"Supporting Information"

A novel approach to construct calix[4]arene-appended rhodamine B based supramolecular system for nematic mesophase and nematode cell imaging

Vinay S. Sharma[a#*], Hitendra Mali[a#], Anuj S. Sharma[a], Unnati Patel[a], Sweta Thakar[b], Pranav S. Shrivastav[a*]

[a]: *Department of Chemistry, School of Science, Gujarat University, Ahmedabad-380008, India.*

[b]: *Department of Zoology, School of Sciences, Gujarat University, Ahmedabad-380009, India.*

***Corresponding author*: vinaysharma3836@gmail.com, pranav_shrivastav@yahoo.com**

#: equally contributed

Contents:

1.	**Synthesis and Characterization**	**2**
2.	**Experimental method**	**3**
3.	**Synthesis procedure**	**4-8**
4.	**UV & Fl datas**	**9-10**
4.	**Characterization datas**	**11-16**
5.	**References**	**17**

Synthesis and Characterization

Rhodamine B, alkyl bromides (R-Br), such as n-propyl bromide, and 1,2-dibromoalkane were purchased from SRL Chemical Ltd in India. Other chemicals, including 4-tert-butyl phenol, formaldehyde, hydrazine hydrate, diphenyl ether, NaH, and anhydrous K_2CO_3 were procured from JSK Chemical Ltd in India. The ^{1}H and ^{13}C NMR spectra were recorded using a Bruker Advance spectrometer operating at 500 MHz for ^{1}H NMR and 126 MHz for ^{13}C NMR. The spectra were acquired in $CDCl_3$ solvent, which is commonly used as a deuterated solvent for NMR experiments. For the IR study, the samples were prepared using KBr pellets. The IR spectra were then analysed using a TENSOR 27 instrument from Bruker. The IR analysis covered a range of 3800-600 cm^{-1}, which corresponds to the typical range for infrared spectroscopy. The investigation of the liquid crystalline phase was conducted using a Polarizing Optical Microscope (POM) model Nikon Eclipse LV-100 POL. The microscope was equipped with a temperature-controlled LTSE 420 heating stage from Linkam Scientific Instruments, located in Tadworth, Surrey, UK. The thermal behavior of the compounds was analysed using a Shimadzu DSC-60 plus differential scanning calorimeter. The DSC-60 plus instrument allows for precise control of heating and cooling rates (10 °C/min) during the thermal analysis process. Elemental analysis was carried out using GmbH Vario Micro cube elemental analyser. The XRD measurement study was conducted using a Rigaku-Ultima IV powder diffractometer, which was equipped with a Cu Kα (λ=1.5418 Å) based X-ray tube operating at an applied voltage of 40 kV and a current of 30 mA at variable temperatures. The data were collected using Philips X'PERT MPD software. The absorption and fluorescence spectra were studied on a Jasco V-570 UV-Vis spectrophotometer and Jasco FP-6500 spectrofluorometer. To perform the bio-imaging experiment on selected nematodes, the nematodes were cultivated in a laboratory environment on NGM (Nematode Growth Medium) agar plates. NGM agar is a commonly used nutrient-rich medium that provides a

suitable environment for nematode growth and development. Once the nematodes reached the desired stage, they were collected from the NGM plates using M9 buffer. After collection, the nematodes were centrifuged at 3000 rpm (revolutions per minute). Centrifugation at this speed helps separate the nematodes from the M9 buffer solution. M9 buffer is a buffered saline solution that provides a controlled environment for handling and processing nematodes. The standard sample of rhodamine B and the calixarene functionalized rhodamine B derivative **(3c)** were added to the separate nematode pellet. After the addition of the staining compounds, the sample was incubated at room temperature for 30 minutes in the dark. The incubation period allows for the staining compounds to interact with the nematodes, enabling the dyes to bind to specific structures or targets within the nematode cells. The nematodes were washed twice with M9 buffer, and the stained nematodes were observed under Lynx LM-52-3001 Fluorescence Microscope under RGB filters and images were captured with the help of motic image plus software.

Preparation of *p-tert*-butylcalix[4]arene (1a)

The synthesis of *p-tert*-butylcalix[4]arene **(1a)** was synthesized by previously reported method from the literature,[1] white precipitate, yield 87 %.

Preparation of dipropyl calix[4]arene (1b)

To synthesize compound **(1b)**, a solution containing p-*tert*-butylcalix[4]arene (1.0 mmol) in 10 ml of CH_3CN was prepared, and anhydrous K_2CO_3 (2.5 mmol) was added. The resulting solution was stirred at room temperature for 2 hours. Once a cloudy solution formed, n-propyl bromide (3.0 mmol) was added dropwise, and stirring was continued for an additional 1 h at room temperature. The reaction mixture was then poured into ice cold water and extracted with dichloromethane. Further, the DCM layer was subsequently washed with water and dried using Na_2SO_4. Evaporation yielded a white solid, which was purified using column chromatography, off white precipitates, yield 74 %.[2]

Preparation of compound (RBN) (1c)

To obtain the compound (RBN), the excess amount of hydrazine hydrate was treated with rhodamine **B** (1 mmol) in ethanol as the solvent. The reaction was carried out at 78 ºC for 4 hours. The reaction mixture was then poured into ice cold water to obtained the off pink type solid product, yield 87 %.[3]

Preparation of compounds (2a-2d) with alkyl arm

Compound **(RBN)** (1 mmol) was combined with 1,2-dibromoalkane (3 mmol) in a solution of dimethylformamide (DMF), along with K_2CO_3 (3.8 mmol). The mixture was heated at 80 °C for a period of 4 hours while being stirred. After cooling, the reaction mixture was extracted with diethyl ether. Wash the combined extracts with water, followed by drying over Na_2SO_4. Concentrate the residue to dryness and separate the crude product. The crude product was further purified through column chromatography using silica gel as the stationary phase and eluted with a mixture of ethyl acetate/hexane (6:4 v/v). This purification step led to the isolation of the desired compound **(2a-2d)**.[4]

Preparation of final targeted compounds (3a-3d)

A solution of compound **(1b)** (1.0 mmol) in DMF (10 mL) was prepared, and K_2CO_3 (2.5 mmol) was added to initiate the reaction. The resulting solution was stirred for 2 h at room temperature until a cloudy solution formed. After that, compounds **(2a-2d)** (3.0 mmol) were added and stirred continuously for 1 h at room temperature and further reflux to 6 h. The reaction was monitored using the TLC method, confirming the completion of the starting material. To quench the reaction, the reaction mixture was carefully poured into the ice-cold water and extracted by using dichloromethane (DCM). The DCM layer was separated and washed with water, followed by drying over Na_2SO_4. Upon evaporation of the solvent, a pinkish-purple solid was obtained as a crude product. The crude product was further purified by using column chromatography. Elution with a mixture of ethyl acetate: hexane (2:8 v/v) allowed for the separation to obtain the desired compounds **(3a-3d)**.[5]

Quantum yield measurements

The quantum yield was measured following the established procedure, utilizing rhodamine 6G in ethanol as the standard. Absolute values were calculated using the following equation:

$$\mathbf{Q_S = Q_R \times (m_S/m_R) \times (n_S/n_R)^2}$$

Where, Q: Quantum yield, m: Slope of the plot of integrated fluorescence intensity vs absorbance (Calculated from Fig.S5), n: refractive index (1.361 for ethanol and 1.4073 for tetrahydrofuran). The subscript R refers to the reference fluorophore i.e. rhodamine 6G solution in EtOH and subscript S refers to the sample under investigation. To minimize reabsorption effects, the absorbance was maintained below 0.15 at the excitation wavelength of 560 nm for compounds **(3a-3d)**. The quantum yield of rhodamine 6G in EtOH is 0.95. After substituting the appropriate values, the simplified equation for calculation is provided below, and the obtained values are listed in the table below.[6,7]

$$\mathbf{Q_S = 0.95 \times (m_S / m_R) \times (1.4073/1.361)^2}$$

Compounds	m_S	m_R	Q_S
3a	7.9331×10^8	9.9325×10^8	0.811
3b	7.9205×10^8	9.9325×10^8	0.809
3c	8.3711×10^8	9.9325×10^8	0.856
3d	8.2051×10^8	9.9325×10^8	0.839

Compound 3a: Yield: 73 %, purple solid. IR in cm^{-1}: 858 Poly(-CH_2-)$_n$ group, 1016 (-C-H def. hydrocarbon), 1247 (-C-O- Str.), 1385 (-C-O- str. in (-CH_2-)$_n$ alkyl chain group), 1557 (-C-C- def. in -CH_2-unit in alkyl chain), 2846 and 2930 (-C-H str. in -CH_3), 3142 (-C-H str. In aromatic ring), 3321 (-N-H str.). ^{1}H NMR in δ ppm: 8.12 (d, 2H, Ar-H), 7.47 (m, 2H, Ar-H), 7.22 (d, 2H, Ar-H), 7.14 (s, 8H, Ar-H), 7.03 (d, 2H, Ar-H), 6.54 (d, 4H, Ar-H), 6.40 (d, 4H, Ar-H), 6.28 (s, 4H, Ar-H), 4.54 (s, 2H, -NH-), 4.20 (d, 4H, Ar-CH_2-Ar), 4.04 (t, 4H, -OCH_2-), 3.99 (t, 4H, -OCH_2-), 3.82 (d, 4H, Ar-CH_2-Ar), 3.53 (m, 16H, -CH_2-), 2.55 (t, 4H, -CH_2-), 1.80 (m, 8H, -CH_2-), 1.37 (m, 4H, -CH_2-), 1.26 (s, 36H, -$C(CH_3)_3$), 1.09 (t, 24H, -CH_3), 0.92 (t, 6H, -CH_3). ^{13}C NMR in δ ppm: 161.22, 154.76, 152.53, 150.90, 149.31, 144.33, 142.70,

139.47, 133.77, 132.00, 130.65, 129.98, 129.75, 128.52, 127.93, 126.33, 123.43, 113.86, 111.26, 106.40, 97.75, 69.50, 68.96, 68.45, 49.70, 47.12, 34.49, 31.29, 29.71, 26.01, 23.46, 21.20, 12.60, 10.79. Elemental analysis: calculated for $C_{114}H_{144}N_8O_8$ (%): C, 78.04; H, 8.27; N, 6.39; O, 7.30; found: C, 78.08; H, 8.24; N: 6.41; O, 7.28. HRMS: m/z calculated: 1753.112 $[M+H]^+$, found 1754.351.

Compound 3b: Yield: 71 %, light purple solid. IR in cm^{-1}: 860 Poly(-CH_2-)$_n$ group, 1011 (-C-H def. hydrocarbon), 1240 (-C-O- Str.), 1382 (-C-O- str. in (-CH_2-)$_n$ alkyl chain group), 1561 (-C-C- def. in -CH_2-unit in alkyl chain), 2842 and 2934 (-C-H str. in -CH_3), 3143 (-C-H str. In aromatic ring), 3323 (-N-H str.). ^{1}H NMR in δ ppm: 8.12 (d, 2H, Ar-H), 7.48 (m, 2H, Ar-H), 7.22 (d, 2H, Ar-H), 7.16 (s, 8H, Ar-H), 7.03 (d, 2H, Ar-H), 6.54 (d, 4H, Ar-H), 6.40 (d, 4H, Ar-H), 6.29 (s, 4H, Ar-H), 4.55 (s, 2H, -NH-), 4.20 (s, 4H, Ar-CH_2-Ar), 4.04 (t, 4H, -OCH_2-), 4.00 (t, 4H, -OCH_2-), 3.81 (d, 4H, Ar-CH_2-Ar), 3.53 (m, 16H, -CH_2-), 2.58 (t, 4H, -OCH_2-), 1.80 (m, 8H, -CH_2-), 1.40 (m, 8H, -CH_2-), 1.26 (s, 36H, -$C(CH_3)_3$), 1.09 (t, 24H, -CH_3), 0.92 (t, 6H, -CH_3). ^{13}C NMR in δ ppm: 160.11, 154.51, 151.99, 149.48, 143.56, 142.49, 139.35, 133.77, 132.61, 132.00, 131.54, 129.75, 128.81, 128.40, 126.70, 125.32, 122.08, 114.76, 112.48, 106.47, 97.73, 68.45, 67.46, 49.20, 46.48, 35.08, 31.94, 29.71, 29.07, 27.42, 26.16, 25.20, 21.20, 12.82, 10.33. Elemental analysis: calculated for $C_{116}H_{148}N_8O_8$ (%): C, 78.16; H, 8.37; N, 6.29; O, 7.18; found: C, 78.10; H, 8.35; N: 6.34; O, 7.21. HRMS: m/z calculated: 1781.147 $[M+H]^+$, found 1782.451.

Compound 3c: Yield: 75 %, purple solid. IR in cm^{-1}: 861 Poly(-CH_2-)$_n$ group, 1012 (-C-H def. hydrocarbon), 1251 (-C-O- Str.), 1390 (-C-O- str. in (-CH_2-)$_n$ alkyl chain group), 1553 (-C-C- def. in -CH_2-unit in alkyl chain), 2849 and 2929 (-C-H str. in -CH_3), 3156 (-C-H str. In aromatic ring), 3319 (-N-H str.). ^{1}H NMR in δ ppm: 8.11 (d, 2H, Ar-H), 7.47 (m, 2H, Ar-H), 7.22 (d, 2H, Ar-H), 7.15 (s, 8H, Ar-H), 7.03 (d, 2H, Ar-H), 6.53 (d, 4H, Ar-H), 6.41 (d, 4H, Ar-H), 6.24 (s, 4H, Ar-H), 4.54 (s, 2H, -NH-), 4.21 (d, 4H, Ar-CH_2-Ar), 4.04 (t, 4H, -OCH_2-

), 3.99 (t, 4H, -OCH_2-), 3.82 (d, 4H, Ar-CH_2-Ar), 3.54 (m, 16H, -CH_2-), 2.55 (t, 4H, -OCH_2-), 1.80 (m, 8H, -CH_2-), 1.37 (m, 12H, -CH_2-), 1.26 (s, 36H, -$C(CH_3)_3$), 1.09 (t, 24H, -CH_3), 0.92 (t, 6H, -CH_3). ^{13}C NMR in δ ppm: 160.22, 153.06, 152.56, 151.71, 149.14, 143.67, 142.51, 139.41, 135.31, 132.43, 131.94, 130.04, 128.97, 128.56, 128.39, 126.18, 125.23, 124.19, 114.41, 110.66, 106.60, 96.58, 69.20, 68.40, 49.30, 47.19, 34.54, 31.81, 30.62, 29.33, 29.23, 29.09, 27.90, 25.99, 22.67, 21.03, 12.34, 10.20. Elemental analysis: calculated for $C_{118}H_{152}N_8O_8$ (%): C, 78.28; H, 8.46; N, 6.19; O, 7.07; found: C, 78.31; H, 8.45; N: 6.17; O, 7.07. HRMS: m/z calculated: 1809.175 $[M+H]^+$, found 1810.234.

Compound 3d: Yield: 70%, pinkish purple solid. IR in cm^{-1}: 863 Poly(-CH_2-)$_n$ group, 1018 (-C-H def. hydrocarbon), 1249 (-C-O- Str.), 1391 (-C-O- str. in (-CH_2-)$_n$ alkyl chain group), 1560 (-C-C- def. in -CH_2-unit in alkyl chain), 2836 and 2938 (-C-H str. in -CH_3), 3131 (-C-H str. In aromatic ring), 3329 (-N-H str.). ^{1}H NMR in δ ppm: 8.11 (d, 2H, Ar-H), 7.46 (m, 2H, Ar-H), 7.21 (d, 2H, Ar-H), 7.12 (s, 8H, Ar-H), 7.03 (d, 2H, Ar-H), 6.55 (d, 4H, Ar-H), 6.41 (d, 4H, Ar-H), 6.27 (s, 4H, Ar-H), 4.52 (s, 2H, -NH-), 4.19 (d, 4H, Ar-CH_2-Ar), 4.02 (t, 4H, -OCH_2-), 3.99 (t, 4H, -OCH_2-), 3.83 (d, 4H, Ar-CH_2-Ar), 3.54 (m, 16H, -CH_2-), 2.54 (t, 4H, -OCH_2-), 1.80 (m, 8H, -CH_2-), 1.37 (m, 20H, -CH_2-), 1.25 (s, 36H, -$C(CH_3)_3$), 1.09 (t, 24H, -CH_3), 0.92 (t, 6H, -CH_3). ^{13}C NMR in δ ppm: 160.00, 154.10, 152.56, 151.71, 149.29, 143.32, 142.88, 139.52, 135.30, 132.43, 131.05, 130.04, 129.72, 128.97, 128.52, 126.18, 125.23, 124.19, 114.40, 110.66, 106.58, 97.45, 81.91, 70.10, 69.39, 68.40, 49.52, 47.00, 34.60, 31.94, 29.71, 25.99, 25.39, 22.71, 22.33, 13.65, 10.22. Elemental analysis: calculated for $C_{122}H_{160}N_8O_8$ (%): C, 78.50; H, 8.64; N, 6.00; O, 6.86; found: C, 78.47; H, 8.70; N, 6.03; O, 6.80. HRMS: m/z calculated: 1865.247 $[M+H]^+$, found 1866.124.

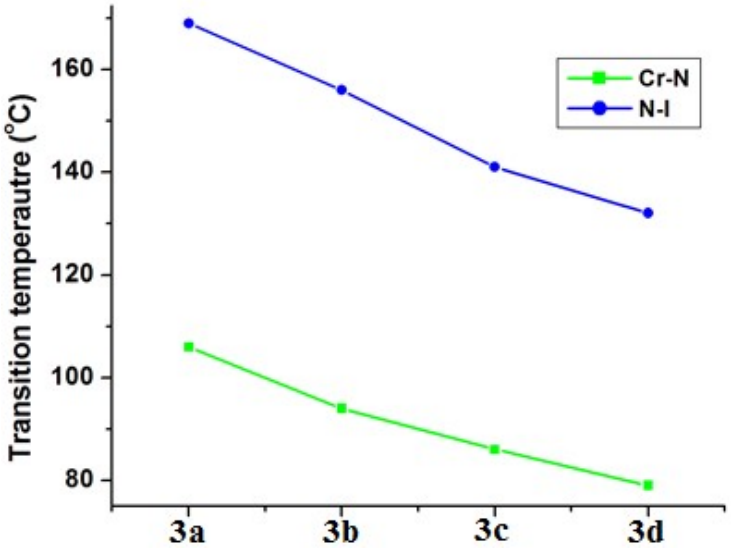

Figure S1. Phase behavior of the compounds **(3a-3d)** on heating condition.

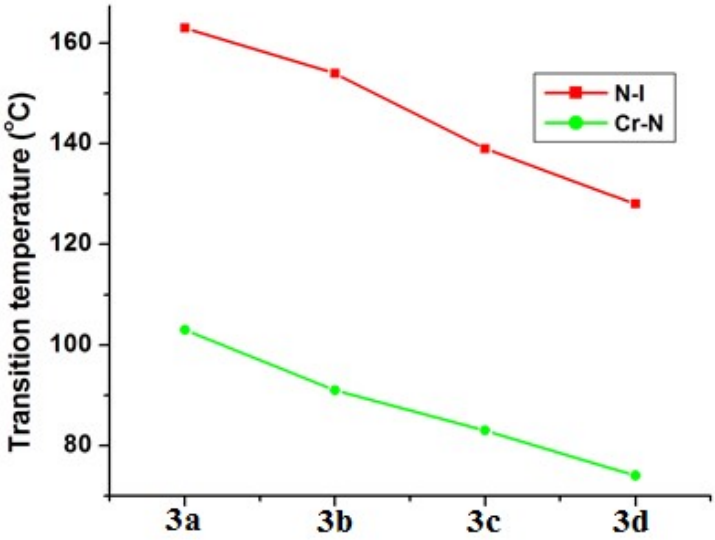

Figure S2. Phase behavior of the compounds **(3a-3d)** on cooling condition.

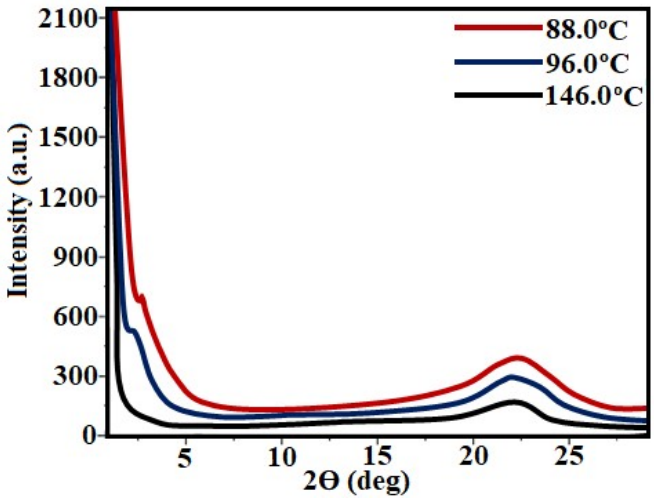

Figure S3. X-ray diffraction pattern of the compound **3c** with hexyloxy side group at isotropic to mesomorphic state.

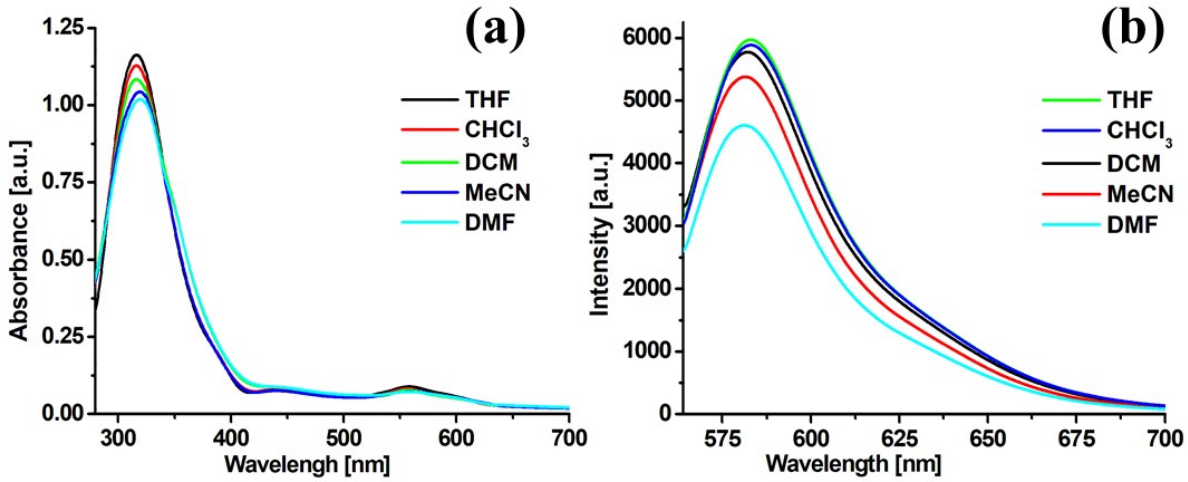

Figure S4. Absorption and fluorescence spectra of compounds **3a** in different solvent (5 μM, λex = 560 nm) (a-b).

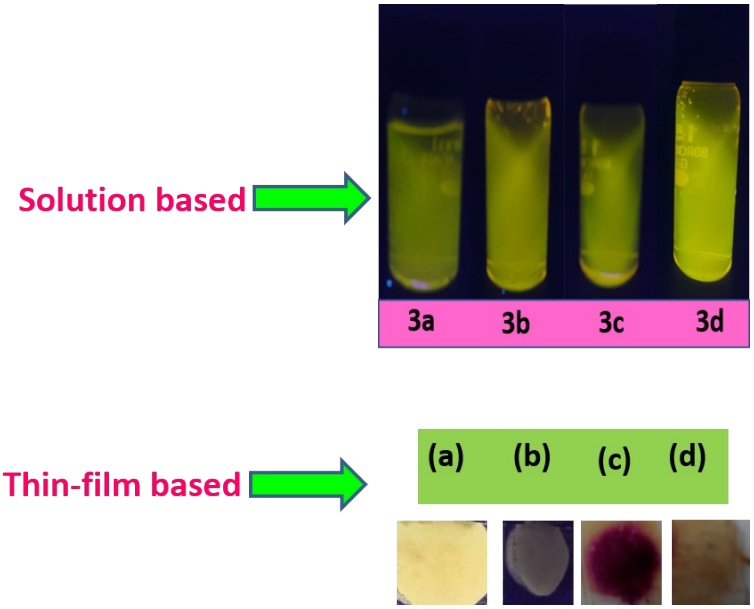

Figure S5. The observed color in solution and thin film under long UV-light.

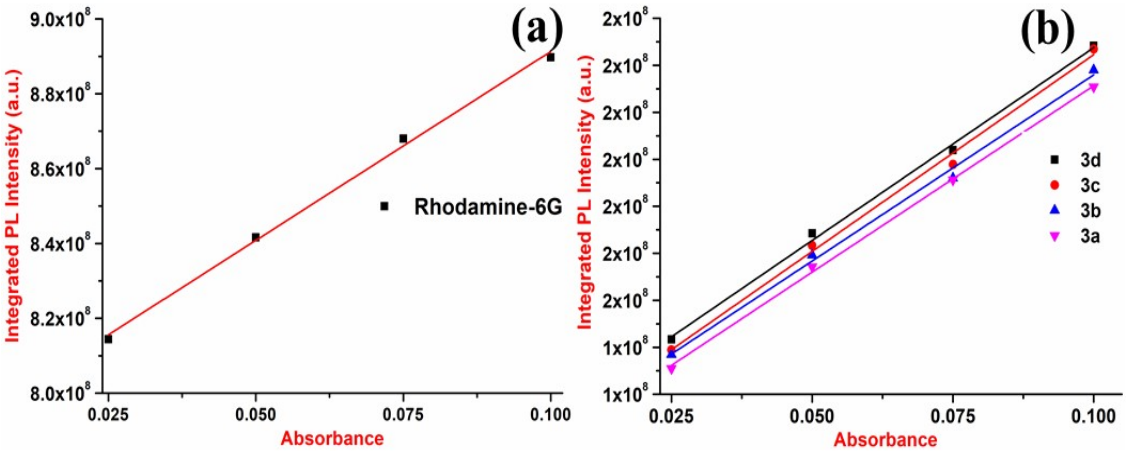

Figure S6. Plots of integrated photoluminescence intensity vs absorbance of reference rhodamine 6G (a) and compound 3a-3d (b).

Table S1. Photophysical behavior of calixarene nematic LCs (**3a-3d**) with solution and thin film state.

Comp.	Absorption[a] (nm)	Emission[a] (nm)	Stoke shift (nm)	($\varepsilon/10^6$ L.mol^{-1} cm^{-1})	Absorption[b] (nm)	Emission[b] (nm)
3a	326, 556	581	26	21.2	321, 551	578
3b	324, 558	588	30	22.4	322, 552	579
3c	325, 560	584	24	23.4	320, 554	581
3d	323, 561	586	25	23.8	318, 556	582

[a]: Micromolar THF solution; **[a]:** Absorption maxima during excitation; **[b]:** Emission in thin films

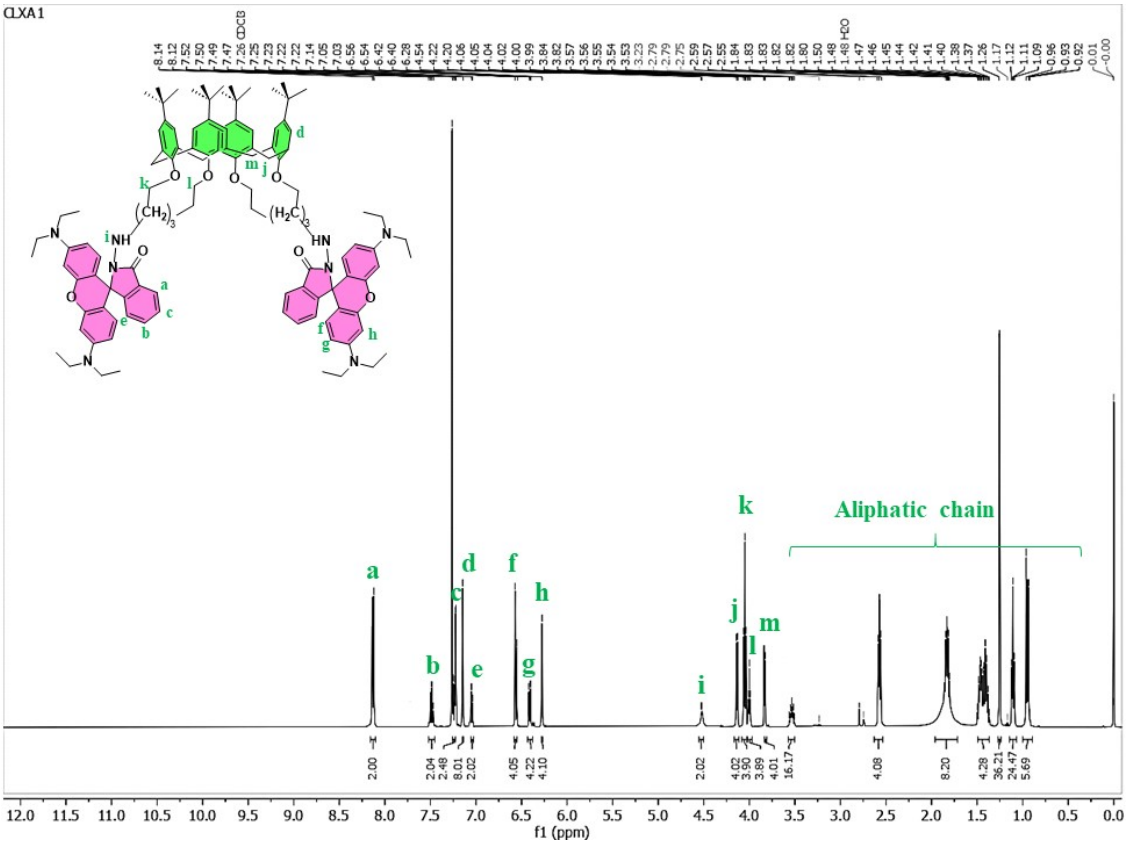

Fig. S7. The ^{1}H NMR spectrum of compound **3a**.

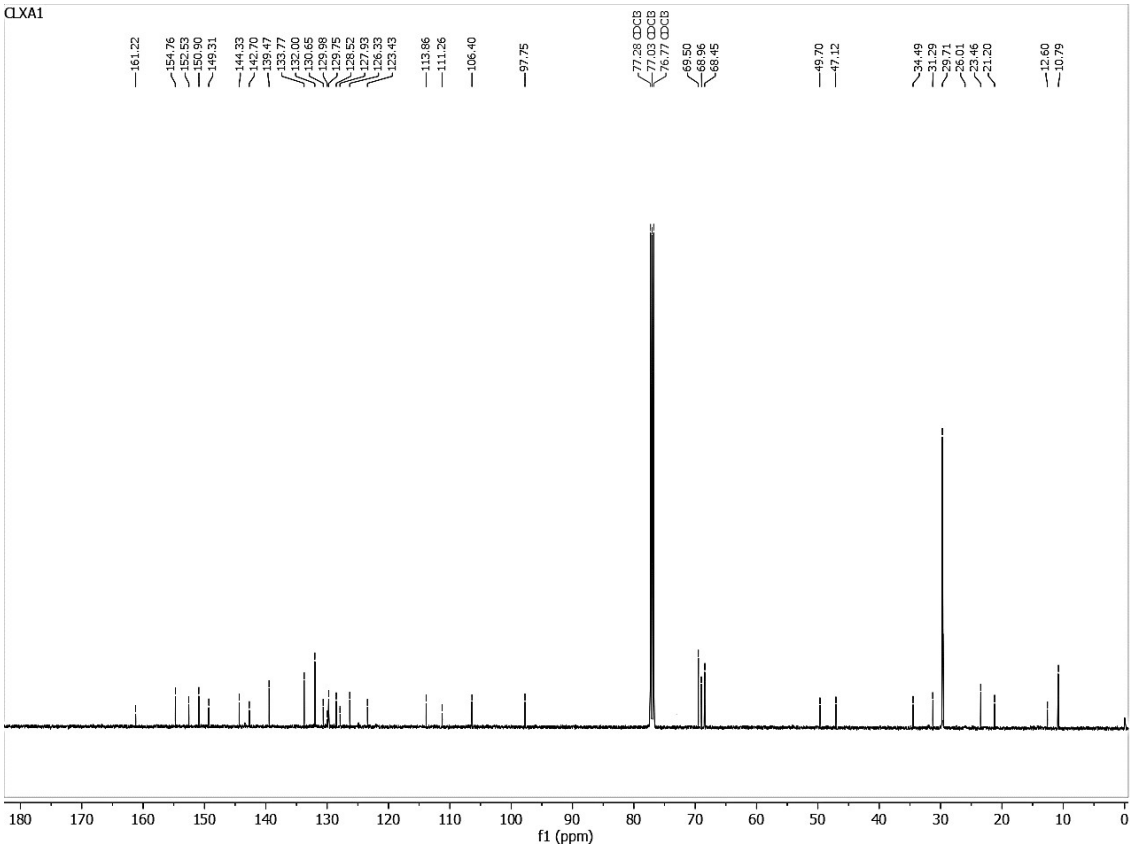

Fig. S8. The ^{13}C NMR spectrum of compound **3a**.

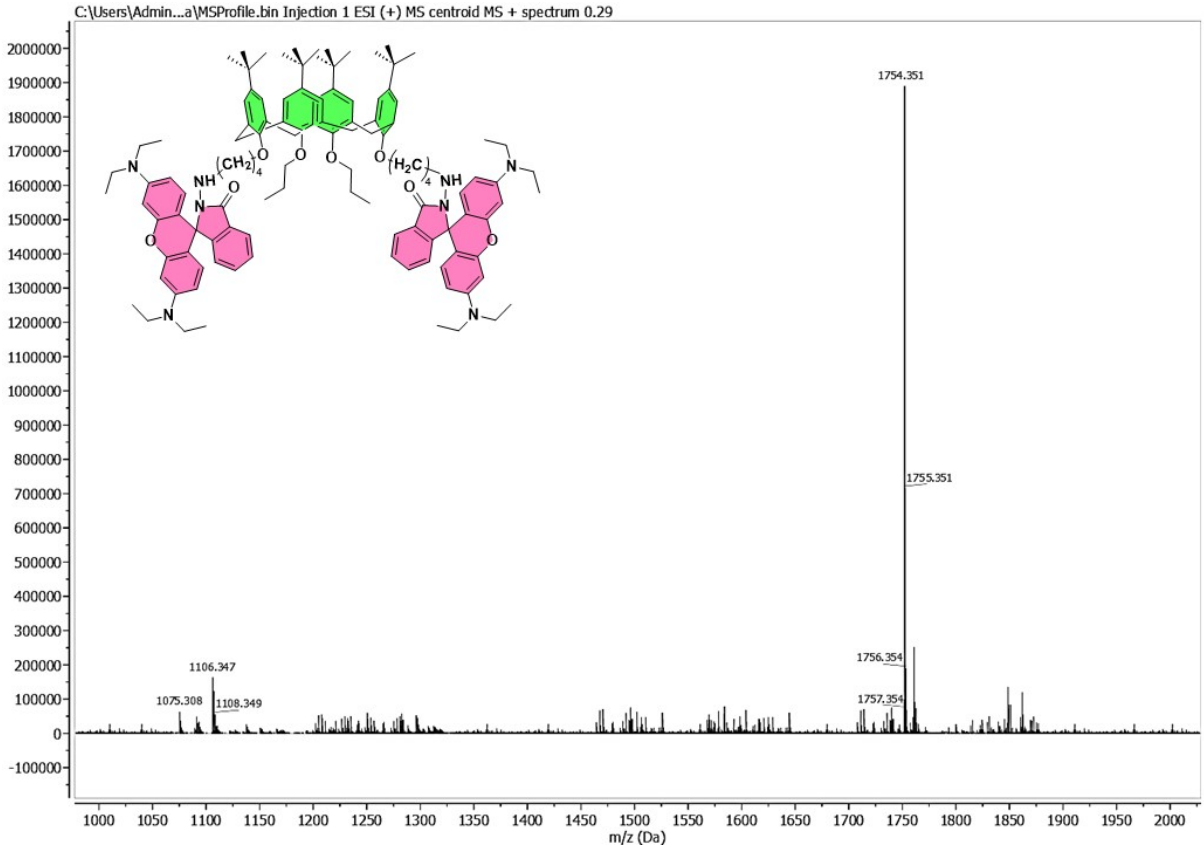

Figure S9. Mass spectra of compound **3a**.

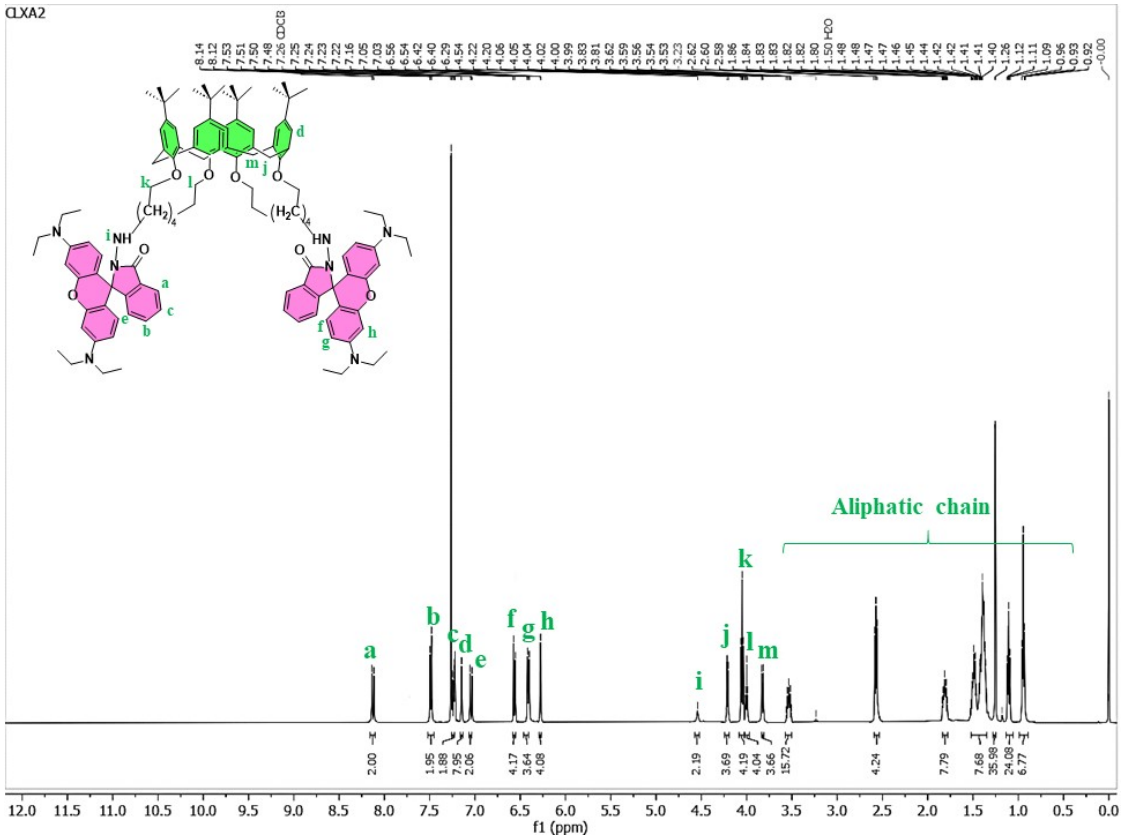

Fig. S10. The ^{1}H NMR spectrum of compound **3b**.

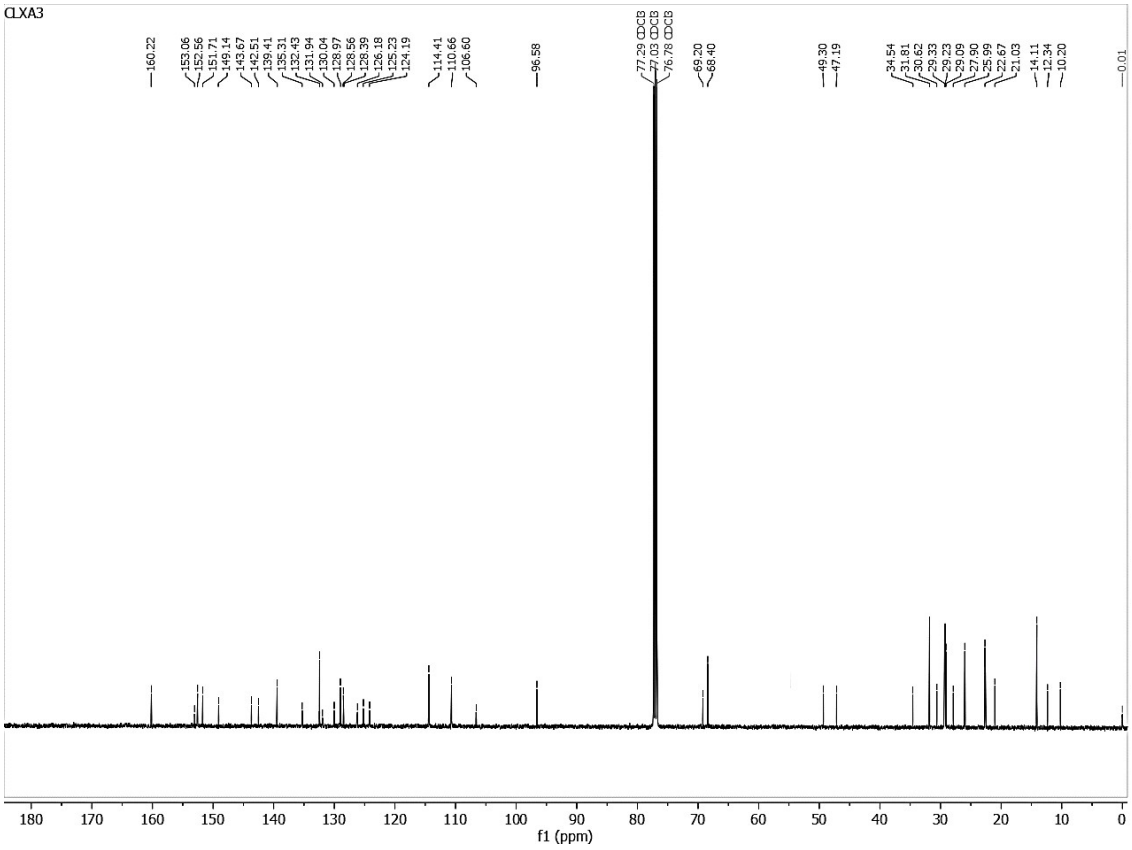

Fig. S11. The ^{13}C NMR spectrum of compound **3b**.

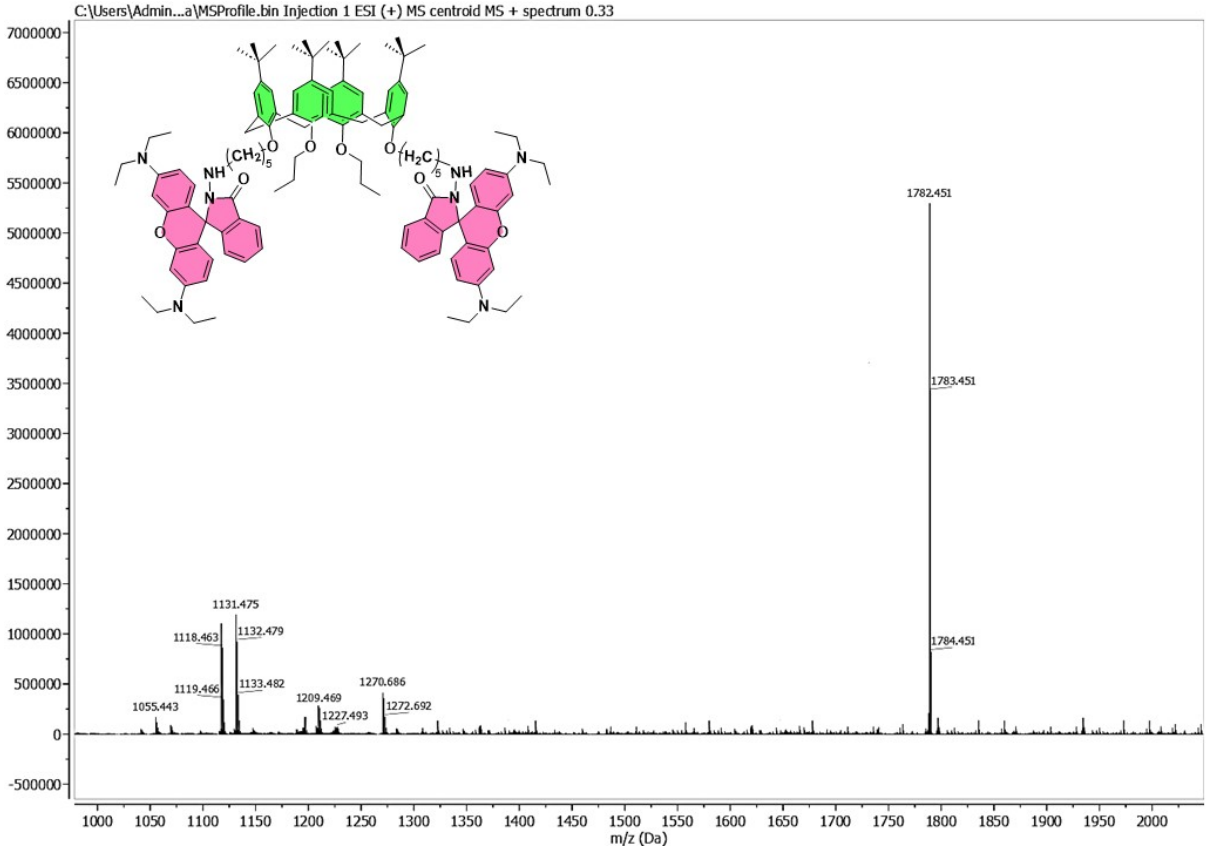

Fig. S12. Mass spectra of compound **3b**.

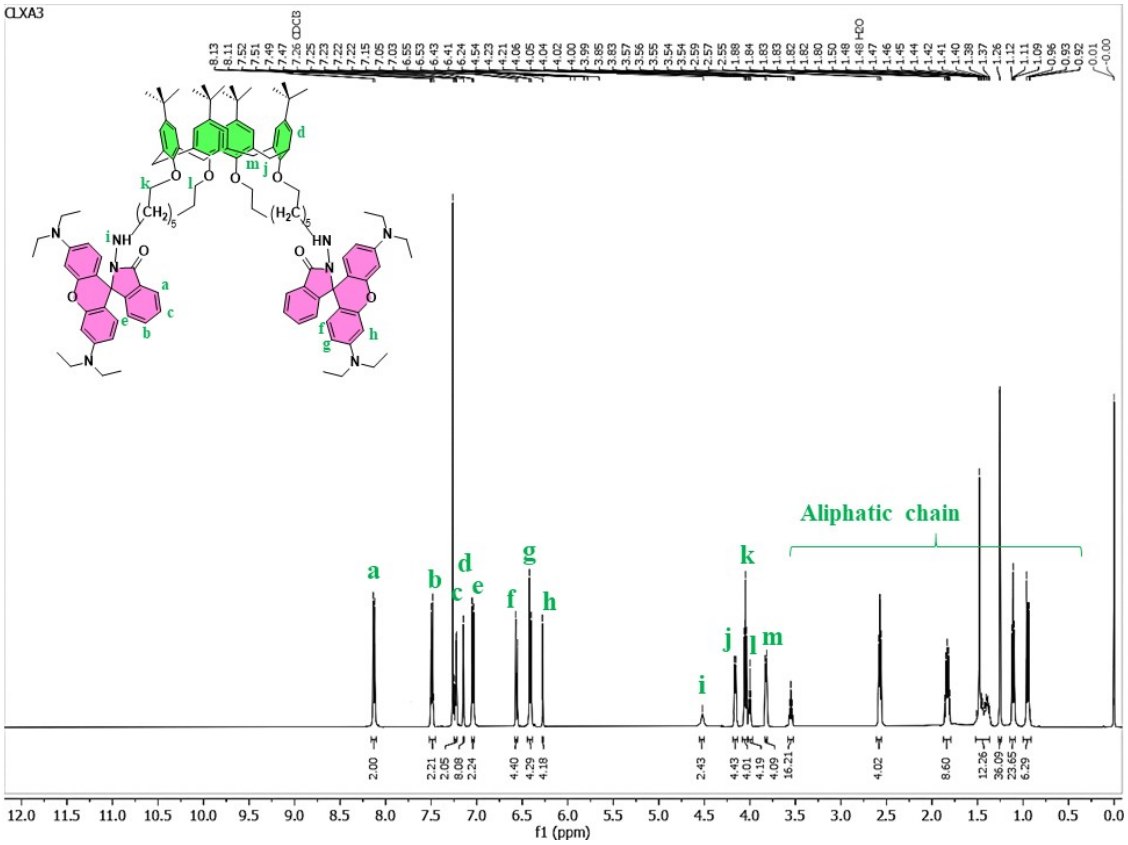

Fig. S13. The ^{1}H NMR spectrum of compound **3c**.

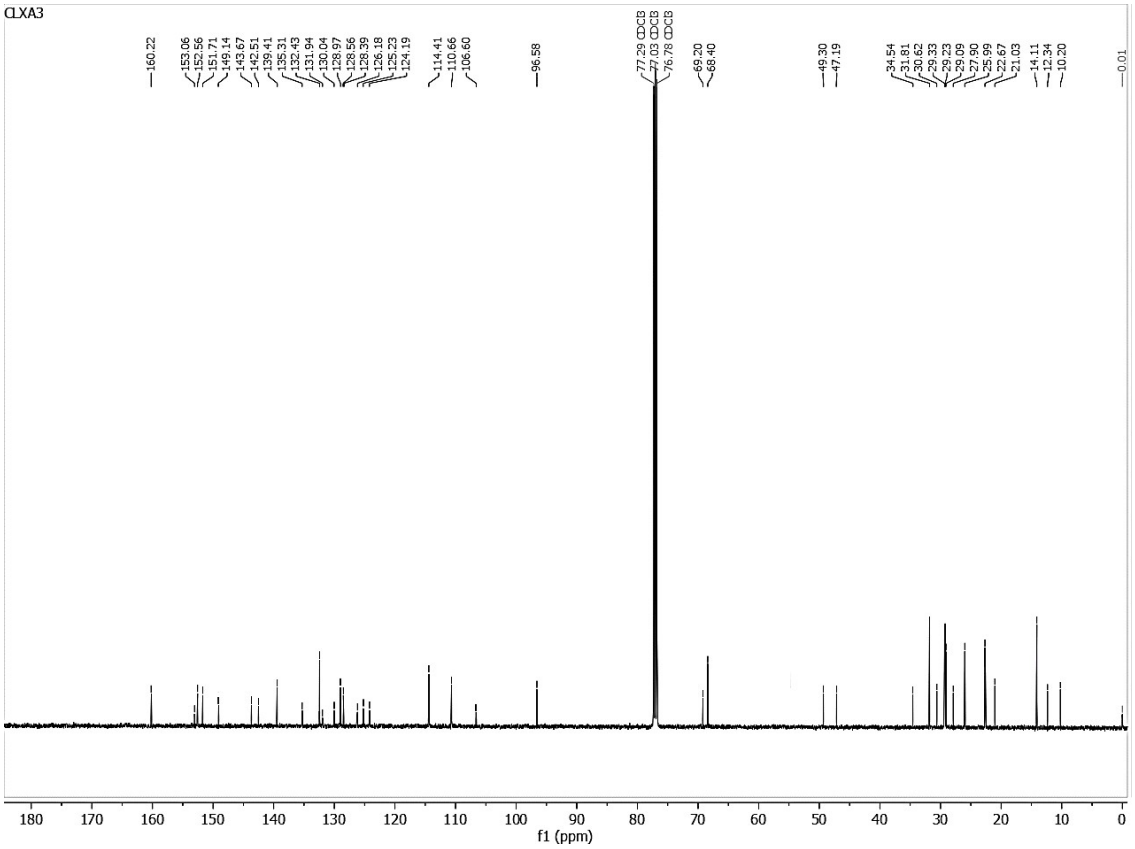

Fig. S14. The ^{13}C NMR spectrum of compound **3c**.

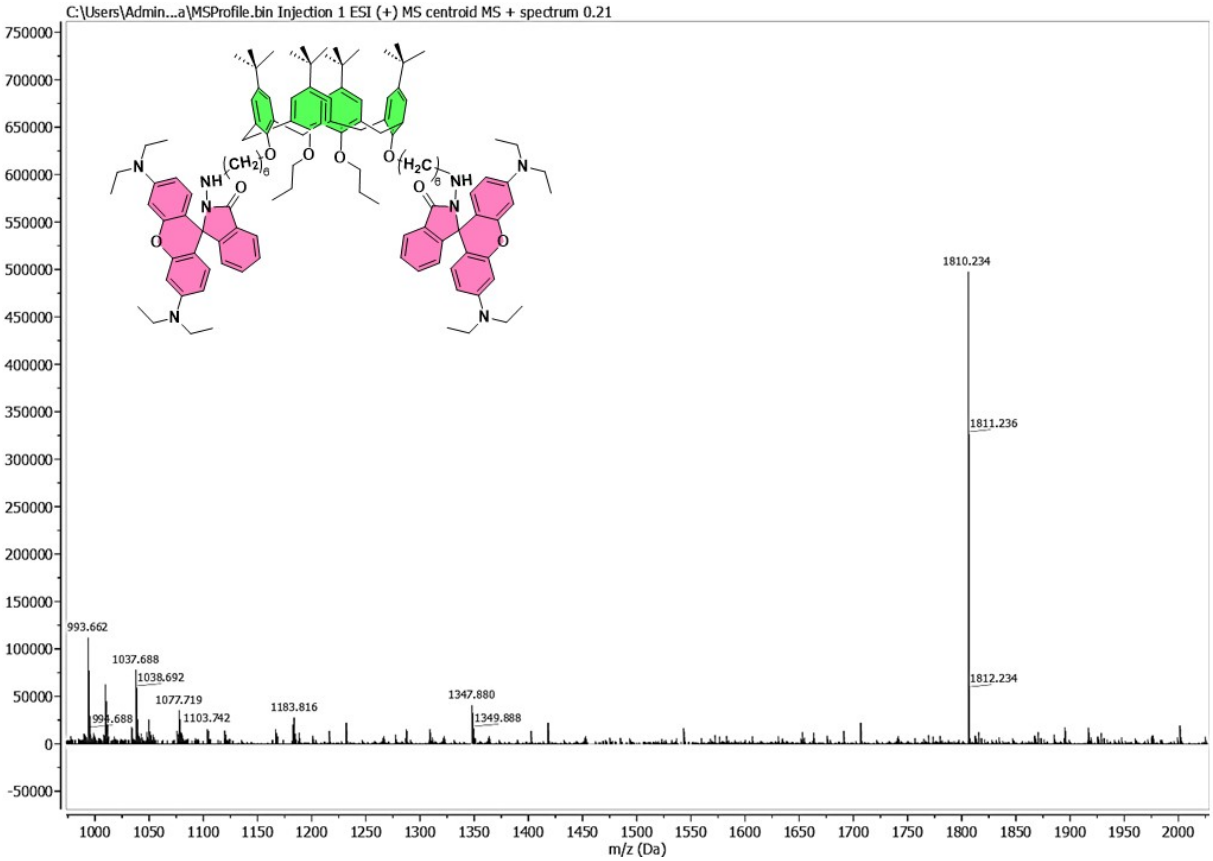

Fig. S15. Mass spectra of compound **3c**.

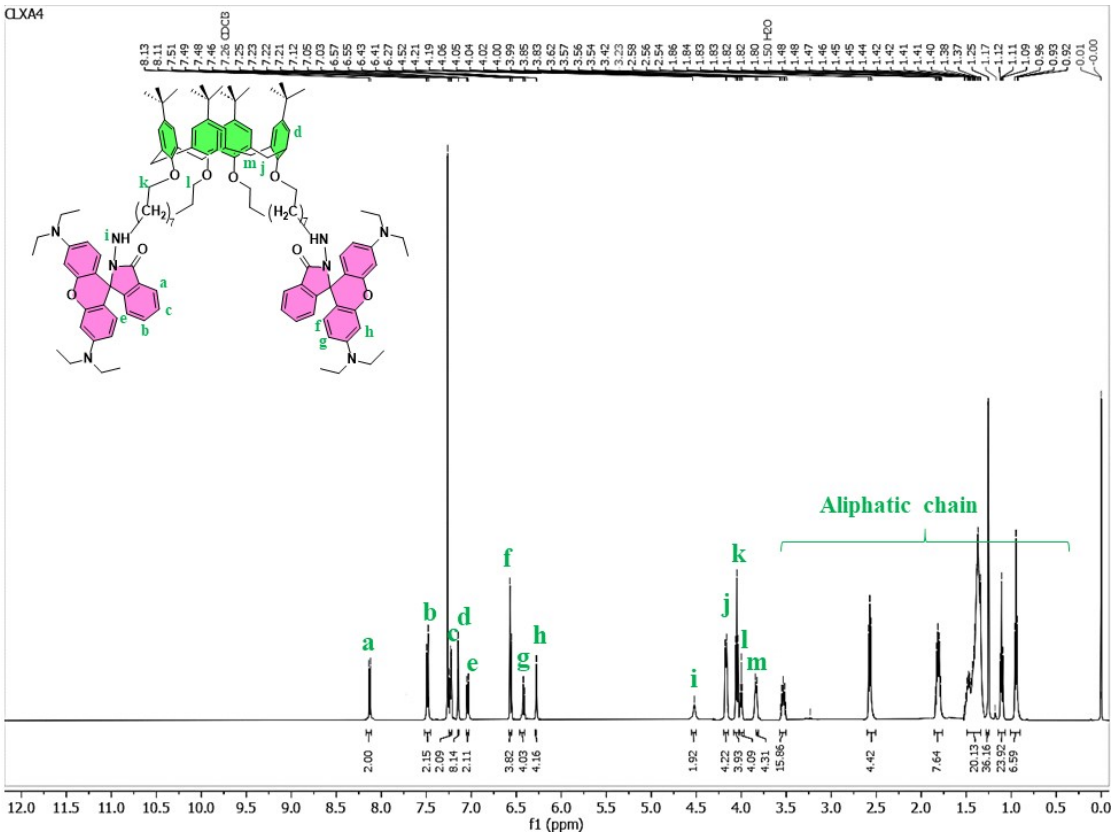

Fig. S16. The ^{1}H NMR spectrum of compound **3d**.

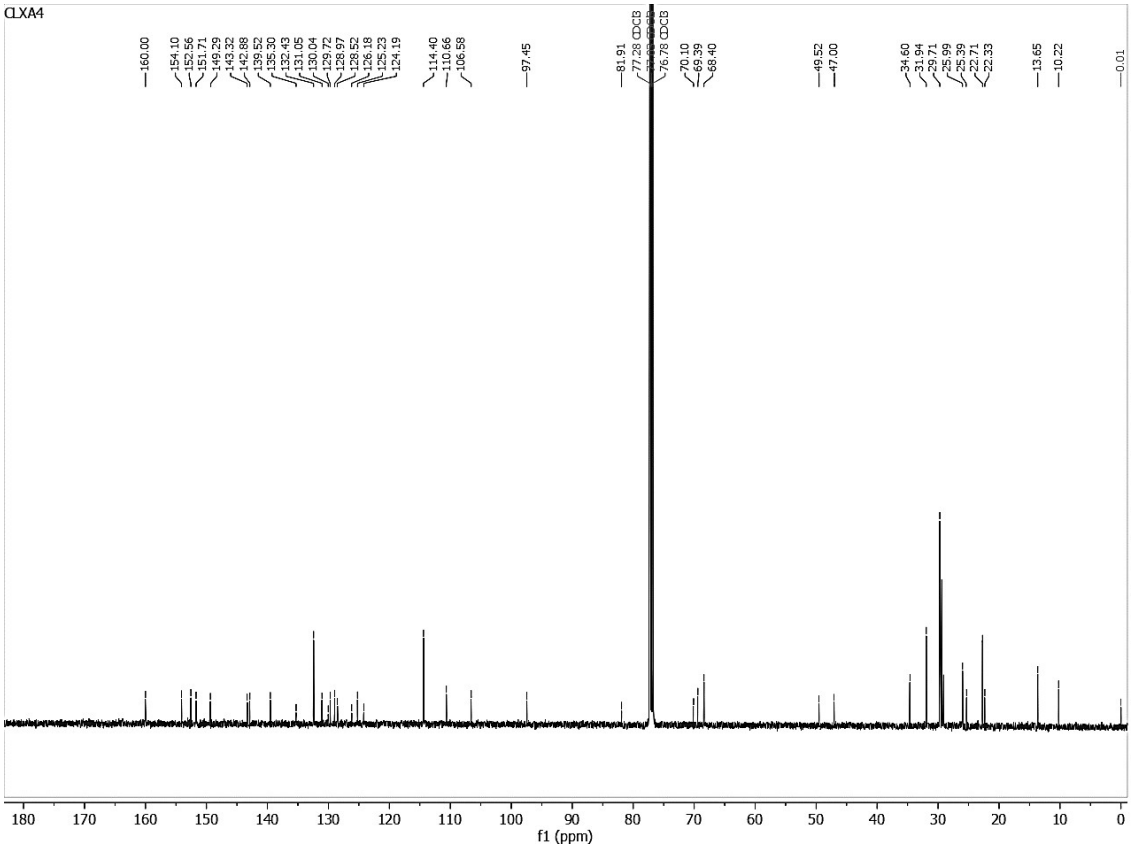

Fig. S17. The ^{13}C NMR spectrum of compound **3d**.

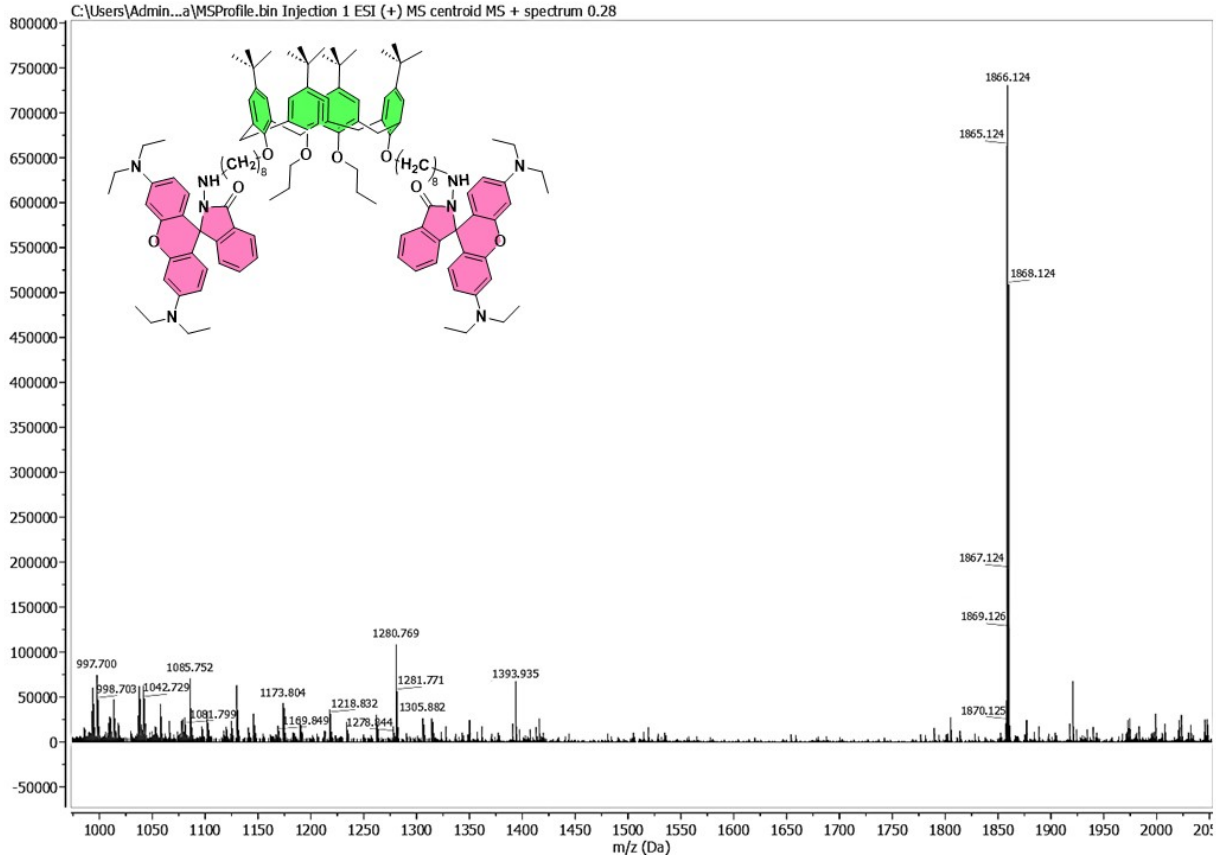

Fig. S18. Mass spectra of compound **3d**.

References

1. M.B. Patel, N.R. Modi, J.P. Raval, S.K. Menon, *Org. Biomol. Chem*., **2012**, 10, 1785.

2. M.M. Nigra, A.J. Yeh, A. Okrut, A.G. DiPasquale et al. *Dalton Transactions*., **2013**, 42, 12762-12771.

3. A. Dhara, A. Jana, S. Konar, S.K. Ghatak et al. *Tetrahedron Letters*., **2013**, 54, 3630-3634.

4. V.S. Sharma, R.B. Patel, *Mol.Cryst.Liq.Cryst*., **2016**, 630, 58-68.

5. A. Patel, V.S. Sharma, V.G. Rao, V.A. Rana et al. *J.Mol.Liq*., **2022**, 366, 120261.

6. P.K. Behera, F.R. Chen, P. Gautam, M. Roy, D.S.S. Rao, C.V. Yelamaggad, J.H. Jou and A. Ammathnadu Sudhakar, *Chem. - Eur. J.,* **2024**, e202304333.

7. V.K. Vishwakarma, M.R. Nagar, N. Lhouvum, J.H. Jou and A. Ammathnadu Sudhakar, *Adv. Opt. Mater.*, **2022**, 10, p.2200241.