

Easy Access to 2,1,3-Benzothiadiazole-based Symmetric Deep Red-AIEgens for Concurrent Staining of Lipid Droplets and Lysosomes

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Contents

1. Materials and Methods-----	S1
2. PXRD patterns/TGA profile of MBM , DBD and TBT -----	S2
3. Solvatochromism of MBM , DBD and TBT -----	S2-S3
4. AIE/VIE and Lifetime study of MBM , DBD and TBT -----	S3-S5
5. pH dependent study of MBM , DBD and TBT -----	S5
6. Solid state photophysical of MBM , DBD and TBT -----	S6
7. Cytotoxicity and colocalization study of the designed probes in (a) A549 cells, and (b) NRK cells	S6
8. Oleic acid treatment for (a) MBM (b) DBD and (c) TBT -----	S7
9. Cellular localisation of MBM in A549 cells	S7
10. Photostability of MBM -----	S8
11. Synthesis and Characterisation -----	S8-S12

Materials and Methods

General Experimental Information

All the required chemicals were purchased from various companies and used without purification. All the products were characterized by ¹H, and ¹³C NMR spectroscopy. NMR spectra were recorded on a Bruker 400 MHz instrument (400 MHz for ¹H NMR, and 101 MHz for ¹³C NMR). ¹H NMR experiments are reported in units, parts per million (ppm), and were measured relative to residual DMSO (2.50 ppm) in the deuterated solvent. ¹³C NMR spectra are reported in ppm relative to deuterated DMSO (39.52 ppm) and all were obtained with ¹H decoupling. Coupling constants were reported in Hz. Reactions were monitored by thin-layer chromatography (TLC). liquid chromatography-mass spectrometry (LC-MS) was obtained by

the electron spray ionization (ESI) technique using the Q-TOF mass analyser and was reported as m/z (relative intensity). Melting points of compounds were recorded on a KRUSS Optronic M3000 apparatus.

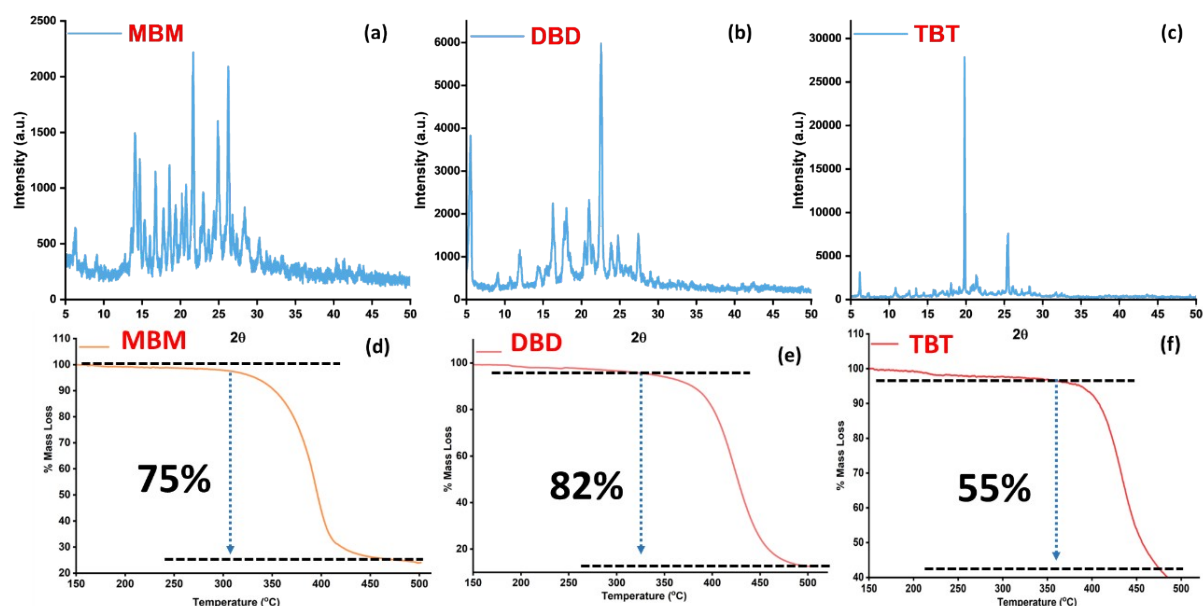


Fig S1. PXRD patterns of (a) MBM (b) DBD and (c) TBT, TGA profile of (d) MBM (e) DBD and (f) TBT

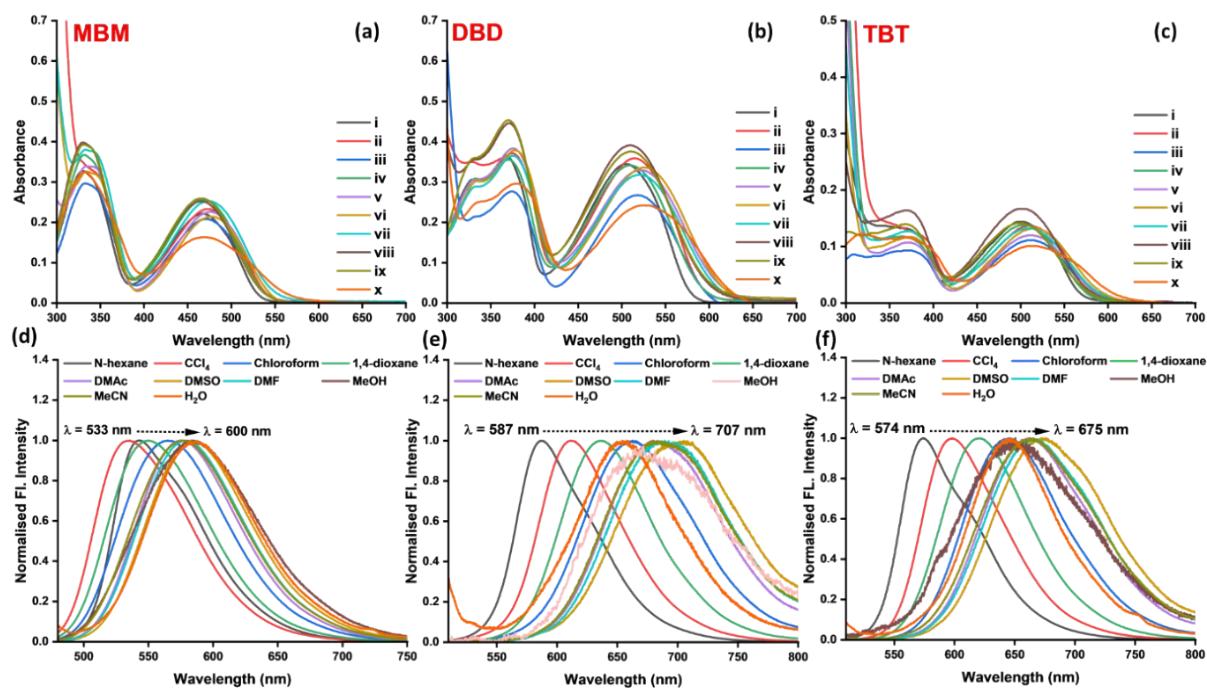


Fig S2. UV-Vis spectrum of (a) MBM (b) DBD and (c) TBT in different solvents. i- N-hexane, ii- CCl₄, iii- chloroform, iv- I,4-dioxane, v- DMAc, vi-DMSO, vii-DMF, viii-MeOH, ix-MeCN, x- H₂O ,Fl. spectrum of (d) MBM, (e) DBD, and (f) TBT in different solvents.

Table S1. Tabulated photophysical parameters of **MBM**, **DBD** and **TBT** in different solvents (10 uM)

Solvents	MBM			DBD			TBT		
	λ_{abs}	λ_{emi}	Φ_f	λ_{abs}	λ_{emi}	Φ_f	λ_{abs}	λ_{emi}	$\Phi_f(\%)$
<i>n</i> -hexane	467	533	24.08	500	587	15.91	500	574	16.19
CCl ₄	473	565	36.04	500	611	24.99	500	597	34.23
Chloroform	471	595	20.04	520	663	2.58	513	645	7.37
1,4-dioxane	464	580	29.09	526	636	13.42	513	620	15.48
DMAc	478	606	24.83	526	679	2.43	513	660	4.95
DMSO	478	614	19.69	526	705	0.97	497	676	2.75
DMF	471	607	20.22	521	689	1.34	497	665	3.39
MeOH	478	615	1.11	505	660	0.07	497	665	0.13
MeCN	465	606	15.80	508	685	1.01	499	663	2.49
H ₂ O	468	612	2.32	526	654	0.65	497	675	1.35

The relative quantum yield of the probe was measured with respect to Nile Red (in Ethanol) using formula,

$$\Phi_f = \Phi_{ref} \times \frac{a_{ref}}{a_{sam}} \times \frac{A_{sam}}{A_{ref}} \times \left(\frac{n_{sam}}{n_{ref}}\right)^2$$

Where, Φ_f = quantum yield of probe Φ_{ref} = quantum yield of quinine sulfate (0.12)

A_{ref} = area under curve of emission spectra of Nile Red in Ethanol, A_{sam} = area under curve of emission spectra of probe, a_{sam} = Absorbance of probe, a_{ref} = Absorbance of quinine sulfate in 0.1M H₂SO₄, n_{sam} = refractive index of respective solvents, n_{ref} = refractive index of ethanol.

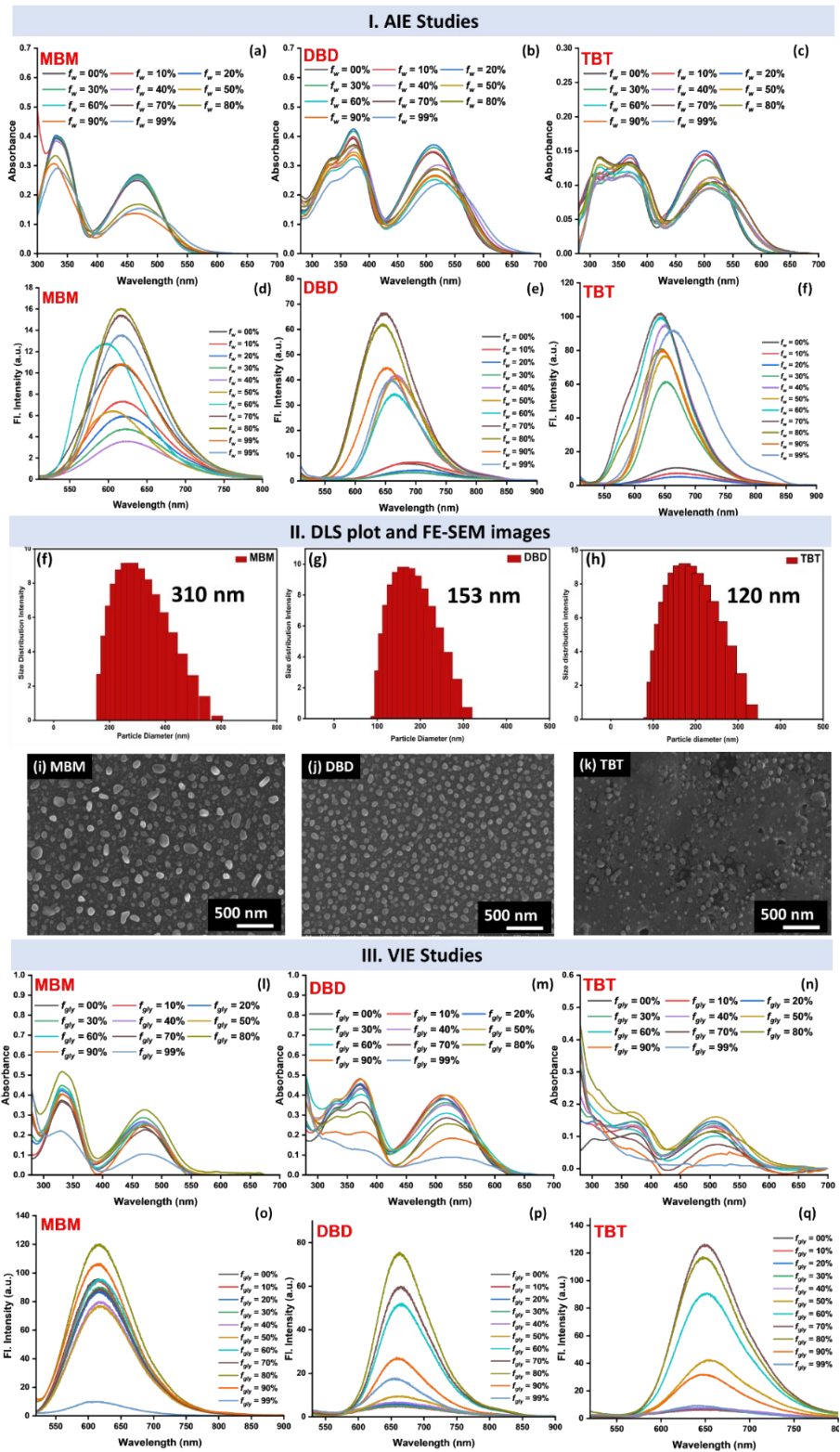


Fig S3. (I) UV-Vis (a-c) and Fl. spectrum (d-f) with different (f_w), (II) DLS plot (f-h) and FE-SEM images (i-k) of **MBM** ($f_w = 80\%$), **DBD** ($f_w = 70\%$), and **TBT** ($f_w = 70\%$), (III) UV-Vis (l-n) and Fl. spectrum (o-q) with different fractions of glycerol of **MBM**, **DBD** and **TBT**.

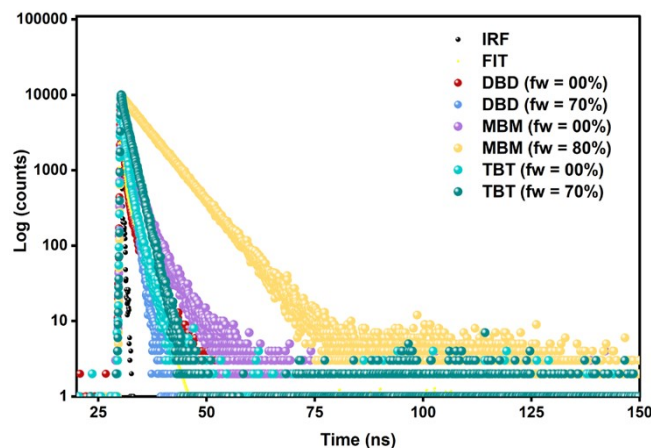


Fig S4 lifetime decay plot for MBM, DBD and TBT in AIE state

Table S2 Lifetime parameters for MBM, DBD and TBT in AIE state

Sample	τ_1	τ_2	α_1	α_2	Avg. $\langle\tau\rangle$ ns	χ^2
MBM ($f_w = 00\%$)	0.63	2.84	0.80	0.20	1.80	1.1
MBM($f_w = 80\%$)	0.15	6.17	0.44	0.56	6.06	1.0
DBD ($f_w = 00\%$)	0.23	2.31	0.96	0.04	0.76	1.0
DBD ($f_w = 70\%$)	0.24	0.98	0.48	0.52	0.84	1.0
TBT ($f_w = 00\%$)	1.69	0.41	0.85	0.15	0.94	1.0
TBT($f_w = 70\%$)	0.21	1.73	0.45	0.55	1.60	1.0

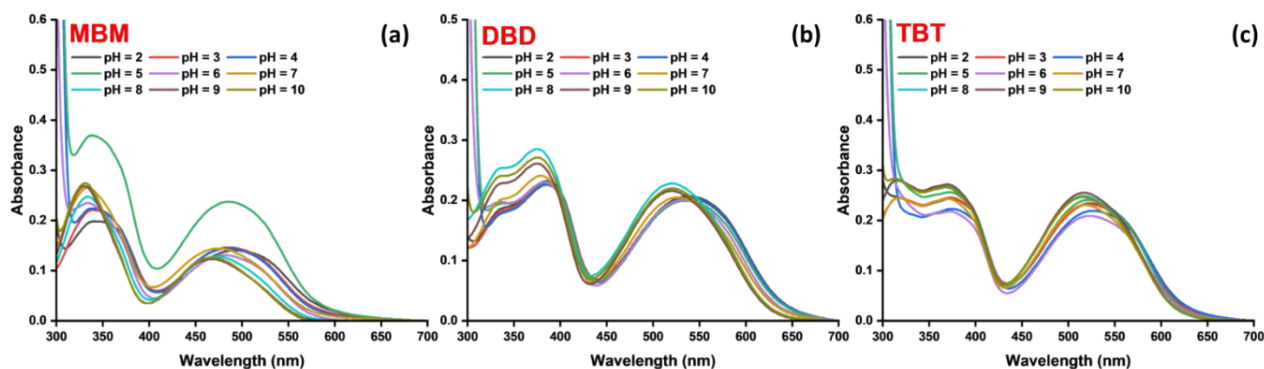


Fig S5. pH-dependent UV-Vis spectrum of (a) MBM, (b) DBD, and (c) TBT

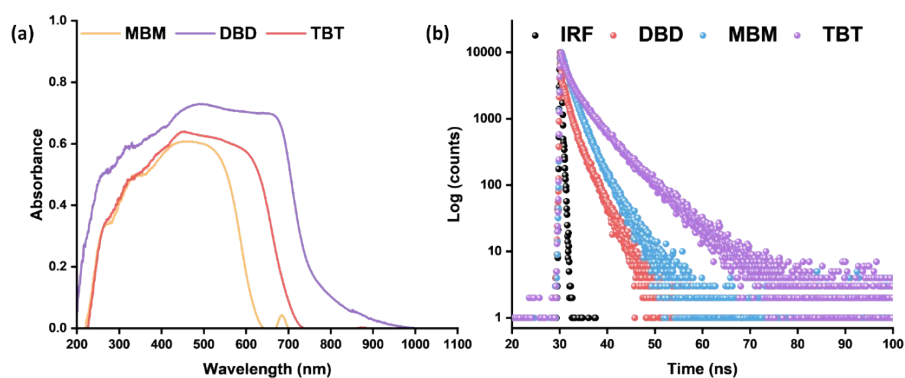
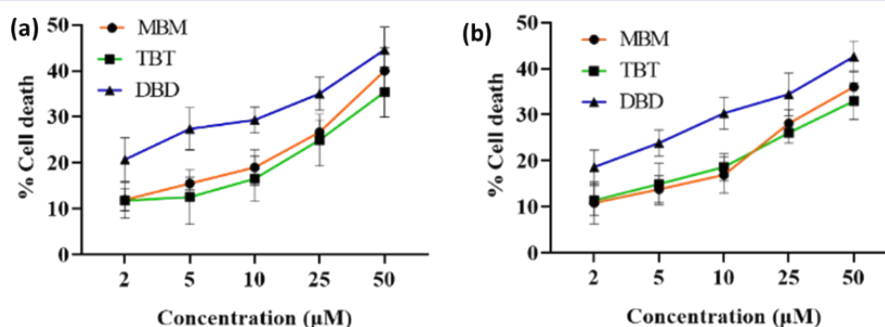


Fig S6. Solid state (a) UV-Vis spectrum (b) lifetime decay curve of **MBM**, **DBD** and **TBT**.

Table S3. Lifetime parameters for **MBM**, **DBD** and **TBT** in solid state

Sample	λ_{emi} (nm)	Φ_f (%)	τ_1	τ_2	a_1	a_2	Avg. $\langle\tau\rangle$ (ns)	χ^2	$k_r \times 10^6$ s^{-1}	$k_{nr} \times 10^6$ s^{-1}
MBM	630	12	0.92	2.83	0.66	0.34	2.09	1.0	57.4	421.05
DBD	680	3	0.04	2.24	0.98	0.02	1.15	1.1	26.0	843.5
TBT	730	8	1.32	5.62	0.71	0.29	4.03	1.0	19.8	228.3

I. Cytotoxicity studies



II. Colocalization studies

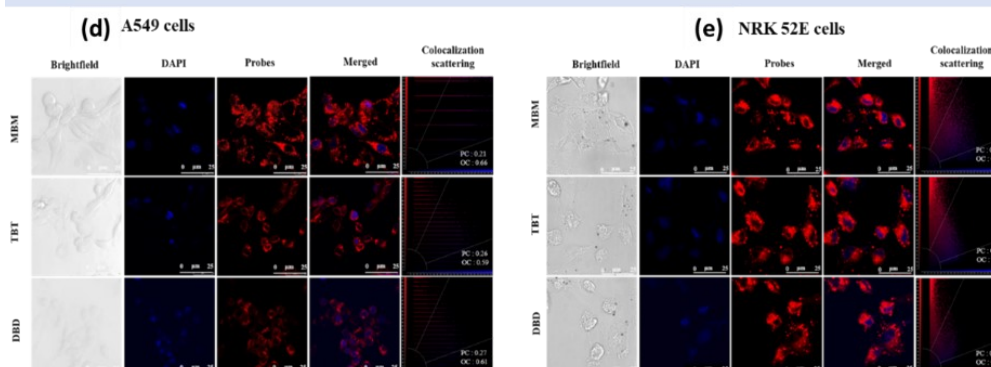


Fig S7.(I) Cytotoxicity study of the designed probes in (a) A549 cells, and (b) NRK cells, the MTT assays were carried out in A549 cells and NRK cells using the probes with different concentration 2, 5, 10, 25, 50 μM for 24 h. (II) Colocalization studies of **MBM**, **DBD** and **TBT**

with nucleus staining dye 4',6-diamidino-2-phenylindole (DAPI) for (d) A549 and (e) NRK 52E cells (fixed).

Table S4. Respective Pearson's correlation coefficient for **MBM**, **DBD** and **TBT** for lysosome and LD in the fixed cells

Probes	Pearson correlation for Lysosome	Pearson correlation for LDs
MBM	0.94	0.92
DBD	0.91	0.89
TBT	0.89	0.92

Oleic acid study (a) MBM (b) DBD and (c) TBT

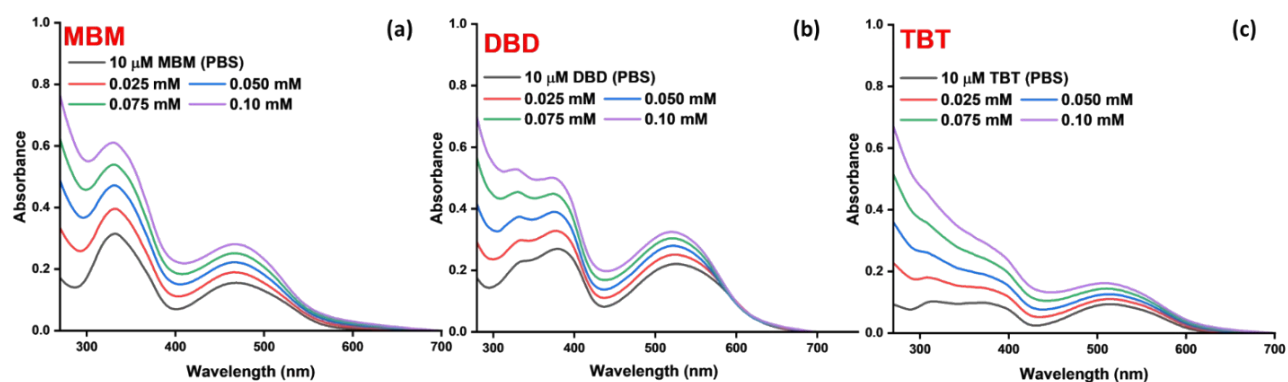


Fig S8. UV-Vis spectrum of (a) **MBM** (b) **DBD** and (c) **TBT** upon treatment of oleic acid

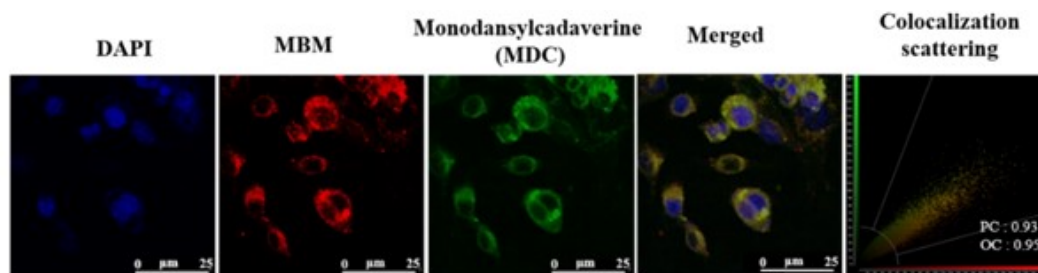


Fig S9. Cellular localisation of **MBM** in A549 cells. Merged image shows the co-localisation of **MBM** probe with MDC (a standard dye for APGs).

Photostability of probes

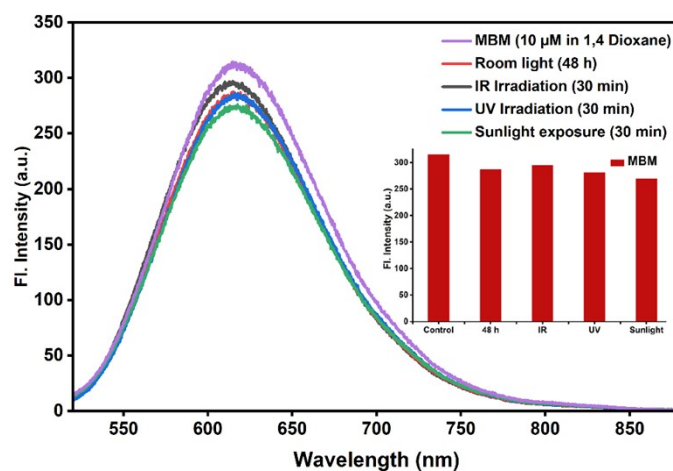


Fig S10. Fl. spectrum of MBM upon UV, IR, Sunlight irradiation and after 48h

Synthesis and Characterisation

HRMS, ^1H NMR, ^{13}C NMR and IR spectrum

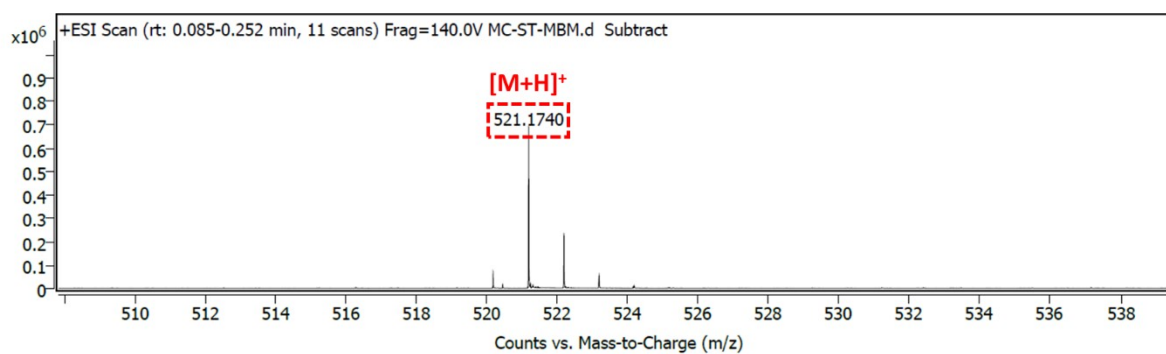


Fig S11. HRMS spectra for MBM

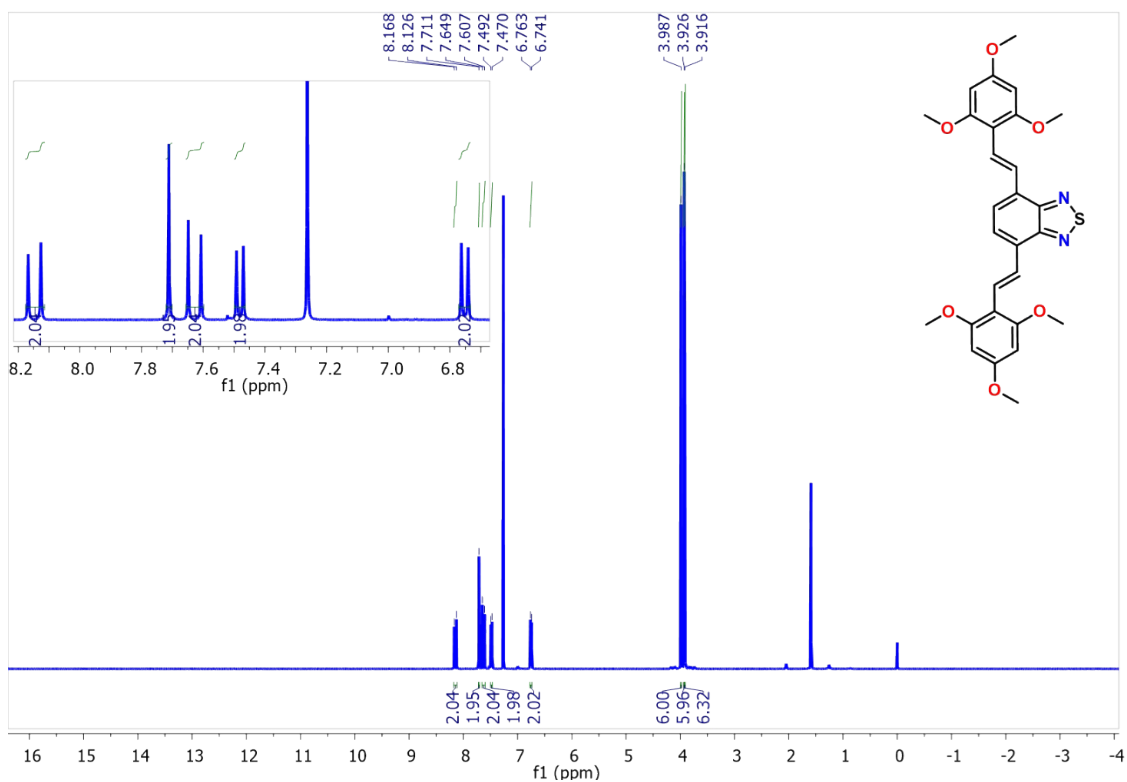


Fig S12 ^1H NMR spectra for MBM in CDCl_3

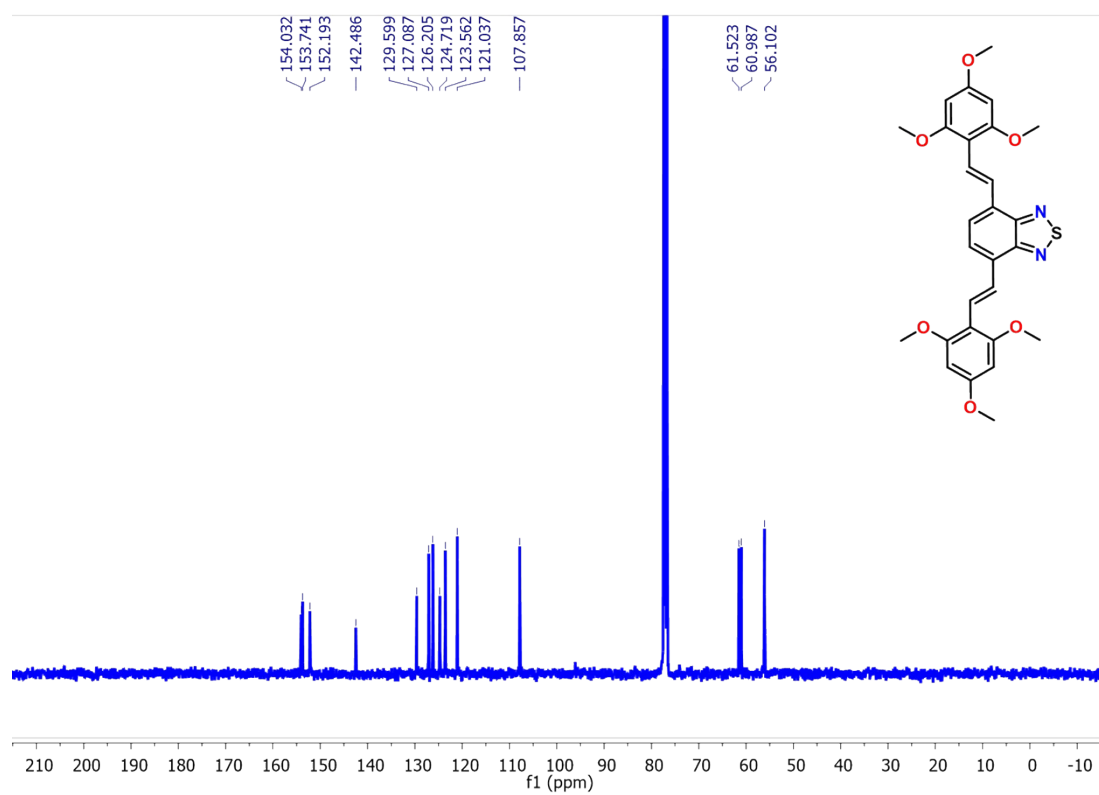


Fig S13. ^{13}C NMR spectra for MBM in CDCl_3

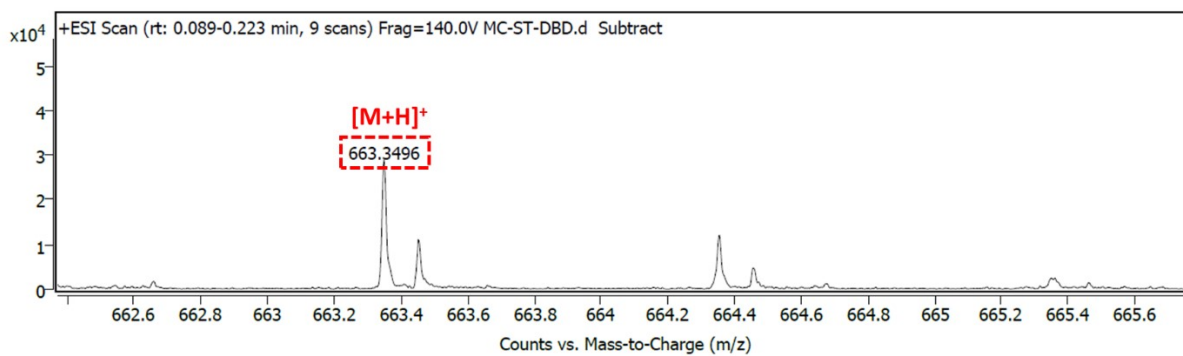


Fig S14. HRMS spectra for DBD

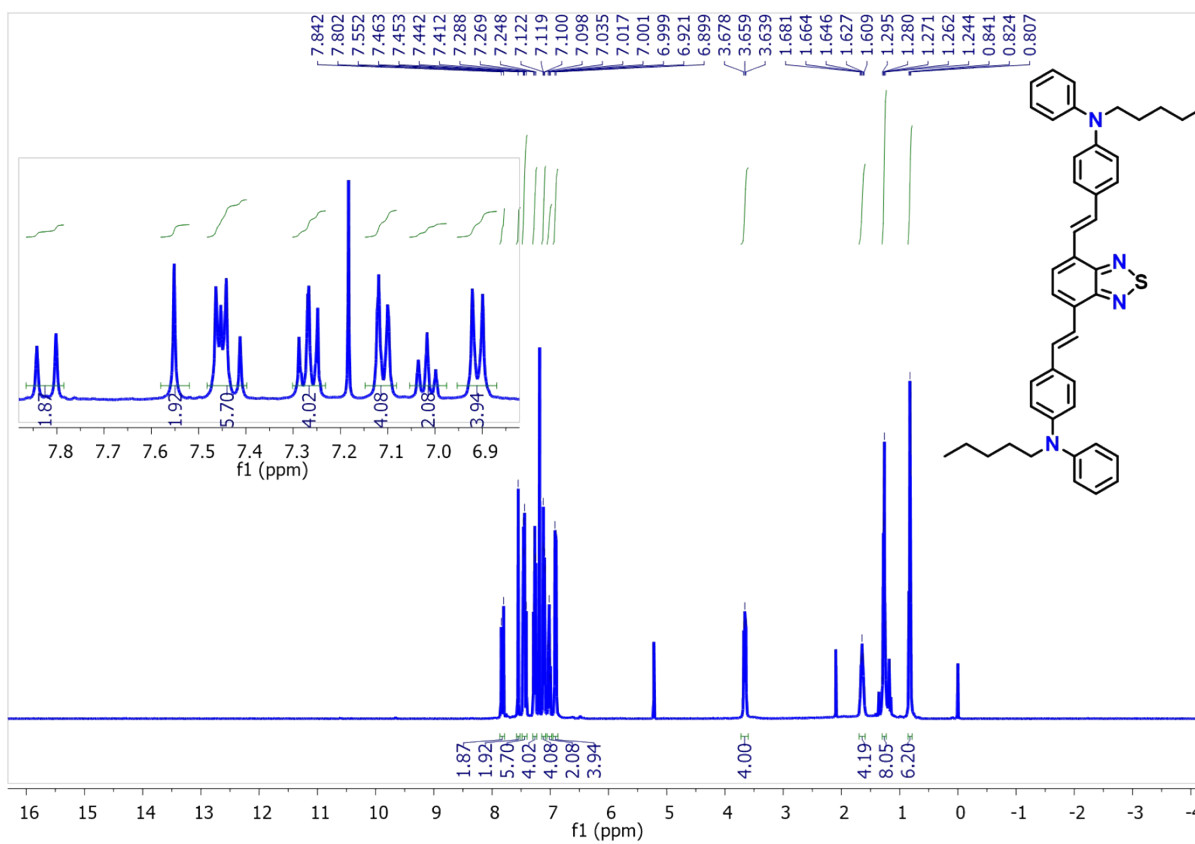


Fig S15. ^1H NMR spectra for **DBD** in CDCl_3

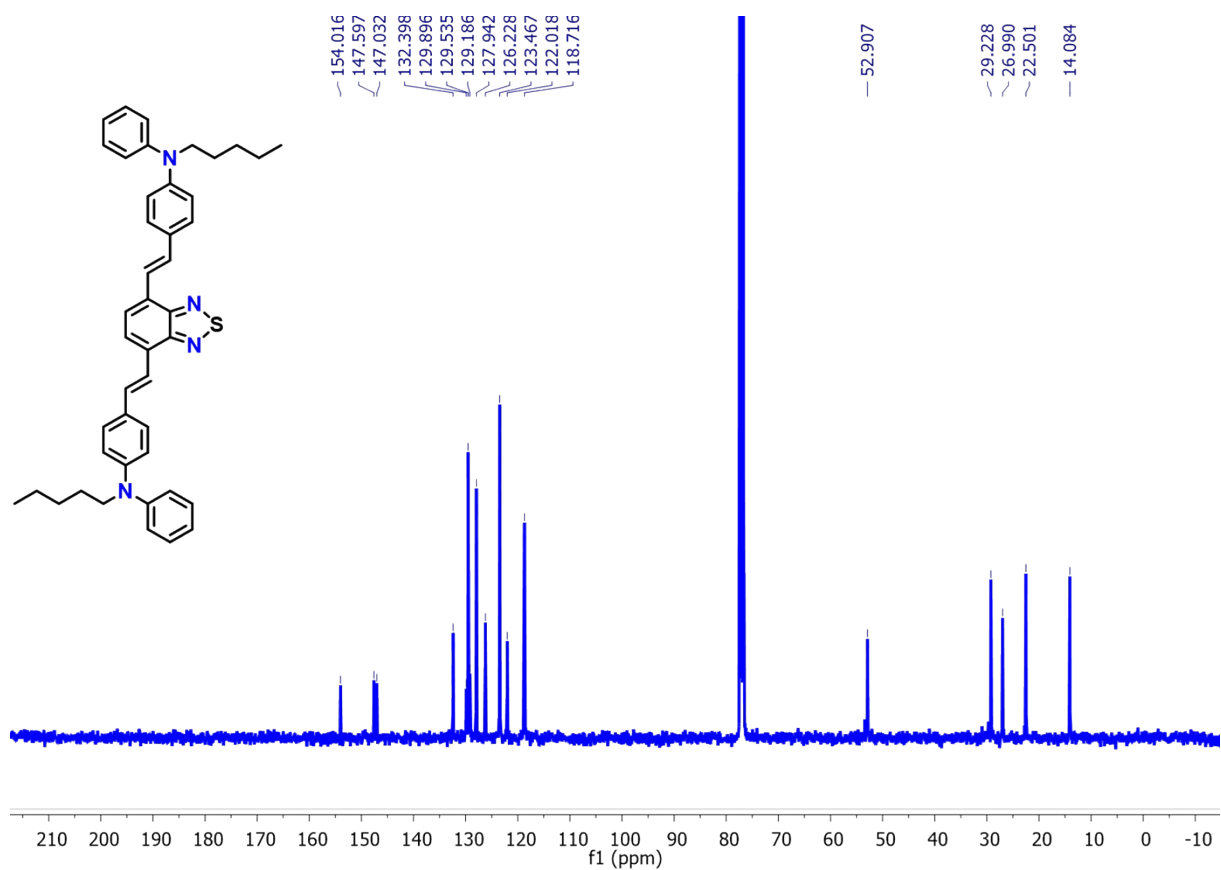


Fig S16. ^{13}C NMR spectra for **DBD** in CDCl_3

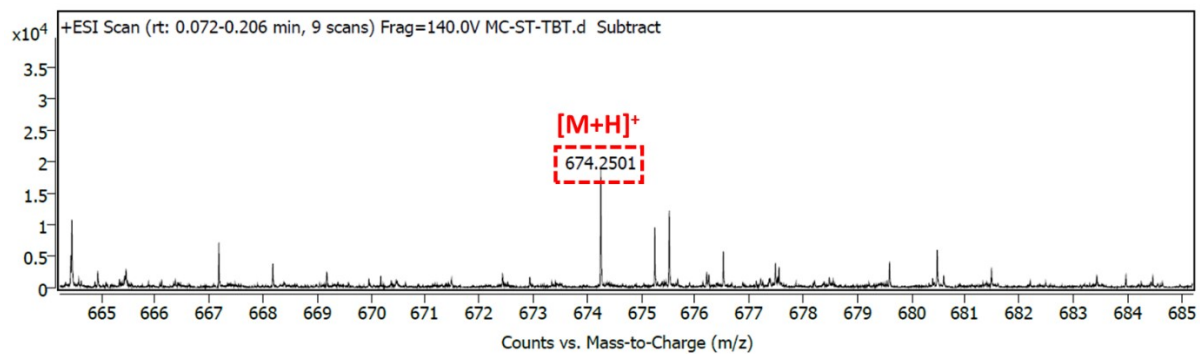


Fig S17. HRMS spectra for **TBT**

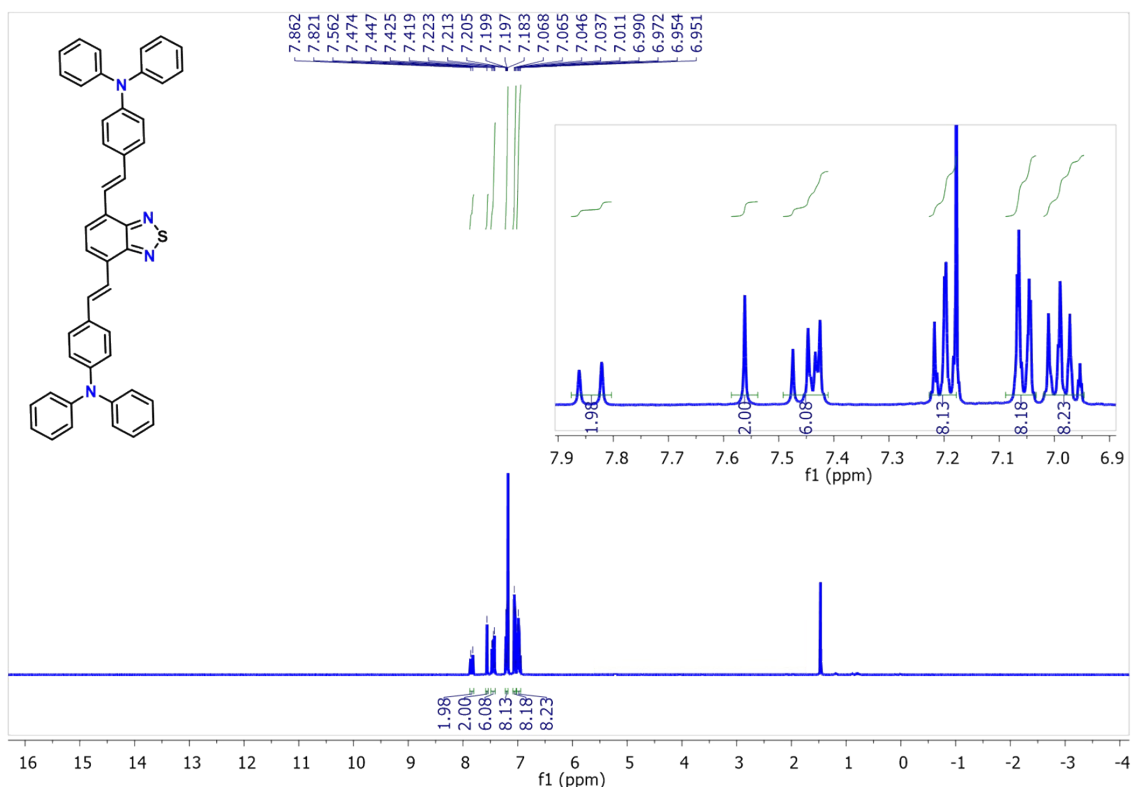


Fig S18. ¹H NMR spectra for TBT in CDCl₃

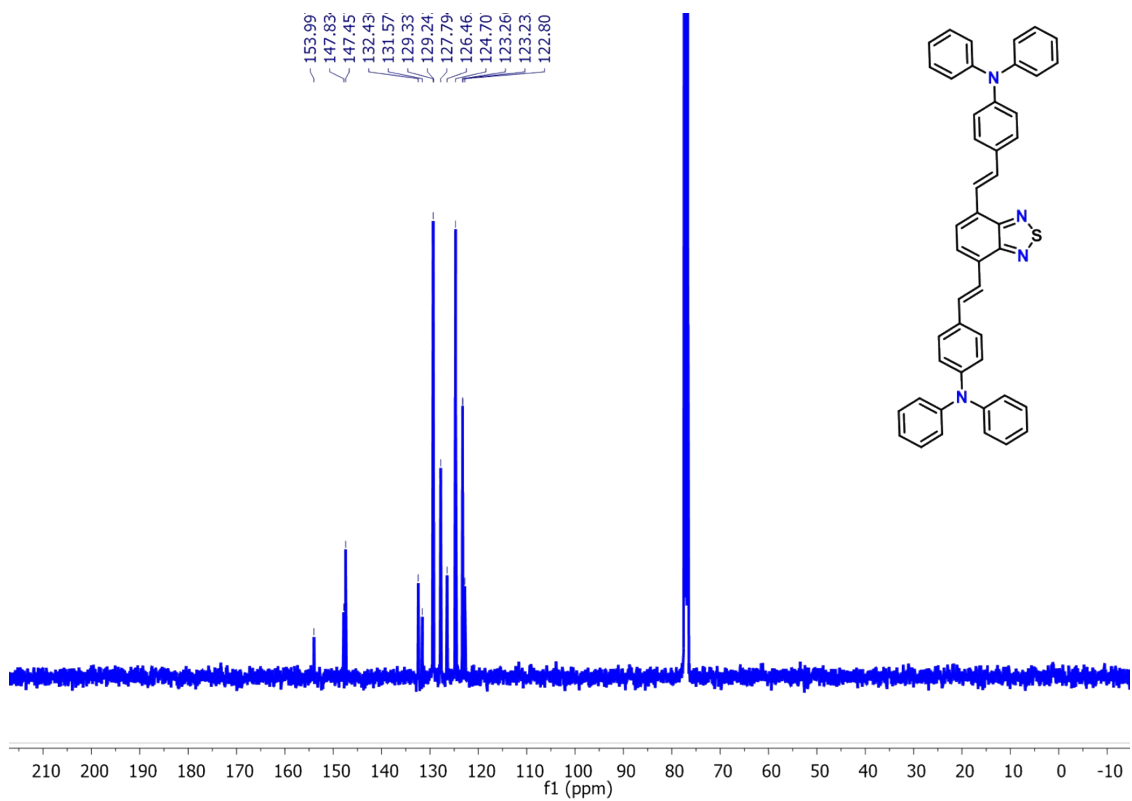


Fig S19. ¹³C NMR spectra for TBT in CDCl₃