J. Bian, Y. Li, J. Zhang, X. Wang and B. Tang, *Anal. Chem.*, 2022, **94**, 10213-10220.