Supplementary data

PET-Driven Fluorescence Modulation in Halochromic Styryl Hemicyanine Dyes Targeting DNA Minor Groove

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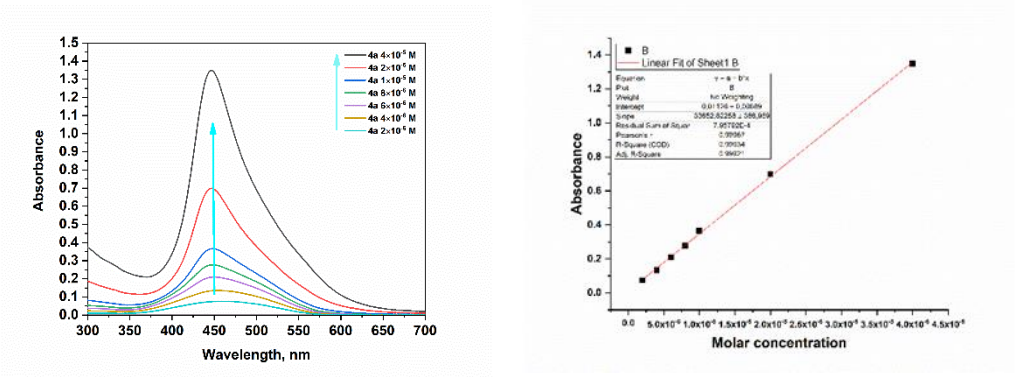
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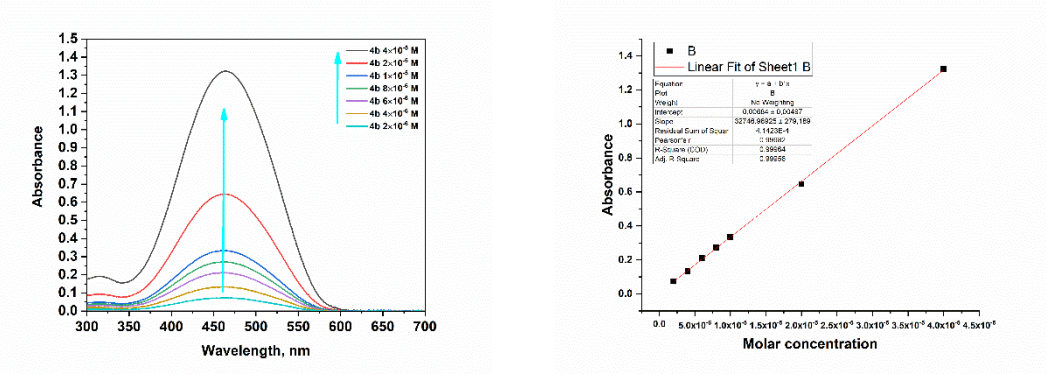
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UV/VIS spectra and calibration curves for molar extinction coefficients (ε)



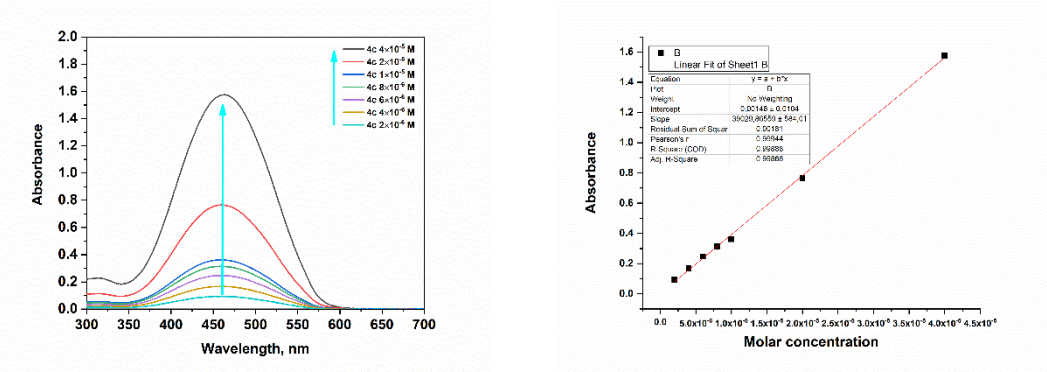
(**a**) **4a** (**b**) **4a**

Figure S1. UV/VIS spectra and calibration curve of dye **4a**, c= 0.2 – 4×10^{-5} M in TE buffer.



(**a**) **4b** (**b**) **4b**

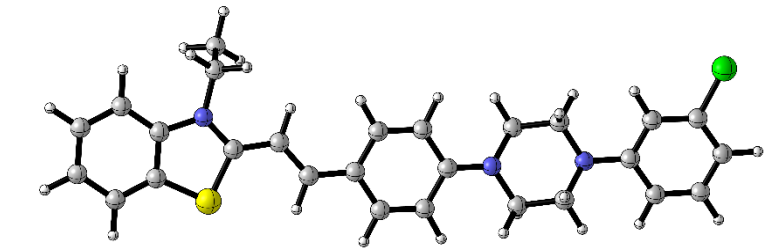
Figure S2. UV/VIS spectra and calibration curve of dye **4b**, c= 0.2 – 4×10^{-5} M in TE buffer.



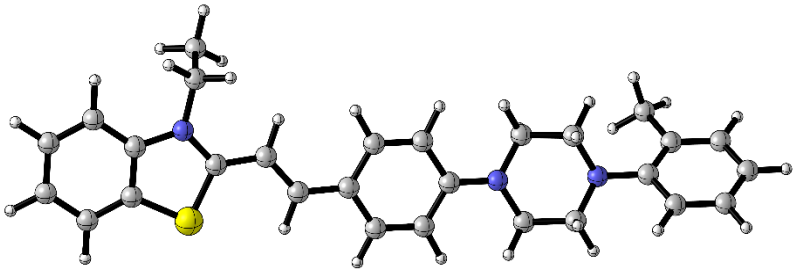
(**a**) (**b**)

Figure S3. UV/VIS spectra and calibration curve of dye **4c**, c= 0.2 – 4×10^{-5} M in TE buffer.

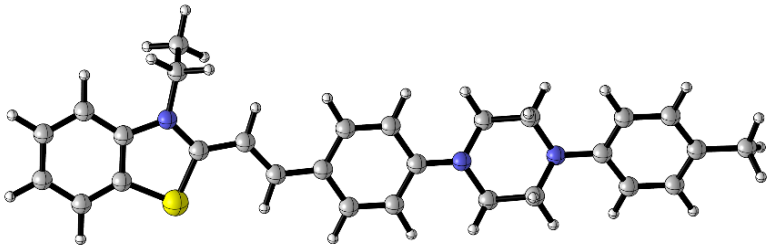
Geometry optimization of the trans isomers of compounds 4a-4c



4a



4b



4c

Figure S4. B3LYP/6-31+G(d,p) optimized geometries of trans isomers of **4a**-**4c**. Color scheme: C–gray, S–yellow, H–white, N–blue.

Solvatochromic measurements in solvents with different polarity

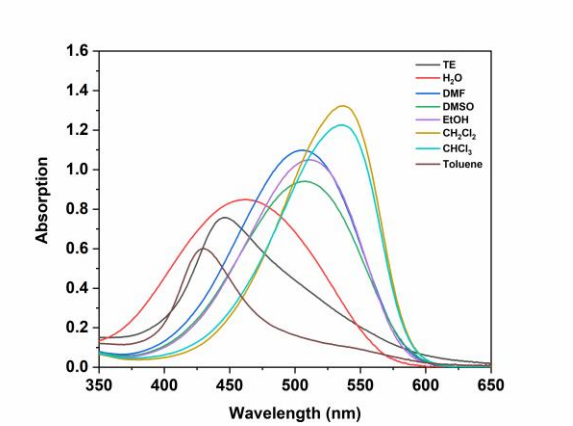


Figure S5. Position and intensity of the CT absorption maxima for dye **4a** (2×10^{-5} M) in solvents with different polarity.

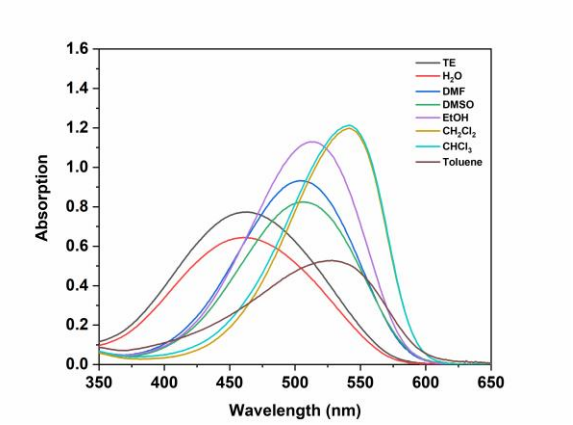


Figure S6. Position and intensity of the CT absorption maxima for dye **4b** (2×10^{-5} M) in solvents with different polarity.

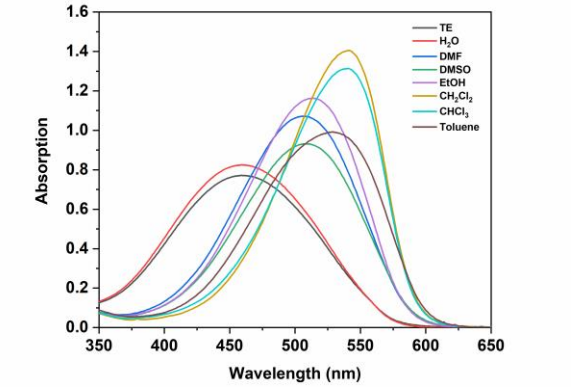


Figure S7. Position and intensity of the CT absorption maxima for dye **4c** (2×10^{-5} M) in solvents with different polarity.

Table S1. Kamlet–Taft parameters for different solvents (α, β, π^*).

Solvent	α	β	π^*
Water	1.17	0.47	1.09
Ethanol	0.86	0.75	0.54
Dimethylformamide	0.00	0.69	0.88
Dimethyl sulfoxide	0.00	0.76	1.00
Dichloromethane	0.13	0.10	0.82
Chloroform	0.44	0.00	0.58
Toluene	0.00	0.11	0.54

UV-vis spectra of dyes 4a and 4b in the presence of HCl

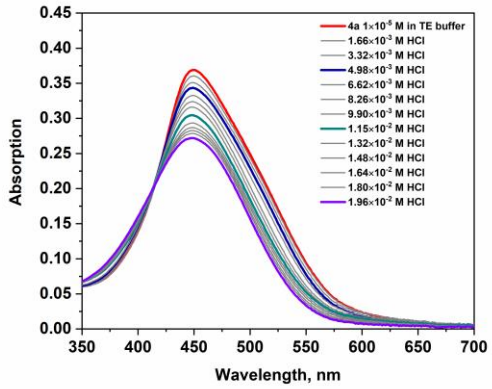


Figure S8. The changes in absorption spectrum of **4a** upon titration with HCl.

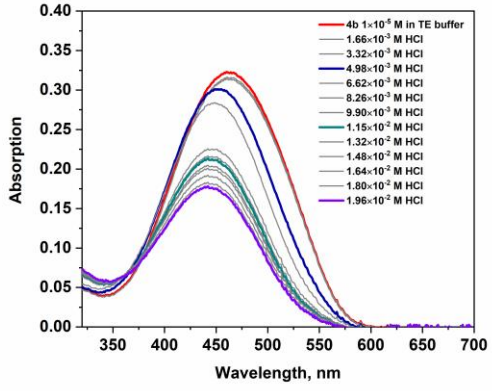


Figure S9. The changes in absorption spectrum of **4b** upon titration with HCl.

Absorption spectra of dyes 4a-4c in free state and in the presence of DNA and HCl

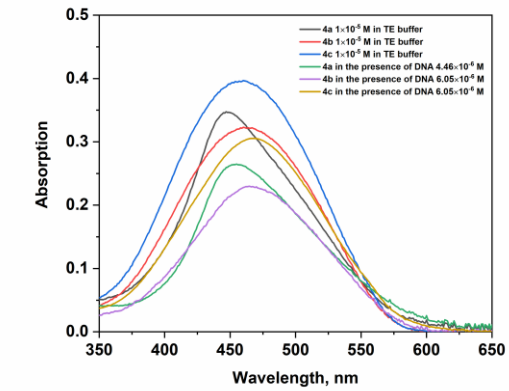


Figure S10. Absorption spectra of dyes **4a-4c** in solution and their complexes with DNA in TE buffer.

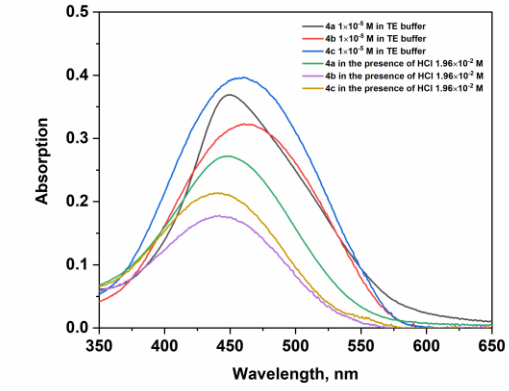
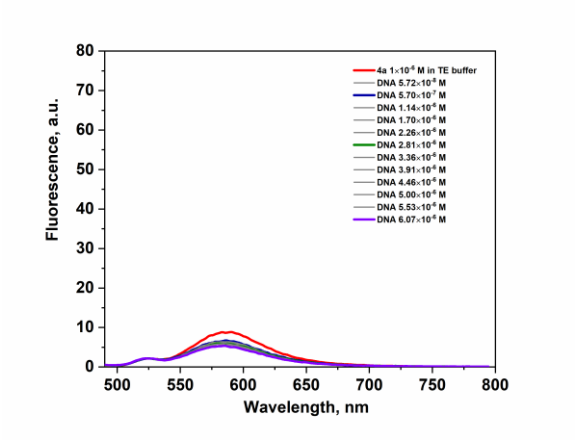
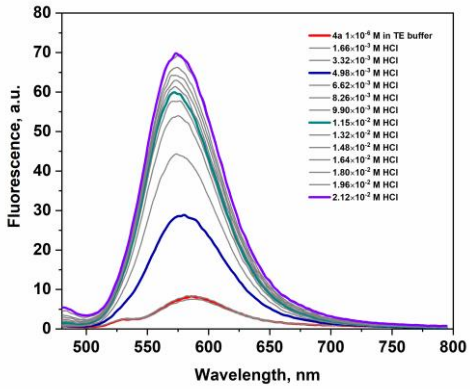


Figure S11. Absorption spectra of dyes **4a-4c** in solution and in the presence of HCl.

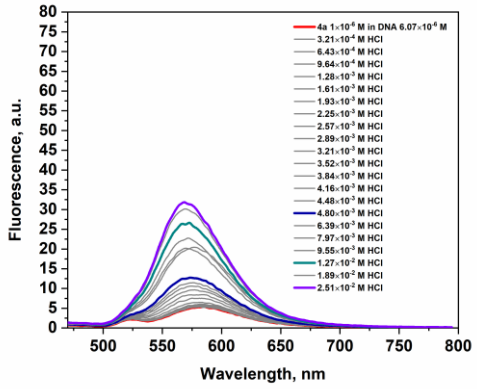
Fluorescence spectra of dyes 4a and 4b in solution and in the presence of DNA and HCl



A)



B)



C)

Figure S12. Emission spectra of: **A) 4a** upon titration with DNA **B) 4a** upon titration with HCl; **C) 4a-dye-DNA complex** upon titration with HCl.

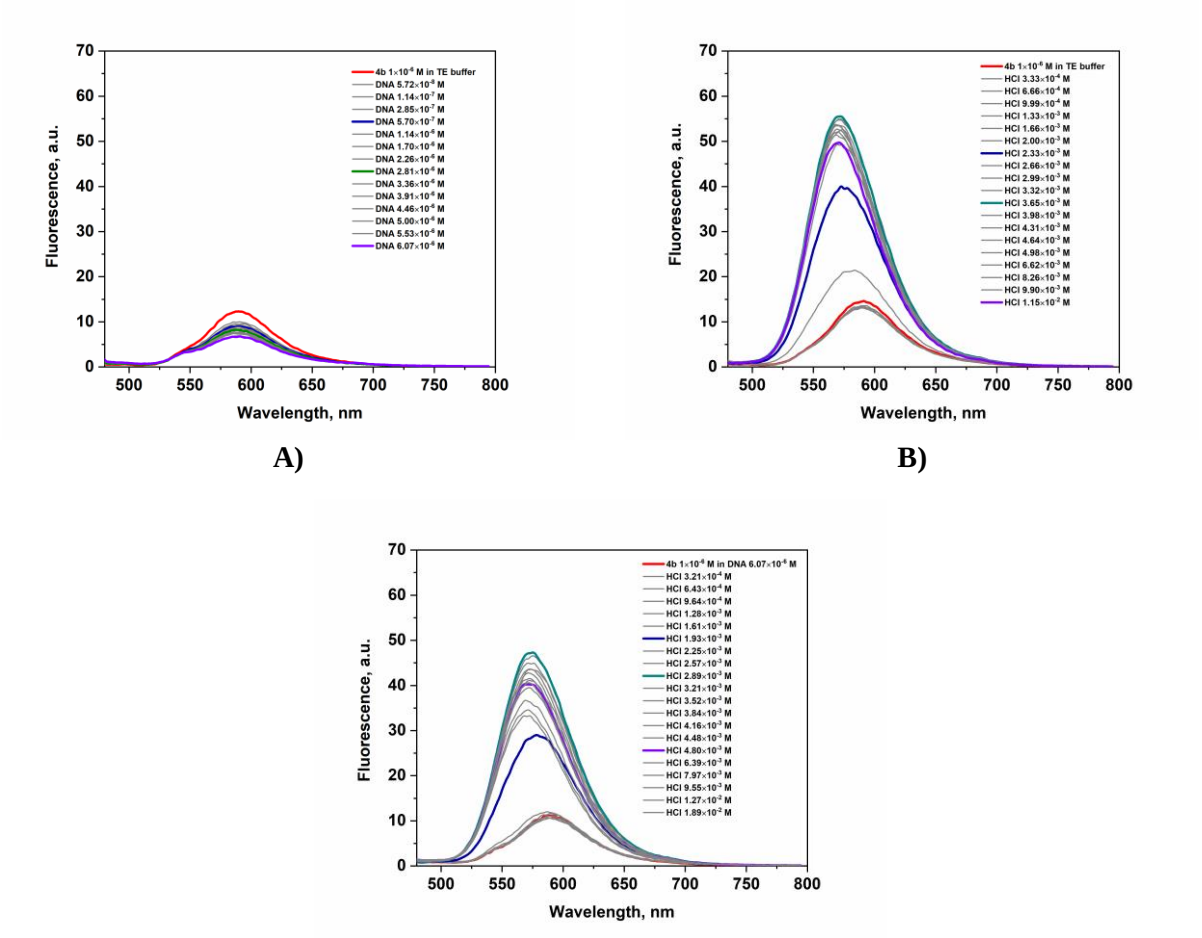
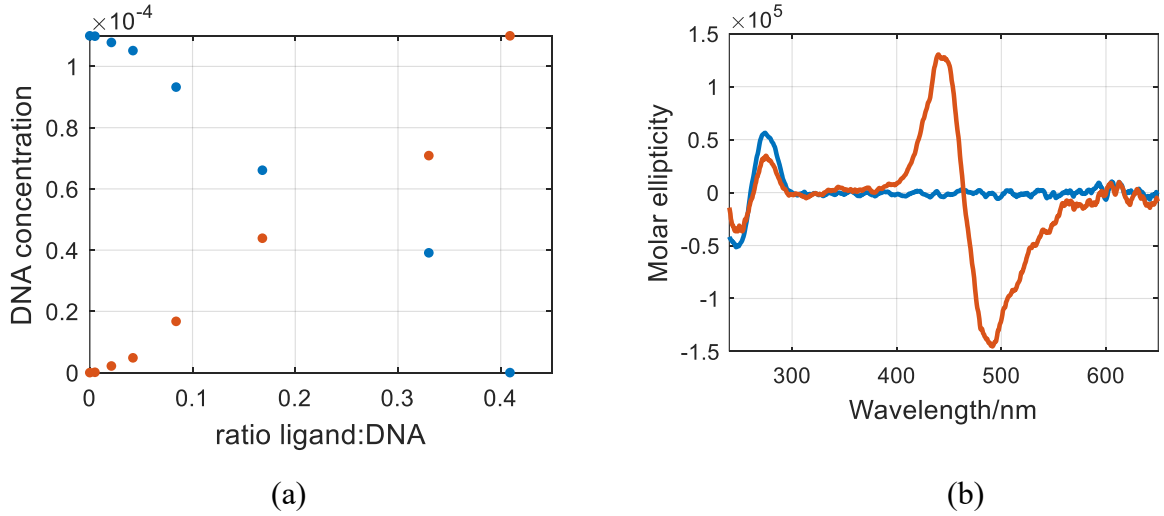


Figure S13. Emission spectra of: **A) 4b** upon titration with DNA **B) 4b** upon titration with HCl; **C) 4b-dye-DNA complex** upon titration with HCl.

Molecular docking Table S2. Binding energy of the most favourable docking modes.

Conformer	Binding energy, kcal/mol, compound 4a	Binding energy, kcal/mol, compound 4b	Binding energy, kcal/mol, compound 4c	Binding energy, kcal/mol, thiazole orange
	Groove binder	Groove binder	Groove binder	intercalator
1	-9.4	-8.7	-9.2	-8.1
2	-8.6	-8.4	-8.5	-7.9
3	-8.4	-8.3	-8.4	-7.6
4	-8.4	-8.3	-8.4	-7.3
5	-8.1	-8.1	-8.2	-7.3
6	-8.1	-8.0	-8.0	-7.0
7	-8.1	-8.0	-8.0	-7.0
8	-8.0	-7.9	-7.9	-6.9
9	-7.9	-7.9	-7.8	-6.9

CD Spectra

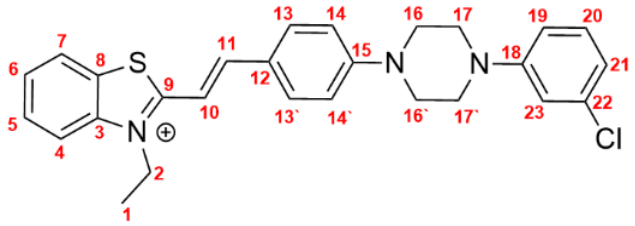


(a) (b)

Figure S14. Deconvolution results of the CD spectra shown in Figure 11, obtained using Multivariate Curve Resolution based on Alternating Least Squares (MCR-ALS).

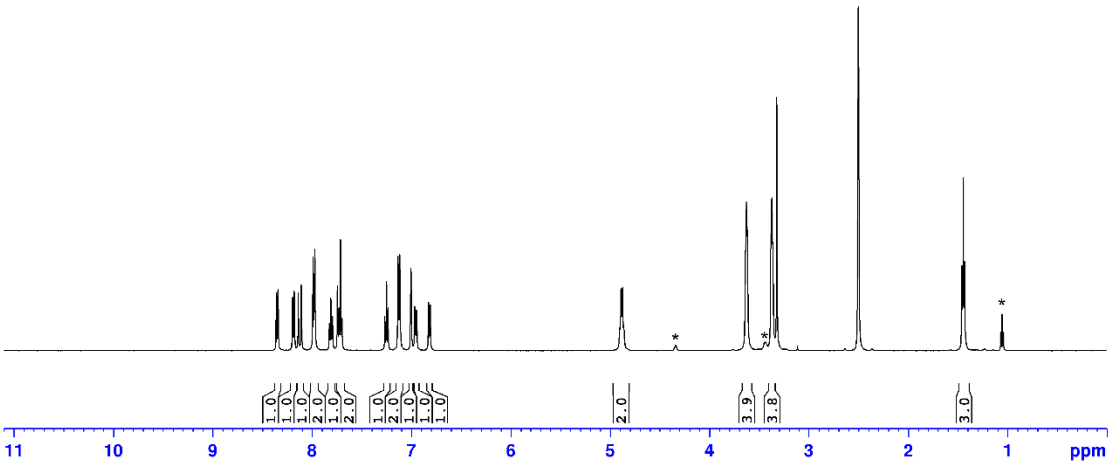
NMR characteristics

NMR spectra of dye **4a**



[1]H-NMR:



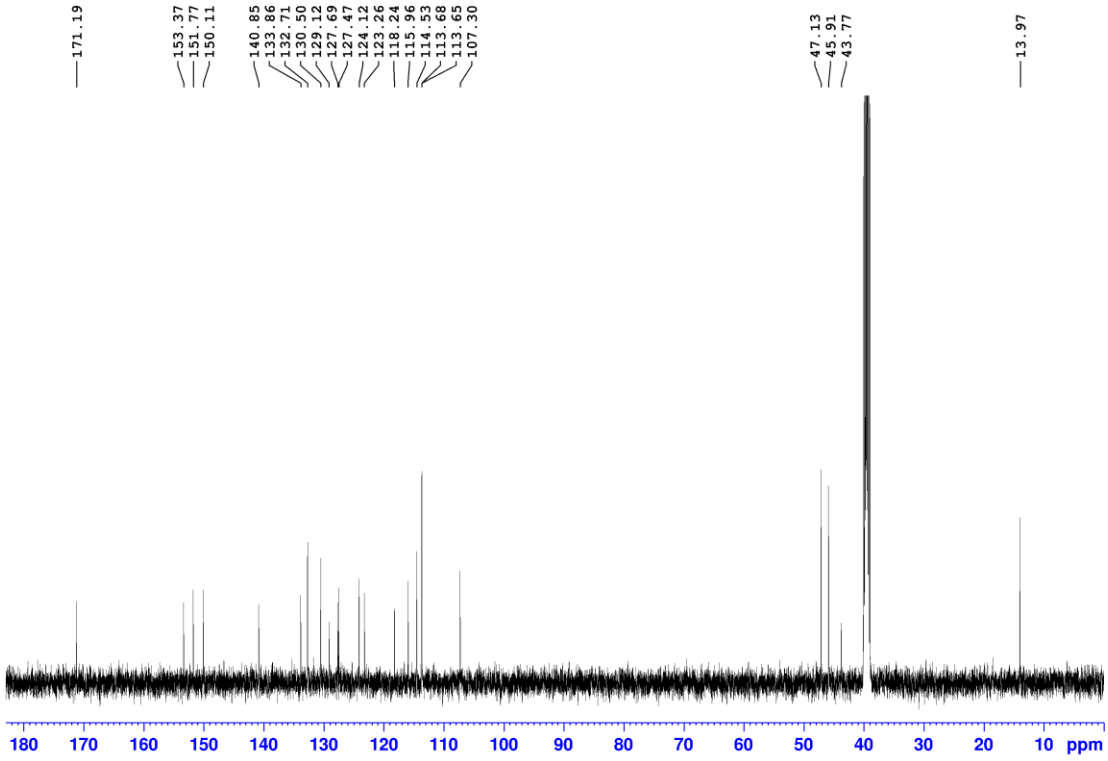


[1]H-NMR spectrum of **4a**, residual solvent signals from ethanol are labelled with an asterisk (*)

[1]H-NMR (DMSO-d_6, δ (ppm)): 1.44 (3H, t, H-1, *J*=7.1 Hz); 3.37 (4H, t, H-17, H-17`, *J*=5.0 Hz); 3.63 (4H, t, H-16, H-16`, *J*=5.1 Hz); 4.88 (2H, q, H-2, *J*=7.1 Hz); 6.82 (1H, dd, H-21, *J*=1.5 Hz, *J*=7.8 Hz); 6.96 (1H, dd, H-19, *J*=2.2 Hz, *J*=8.4 Hz); 7.00 (1H, t, H-23, *J*=2.0 Hz); 7.12 (2H, d, H-14, H-14`, *J*=9.0

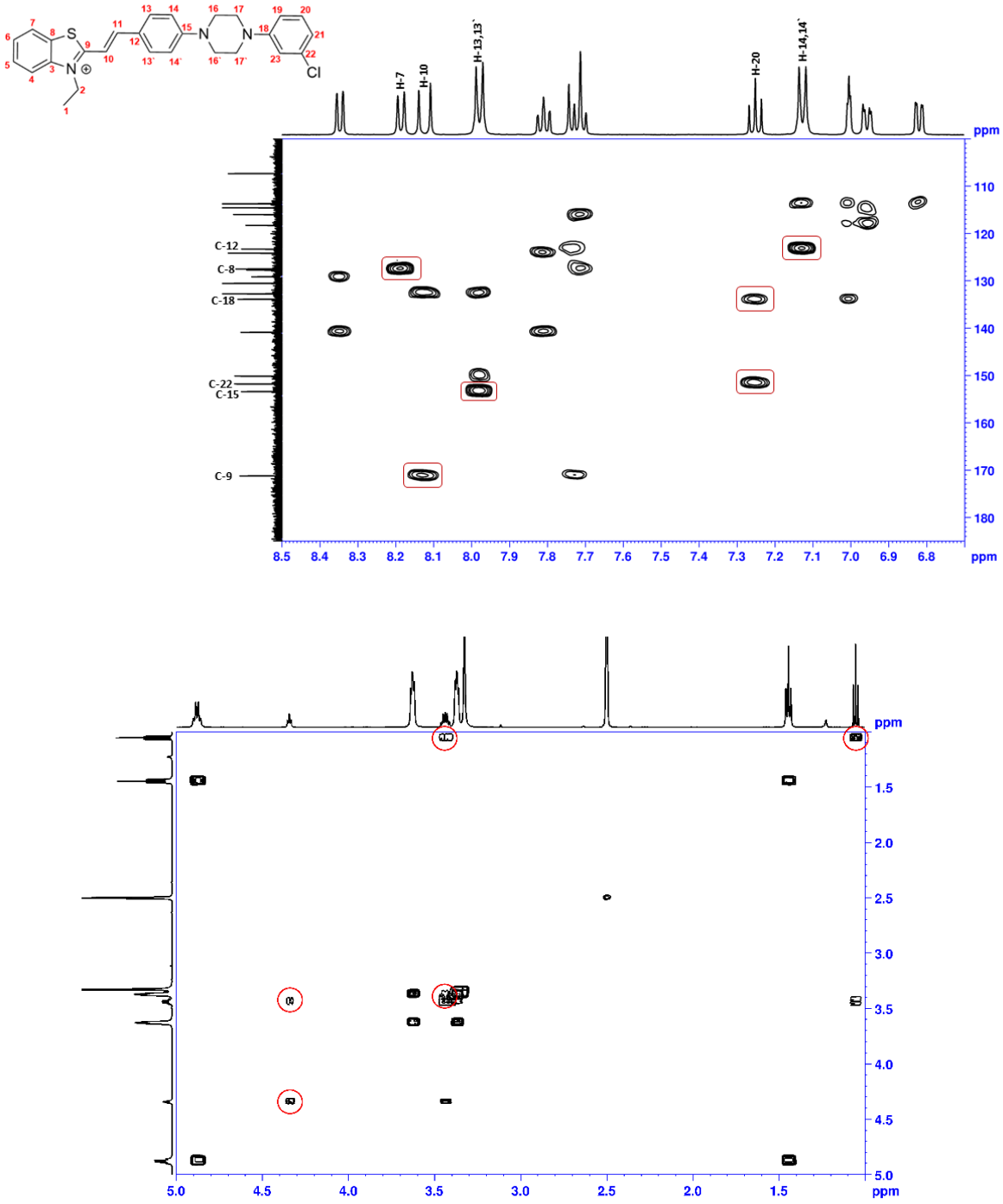
Hz); 7.25 (1H, t, H-20, *J*=8.1 Hz); 7.70-7.74 (2H, m, H-5, *J*=7.7 Hz, H-11, *J*=15.3 Hz); 7.81 (1H, t, H-6, *J*=7.9 Hz); 7.98 (2H, d, H-13, H-13`, *J*=8.9 Hz); 8.12 (1H, d, H-10, *J*=15.3 Hz); 8.19 (1H, d, H-7, *J*=8.5 Hz); 8.35 (1H, d, H-4, *J*=8.0 Hz).

^{13}C-NMR:



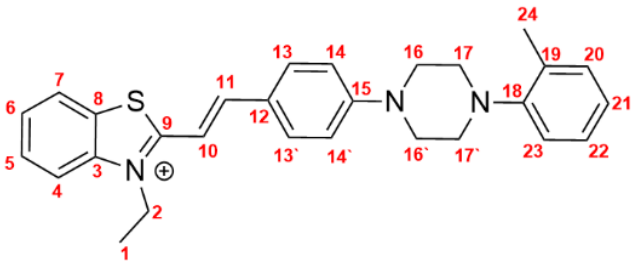
^{13}C-NMR (DMSO-d$_6$, δ (ppm)): 13.97 (1C, C-1); 43.78 (1C, C-2); 45.91 (2C, C-16, C-16`); 47.13 (2C, C-17, C-17`); 107.30 (1C, C-11); 113.65 (1C, C-19); 113.68 (2C, C-14, C-14`); 114.53 (1C; C-23); 115.96 (1C, C-7); 118.24 (1C, C-21); 123.26 (1C, C-12); 124.12 (1C, C-4); 127.47 (1C, C-5); 127.69 (1C, C-8); 129.12 (1C, C-6); 130.50 (1C, C-20); 132.71 (2C, C-13, C-13`); 133.86 (1C, C-18); 140.85 (1C, C-3); 150.11 (1C, C-10); 151.77 (1C, C-22); 153.37 (1C, C-15); 171.19 (1C, C-9).

HMBC:

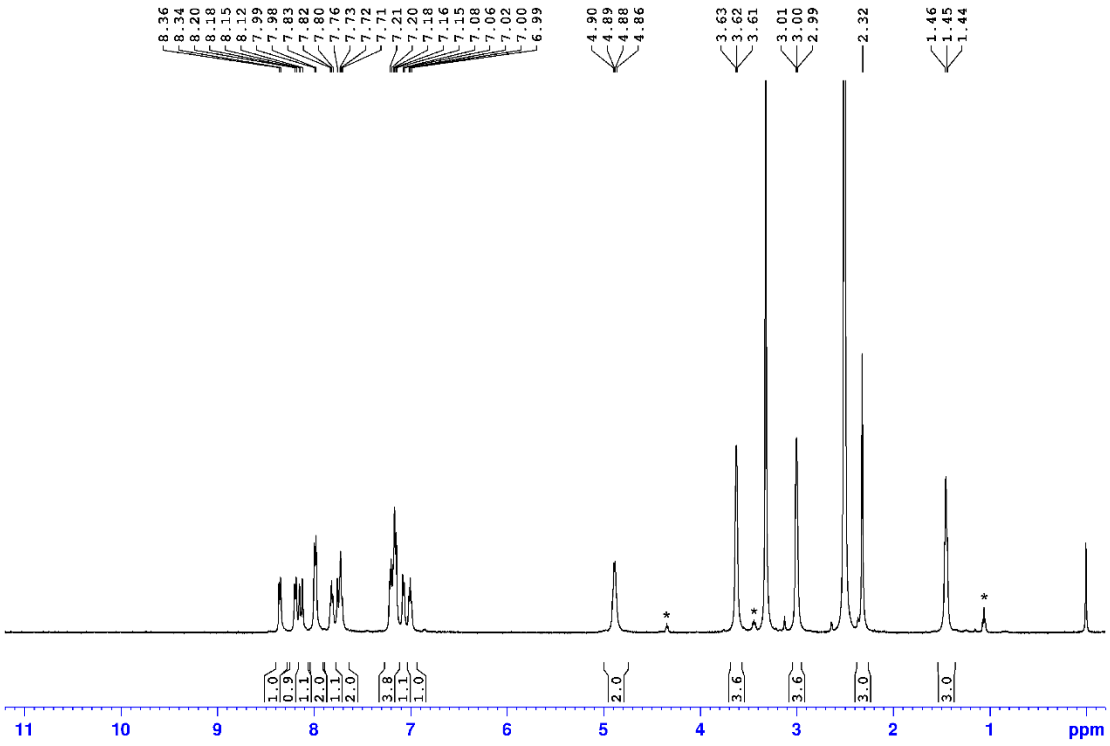


COSY spectrum of **4a**, residual solvent signals from ethanol are marked in red

NMR spectra of dye **4b**



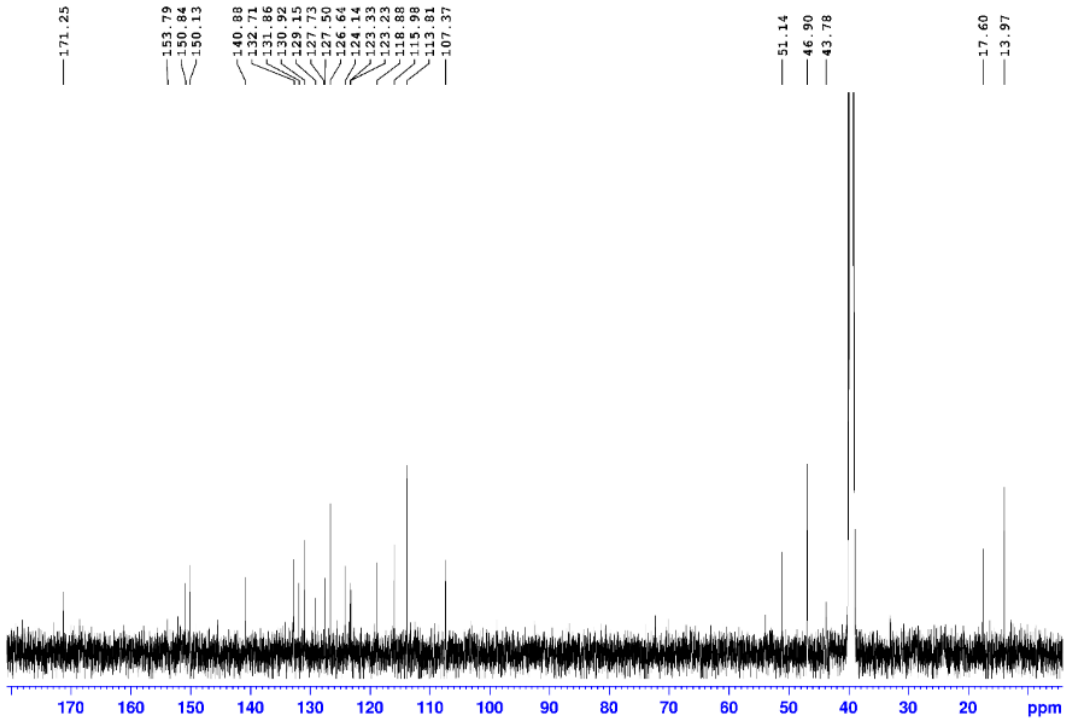
[1]H-NMR:



[1]H-NMR spectrum of **4b**, residual signals from ethanol are are labelled with an asterisk (*)

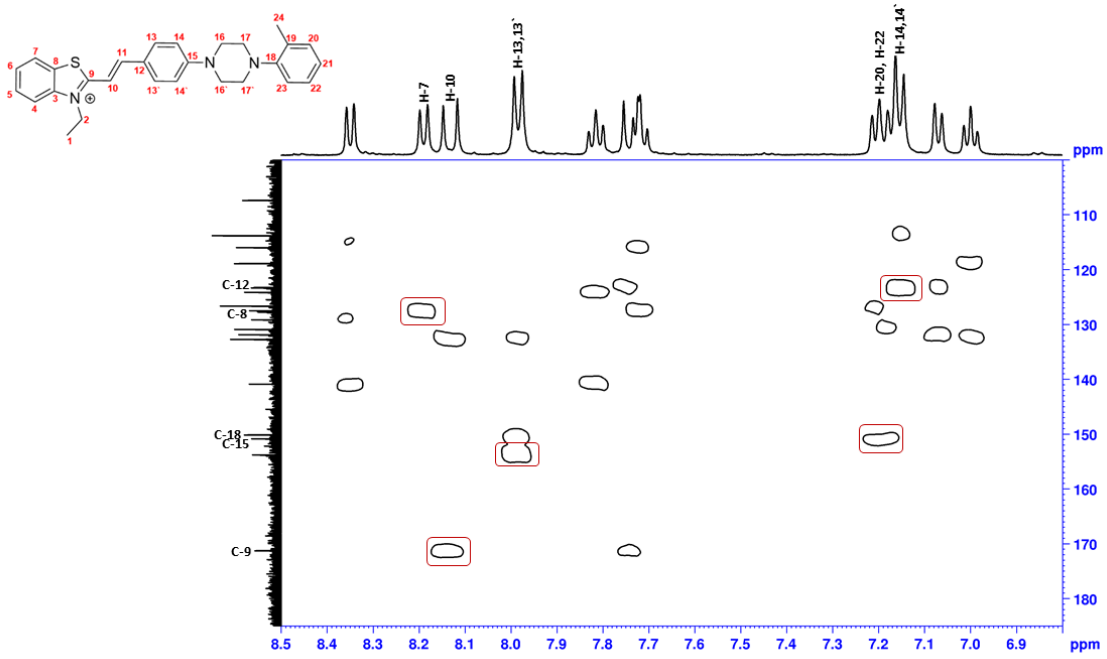
[1]H-NMR (DMSO-d_6, δ (ppm)): 1.45 (3H, t, H-1, *J*=7.2 Hz); 2.32 (3H, s, H-24); 3.00 (4H, t, H-17, H-17`, *J*=4.5 Hz); 3.62 (4H, t, H-16, H-16`, *J*=4.4 Hz); 4.88 (2H, q, H-2, *J*=7.0 Hz); 7.00 (1H, t, H-21, *J*=7.4 Hz); 7.07 (1H, d, H-23, *J*=7.8 Hz); 7.14-7.21 (4H, m, H-14, H-14`, *J*=9.0 Hz, H-20, H-22); 7.70-7.75 (2H, m, H-11, *J*=15.6 Hz; H-5, *J*=7.6 Hz); 7.82 (1H, t, H-6, *J*=7.7 Hz); 7.98 (2H, d, H-13, H-13`, *J*=8.8 Hz); 8.13 (1H, d, H-10, *J*=15.4 Hz); 8.19 (1H, d, H-7, *J*=8.4 Hz); 8.35 (1H, d, H-4, *J*=8.0 Hz).

^{13}C-NMR:

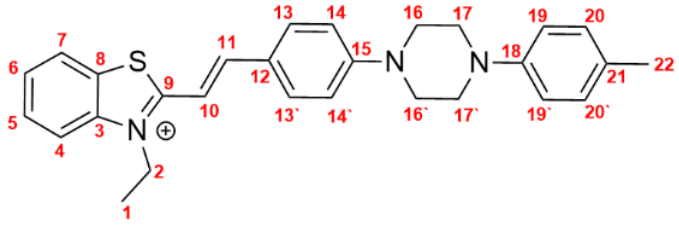


^{13}C-NMR (DMSO-d_6, δ (ppm)): 13.97 (1C, C-1); 17.60 (1C, C-24); 43.78 (1C, C-2); 46.90 (2C, C-16, C-16`); 51.14 (2C, C-17, C-17`); 107.37 (1C, C-11); 113,81 (2C, C-14, C-14`); 115.98 (1C, C-7); 118.88 (1C, C-23); 123.23 (1C, C-12); 123.33 (1C, C-21); 124.14 (1C, C-4); 126.64 (1C, C-20); 127.50 (1C, C-5); 127.73 (1C, C-8); 129.15 (1C, C-6); 130.92 (1C, C-22); 131.86 (1C, C-19); 132.71 (2C, C-13, C-13`); 140.88 (1C, C-3); 150.13 (1C, C-10); 150.84 (1C, C-18); 153.79 (1C, C-15); 171.25 (1C, C-9).

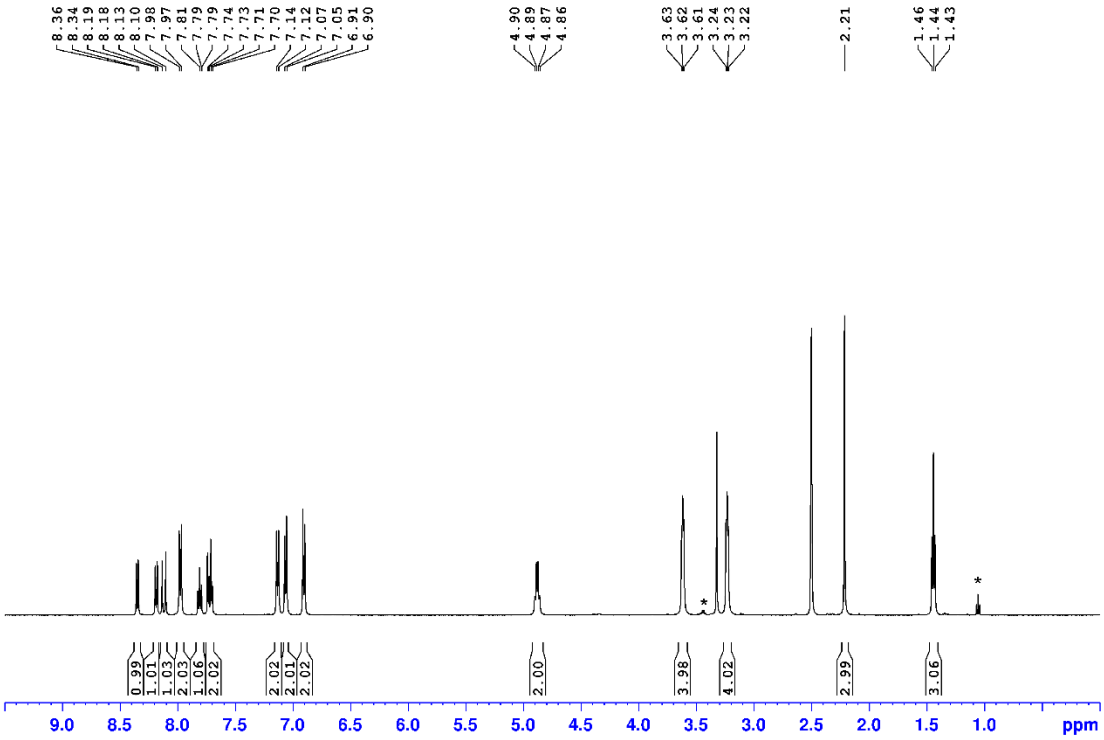
HMBC:


H-7
H-10
H-13,13`
H-20, H-22
H-14,14`
ppm
C-12
C-8
C-18
C-15
C-9
8.5
8.4
8.3
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0
6.9
110
120
130
140
150
160
170
180

NMR spectra of dye **4c**



[1]H-NMR:

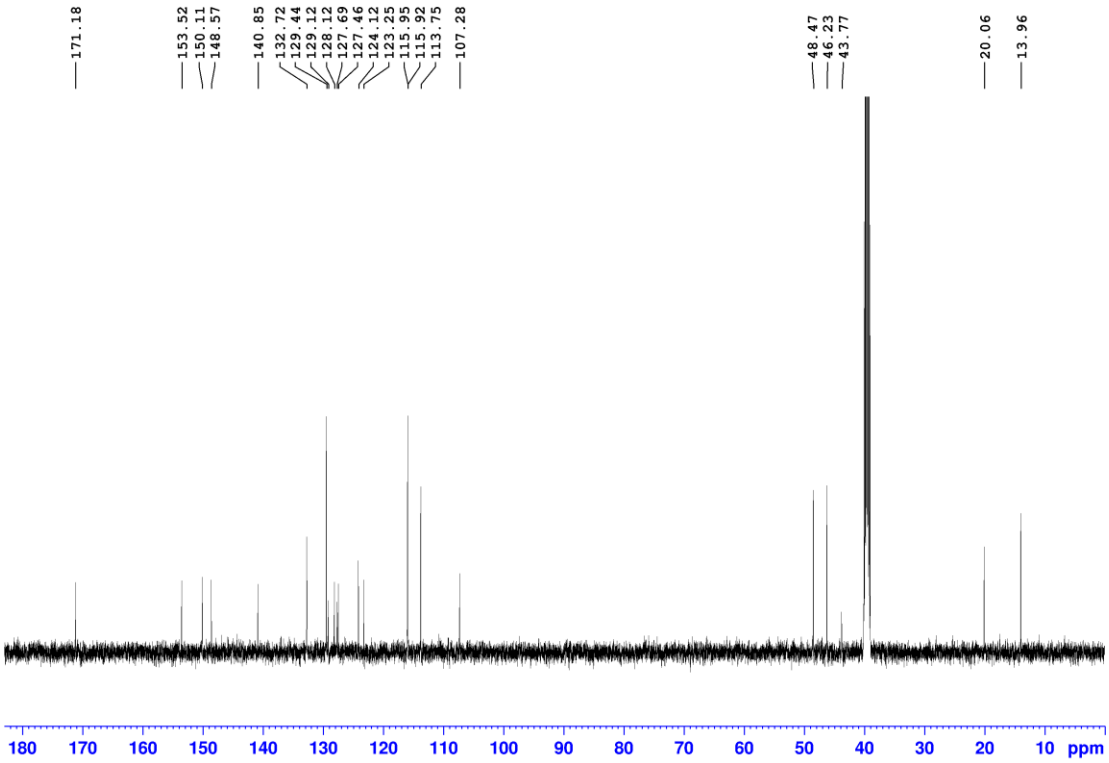


[1]H-NMR spectrum of **4c**, residual signals from ethanol are labelled with an asterisk (*)

[1]H-NMR (DMSO-d_6, δ (ppm)): 1.44 (3H, t, H-1, *J*=7.2 Hz); 2.21 (3H, s, H-22); 3.23 (4H, t, H-17, H-17`, *J*=5.1 Hz); 3.62 (4H, t, H-16, H-16`, *J*=5.1 Hz); 4.88 (2H, q, H-2, *J*=7.2 Hz); 6.90 (2H, d, H-19, H-19`, *J*=8.5 Hz); 7.06 (2H, d, H-20, H-20`, *J*=8.5 Hz); 7.13 (2H, d, H-14, H-14`, *J*=9.0 Hz); 7.70-7.74 (2H,

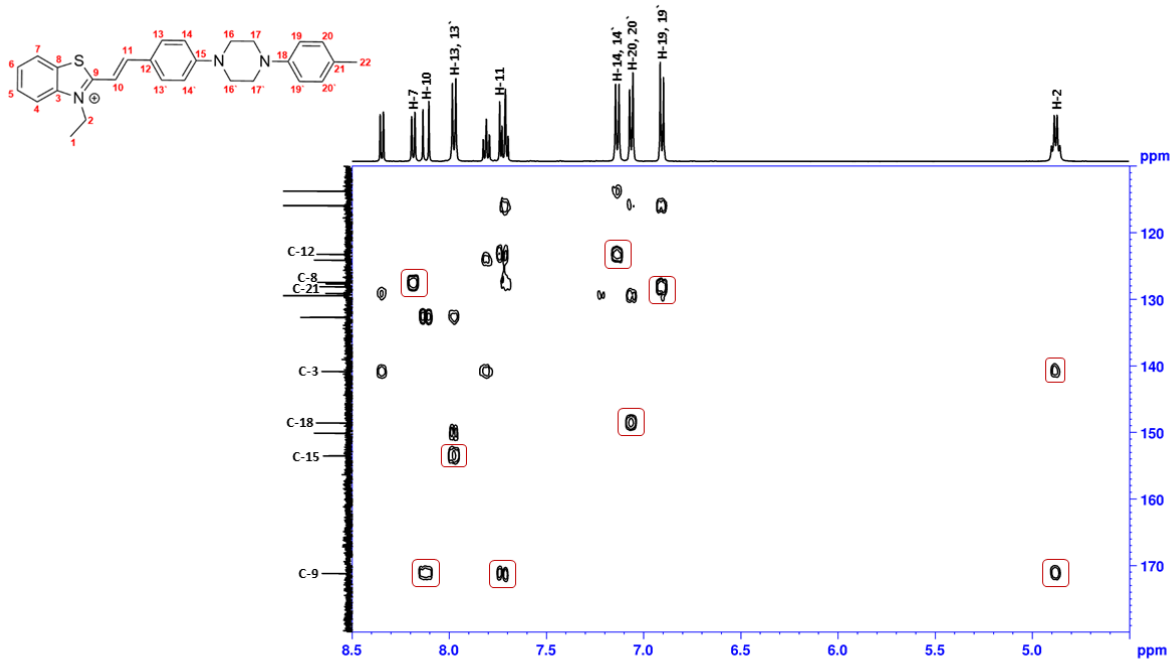
m, H-11, *J*=15.0 Hz, H-5, *J*=7.7 Hz); 7.8 (1H, dt, H-6, *J*=7.9 Hz, *J*=0.8 Hz); 7.98 (2H, d, H-13, H-13`, *J*=9.0 Hz); 8.12 (1H, d, H-10, *J*=15.3 Hz); 8.19 (1H, d, H-7, *J*=8.4 Hz); 8.35 (1H, d, H-4, *J*=8.0 Hz).

^{13}C-NMR:



^{13}C-NMR (DMSO-d_6, δ (ppm)): 13.96 (1C, C-1); 20.06 (1C, C-22); 43.77 (1C, C-2); 46.23 (2C, C-16, C-16`); 48.47 (2C, C-17, C-17`); 107.28 (1C, C-11); 113.75 (2C, C-14, C-14`); 115.92 (2C, C-19, C-19`); 115.95 (1C, C-7); 123.25 (1C, C-12); 124.12 (1C, C-4); 127.46 (1C, C-5); 127.69 (1C, C-8); 128.12 (1C, C-21); 129.12 (1C, C-6); 129.44 (2C, C-20, C-20`); 132.72 (2C, C-13, C-13`); 140.85 (1C, C-3); 148.57 (1C, C-18); 150.11 (1C, C-10); 153.52 (1C, C-15); 171.18 (1C, C-9).

HMBC:


H-7
H-10
H-13, 13'
H-11
H-14, 14'
H-20, 20'
H-19, 19'
H-2
ppm
C-12
C-8
C-21
C-3
C-18
C-15
C-9
120
130
140
150
160
170
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
ppm