Supporting Information

Fluorescent Styryl Pyridine-N-Oxide Probes for Imaging Lipid Droplets

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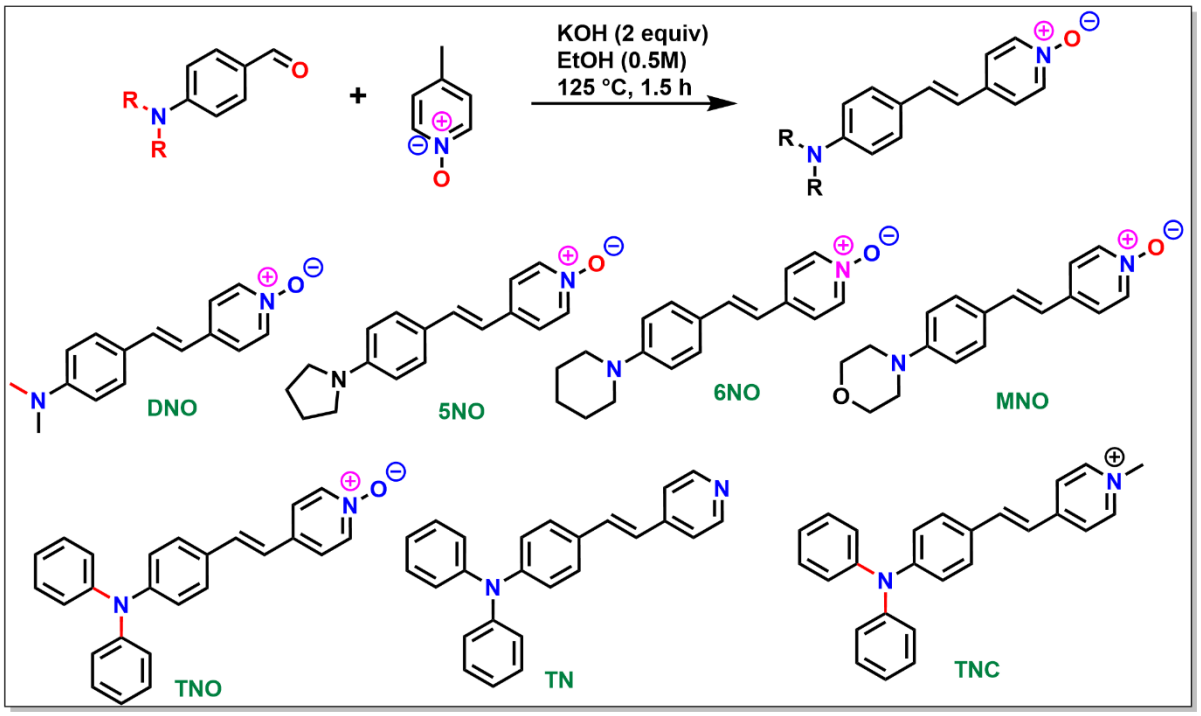
S1. Synthetic procedure



General procedure for the synthesis of DNO, 5NO and MNO:

In a dry round bottom flask equipped with a magnetic bead, p-amino benzaldehyde (1 equiv), 4-methylpyridine-N-oxide (1 equiv.) and potassium hydroxide (2 equiv) were added. The reaction was purged with argon gas and added ethanol (0.5M). The reaction was heated at 125° C for 2 hours, and the progress of the reaction was monitored by TLC. The reaction mixture was filtered, and the insoluble solid was collected, washed multiple times with diethyl ether, dried and weighed.

General procedure for the synthesis of TNO and 6NO:

In a dry round bottom flask equipped with a magnetic bead, *p*-amino benzaldehyde (1 equiv), 4-methylpyridine-N-oxide (1 equiv), and potassium hydroxide (2 equiv) were added. The reaction was purged with argon gas and added ethanol (0.5M). The reaction was heated at 125° C for 2 hours and the progress of the reaction was monitored by TLC. The reaction was concentrated on a rotary evaporator and purified by column chromatography on 100-200 mesh size silica gel with gradient elution from 1 to 5 % methanol in DCM.

Synthesis and optical properties of TN and TNC were referred from literature[1,2.]

Figure S2. Absorption spectral data for 5NO, 6NO, MNO and TNO

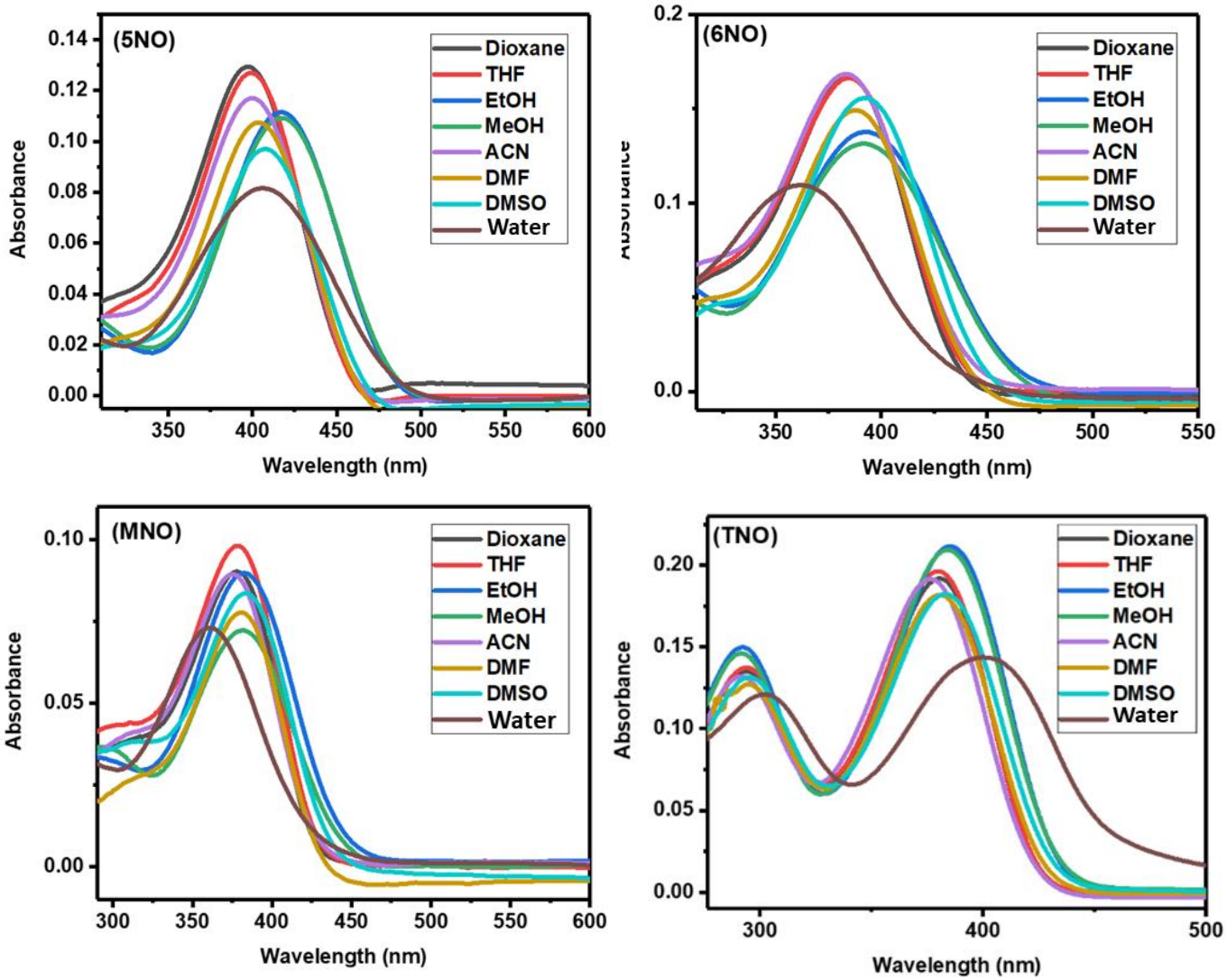


Figure S3. Emission spectral data of compounds 5NO, 6NO, and MNO in different solvents.

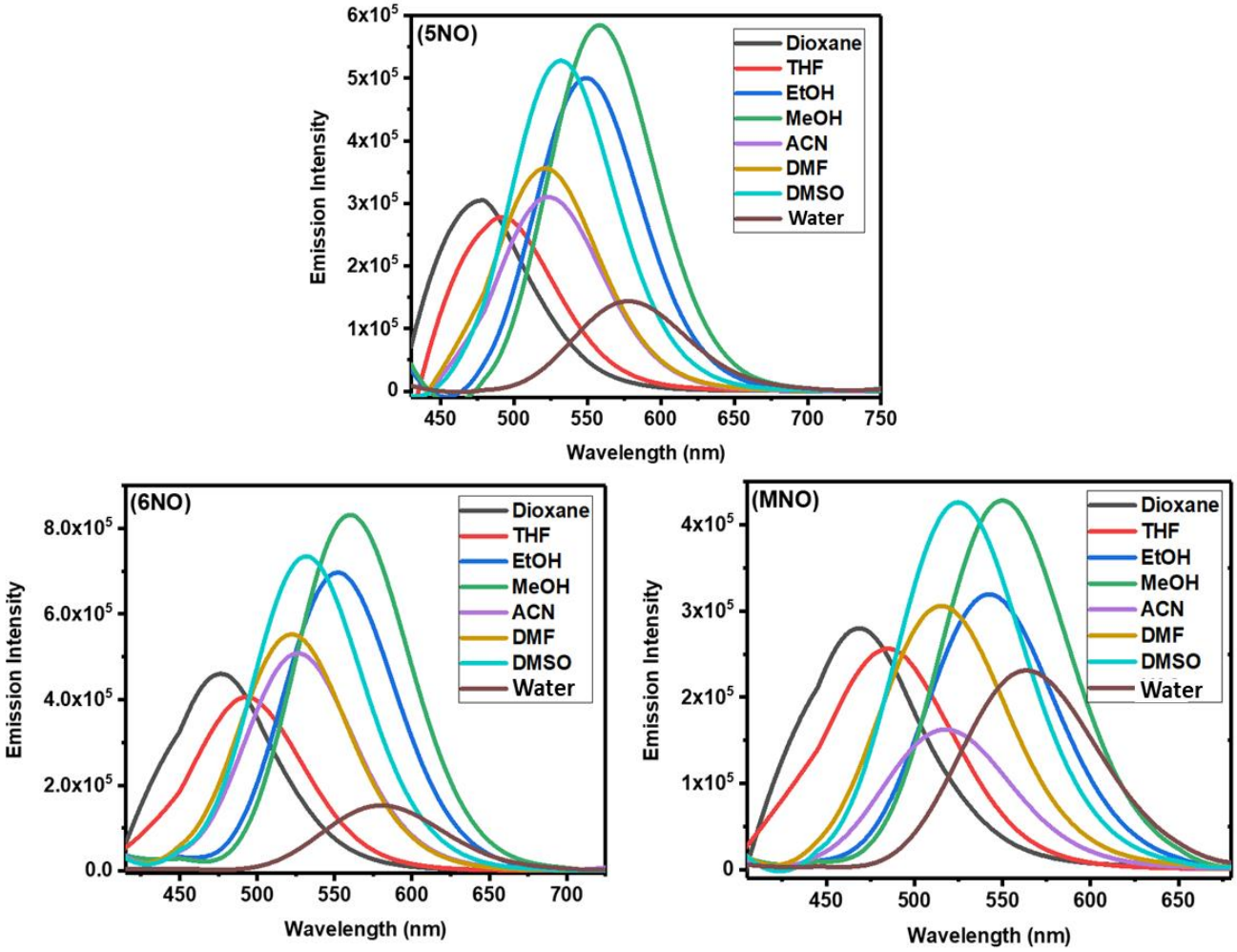


Figure S4. Fluorescence lifetime decay of the compounds

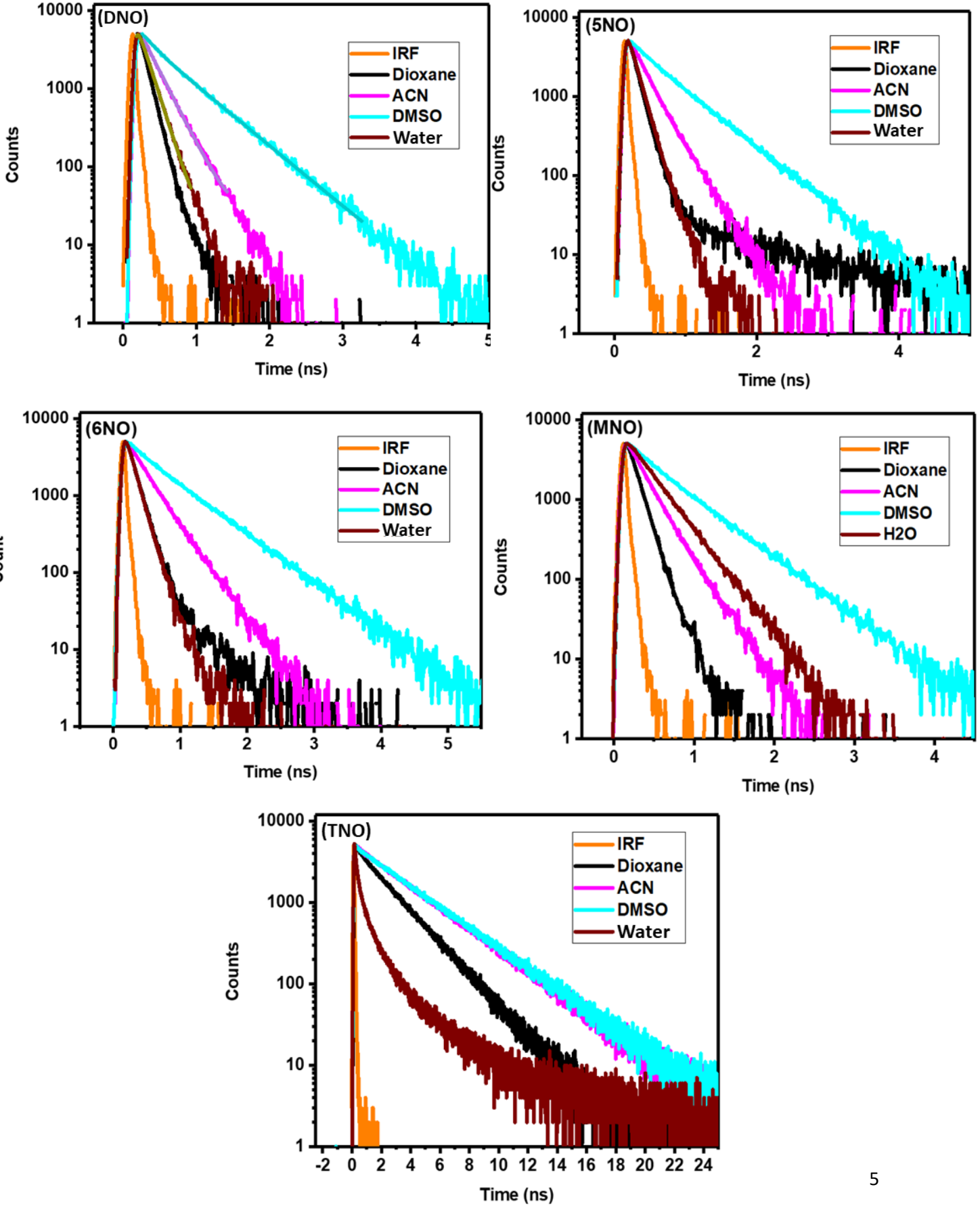
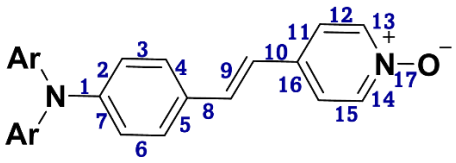


Table S1: Lifetime properties of compounds in various solvents

Compound	Solvents	τ_1 (ns)	*A*1 (%)	τ_2 (ns)	*A*2 (%)	τ_3 (ns)	*A*3 (%)	Average lifetime (τ) (ns)	χ^2
DNO	**Dioxane**	0.10	100	-	-	-	-	0.10	0.99
	ACN	0.23	100	-	-	-	-	0.23	1.18
	DMSO	0.13	9.36	0.55	90.64	-	-	0.43	1.10
	water	0.14	100	-	-	-	-	0.14	1.09
5NO	**Dioxane**	0.11	100	-	-	-	-	0.11	1.06
	ACN	0.23	100	-	-	-	-	0.23	1.19
	DMSO	0.27	13.76	0.62	86.24	-	-	0.52	1.05
	water	0.13	100	-	-	-	-	0.13	1.02
6NO	**Dioxane**	0.13	100	-	-	-	-	0.13	1.16
	ACN	0.30	100	-	-	-	-	0.30	1.23
	DMSO	0.15	6.52	0.67	93.48	-	-	0.55	0.99
	water	0.13	100	-	-	-	-	0.13	1.09
MNO	**Dioxane**	0.12	100	-	-	-	-	0.12	1.23
	ACN	0.23	100	-	-	-	-	0.23	1.31
	DMSO	0.17	9.08	0.58	90.92	-	-	0.48	1.17
	water	0.31	100	-	-	-	-	0.31	1.20
TNO	**Dioxane**	0.43	3.36	2.20	96.64	-	-	1.94	1.06
	ACN	3.30	100	-	-	-	-	3.30	1.13
	DMSO	3.40	100	-	-	-	-	3.40	1.13
	water	0.55	33.65	1.80	30.70	0.13	33.65	0.30	1.08

Table S2 Geometrical coordinates of the N-oxide derivatives



	5NO	6NO	DNO	PNO	TNO
N1-C2	1.365	1.438	1.374	1.411	1.397
C2-C3	1.420	1.400	1.416	1.404	1.414
C3-C4	1.382	1.390	1.385	1.386	1.387
C4-C5	1.410	1.407	1.407	1.406	1.407
C5-C6	1.408	1.407	1.409	1.408	1.409
C6-C7	1.384	1.388	1.382	1.384	1.384
C7-C2	1.416	1.400	1.420	1.412	1.408
C5-C8	1.454	1.463	1.455	1.457	1.458
C8-C9	1.352	1.348	1.352	1.351	1.350
C9-C10	1.454	1.457	1.455	1.456	1.456
C10-C11	1.408	1.408	1.410	1.407	1.409
C11-C12	1.377	1.374	1.375	1.378	1.374
C12-N13	1.367	1.372	1.371	1.367	1.371
N13-C14	1.371	1.367	1.367	1.37	1.367
C14-C15	1.374	1.378	1.377	1.374	1.377
C15-C16	1.410	1.406	1.407	1.409	1.406
N14-O17	1.288	1.284	1.287	1.286	1.285

Table S3 TDDFT results of the TN, TNC and TNO derivatives provided with computed excitation energy, oscillator strength, major transitions involved in terms of frontier molecular Orbitals, and dipole moments.

	$\lambda_{\omega X97BD}$[a] (nm)	$f_{\omega X97BD}$[a]	Major transitions	μ_g[b] (Debye)	μ_e[c] (Debye)
TN	361.3	1.34	HOMO->LUMO (86%), H-1->LUMO (11%)	5.76	5.26
TNC	430.2	1.23	HOMO(A)->LUMO(A) (24%), HOMO(B)->LUMO(B) (55%) H-1(B)->LUMO(B) (9%),	8.48	8.53
TNO	375.2	1.89	HOMO->LUMO (91%), H-1->L+4 (2%), HOMO->L+4 (3%)	7.8	7.8

[a]TDDFT simulated absorption maximum and oscillator strength using ωX97BD/6-31G**/C-PCM(THF)level of theory; [b]$\mu_{g:}$ ground state dipole moment; [c]$\mu_{e:}$ transient dipole moment.

Table S4 Comparison of absorption properties of the molecules with computed excitation energy, oscillator strength, major transitions involved in terms of frontier molecular Orbitals, and dipole moments.

	λ_{exp}[a] (nm)	$\lambda_{CAM\text{-}B3LYP}$[b] (nm)	$f_{CAM\text{-}B3LYP}$[b]	Major transitions	μ_g[c] (Debye)	μ_e[d] (Debye)
5NO	403	361.8	1.91	HOMO->LUMO (93%)	12.0	11.6
6NO	383	322.5	1.78	HOMO->LUMO (91%), H-2->LUMO (2%)	6.5	6.7
DNO	395	357.5	1.1.85	HOMO->LUMO (93%)	11.3	10.9
MNO	377	349.4	1.88	HOMO->LUMO (93%)	8.1	7.8
TNO	380	365.3	1.89	HOMO->LUMO (89%), H-1->L+4 (2%), HOMO->L+4 (4%)	7.8	7.7

[a]Absorption spectra measured in THF in the concentration of 1×10^{-5} M at ambient temperature; [b]TDDFT simulated absorption maximum and oscillator strength using **B3LYP/6-31G**/C-PCM(THF)** level of theory; [c]$\mu_{g:}$ ground state dipole moment; [d]$\mu_{e:}$ transient dipole moment.

Table S5 Comparison of absorption properties of the molecules with computed excitation energy, oscillator strength, major transitions involved in terms of frontier molecular Orbitals, and dipole moments.

	λ_{exp}[a] (nm)	$\lambda_{CAM\text{-}B3LYP}$[b] (nm)	$f_{CAM\text{-}B3LYP}$[b]	**Major transitions**	μ_g[c] (Debye)	μ_e[d] (Debye)
5NO	403	361.8	1.91	HOMO->LUMO (93%)	12.0	11.6
6NO	383	322.5	1.78	HOMO->LUMO (91%), H-2->LUMO (2%)	6.5	6.7
DNO	395	357.5	1.1.85	HOMO->LUMO (93%)	11.3	10.9
MNO	377	349.4	1.88	HOMO->LUMO (93%)	8.1	7.8
TNO	380	365.3	1.89	HOMO->LUMO (89%), H-1->L+4 (2%), HOMO->L+4 (4%)	7.8	7.7

[a]Absorption spectra measured in THF in the concentration of 1×10^{-5} M at ambient temperature; [b]TDDFT simulated absorption maximum and oscillator strength using **CAM-B3LYP/6-31G**/C-PCM(THF)** level of theory; [c]$\mu_{g:}$ ground state dipole moment; [d]$\mu_{e:}$ transient dipole moment. Both CAM-B3LYP and B3LYP gave identical results**.**

Figure. S5. pH-dependent absorbance titration for DNO, 5NO, 6NO and MNO

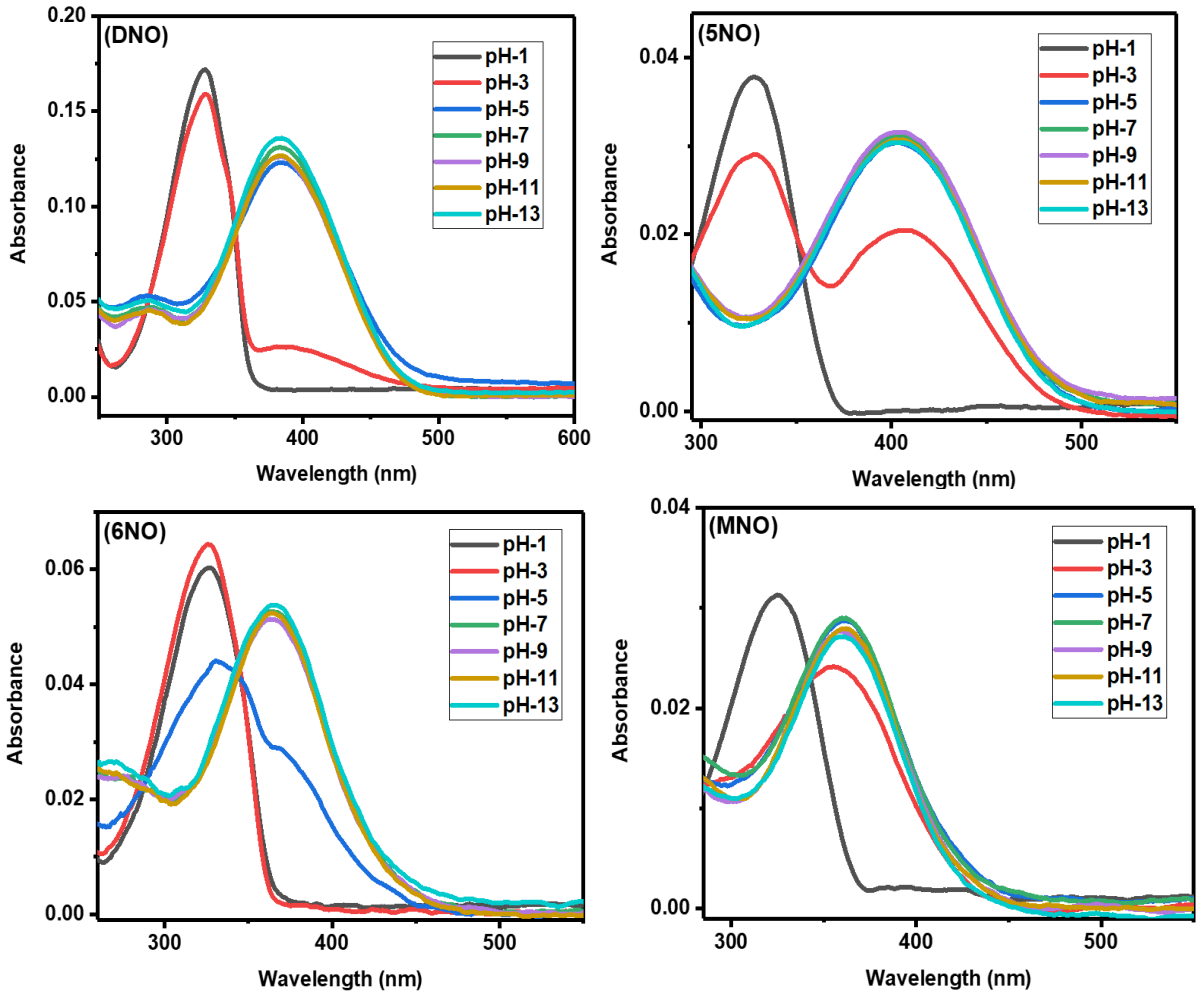


Figure S6. pH-dependent emission spectra for DNO, 5NO, 6NO and MNO

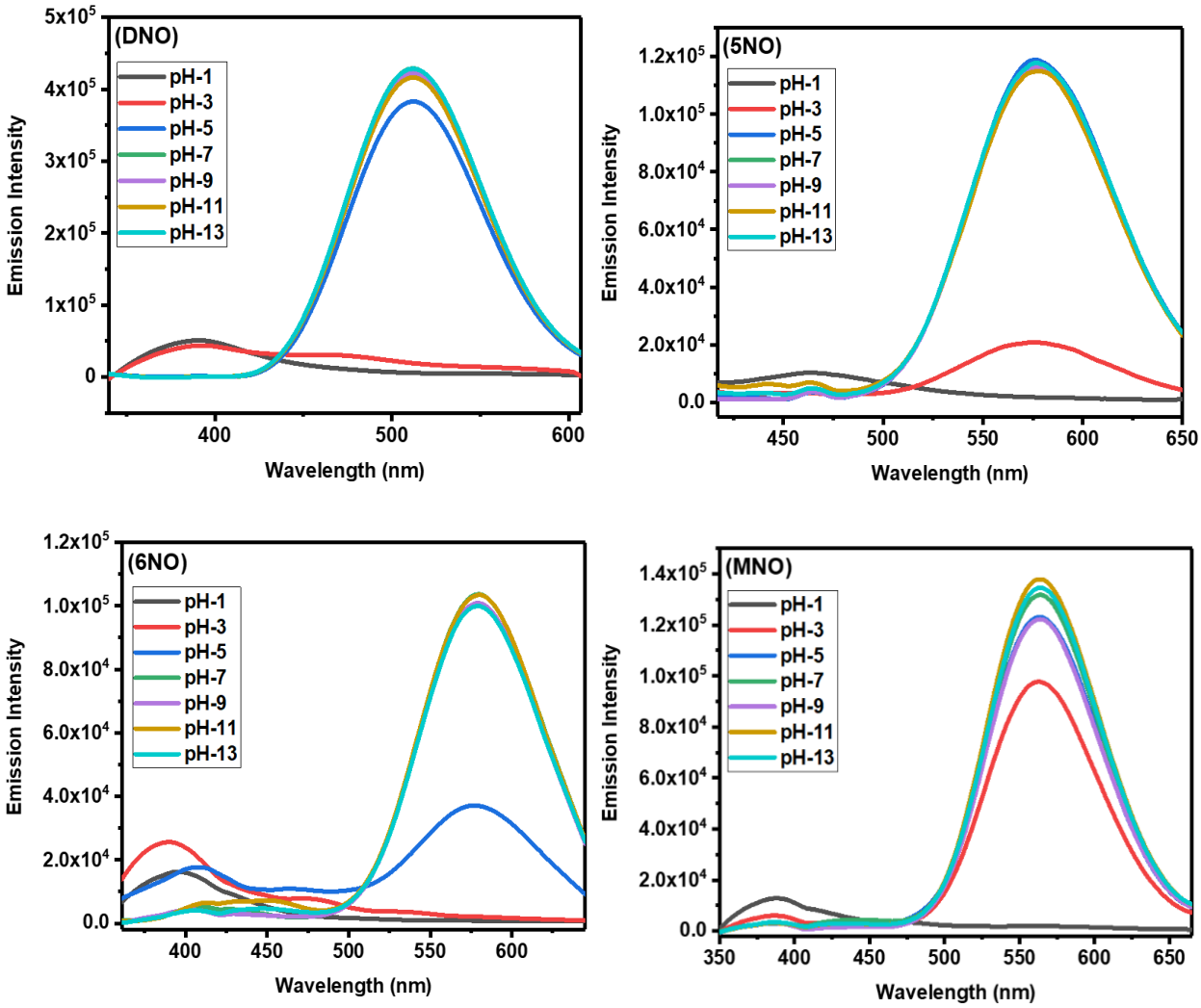
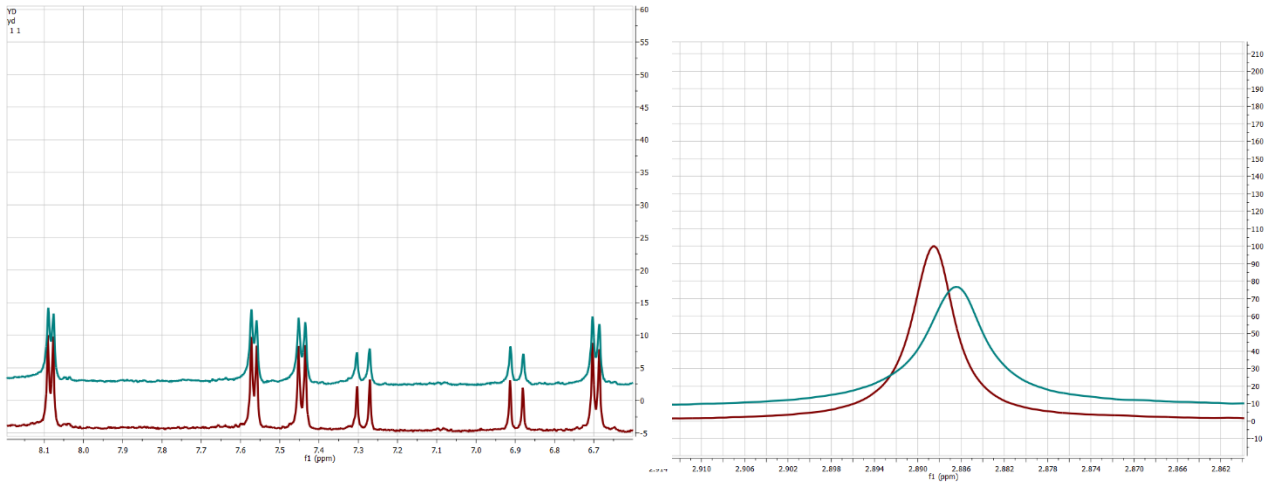
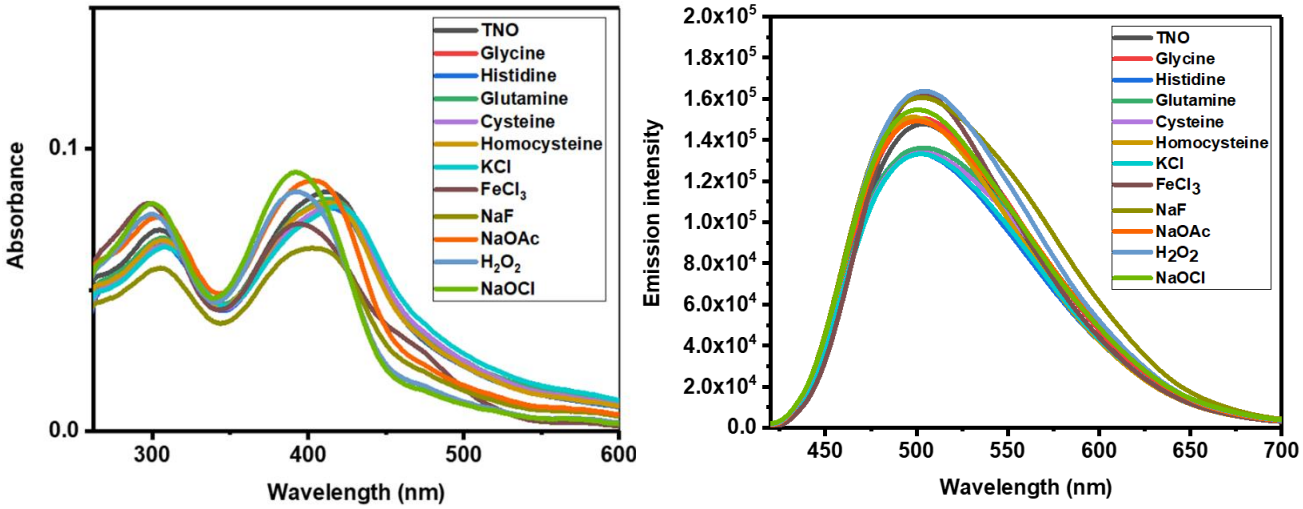


Figure S7. Overlay pH-dependent NMR for DNO



Overlay NMR spectra of DNO in pH-3 (red line) and pH-7 (green line). Calibrated axis - 2.5 ppm (DMSO-d_6).

Figure S8: Influence of added analytes on the absorption and emission of TNO



(Excitation Wavelength 380nm. Absorption and emission is recorded after 5 minutes of addition of the analyte)

Figure S9: Live cell imaging in COS-7 cells: DNO, 5NO, 6NO, and MNO. The data suggests weaker specificity of the molecules Scale bar 10µm

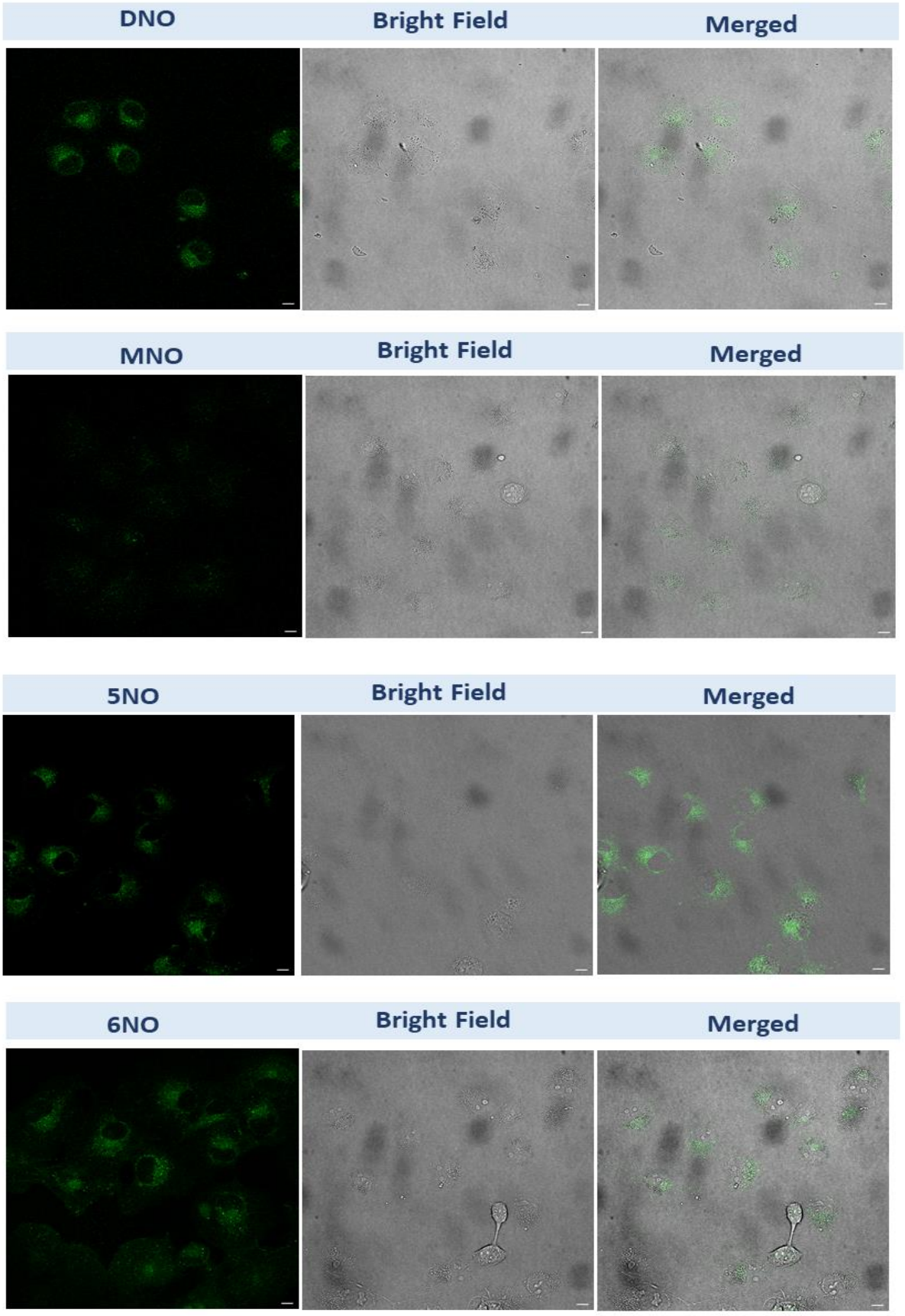


Figure S10: MTT Assay for TNO and TNC. The compounds are good for cellular imaging studies with over 80% cell viability at all concentrations. The imaging is performed at 1 μM,

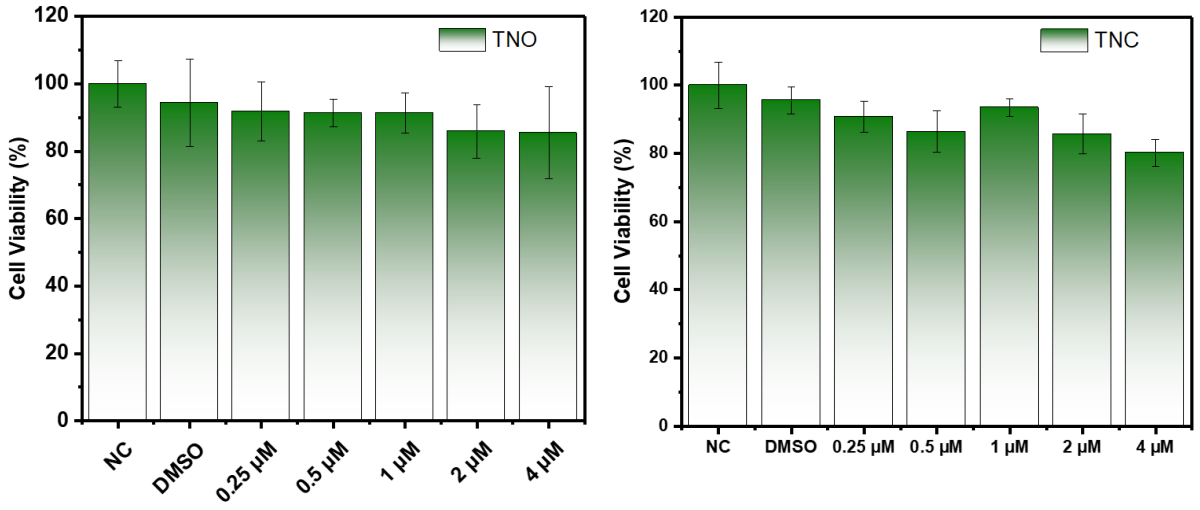


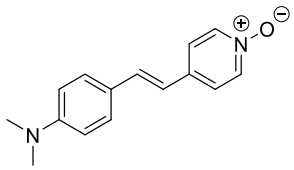
Table S6: Molar absorption coefficient **(*ε*)** of all compounds in different solvents

Solvent	**DNO**	**5NO**	**6NO**	**MNO**	**TNO**
	ε	***ε***	***ε***	***ε***	***ε***
Dioxane	19.7	6.9	10.1	5.6	9.7
THF	19.8	6.8	10.5	7.1	9.9
EtOH	17.1	5.9	7.4	4.5	9.8
MeOH	16.4	5.7	8.2	5.0	10.0
ACN	19.2	6.2	10.5	6.3	9.9
DMF	18.9	5.7	10.2	5.9	9.5
DMSO	18.3	5.2	7.9	5.4	9.0
H_2O	13.2	4.2	5.8	4.1	6.6

ε (M^{-1} cm^{-1}) × 10^3

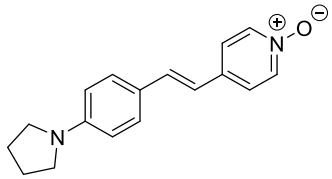
S11. Characterisation data

(E)-4-(4-(dimethylamino)styryl)pyridine 1-oxide (DNO)



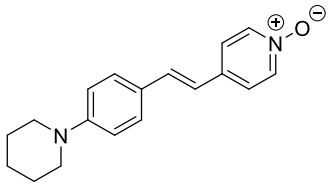
^{1}H NMR (500 MHz, DMSO-d_6): δ 8.11 (d, *J* = 6.5 Hz, 2H), 7.51 (d, J = 7 Hz, 2H), 7.44 (d, J = 8.5 Hz, 2H), 7.27 (d, J=16.5 Hz, 1H), 6.94 (d, J =16.5Hz , 1H), 6.72 (d, J=8.5 Hz, 2H), 2.95 (s, 6H). **^{13}C NMR** (126 MHz; DMSO-d_6) δ 150.92, 138.96, 135.64, 132.56, 128.57, 124.50, 123.09, 119.92, 112.58, 40.58. **HRMS** (ESI) m/z calcd for $C_{15}H_{17}N_2O^+$ $[M + H]^+$ 241.1335, found 241.1320.

(E)-4-(4-(pyrrolidin-1-yl)styryl)pyridine 1-oxide (5NO)



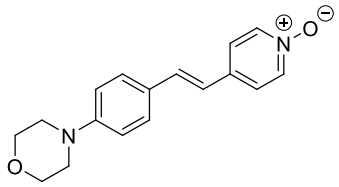
^{1}H NMR (500 MHz, DMSO-d_6): δ 8.10 (d, J= 7 Hz, 2H), 7.5 (d, J= 7 Hz, 2H), 7.43 (d, J= 8.5 Hz, 2H), 7.26 (d, J= 16.5 Hz, 1H), 6.91 (d, J= 16.5 Hz, 1H), 6.55 (d, J= 8.5 Hz, 2H), 3.26 (t, J= 6 Hz, 4H), 1.95 (t, J= 6.5 Hz, 4H) **^{13}C NMR** (126 MHz; DMSO-d_6) δ 148.31, 138.94, 135.82, 132.87, 128.76, 123.76, 122.98, 119.24, 112.22, 47.71, 25.43 **HRMS** (ESI) m/z calcd for $C_{17}H_{19}N_2O^+$ $[M + H]^+$ 267.1492, found 267.1477.

(E)-4-(4-(piperidin-1-yl)styryl)pyridine 1-oxide (6NO)



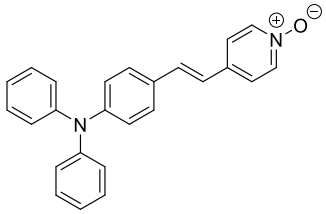
^{1}H NMR (500 MHz, $CDCl_3$): δ 8.00 (d, J= 6.5 Hz, 2H), 7.27 (d, J= 9 Hz, 2H), 7.15 (d, J= 6.5 Hz, 2H), 6.95 (d, J= 16 Hz, 1H), 6.78 (d, J= 8.5 Hz, 2H), 6.63 (d, J= 16.5 Hz, 1H), 3.13 (t, J= 5 Hz, 4H), 1.60-1.49 (m, 7H) **^{13}C NMR** (126 MHz; $CDCl_3$) δ 151.14, 137.90, 135.75, 131.88, 127.17, 124.78, 121.34, 118.93, 114.40, 48.45, 24.50, 23.26 **HRMS** (ESI) m/z calcd for $C_{18}H_{21}N_2O^+$ $[M + H]^+$ 281.1648, found 281.1634.

(E)-4-(4-morpholinostyryl)pyridine 1-oxide (MNO)



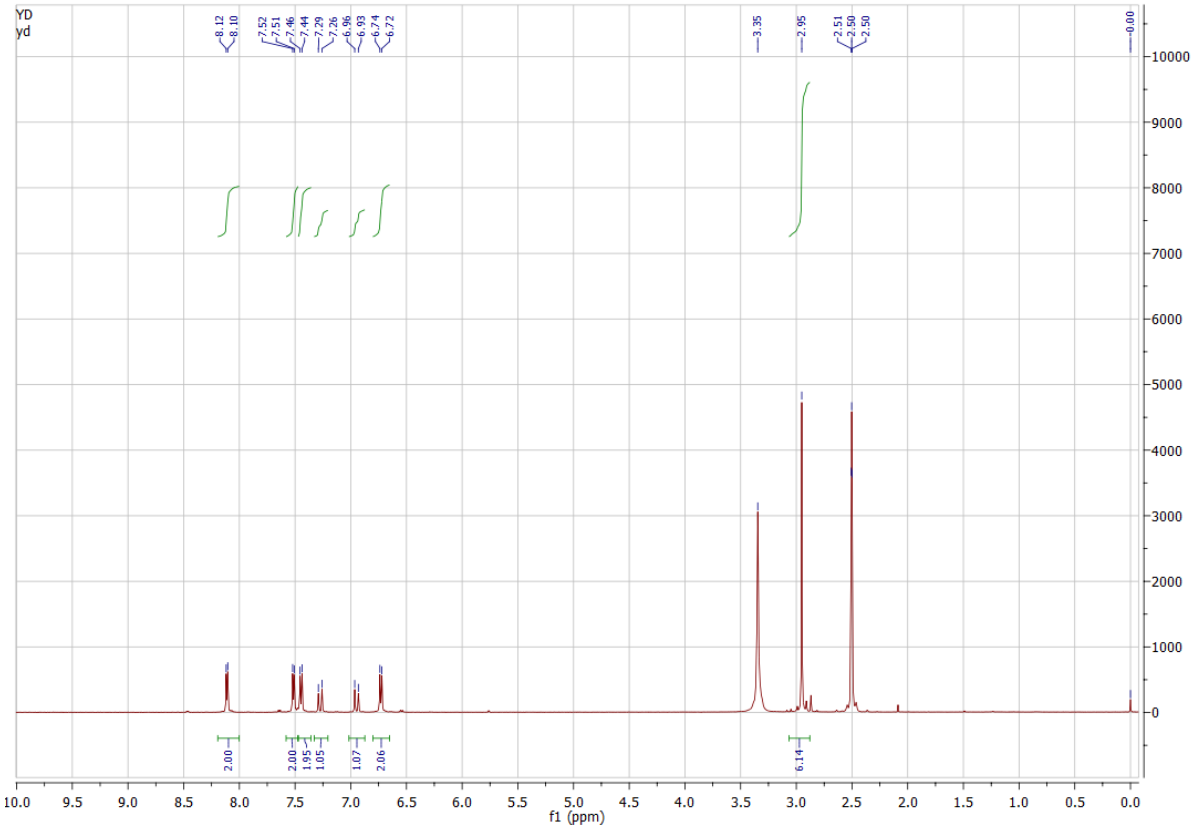
^{1}H NMR (500 MHz, $CDCl_3$): δ 8.12 (d, J= 7 Hz, 2H), 7.43 (d, J= 8.5 Hz, 2H), 7.31 (d, J= 7 Hz, 2H), 7.09 (d, J= 16 Hz, 1H), 6.90 (d, J= 8.5 Hz, 2H), 6.81 (d, J= 16 Hz, 1H), 3.87 (t, J= 4.5 Hz, 4H), 3,23 (t, J= 5 Hz, 4H) **^{13}C NMR** (126 MHz; $CDCl_3$) δ 151.57, 139.03, 136.47, 132.61, 128.20, 127.17, 122.53, 120.83, 115.15, 66.72, 48.45 **HRMS** (ESI) m/z calcd for $C_{17}H_{19}N_2O_2^+$ $[M + H]^+$ 283.1441, found 283.1427.

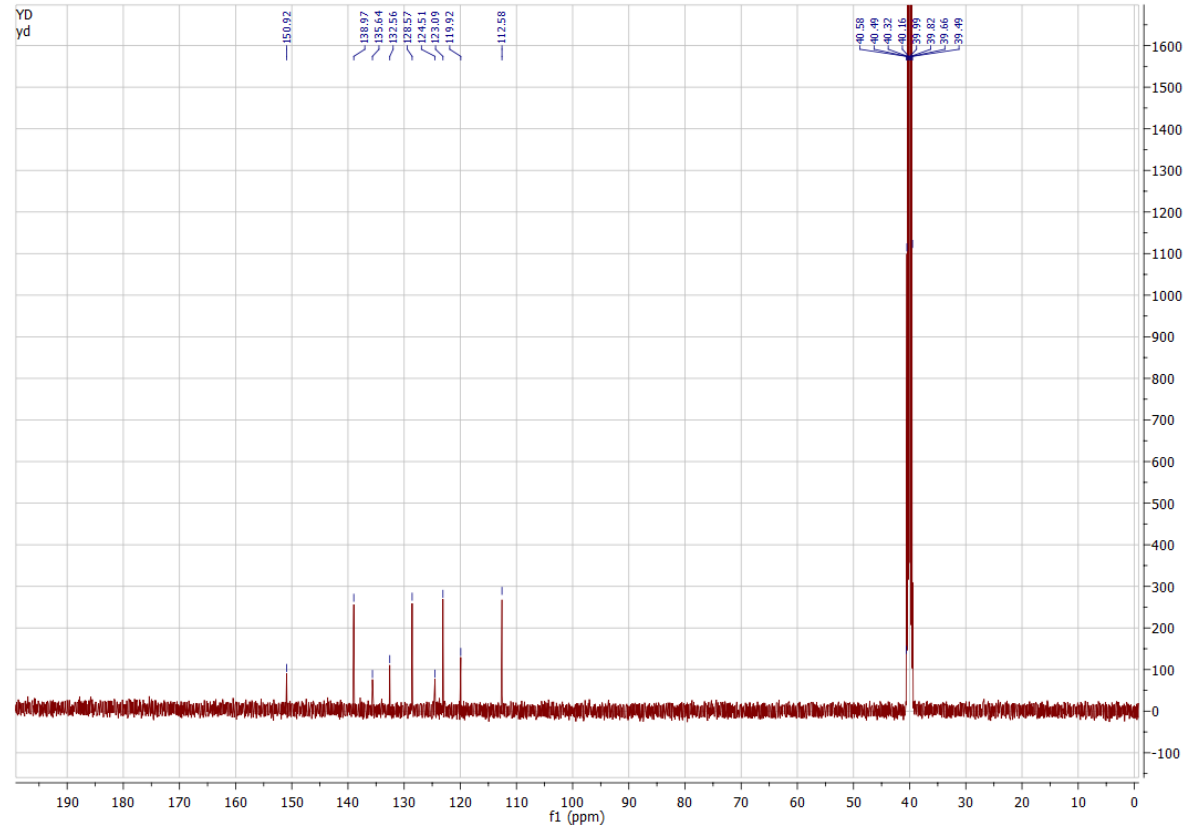
(E)-4-(4-(diphenylamino)styryl)pyridine 1-oxide (TNO)

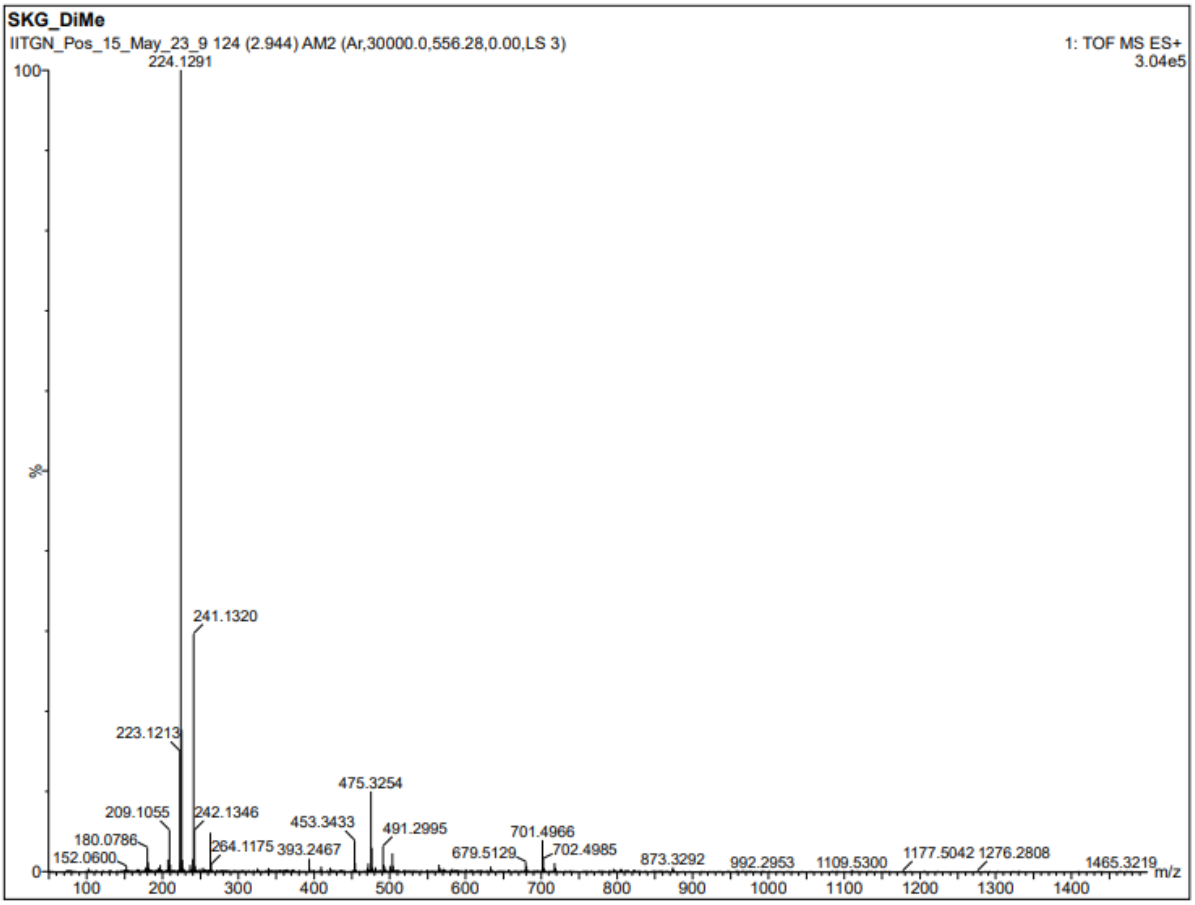


^{1}H NMR (500 MHz, $CDCl_3$): δ 8.39 (d, J= 6 Hz, 2H), 7.24 (d, J= 8.5 Hz, 2H), 7.17-7.07 (m, 7H), 6.98 (d, J= 8 Hz, 4H), 6.93-6.90 (m, 4H), 6.72 (d, J= 16.5 Hz, 1H). **^{13}C NMR** (126 MHz; $CDCl_3$) δ 148.97, 147.35, 146.16, 143.89, 131.60, 128.69, 128.33, 126.91, 123.80, 122.77, 122.42, 121.71, 119.56 **HRMS** (ESI) m/z calcd for $C_{25}H_{21}N_2O^+$ $[M + H]^+$ 365.1648, found 365.1635.

(E)-4-(4-(dimethylamino)styryl)pyridine 1-oxide (DNO)







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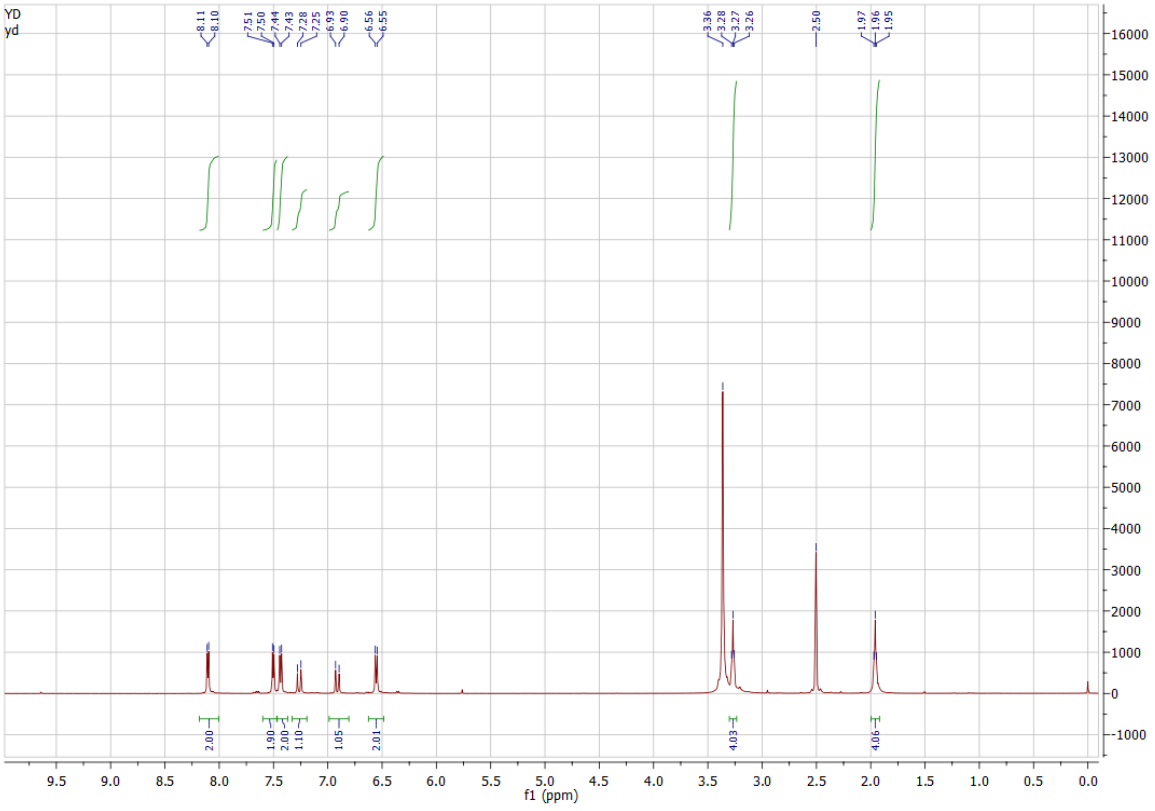

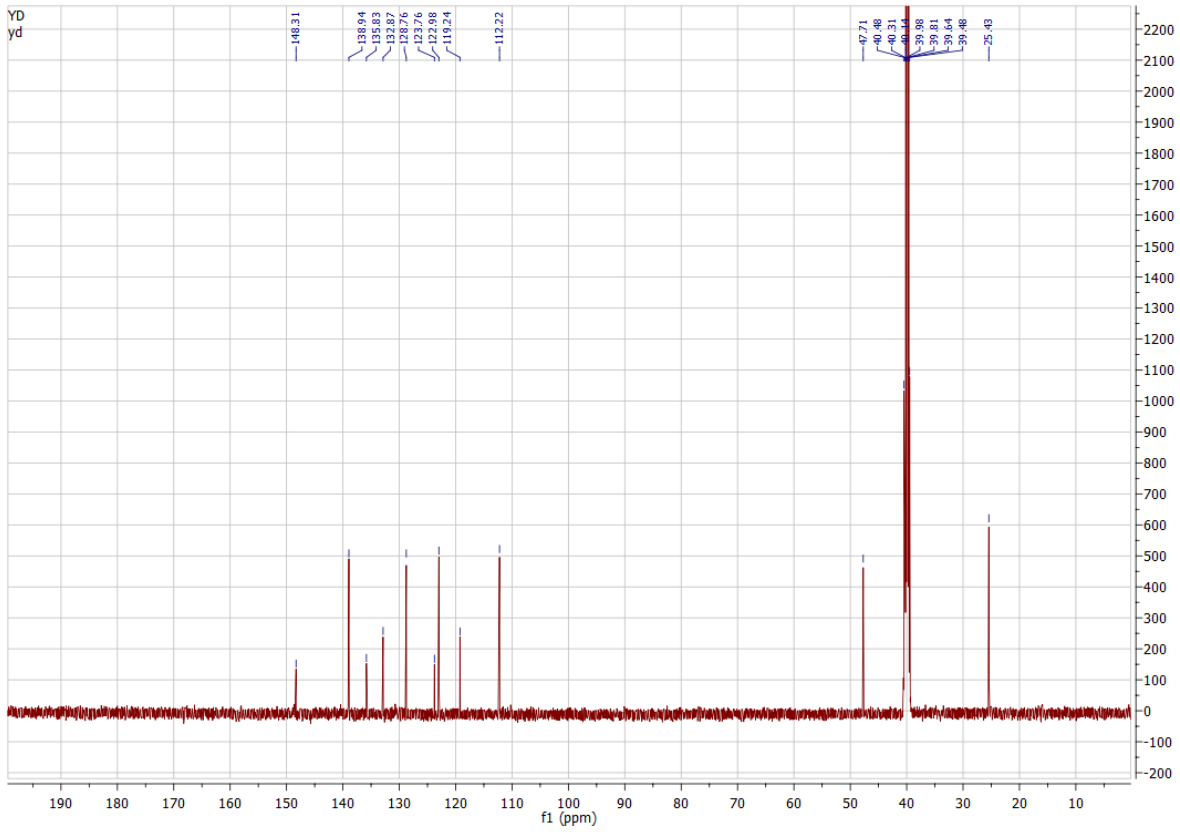
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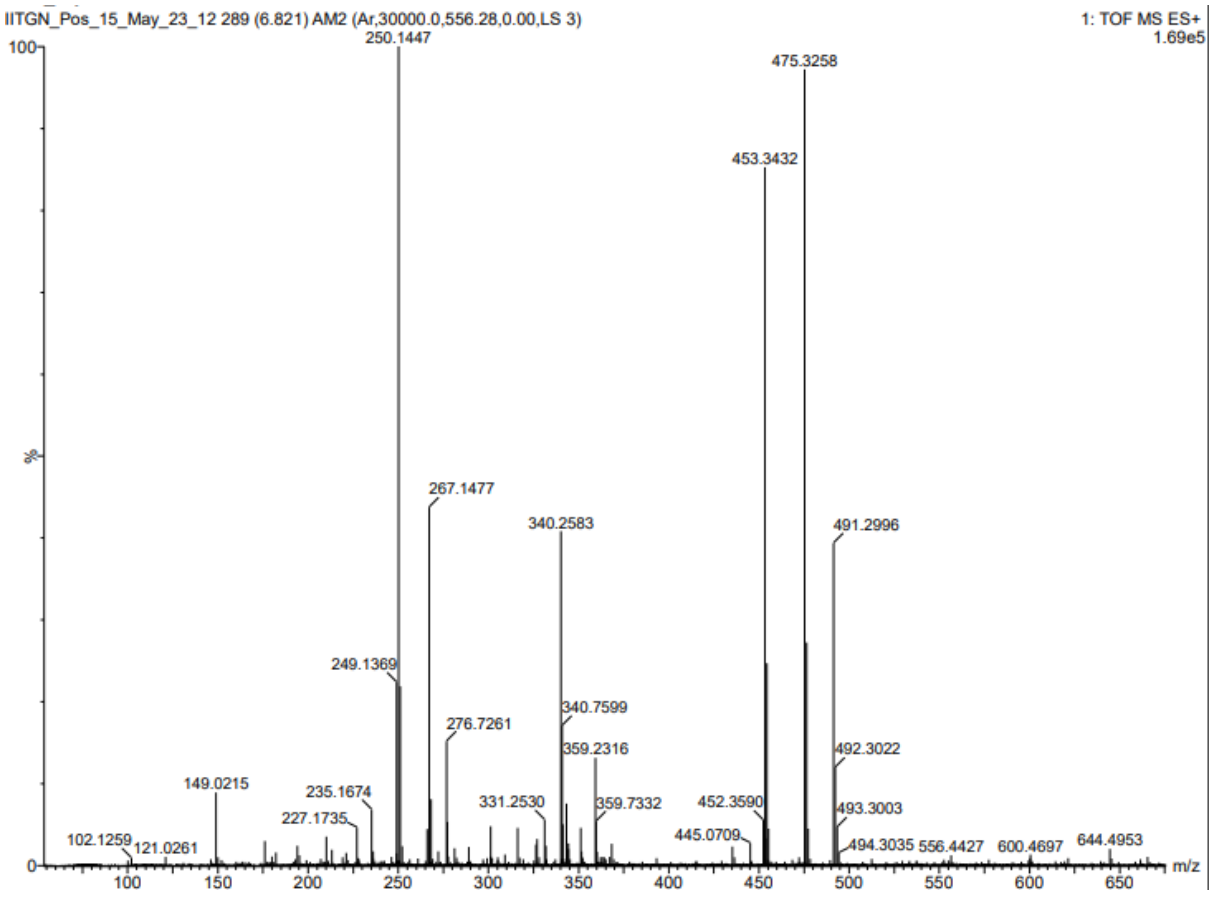
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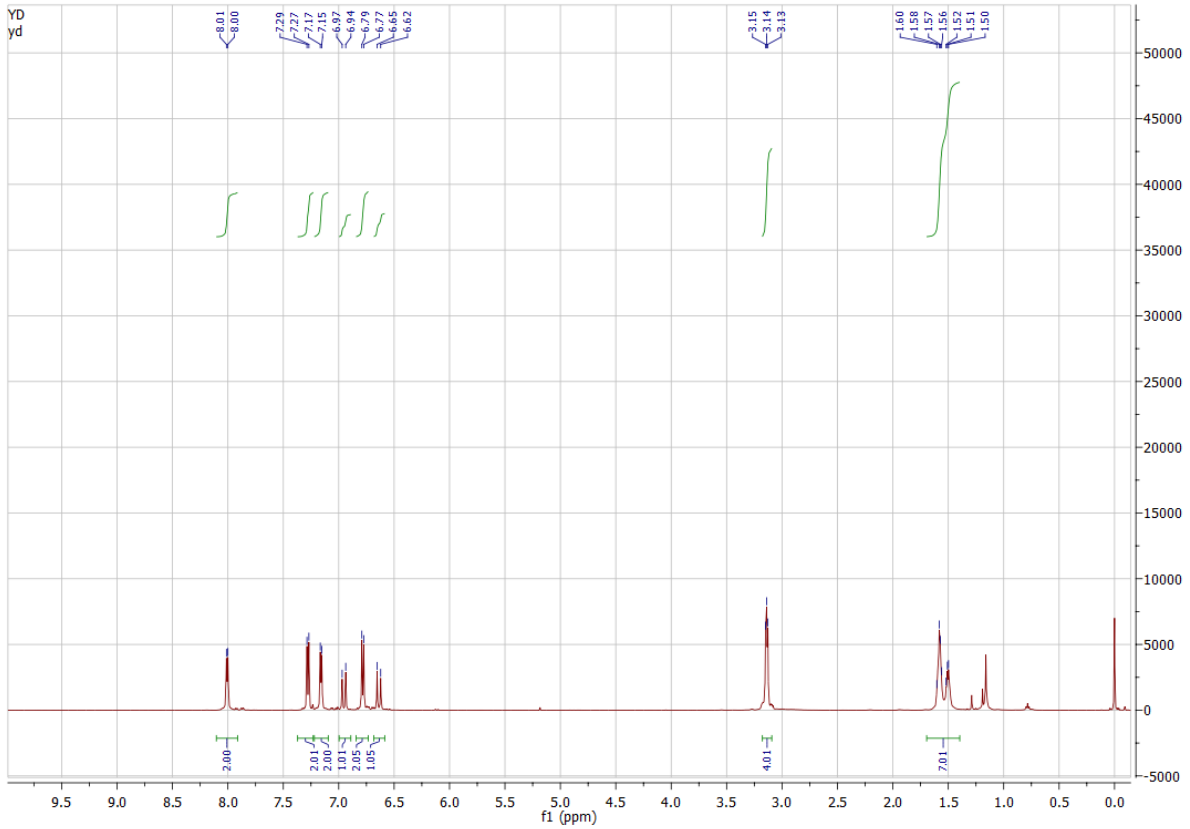
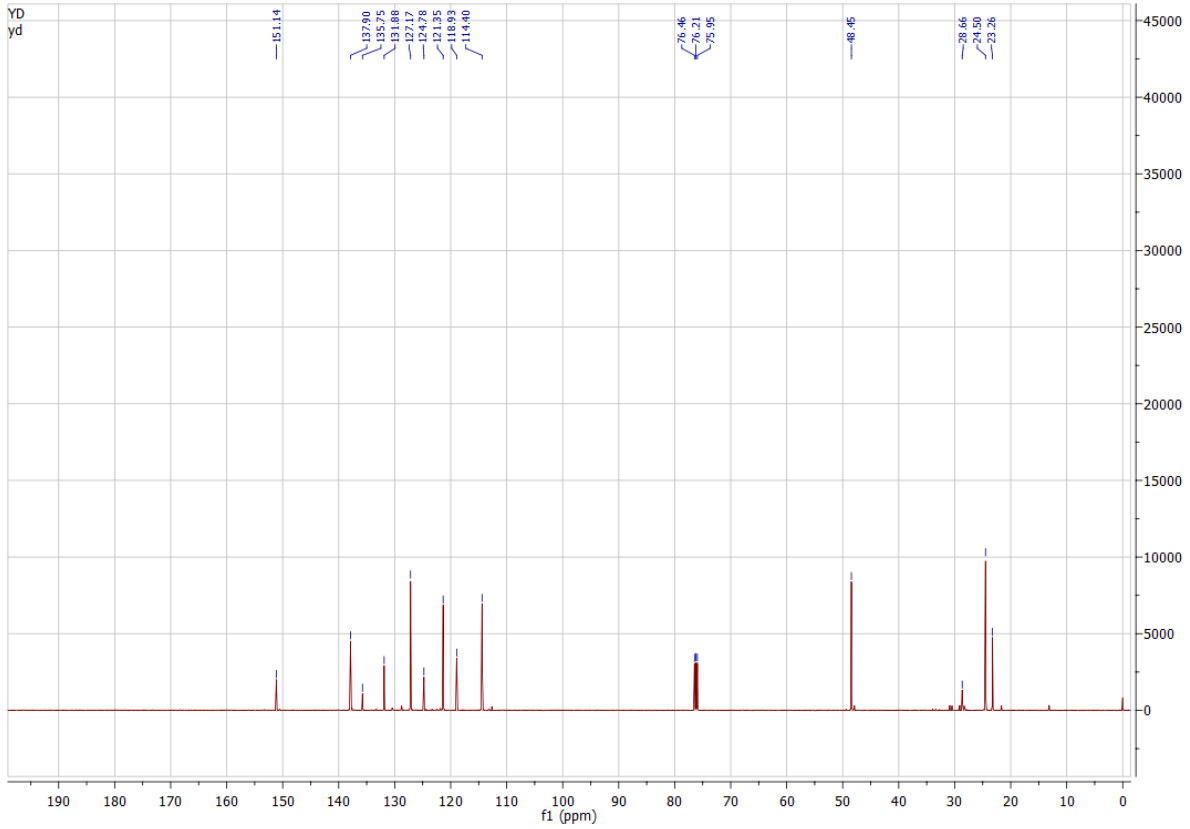
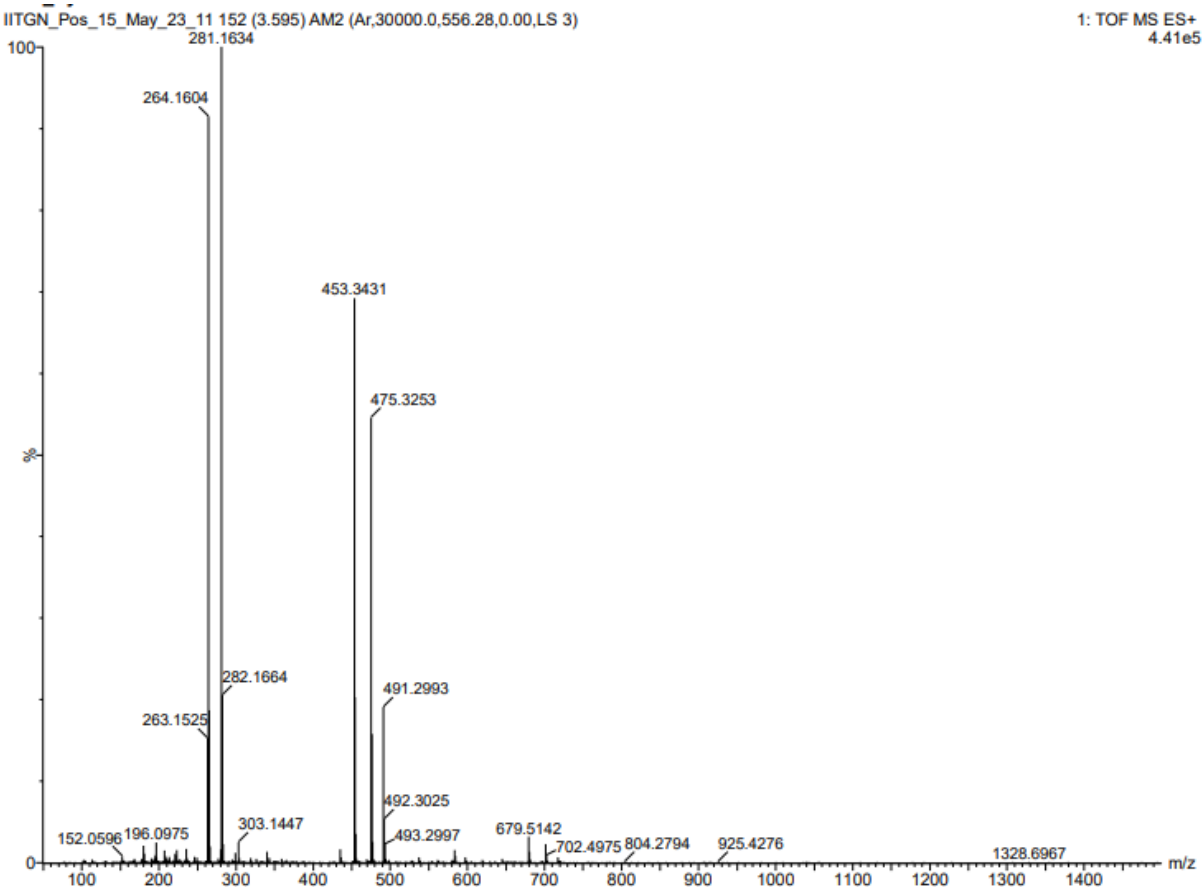
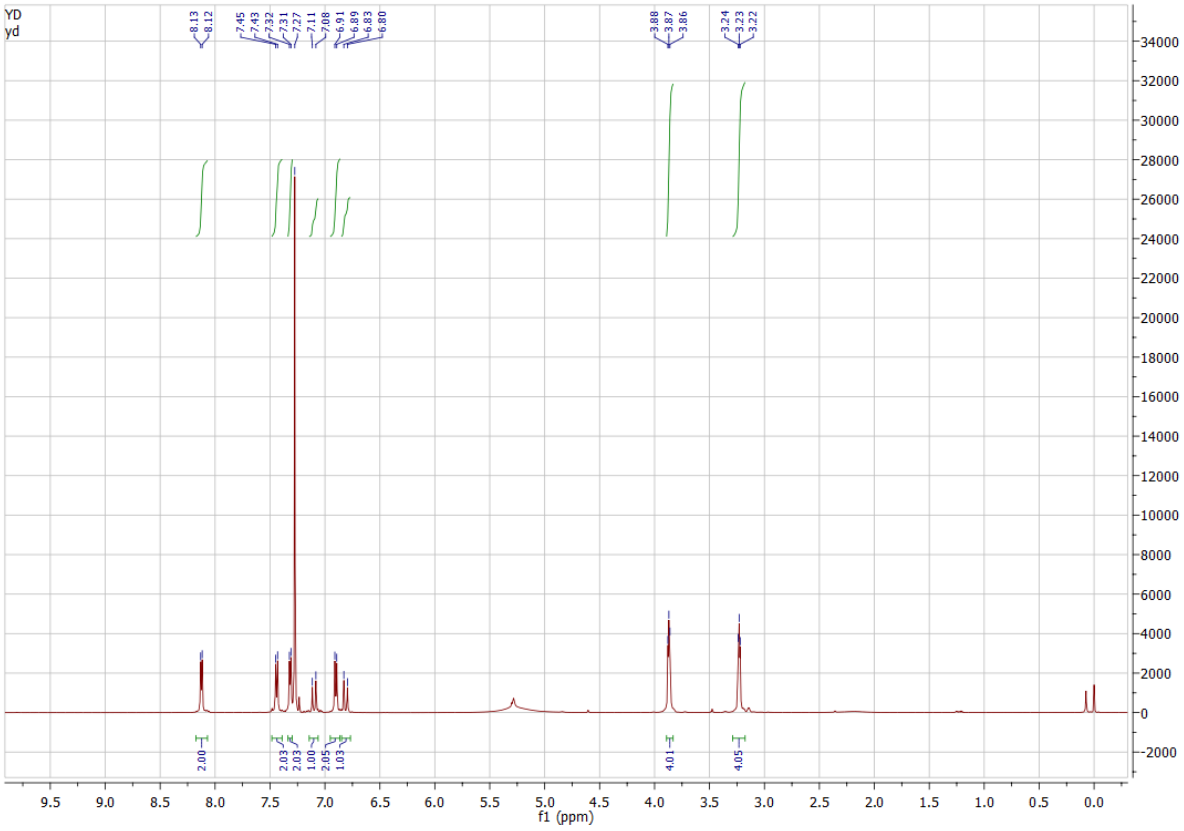
(E)-4-(4-(piperidin-1-yl)styryl)pyridine 1-oxide (6NO)

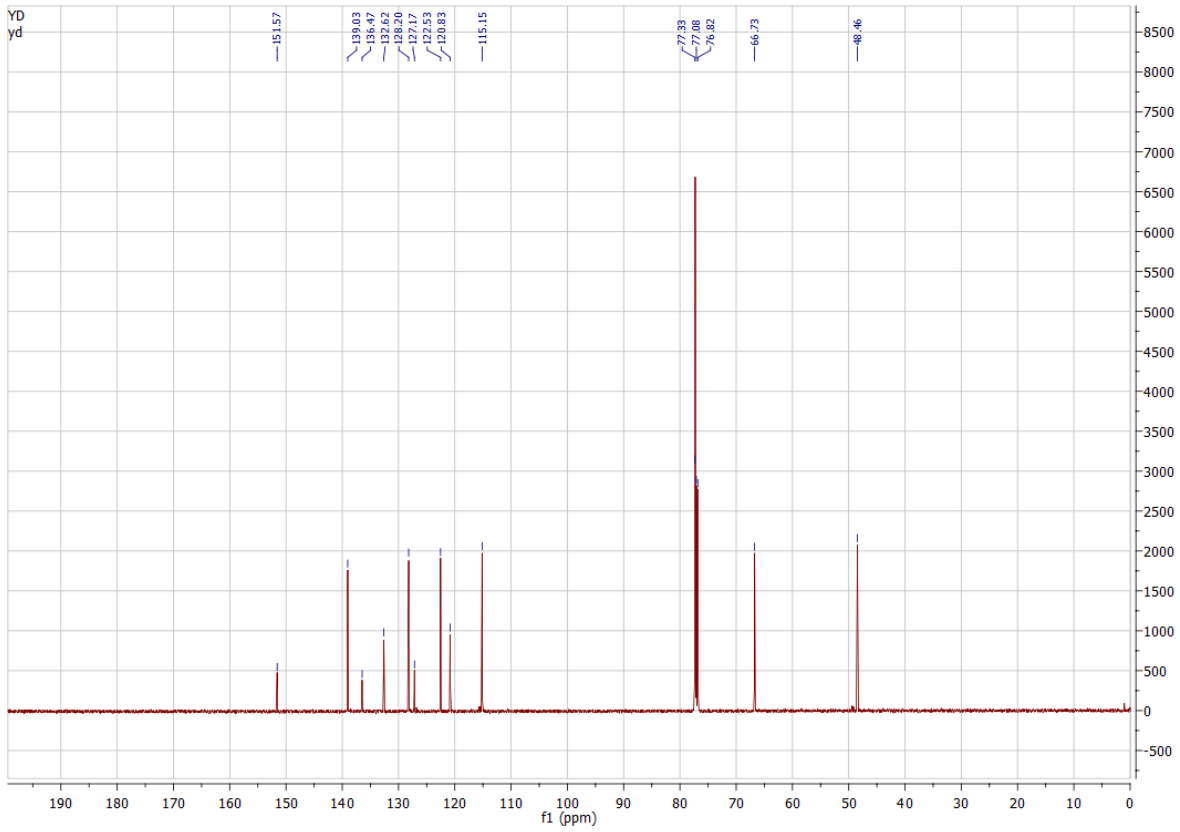


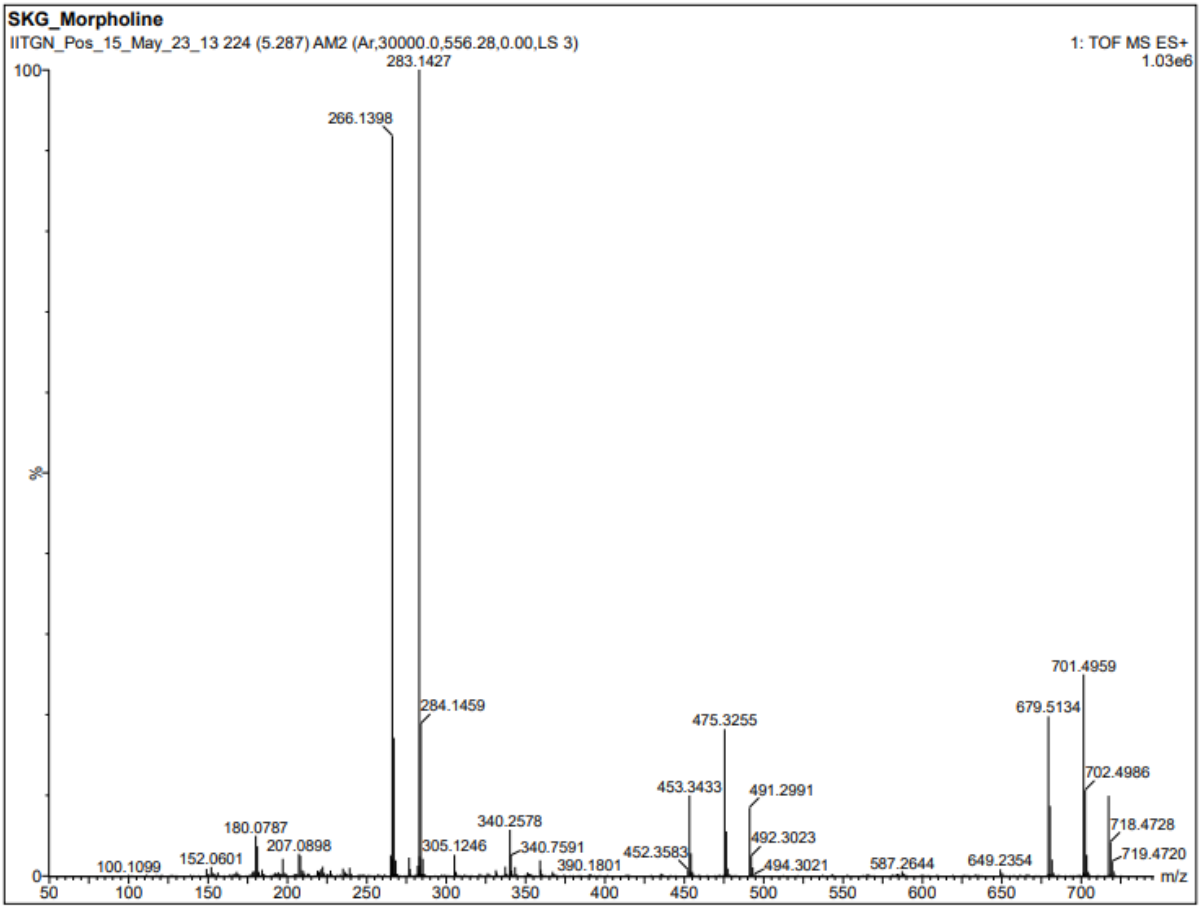




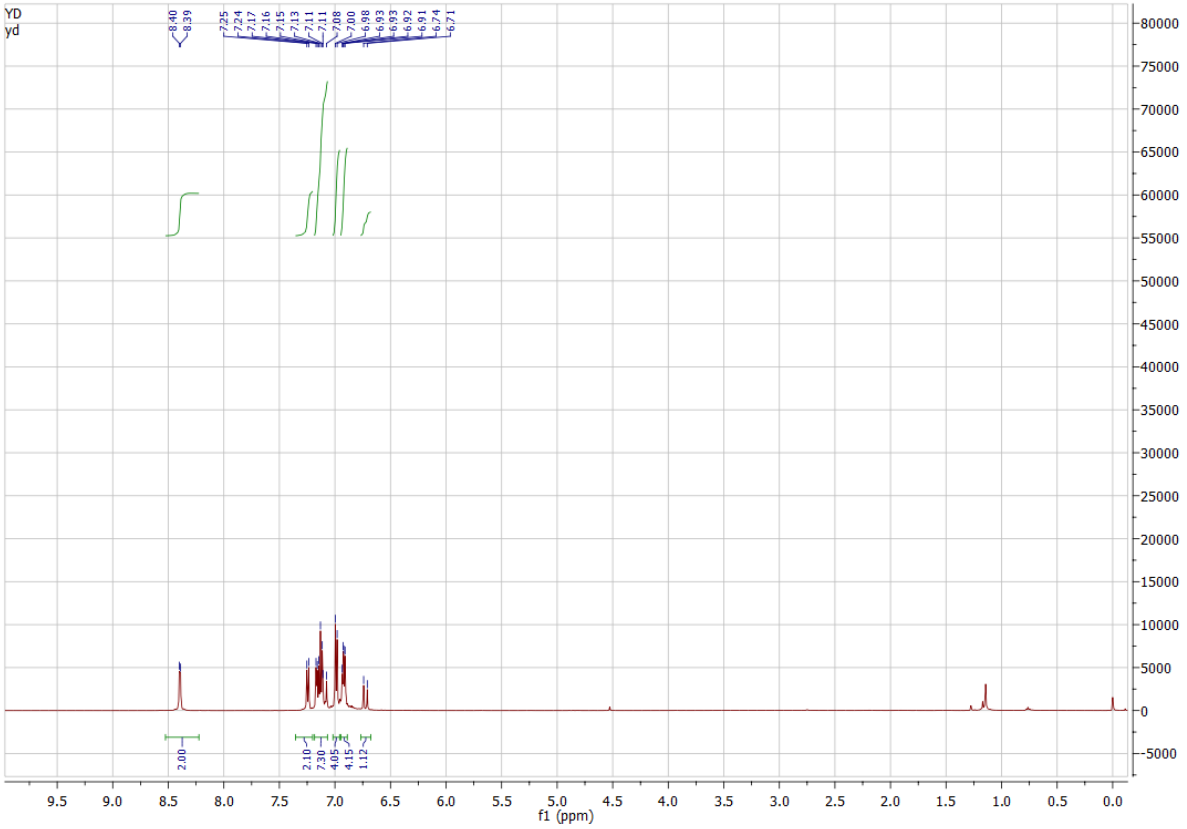
(E)-4-(4-morpholinostyryl)pyridine 1-oxide (MNO)

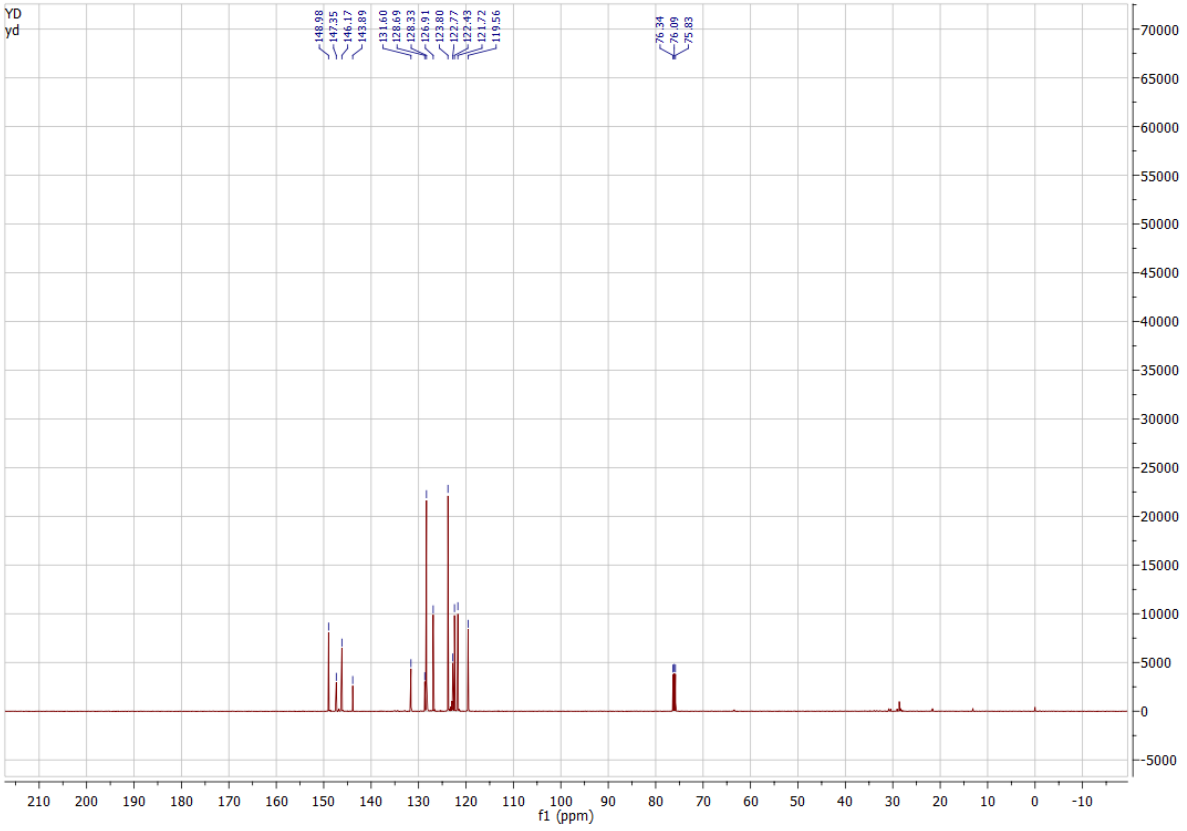


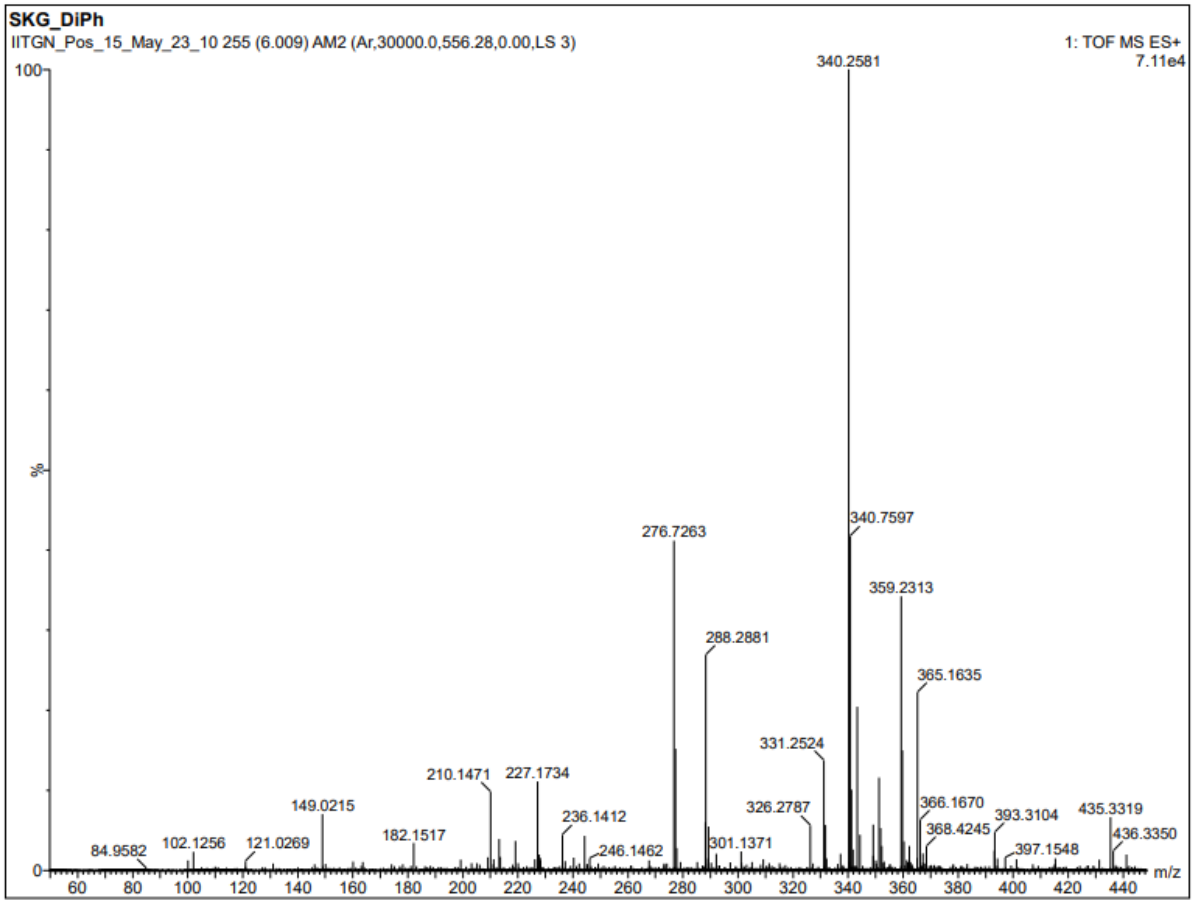

YD
yd
151.57
139.03
136.47
132.62
128.20
127.17
122.53
120.83
115.15
77.33
77.08
76.82
66.73
48.46
f1 (ppm)


SKG_Morpholine
IITGN_Pos_15_May_23_13 224 (5.287) AM2 (Ar,30000.0,556.28,0.00,LS 3)
1: TOF MS ES+
1.03e6
283.1427
266.1398
284.1459
475.3255
701.4959
679.5134
702.4986
453.3433
491.2991
718.4728
719.4720
340.2578
180.0787
207.0898
305.1246
340.7591
492.3023
152.0601
390.1801
452.3583
494.3021
100.1099
587.2644
649.2354
%
m/z

(E)-4-(4-(diphenylamino)styryl)pyridine 1-oxide (TNO)







References

1. Tydlitát, J., Achelle, S., Rodríguez-López, J., Pytela, O., Mikýsek, T., Cabon, N., ... & Bureš, F. *Dyes and Pigments*, 2017, ***146***, 467-478.
2. Kumari, B., Singh, A., Jana, P., Radhakrishna, M., & Kanvah, S. *New Journal of Chemistry*, 2019, ***43*****(29),** 11701-11709.