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In-depth Photo-physical Properties, Characterization and Cell Imaging Applications of 2, 6-dicyanoanilines Based on Iso-phthalaldehyde and Tere-phthalaldehyde Frame

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

In current manuscript we have reported the interesting fluorescence properties, characterization and cell imaging applications of 2, 6-dicyanoanilines based on iso-phthalaldehyde and tere-phthalaldehyde frame. Earlier preliminary reported work was further extended to in-depth study of photo-physical properties like quantum yield, Fluorescence decay, stock shifts etc, and screening of representative compounds (1b and 2b) for use in fluorescent cell imaging applications. The 2, 6-dicyanoanilines based on iso-phthalaldehyde and tere-phthalaldehyde frame were showing very interesting photo-physical properties and promising prospectus for cell imaging applications.

Keywords: 2; 6-dicyanoanilines; cell imaging; phthalaldehyde; malononitrile; aliphatic aldehyde.

1. INTRODUCTION

Array of fluorescent organic compounds and their properties have been utilized in various chemo sensing applications, fluorescence cell imaging study *etc* [1,2] and there is always need for the design, synthesis and screening of various such molecules for essential applications.

The 2,6-dicyanoanilines and their analogues are known for their significant fluorescent properties [3,4] and have been studied and exploited in many different areas of science like nonlinear optical materials [5, 6, 7], molecular electronic devices [8] and have been reported to exhibit biological activities like antileishmanial [8], inhibitor of a-amylase and a-glucosidase enzymes [9], and antifungal [10] activity. “Also, the flexibility of conversion of cyano and amino groups in to other functional groups makes these compounds versatile for utilization as intermediates in the preparation of many diverse substrates for comprehensive use”.

“There are number of such compounds and methods of their preparations are reported in the literature” [11,12,13-16]. Most of the reported 2, 6-dicyanoanilines and their related compounds are based on single acceptor-donor-acceptor (A-D-A) moiety on the aromatic skeleton, except for a few synthetic strategies reported for tri-substituted 2,6-dicyanoanilines by Klebe [17], Webster et al.[18] and Wallenfels et al.[19] where single aromatic ring bears two A-D-A systems.

The importance of fluorescence bio-imaging has been reflected in number of scientific publications [20-22,23]. The fluorescence bio-imaging applications of dicyanoanilnes have been recently reported by the Borate et al. [24] and Yalcin et al*.* [25].

These references [24,25] forms the basis of this paper to study fluorescence properties and cell imaging trials of newly synthesized 4-alkyl-3-aryl-2,6-dicyanoanilines against human oral mucosa cells.

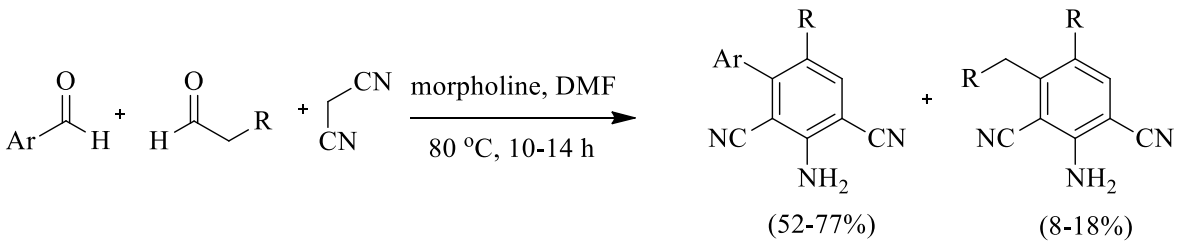
1.1 Literature Review

In the beginning of the research work literature survey was undertaken [11] to design new chemical entities of the 4-alkyl-3-aryl-2,6-dicyanoanilines which reveal that though there are variously substituted 2,6-dicyanonilines are reported in the literature, there is no report on the multicomponent reaction of aromatic di-aldehyde (phthalaldehyde, isophthalaldehyde or terephthalaldehyde) with malononitrile and aliphatic aldehyde in the presence of base which can deliver a number of very interesting new chemical entities and the structure and property relationship of this type of molecules can be studied [7] and further they can be screened for their potential applications like fluorescence cell imaging *etc*.

In the previous work [26] we have just designed, synthesized, characterized and formally photo-physical responses of the new molecules were reported. Due to short of time and resources’ we were not able to fully elaborate the characterization, plausible mechanism of synthesis, further study various photo-physical properties and possible applications of these new chemical entities. Thus, we have decided to further re-structure the study and present our observations with additional study that we have steered on these new molecules.

2. MATERIALS AND METHODS

The synthetic methodology reported by H. B. Borate et al. [27] for the synthesis of 3-aryl,4-alkyl-2,6-dicyanoanilines in good yield (Scheme 1) were chosen for the synthesis of newly designed molecules.



Ar = (Un)substituted phenyl, naphthyl, thienyl; R = Me, n-Pr, n-Hex

Scheme 1. Synthesis of 3-aryl,4-alkyl-2,6-dicyanoanilines in good yield

The novel class of 3-aryl,4-alkyl-2,6-dicyanoanillnes based on isophthalaldehyde or terephthalaldehyde skeleton and synthesized molecule were characterized by ^{1}H NMR, ^{13}C NMR, FT-IR, HR-MS spectroscopy. In additional work we have explored the photo-physical characteristics like quantum yield; Stokes' shift and fluorescence lifetime of new molecules were studied. Further, the fluorescence cell imaging potential of these molecules were evaluated on human oral mucosa cells.

3. RESULTS AND DISCUSSION

3.1 Synthesis of New Dicyanoanilines Based on Isophthalaldehyde or Terephthalaldehyde

We performed multicomponent reactions [27,28] of isophthalaldehyde or terephthalaldehyde with malononitrile and aliphatic aldehyde (butanal or hexanal or nonanal) in the presence of morpholine in dimethylformamide at 80 °C for 8 h to build up new chemical entities bearing two A-D-A systems (Scheme 2 and 3, Table 1).

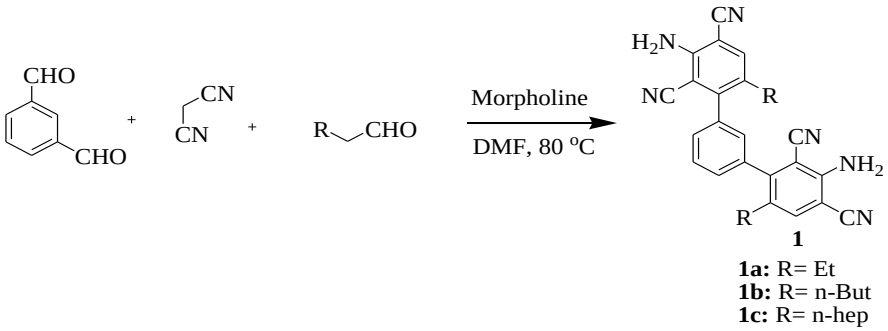
Being multi-component reaction and two reactive sites present on the single aromatic skeleton as expected, the reaction gave mixture of products and it was apparent difficult to isolate the desired fluorescent compound by column chromatography. While resolving the purification issue it was observed that various by-products formed in the reaction has good solubility in the 20 % ethyl acetate and petroleum ether combination. So, the crude reaction mixture extracted from ethyl acetate was poured drop by drop in the petroleum ether and product was precipitated. After taking TLC of supernent solution and crude products it was observed that precipitated crude products has major proportion of the desired product. Then, crude product was further purified by column chromatography. "All the products were characterized by ^{1}H NMR, ^{13}C NMR, IR and High-Resolution Mass Spectroscopy. These compounds were found to be soluble in DMF and DMSO and sparingly soluble in methanol, acetonitrile, chloroform, acetone, dichloromethane, ethyl acetate ***etc"***. [16].

3.2 Possible Mechanism of the Reaction

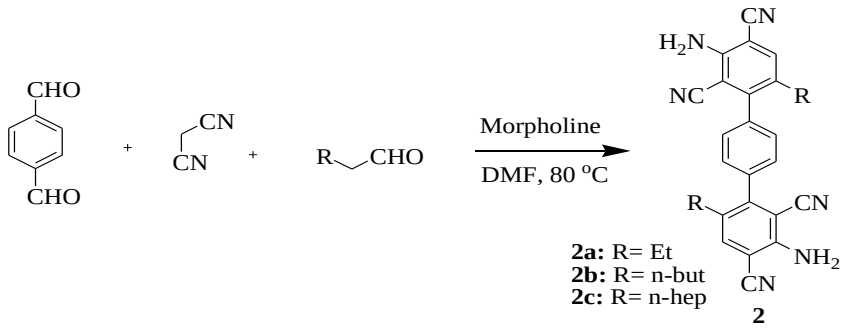
The plausible mechanism [4] of the reaction which yields two acceptor-donor-acceptor features on single aromatic ring is depicted in Scheme 4. Initially, base catalyzed knoevenagel reaction of aromatic aldehyde and aliphatic aldehyde with malononitrile yields respective knoevenagel products (I). Further, abstraction of proton from the each α-hydrogen from aliphatic knoevenegel products forms carbanion ion which attacks the benzyl carbon of the aromatic knoevenegel product leading to the formation of intermediate (II) which upon removal of hydrogen and cyanide ion undergoes cyclization to form intermediate (III) which then undergo aromatization to form the final product IV.

3.3 Photo-Physical Properties of Newly Synthesized 2,6-Dicyanoanilines

The UV-vis absorption, fluorescence emission and excitation spectra of synthesized compounds were recorded in dimethyl formamide and are presented in Figs. 1 and 2. The wavelength of maximum absorption (λ_max) in the UV-vis was observed at ~365 nm while the wavelength of maximum emission (λ _max.emi) for the compounds 1a-1c was observed at ~417 nm while for compounds 2a-2c it was 421 nm. For evaluation of the quantum yields of newly synthesized compounds quinine sulphate (QS) were chosen as a reference standard.



Scheme 2. Synthesis of dicyanoanilines based on isophthalaldehyde skeleton

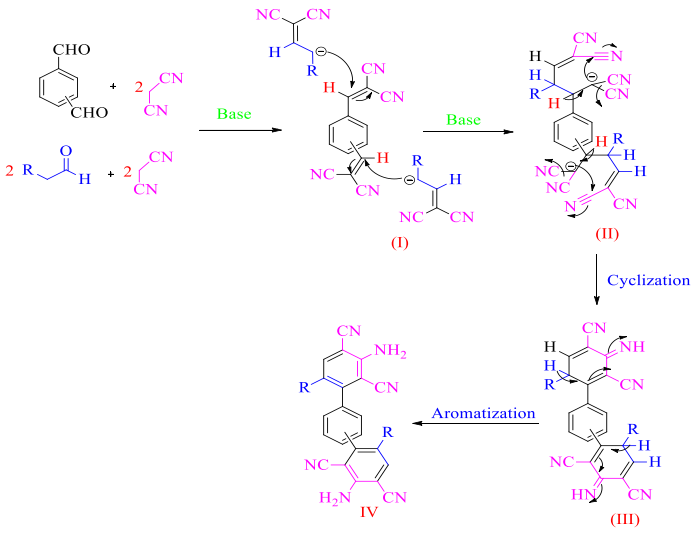


Scheme 3. Synthesis of dicyanoanilines based on terephthalaldehyde skeleton

Table 1. Synthesis of new 2,6-dicyanoanilines

Entry	Aliphatic aldehyde	Aromatic dialdehyde	Product	Yield[a] (%)
1	Butanal	isophthalaldehyde	1a	35
2	Hexanal	isophthalaldehyde	1b	43
3	Nonanal	isophthalaldehyde	1c	50
4	Butanal	terephthalaldehyde	2a	45
5	Hexanal	terephthalaldehyde	2b	48
6	Nonanal	terephthalaldehyde	2c	53

[a] *The yields given are for isolated products*



Scheme 4. Possible mechanism of the reaction

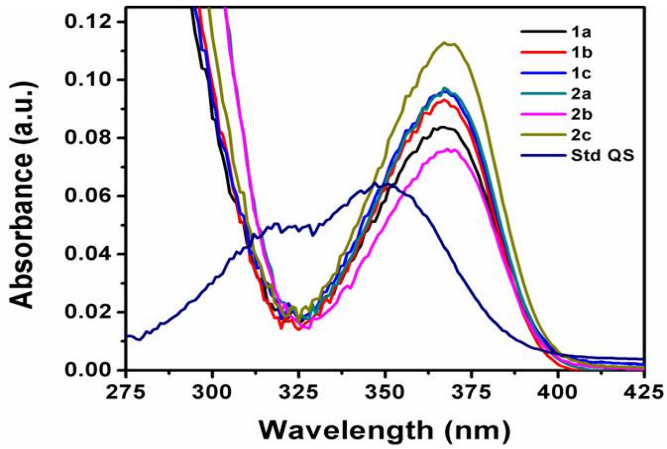


Fig. 1. UV-visible spectra of all compounds and standard QS at concentration of 5 × 10^{-6} M

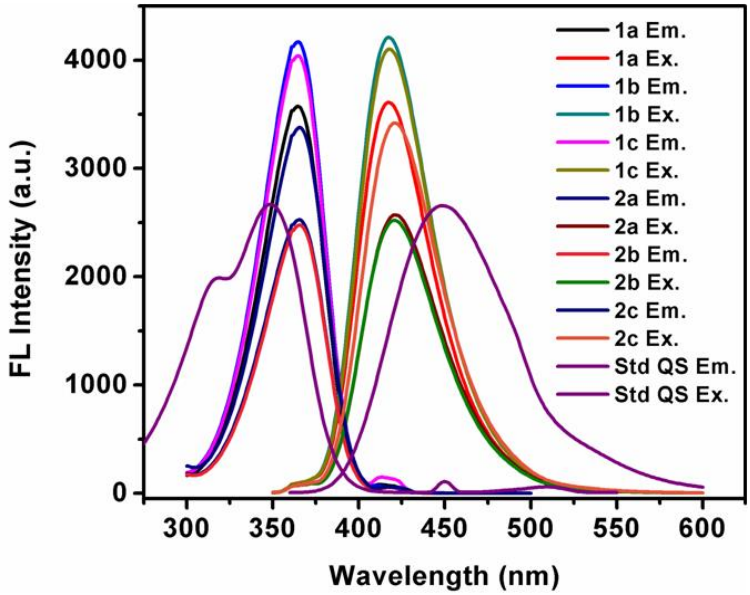


Fig. 2. Fluorescence emission and excitation spectra of all compounds and standard QS at concentration of 5 × 10^{-6} M

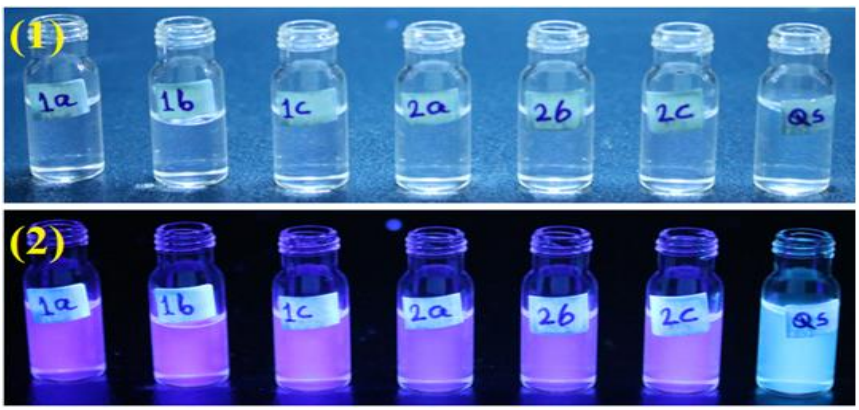


Fig. 3. Photographic images of all of newly synthesized compounds and standard QS under normal (1) and UV light (2) of wavelength 365 nm with sample concentration of 5 × 10^{-6} M

Table 2. Optical properties of new 2, 6-dicyanoanilines set as 5 × 10^{-6} M concentrations in dimethylformamide

Sr. no.	Comp. no.	UV-vis absorption		Fluorescence emission		Quantum yield	$\lambda_{max.ex}$ (nm)	Stokes' shift
		λ_{max} (nm)	Intensity (a.u.)	$\lambda_{max.emi}$ (nm)	Intensity (a.u.)	ϕ_F		
1	1a	367	0.0838	417	3573	0.6418	365	52
2	1b	367	0.0932	417	4170	0.6735	365	52
3	1c	367	0.0959	417	4040	0.6341	365	52
4	2a	367	0.0973	421	2526	0.3907	365	56
5	2b	367	0.0763	421	2477	0.4886	365	56
6	2c	367	0.1129	421	3377	0.4502	365	56
7	Std QS	350	0.064	449	2656	0.5400	349	100

All newley synthesized compounds having 365 nm as a excitation wavelength and fluorescence emission wavelength for compounds 1a-1c at 417 nm while for cmpounds 2a-2c it is 421 nm. The values for fluorescence quantum yield of compounds based on isophthalaldehyde [1a-1c] and terephthaladehyde [2a-2c] with various aliphatic chains lengths are given in Table 2. It is observed that between the two isomers of phthalaldehyde (isophthalaldehyde and terephthaladehyde) used in present study, the compound 1b obtained from the isophthalaldehyde with four carbon chain shows very good fluorescence quantum yield (0.67) which is more than the reference standard compound QS (0.54) and compound 2b obtained from terephthaladehyde with flour aliphatic carbon chain shows fluorescence quantum yield (0.48) which is in close resemblance with standard compound. Rest of the synthesized compounds 1a, 1c, 2a, 2c also have remarkable quantum yield. All these compounds show bright blue luminescence under UV lamp (Fig. 3).

The fluorescence lifetime forms the basis of the fluorescence lifetime imaging microscopy which is an important parameter signifying the lifespan of the fluorophore in the excited state before returning to ground state by emitting a photon. Fluorescence lifetime is an intrinsic property of the fluorophore and it is independent of concentration, intensity etc. [29,30]. The fluorescence lifetime of representative compounds 1b and 2b were recorded in DMF and presented in Fig. 4. The average lifetime of 1b is found to be 2.93 × 10^{-9} ns whereas 2b shows 2.47 × 10^{-9} ns.

The Stokes shift, difference between maxima of emission and excitation wavelength, is also important parameter of fluorescence spectroscopy. Our synthesized compounds also show significant Stokes' shift represented in Table 2. We believe that, the good absorbance, excellent fluorescence quantum yield and significant Stokes' shift exhibited by newly synthesized compound in present study may find applications in various bio-imaging or analytical chemistry for chemo-sensing etc.

3.4 Screening of Fluorescence cell Imaging Potential of Compound 1b and 2b

After the evaluation of fluorescent properties, we proceeded for the screening of the possible biological applications like fluorescence cell imaging. While conducting the cell imaging experiment 4′,6-diamidino-2-phenylindole (DAPI) were taken as reference standard which is a well-known nuclear counter stain used in fluorescence microscopy. The Fig. 5 represents the cell imaging applications of representative compounds 1b and 2b. The initial screening reveals that these types of compounds can be modified for potential bio-imaging applications.

From the Fig. 5 it was observed that the oral mucosa cells incubated with reference DAPI stains the nucleus of the cells (image b) wherein, compounds under study have affinity for the membrane of the cells and cells organelles. Compounds 1b and 2b remarkably stain the cell membrane, nucleus and membranes of other cell organelle suspended in the cell cytoplasm (Image c and d). The cells without the fluorescent material do not exhibit fluorescence and hence there is no visualization of the cells under fluorescence (Image a). Thus, compound in the present study can act as potential bio-imaging probes.

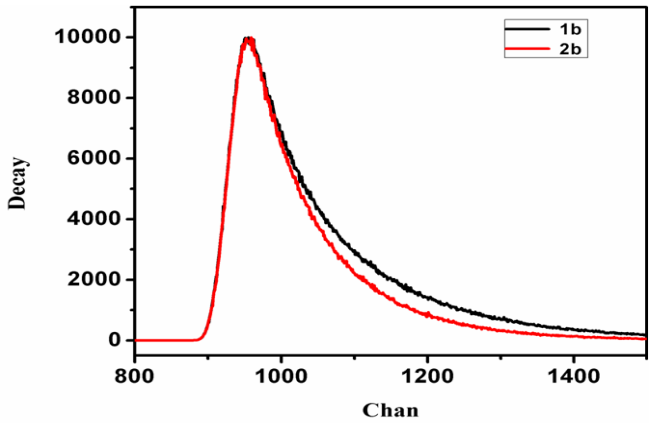


Fig. 4. Fluorescence decay profile of 1b and 2b in DMF

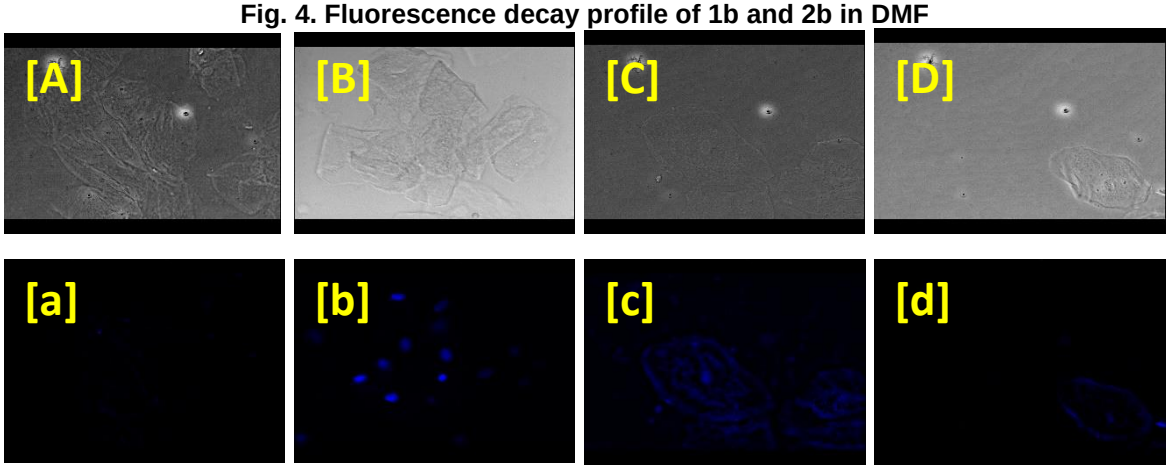
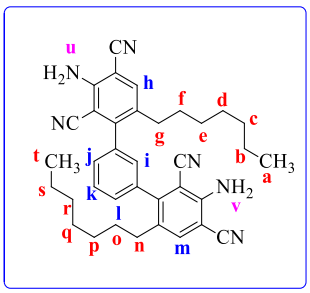


Fig. 5. i) Upper part- bright field microscopic images of human oral mucosa cells incubated with [A]: Blank water, [B] DAPI, [C] 1b and [D] 2b.; ii) Lower part- images under fluorescence microscope incubated with [a]: Blank water, [b] DAPI, [c] 1b and [d] 2b

3.5 Characterization of Representative Compounds

The identity of all the desired products **(Table 1, entries 1-6)** was ascertained on the basis of FT-IR, ^{1}H NMR, ^{13}C NMR, and High-Resolution Mass Spectroscopy.

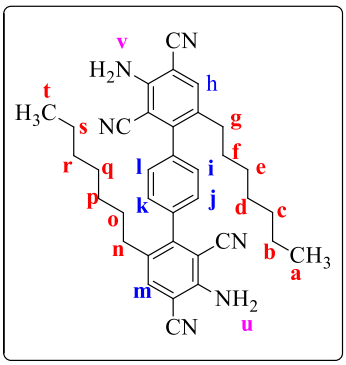
3,3''-diamino-6,6''-diheptyl-[1,1':3',1''-terphenyl]-2,2'',4,4''-tetracarbonitrile (Table 1, entry 3)



The IR spectrum of above compound (Fig. 16) shows characteristic –CN stretching frequency of 2118 cm^{-1}, typical –NH_2 stretching bands at 3336, 3342 cm^{-1}. The aliphatic -C-H stretching frequency at 2853, 2923 and 2953 cm^{-1}. In ^{1}H NMR spectra (Fig. 13) unresolved multiplate at 0.46-0.88 ppm corresponds to six protons indicating terminal two aliphatic –CH_3 groups (a and t), unresolved multiplate at 0.90-1.38 ppm give integration of 20 protons represents ten aliphatic –CH_2 gropus (b, c, d, e, f and o, p, q, r, s), multiplate at 2.25-2.45 ppm shows total 4 proton integration indicates two benzylic –CH_2 gropus (n and g). Moving towards the lower field two peaks at 4.95 and 5.08 ppm collectively corresponds to 4 proton of two –NH_2 gropus (u and v) further in the aromatic region 7.12 (s, 1H) (i), 7.36 (d, 2H, *J*= 8 Hz) (j and l), 7.52 ppm singlet for two proton of dicyanoaniline skeleton (m and h), the triplet at 7.42 (t, 1H, *J*= 8 Hz) (k) supports the proposed structure. ^{13}C NM R spectrum (Fig 14) gives all aliphatic carbon shifts

in ppm at 14.01(2C), 22.51(2C), 28.86(4C), 30.79(2C), 31.59(2C), and 31.90(2C) counting overall 14 aliphatic carbons, peaks at 96.66(2C), 98.80(2C) represents –C attached to the –CN group, peak positions at 115.18(2C), 115.95(2C) for carbon of –CN group, the next all aromatic carbons reflected at 128.42(2C), 128.90(2C), 129.17(2C), 130.50, 131.52, 137.25 (2C), 137.42(2C), 149.26, 149.53 supports the structure and the HR-MS (Fig 15) of compound obtained for M.F. $C_{36}H_{41}N_6$ 557.3400 as (M+H), calculated for M.F. $C_{36}H_{41}N_6$ 557.3393 as (M+H) finally confirms the proposed structure.

3,3''-diamino-6,6''-diheptyl-[1,1':4',1''-terphenyl]-2,2'',4,4''-tetracarbonitrile: (Table 1, entry 6)



IR spectrum of compound (Fig 27) shows typical –CN frequency at 2219 cm^{-1}, aliphatic C-H stretching at 2851, 2921, 2952 cm^{-1} and stretching vibrations of $–NH_2$ at 3378, 3469 cm^{-1} supports the functional groups in the in the compound. ^1H NMR spectrum (Fig 24) shows triplet at 0.84 ppm, integration value for 6 protons with coupling constant *J*= 8 Hz for two terminal $–CH_3$ groups (a, t). The multiplate at 1.03-1.47 ppm corresponds to 20 protons that indicate 10 aliphatic $–CH_2$ groups (b,c,d,e,f and o,p,q,r,s) then triplet at 2.37 ppm for 4 proton with coupling constant of 8 Hz for benzylic $–CH_2$ (g, n) groups. Broad singlet at 5.15 ppm accounting for 4 proton of two $–NH_2$ groups (u,v), due to symmetric 1,4 substitution at middle benzene ring singlet at 7.39 ppm corresponds to 4 aromatic protons (i, j, k, l) further singlet at 7.53 ppm gives 2 proton integration for aromatic proton at the dicyanoaniline moiety supports the structure. The ^{13}C NMR (Fig 25) shows peaks at 13.72 (2C), 21.99 (2C), 28.23, 28.37 (2C), 28.47, 28.62, 30.34, 30.44, 31.09, 31.14, 31.48 ppm accounts for all 14 aliphatic carbons, peaks at 95.85 (2C), 97.68, 97.93 for carbon attached to –CN groups and all peaks at 115.08 (2C), 116.04 (2C), 128.31 (2C), 128.35 (2C), 129.30, 129.48, 137.09, 137.13, 137.35, 137.43, 148.89, 149.13, 150.33, 150.46 ppm supports the structure. The HRMS (Fig 26) obtained for M.F. $C_{36}H_{41}N_6$ 557.3397 as (M+H) and calculated for M. F. $C_{36}H_{41}N_6$ 557.3394 as (M+H) confirms the proposed structure.

5. EXPERIMENTAL DETAILS

Required malononitrile, Morpholine, dimethyl formamide, silica gel for column chromatography (60-120 mesh), ethyl acetate and petroleum ether (boiling range 60-80 °C) were obtained from Spectrochem while all aromatic/aliphatic aldeydes were procured from Sigma-Aldrich and used without further purification. Thin Layer Chromatography analysis was performed on Merck HPTLC Silica gel 60 F_{254} plates. Melting points were determined in an open capillary and are uncorrected. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker-AV 200 MHz spectrometer using $CDCl_3$ DMSO-d_6 as solvent and chemical shifts were reported as δ(inppm). The IR spectra were recorded on a Bruker FT-IR Spectrometer in between the range of 400-4000 cm^{-1}. The High Resolution-Mass Spectra of all compounds were recorded on Thermo Scientific Q-Exactive instrument in ESI mode. Ultraviolet spectra were recorded on Analytik Jena Specord Plus UV/Vis double beam spectrometer and fluorescence spectra were recorded on FP-8300 spectrofluorometer (JASCO, Japan). The fluorescence lifetimes were recorded on Horiba Sci. NL (Japan) in the time scan 500 ps to 1μsec by Time Correlated Single Photon Counting (TCSPC) method at respective emission and excitation wavelengths. Cell imaging experiments were carried out by using Zeiss Axio-scope A 1 trinocular phase contrast microscope with fluorescent attachment.

5.1 Representative Procedure [27] for the Preparation of 3,3''-Diamino-6,6''-Diheptyl- [1,1':3',1''-Terphenyl]-2,2'',4,4''-Tetracarbonitrile (1c)

"To a mixture of isophthalaldehyde (0.500 g, 0.003727 mol), nonanal (1.272 g, 0.008946 mol) and malononitrile (1.08 g, 0.01639 mol) in dry DMF taken in a round bottom flask and equipped with reflux condenser and guard tube, morpholine (1.55 g, 0.01788 mol) was added at 0 °C and the mixture was allowed to cool to room temperature and stirred at 80 °C for 8 h. Progress of the reaction was checked by the

TLC. (Solvent system- Petroleum ether: Ethyl acetate, 80:20). After completion of reaction, the reaction mixture was allowed to cool to room temperature and water was added, then extracted with excess of ethyl acetate (250 mL), dried over sodium sulphate and concentrated on rotavapor. Ethyl acetate (10 mL) was added and the solution of reaction mixture was added slowly drop by drop to 100 mL petroleum ether for recrystallization. After settlement of the precipitate in the beaker, the supernatant solution was decanted in another beaker. The precipitate thus obtained was washed with 10% ethyl acetate and petroleum ether mixture. The residue thus obtained was chromatographed on silica gel (60-120 mesh) using 15-20% ethyl acetate in petroleum ether to afford 3,3''-diamino-6,6''-diheptyl- [1,1':3',1''-terphenyl]-2,2'',4,4''-tetracarbonitrile as off-white flappy solid (1.03 g 50%)" [31].
(In case of **1a** and **2a** the amount of base (Morpholine) used was 3.0 equivalents)

5.2 Photophysical Measurement of the Synthesized Molecules

The UV-visible and fluorescence spectra of the target compounds and QS were recorded at fixed concentration of 5 × 10^{-6} M. While recording emission spectra both the excitation and emission slit width were set at 5-5 nm and low sensitivity. The quantum yield was calculated by using following equation (1)

$$QY = QY_{ref} \frac{\eta^2}{\eta^2_{ref}} \frac{I}{A} \frac{A_{ref}}{I_{ref}} \quad (1)$$

Where, QY = quantum yield of sample, QY_{ref} = quantum yield of reference QS (taken as 0.54), η^2= square of refractive index of solvent used to dissolve samples (dimethylformamide, 2.04633), η^2_{ref} = square of refractive index of solvent used to dissolve standard (0.1 M H_2SO_4, 1.7689), I= fluorescence intensity of sample, A= absorbance of sample, A_{ref}=absorbance of standard, I_{ref} = fluorescence intensity of standard.

5.3 Cell Imaging Experiments

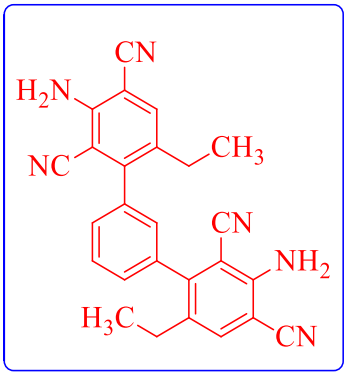
In the present work, we screened imagingpotential of compounds 1b and 2b in dimethylformamide against human oral mucosa epithelial cells. The experiments were performed on oral mucosa cells of two healthy male donors in the age group of 30-35 by informed consents.

5.4 Preparation of Slides for Cell Imaging Experiments

The smear of oral mucosa cells on clean glass slide were prepared by scratching against the oral epithelium of the donor using sterilized steel spoon. The thin smear of oral mucosa cells was prepared on four different clean glass slides and were allowed to dry for five minutes and then 150 µL dilute solutions of DAPI in water were spread over the smear. Similarly, the 150 µL solutions of compounds 1b and 2b in dimethylformamide was spread over the smear and covered with cover glass. After 10 minutes the slides were observed under bright field and FITC spectrum blue (Chroma 3100) comprising 25 mm diameter filters, excitation filter (D350/50), beam splitter (400 DCLP) and emission filter (D460/50) fluorescence microscope at 400× magnification and images were captured.

5.5Physical and Spectral Data for all Newly Synthesized Compounds

3,3''-diamino-6,6''-diethyl-[1,1':3',1''-terphenyl]-2,2'',4,4''-tetracarbonitrile (Table 1, entry 1):



Molecular formula : $C_{26}H_{20}N_6$
Exact mass : 416.1749
Molecular weight : 416.4772
Nature and color : Off-white flappy solid
Melting point : 222 °C

FT-IR (Neat, cm^{-1}): v 3442, 3334 (–NH_2), 2924, 2857 (aliphatic—CH), 2220 (—CN), 1647

(aromatic —C═C).

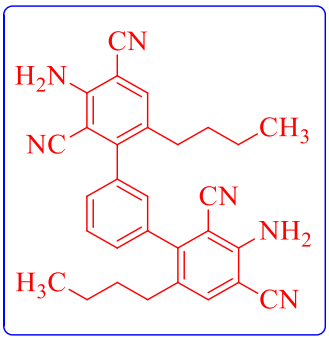
^{1}H NMR (400 MHz, in $CDCl_3$+DMSO-d6, δ ppm): 0.04-0.16 (m, 6H, 2 × –CH_3), 1.39-1.56 (m, 4H, 2 × Ar–CH_2), 5.23 (s, 4H, 2 × –NH_2), 6.27 (s, 1H, Ar–H), 6.45-6.54 (m, 2H, Ar–H), 6.68 (s, 2H, Ar–H), 6.73-6.81 (m, 1H, Ar–H).

^{13}C NMR (100 MHz in $CDCl_3$+DMSO-d6, δ ppm): 13.80 (2C), 23.55 (2C), 94.89 (2C), 96.77 (2C), 114.03 (2C), 114.94 (2C), 127, 127.12 (2C), 127.51

(2C), 129.28, 135.73 (2C), 136.17 (2C), 147.48, 147.73, 149.18, 149.29.

HR-MS (ESI) *m/z*: Obtained for M. F. $C_{26}H_{21}N_6$ 417.1822 as (M+H), Calculated for M. F. $C_{26}H_{21}N_6$ 417.1829 as (M+H).

3,3''-Diamino-6,6''-dibutyl-[1,1':3',1''-terphenyl]-2,2'',4,4''-tetracarbonitrile (Table 1, entry2):



Molecular Formula: $C_{30}H_{28}N_6$
Exact Mass : 472.2375
Molecular Weight : 472.5835
Nature and color : Off-white floppy solid
Melting point : 212 °C

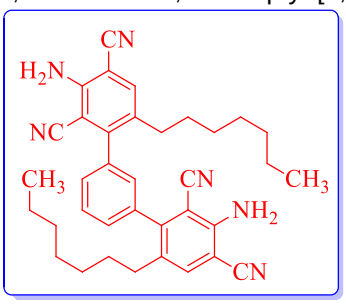
FT-IR (Neat, cm^{-1}): v 3443, 3336 (–NH_2), 2929, 2870 (aliphatic—CH), 2218 (—CN), 1664 (aromatic —C═C).

^{1}H NMR (500 MHz, $CDCl_3$+DMSO-d6, δ ppm): 0.62-0.77 (m, 6H, 2 × –CH_3), 0.98-1.15 (m, 4H, 2 × –CH_2), 2.20-2.37 (m, 4H, 2 × Ar–CH_2), 6.60 (s, 4H, 2 × –NH_2), 7.20 (s, 1H, Ar–H), 7.38-7.47 (m, 2H, Ar–H), 7.62-7.70 (m, 1H, Ar–H), 7.75 (s, 1H, Ar–H), 7.76 (s, 1H, Ar–H).

^{13}C NMR (125 MHz, DMSO-d_6, δ ppm): 13.58 (2C), 21.66 (2C), 30.90 (2C), 32.41 (2C), 95.65 (2C), 97.71 (2C), 115.47 (2C), 116.47 (2C), 128.37, 128.64(2), 128.81, 128.94, 129.31, 137.21, 137.45, 138.41 (2C), 149.33, 149.61, 150.61, 150.76.

HR-MS (ESI) *m/z*: Obtained for M. F. $C_{28}H_{24}N_6$ 473.2457 as (M+H), calculated for $C_{28}H_{24}N_6$ 473.2355 as (M+H).

3,3''-Diamino-6,6''-diheptyl-[1,1':4',1''-terphenyl]-2,2'',4,4''-tetracarbonitrile (Table 1, entry 3):



Molecular Formula : $C_{36}H_{40}N_6$
Exact Mass : 556.3314
Molecular Weight : 556.7430
Nature and color : Off-white floppy solid
Melting point : 190 °C.

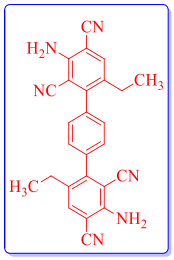
FT-IR (Neat, cm^{-1}): v 3442, 3336 (–NH_2), 2923, 2853 (aliphatic—CH), 2218 (—CN), 1643 (aromatic —C═C).

^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 0.46-0.88 (m, 6H, 2 × $–CH_3$), 0.90-1.38 (m, 20H, 10 × $–CH_2$), 2.25-2.2.45 (m, 4H, 2 × $–CH_2$), 4.95 and 5.08 (2 s, 4H, 2 × $–NH_2$), 7.12 (s, 1H, Ar–H), 7.36 (d, 2H, *J*= 8Hz, Ar–H), 7.52 (s, 2H, Ar–H), 7.42 (t, 1H, *J*= 8Hz, Ar–H).

^{13}C NMR (50 MHz, $CDCl_3$, δ ppm):14.01(2C), 22.51(2C), 28.86(4C), 30.79(2C), 31.59(2C), 31.90(2C), 96.66(2C), 98.80(2C), 115.18(2C), 115.95(2C), 128.42(2C), 128.90(2C), 129.17(2C), 130.50, 131.52, 137.25 (2C), 137.42(2C), 149.26, 149.53.

HR-MS (ESI) *m/z*: Obtained for M. F. $C_{36}H_{41}N_6$ 557.3400 as (M+H), Calculated for M. F. $C_{36}H_{41}N_6$ 557.3393 as (M+H)

3,3''-Diamino-6,6''-diethyl-[1,1':4',1''-terphenyl]-2,2'',4,4''-tetracarbonitrile (Table 1, entry 4):



Chemical formula: $C_{26}H_{20}N_6$
Exact mass : 416.1749
Molecular weight : 416.4772
Natureand color : Off-white flappy solid
Melting point : decomposition around 277-278 °C.

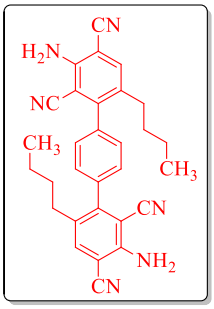
FT-IR (Neat, cm^{-1}): v 3478, 3358 ($–NH_2$), 2930, 2863 (aliphatic—CH), 2215 (—CN), 1632 (aromatic —C═C).

^{1}H NMR (500 MHz, DMSO-d6, δ ppm): 0.81-1.00 (m, 6H, 2 × $–CH_3$), 2.20-2.37 (m, 4H, 2 × $–CH_2$), 6.63 (s, 4H, 2 × $–NH_2$), 7.45 and 7.46 (2s, 4H, Ar–H), 7.79 and 7.80 (2s, 2H, Ar–H).

^{13}C NMR (125 MHz, DMSO-d6, δ ppm): 14.87, 15.02, 24.59, 24.67, 95.77 (2C), 97.44, 97.58, 115.50 (2C), 116.49 (2C), 128.63 (2C), 128.68, 128.76, 130.39 (2C), 137.14, 137.30, 138.00 (2C), 149.39, 149.59, 150.73, 150.84.

HR-MS (ESI) *m/z*: Obtained for M. F. $C_{26}H_{21}N_6$ 417.1822 as (M+H), Calculated for M. F. $C_{26}H_{21}N_6$ 417.1829 as (M+H).

3,3''-Diamino-6,6''-dibutyl-[1,1':4',1''-terphenyl]-2,2'',4,4''-tetracarbonitrile (Table 1, entry 5):



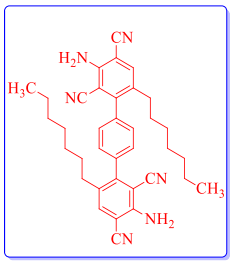
Molecular formula : $C_{30}H_{28}N_6$
Exact mass : 472.2375
Molecular weight : 472.5835
Nature and color : Off-white flappy solid
Melting point : decomposition around 267-270 °C.

FT-IR (Neat, cm^{-1}): v 3477, 3358 ($–NH_2$), 2928, 2865 (aliphatic—CH), 2216 (—CN), 1632 (aromatic —C═C).

^{1}H NMR (500 MHz, DMSO-d6, δ ppm): 0.69 (bs, 6H, 2 × $–CH_3$), 1.06 (bs, 4H, 2 × $–CH_2$), 1.24 (bs, 4H, 2 × $–CH_2$), 2.27 (bs, 4H, 2 × Ar–CH_2), 6.62 (bs, 4H, 2 × $–NH_2$), 7.44 (bs, 4H, Ar–H), 7.78 (bs, 2H, Ar–H).

^{13}C NMR (125 MHz, DMSO-d_6): 13.48 (2C), 21.69 (2C), 30.95 (2C), 32.48 (2C), 95.64 (2C), 97.47, 97.71, 115.43 (2C), 116.48 (2C), 128.62(2C), 128.71(2C), 129.09, 129.20, 137.19, 137.32, 138.50, 138.53, 149.57, 149.83, 150.70, 150.81.

HR-MS (ESI) *m/z*: Obtained for M. F. $C_{30}H_{29}N_6$**:** 473.2448 as (M+H), Calculated for M. F. $C_{30}H_{29}N_6$**:** 473.2455 as (M+H).
3,3''-Diamino-6,6''-diheptyl-[1,1':4',1''-terphenyl]-2,2'',4,4''-tetracarbonitrile (Table 1, entry 6):



Molecular formula : $C_{36}H_{40}N_6$
Exact mass **:** 556.3314
Molecular weight **:** 556.7430
Nature and color **:** Off-white floppy solid
Melting point **:** decomposition around 240-243 ^oC.

FT-IR (Neat, cm^{-1}): v 3469, 3378 (–NH_2), 2921, 2851 (aliphatic—CH), 2219 (—CN), 1644 (aromatic —C═C).

^{1}H NMR (200 MHz, $CDCl_3$, δ ppm): 0.84 (t, *J*= 6Hz, 6H, 2 × –CH_3), 1.03-1.47 (m, 20H, 10 × –CH_2), 2.37 (t, *J*= 8Hz, 4H), 5.15 (bs, 4H, 2 × –NH_2), 7.39(s, 4H, Ar–H), 7.53(s, 2H, Ar–H).

^{13}C NMR (125 MHz, $CDCl_3$+DMSO-d6, δ ppm): 13.72 (2C), 21.99 (2C), 28.23, 28.37 (2C), 28.47, 28.62, 30.34, 30.44, 31.09, 31.14, 31.48, 95.85 (2C), 97.68, 97.93, 115.08 (2C), 116.04 (2C), 128.31 (2C), 128.35 (2C), 129.30, 129.48, 137.09, 137.13, 137.35, 137.43, 148.89, 149.13, 150.33, 150.46.

HR-MS (ESI) *m/z*: Obtained for M. F. $C_{36}H_{41}N_6$ 557.3397 as (M+H), Calculated for M. F. $C_{36}H_{41}N_6$557.3394 as (M+H).

5.6 Copies of ^{1}H NMR, ^{13}C NMR, HR-MS and FT-IR of newly synthesized compounds

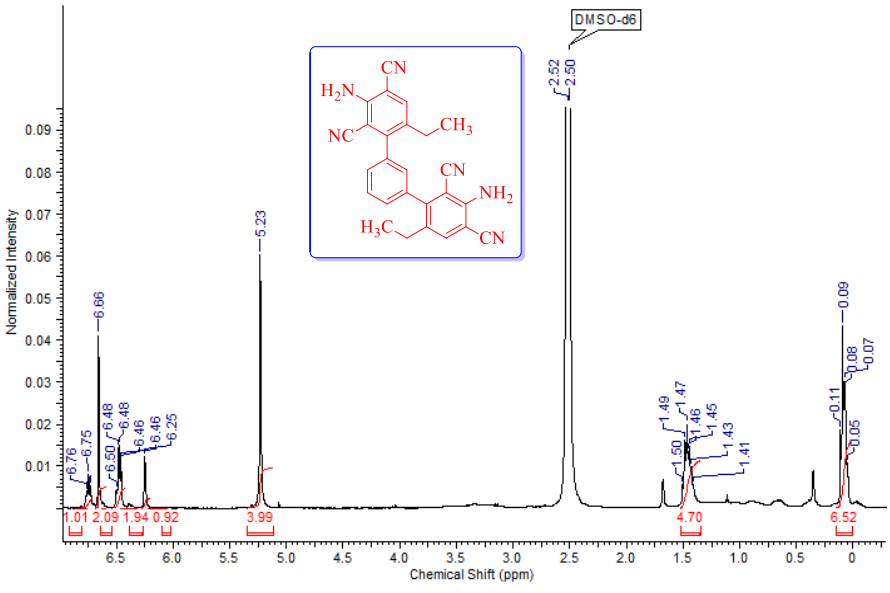


Fig. 6.^{1}H NMR spectrum of compound 1a (Table 1, entry 1)

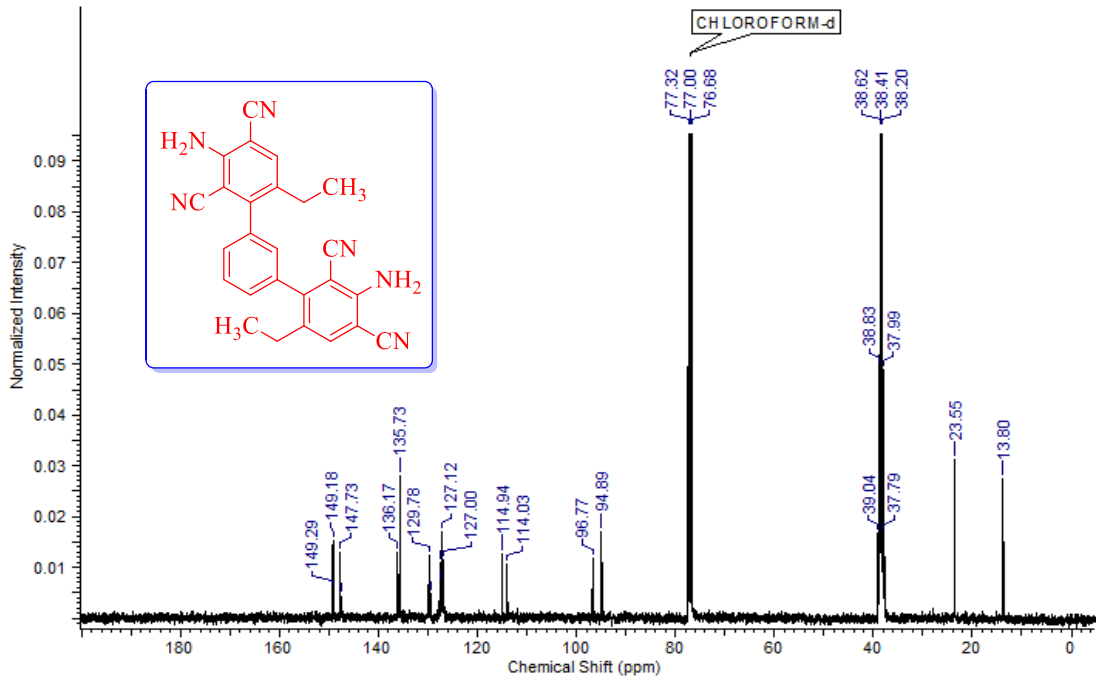


Fig. 7.^{13}C NMR spectrum of compound 1a (Table 1, entry 1)

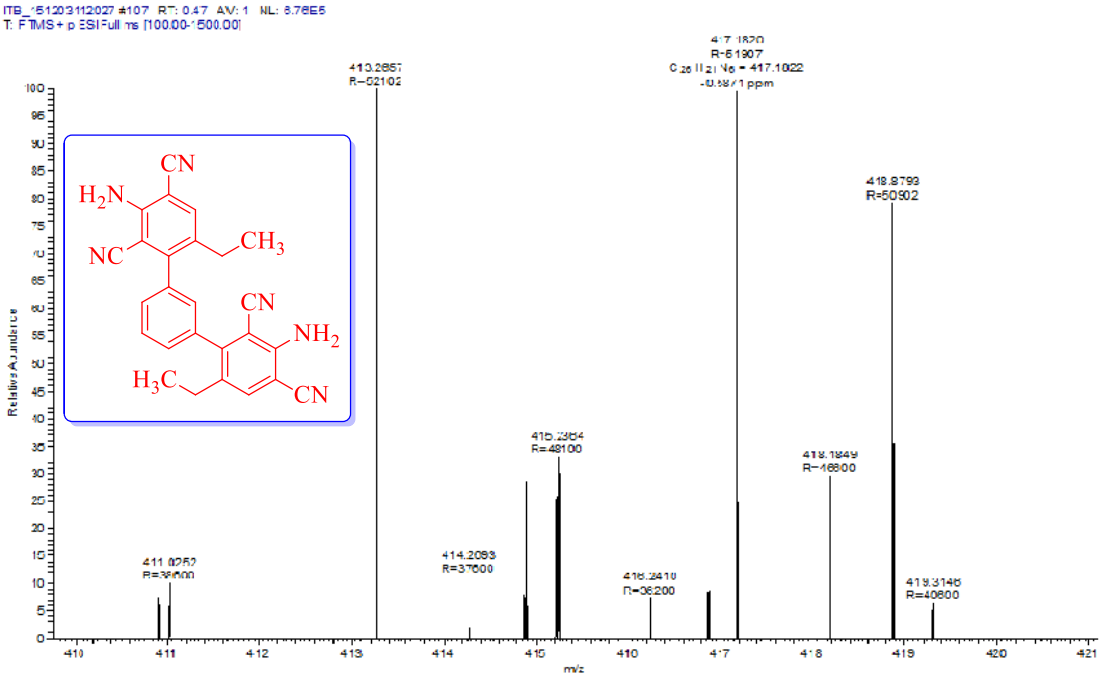


Fig. 8. HR-MS spectra of compound 1a (Table 1, entry 1)

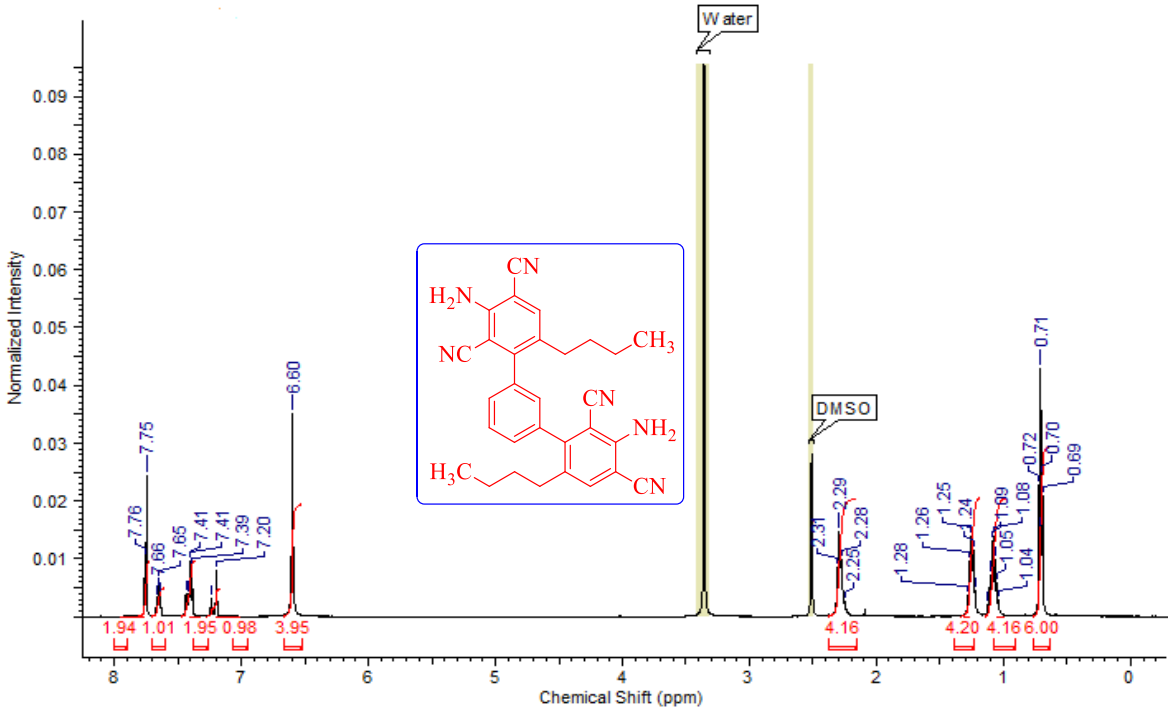


Fig. 9.[1]H NMR spectra of compound 1b (Table 1, entry 2)

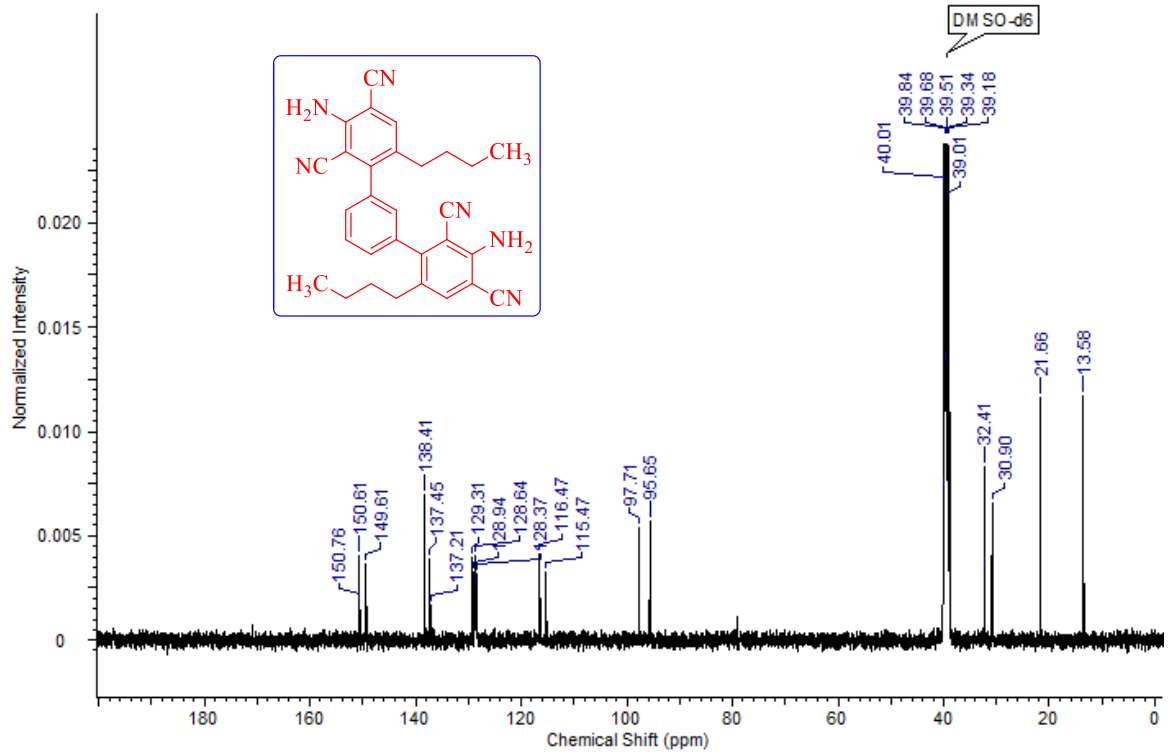


Fig. 10: [13]C NMR spectrum of compound 1b (Table 1, entry 2)

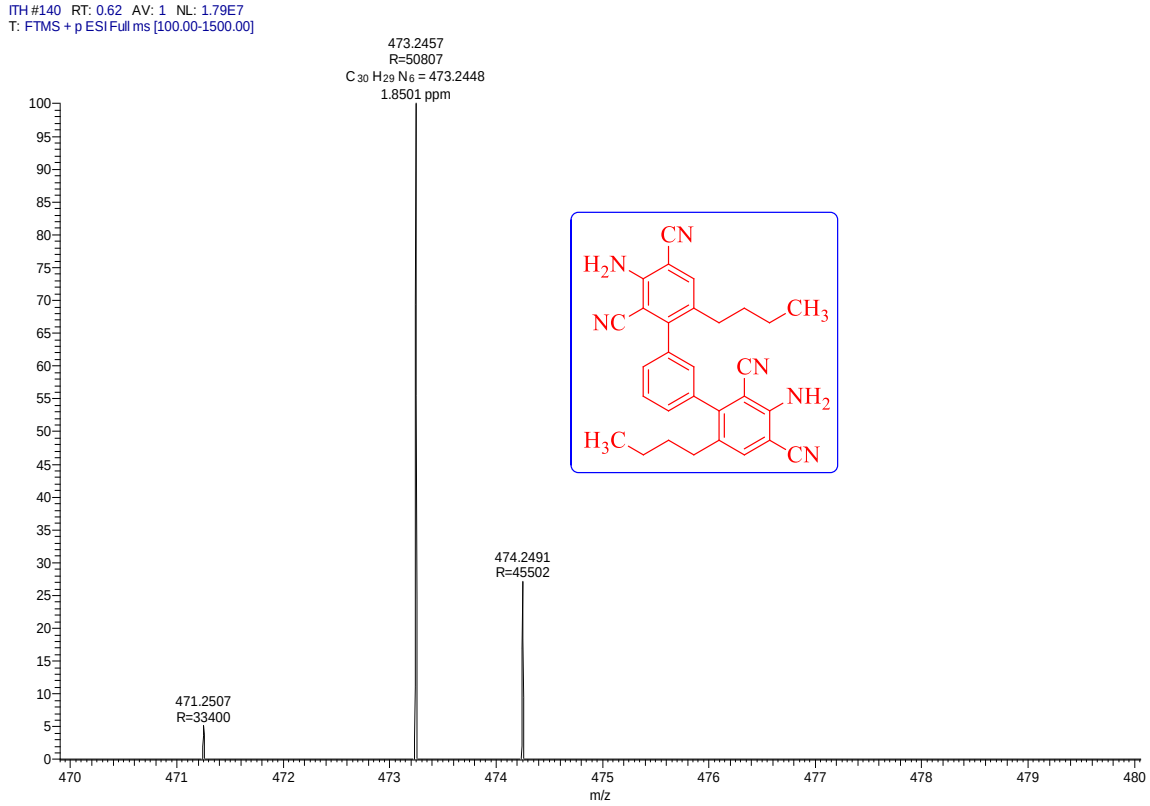


Fig. 11. HR-MS spectra of compound 1b (Table 1, entry 2)

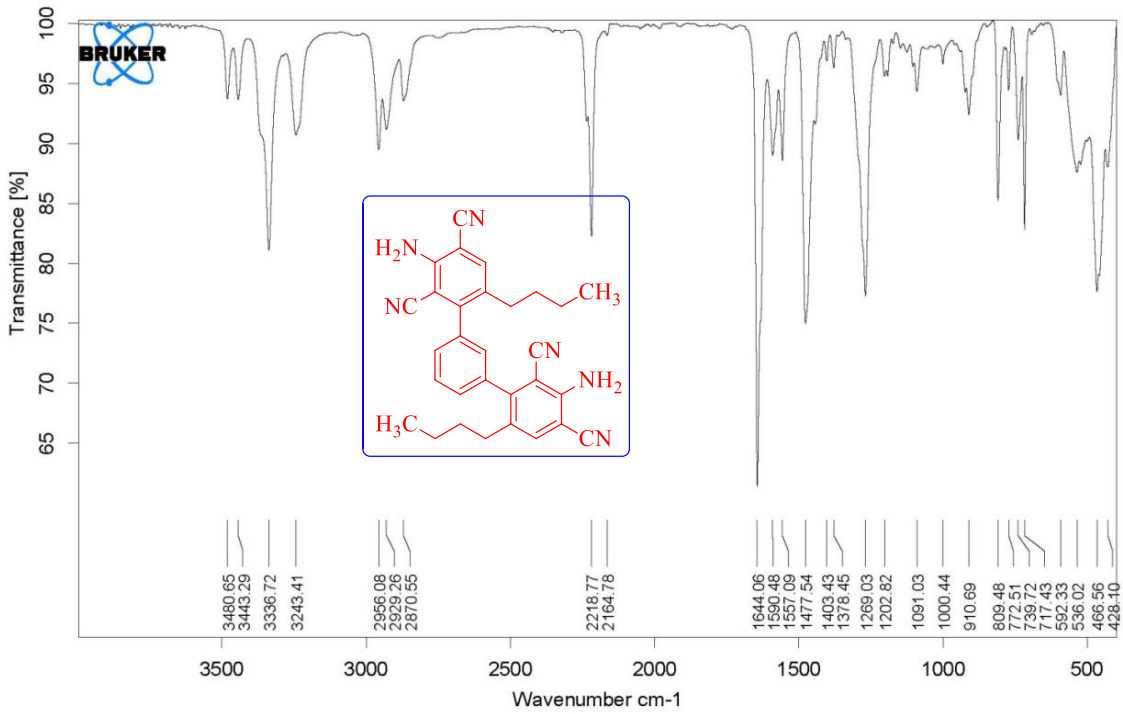


Fig. 12. FT-IR spectra of compound 1b (Table 1, entry 2)

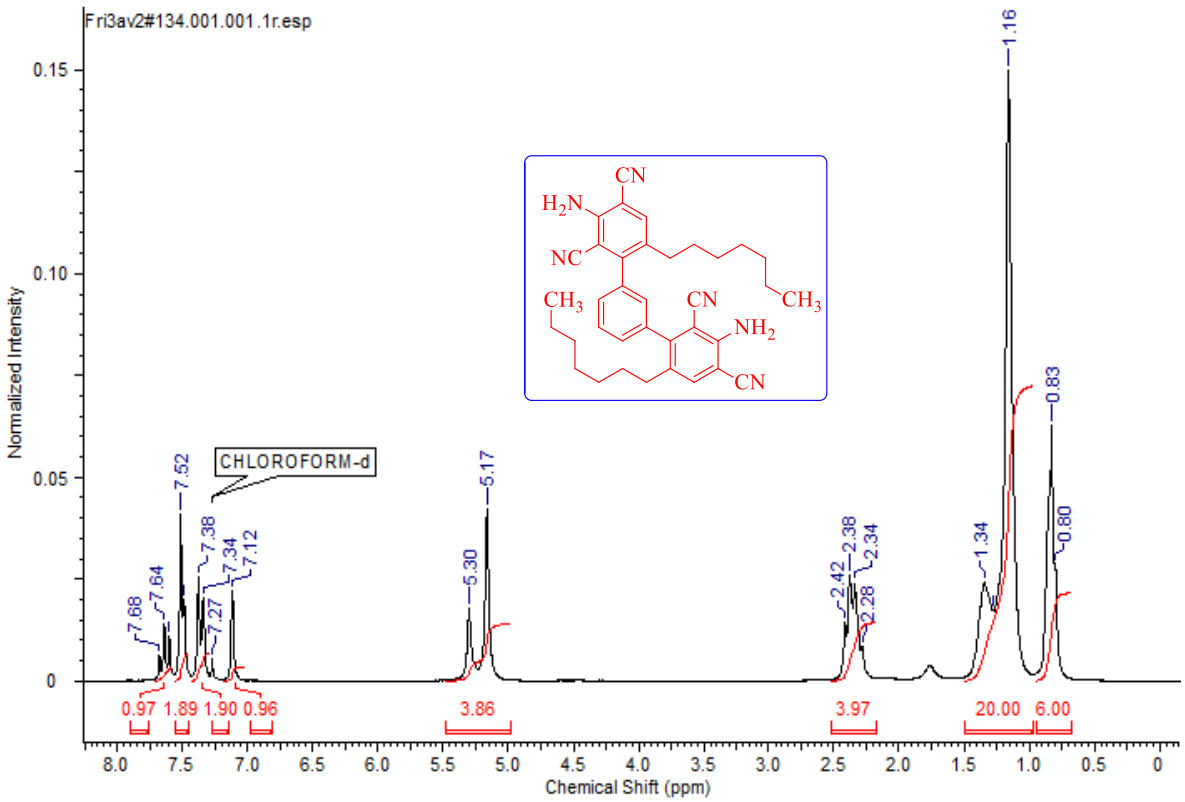


Fig. 13.^{1}H NMR spectrum of compound 1c (Table 1, entry 3)

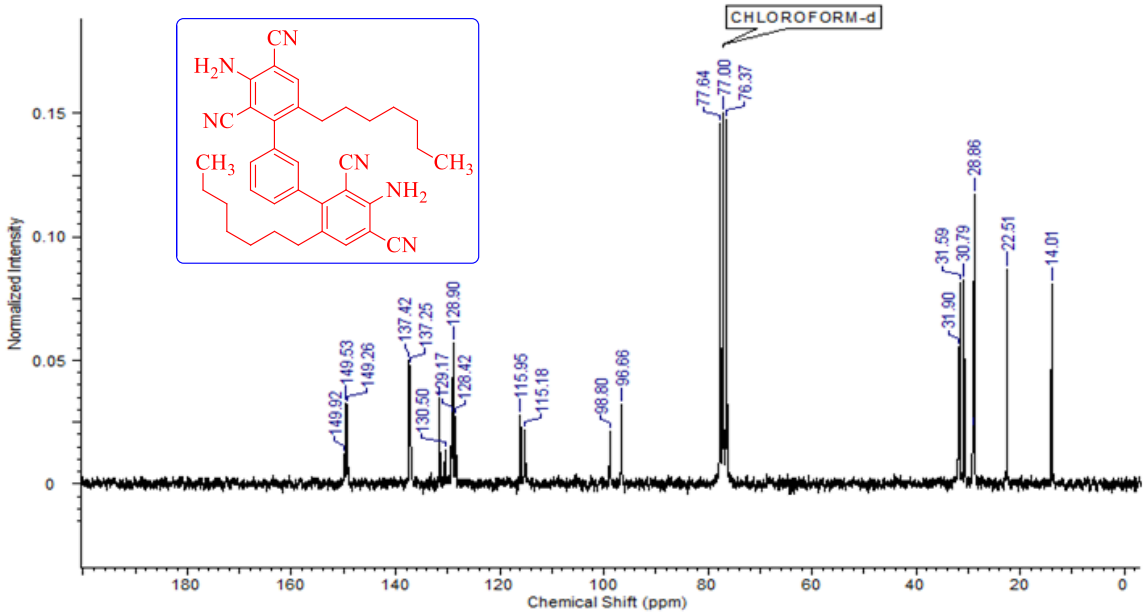


Fig. 14.^{13}C NMR spectrum of compound 1a (Table 1 entry 3)

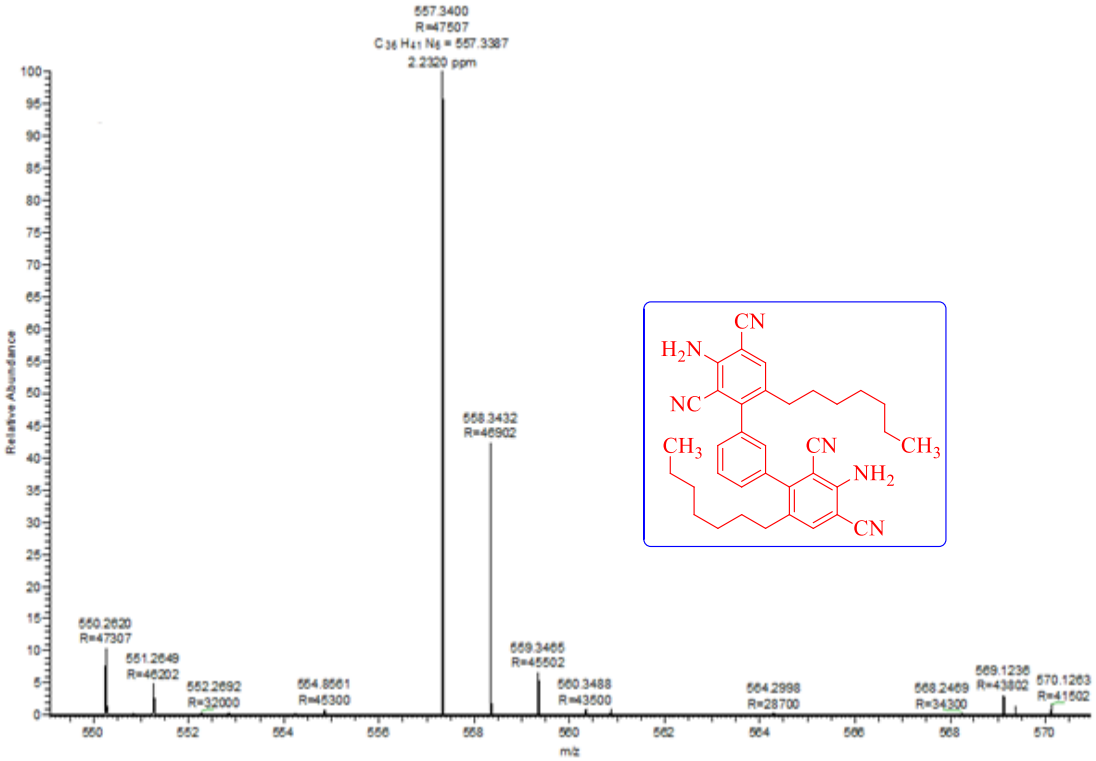


Fig. 15. HR-MS spectrum of compound 1c (Table 1, entry 3)

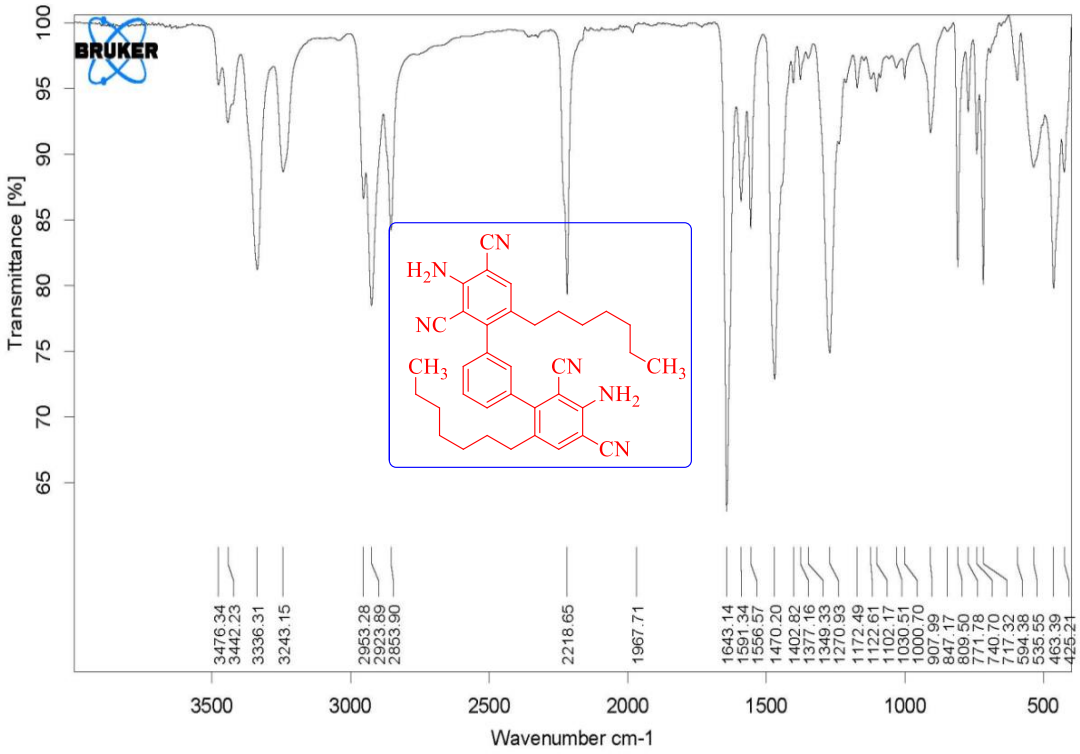


Fig. 16. FT-IR spectrum of compound 1c (Table 1, entry 3)

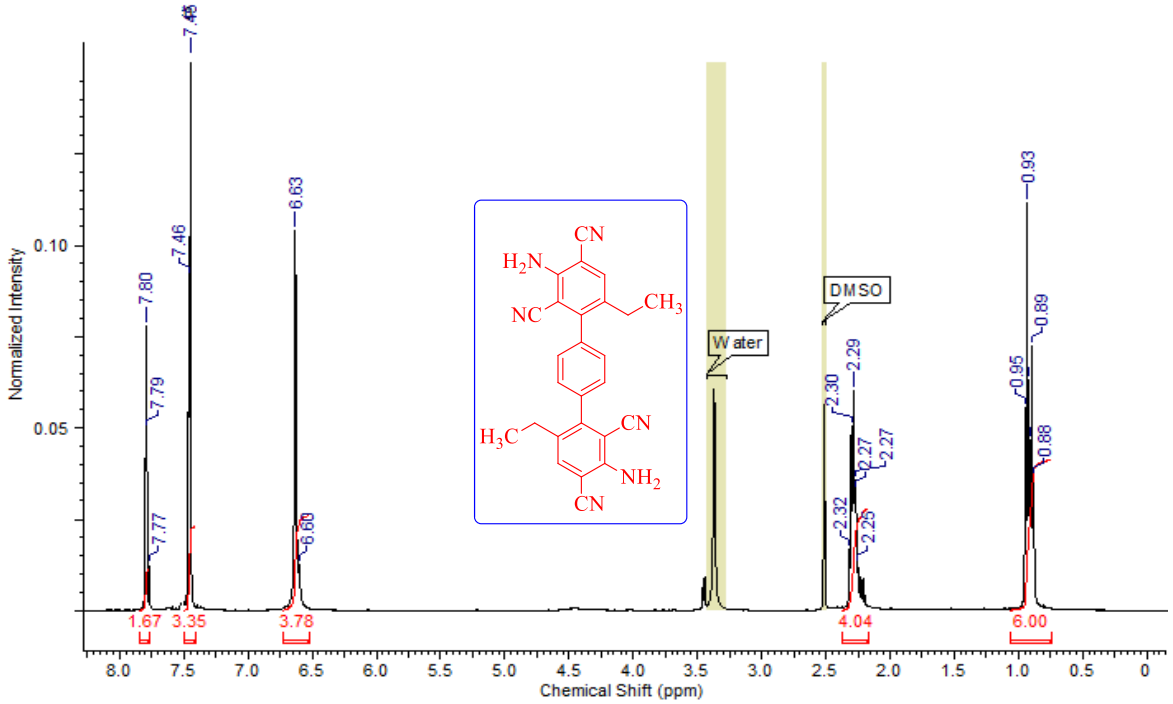


Fig. 17.[1]H NMR spectrum of compound 2a (Table 1, entry 4)

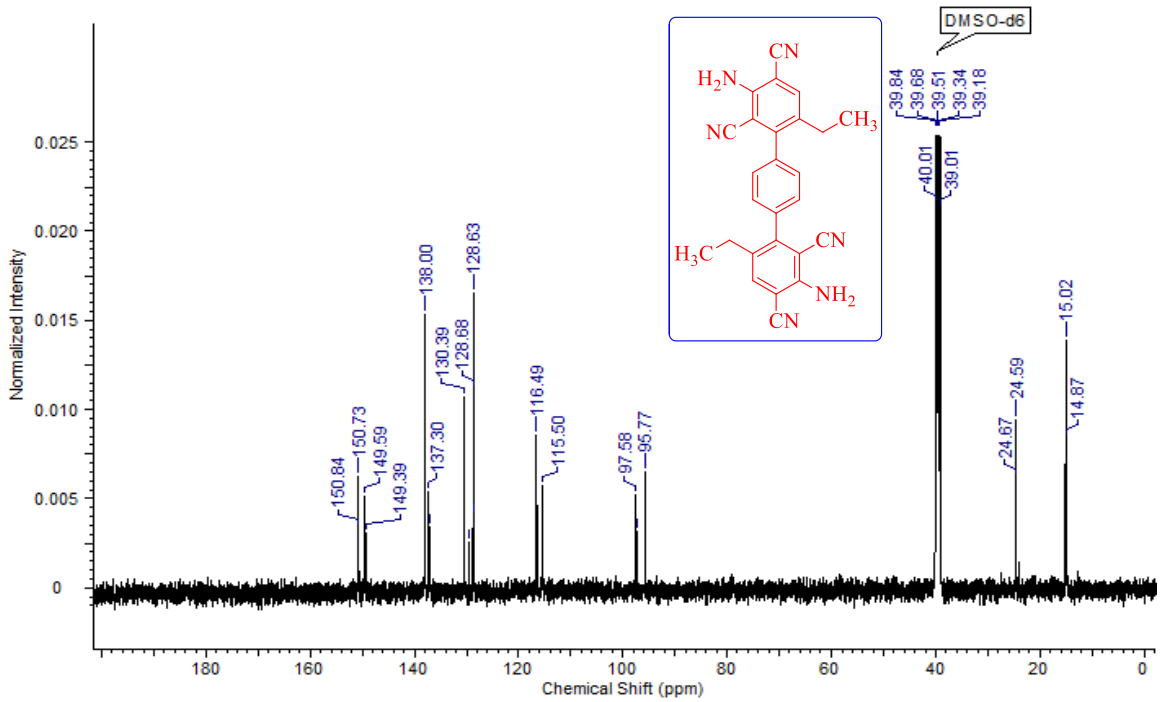


Fig. 18.[13]C NMR spectrum of compound 2a (Table 1, entry 4)

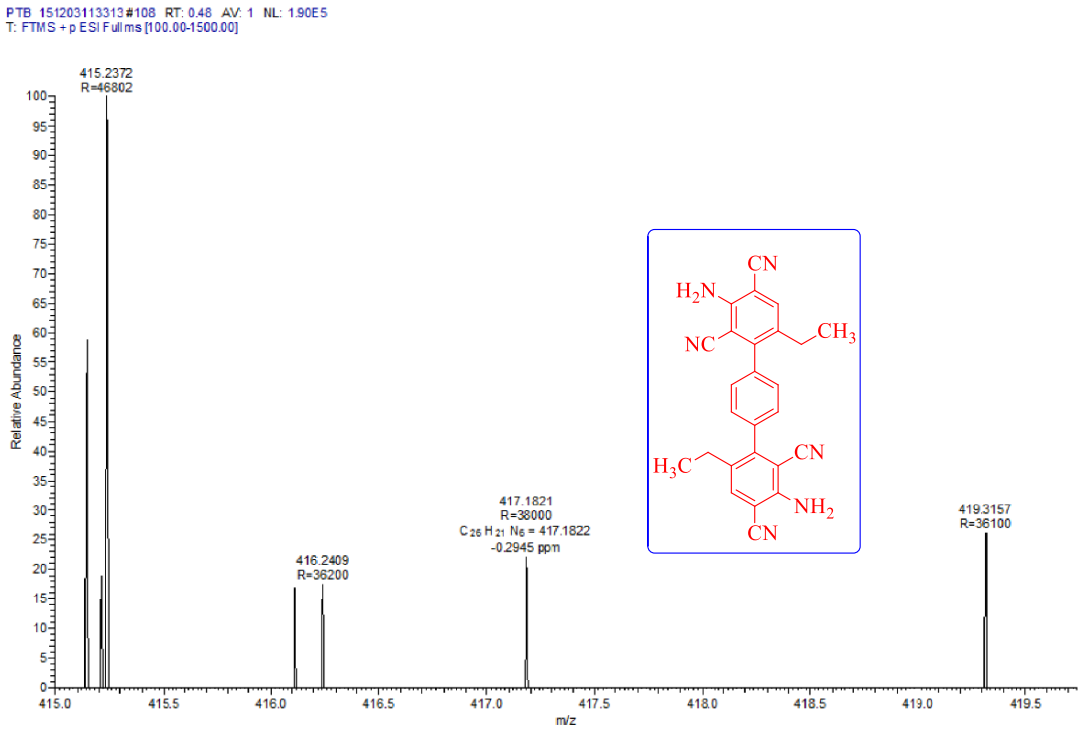


Fig. 19. HR-MS spectrum of compound 2a (Table 1, entry 4)

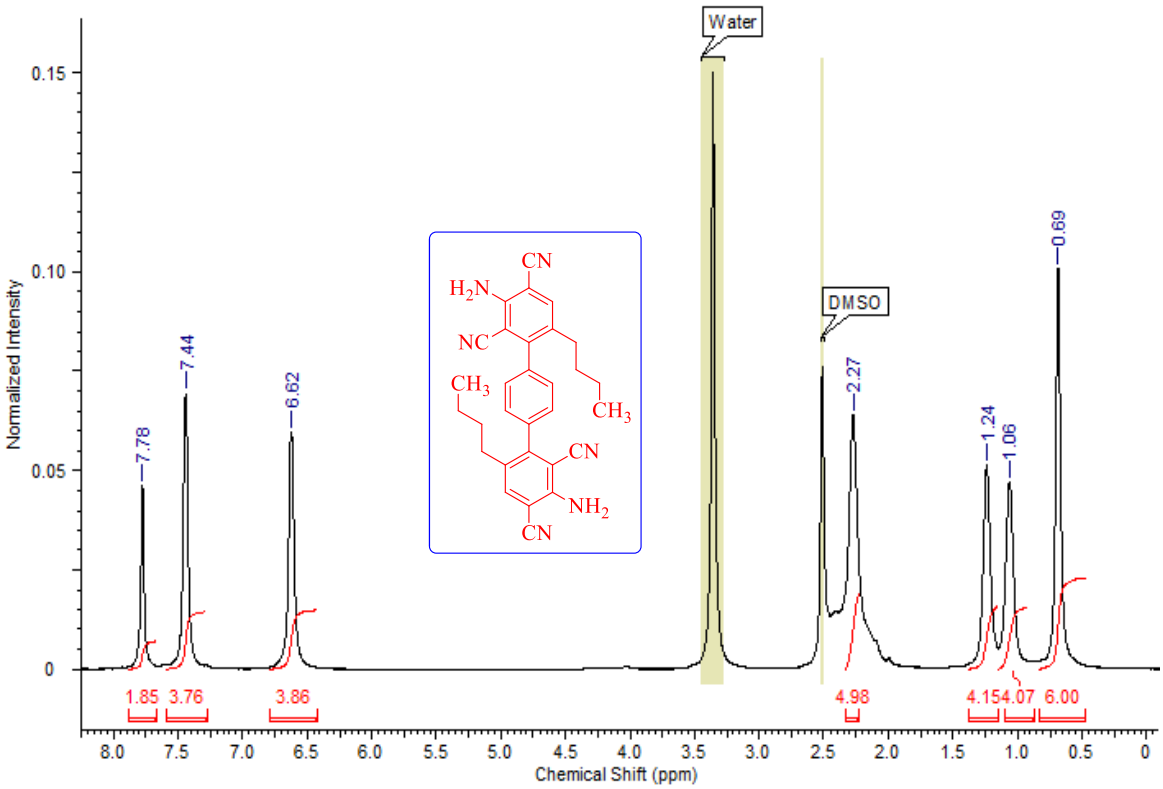


Fig. 20.^{1}H NMR spectrum of compound 2b (Table 1, entry 5)

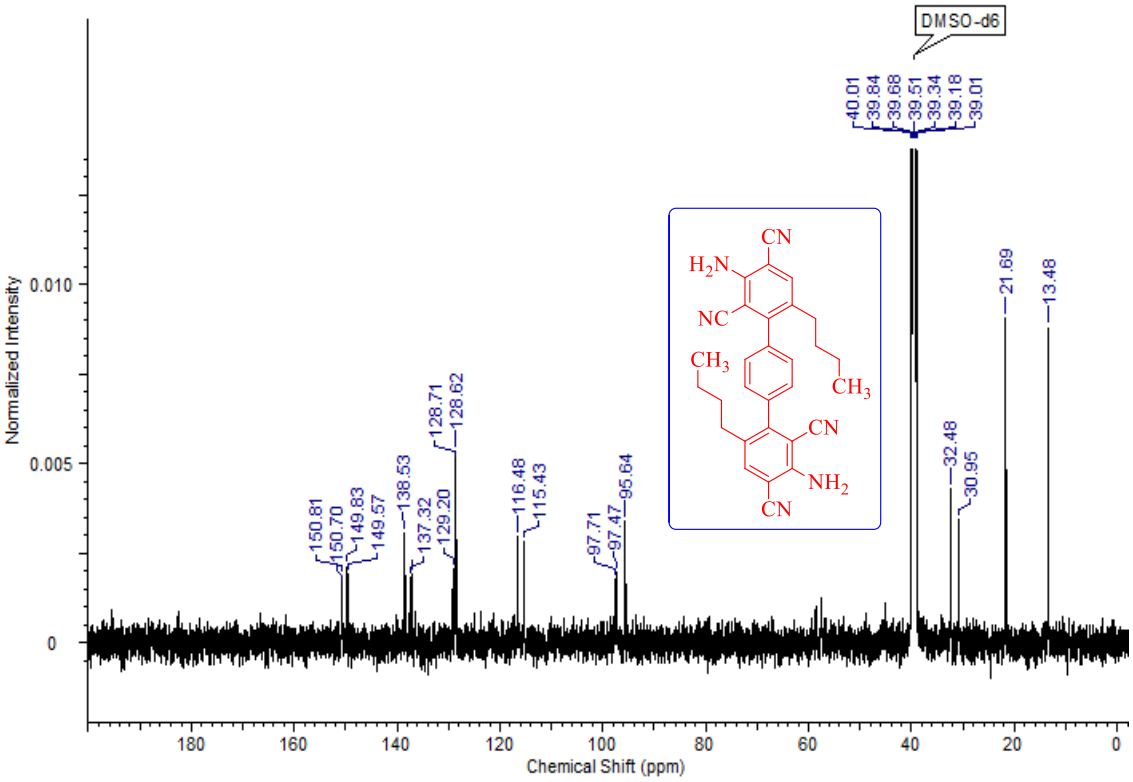


Fig. 21.^{13}C NMR spectrum of compound 2b (Table 1, entry 5)

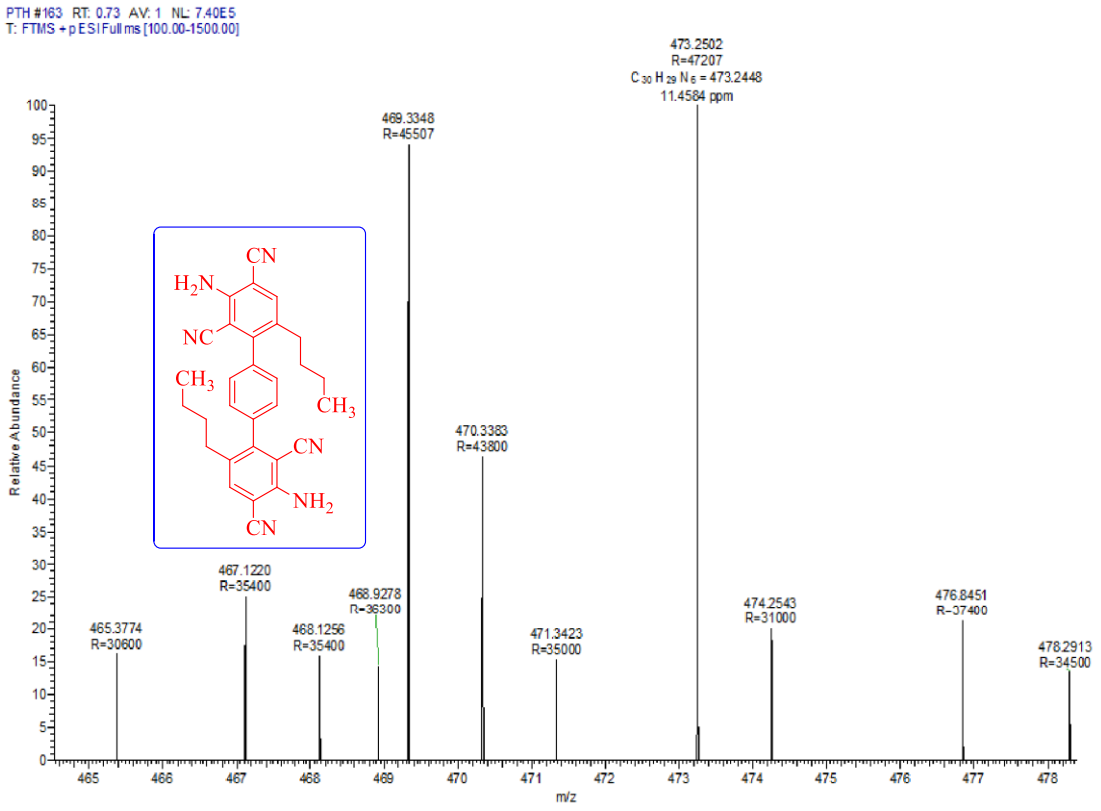


Fig. 22. HR-MS spectrum of compound 2b (Table 1, entry 5)

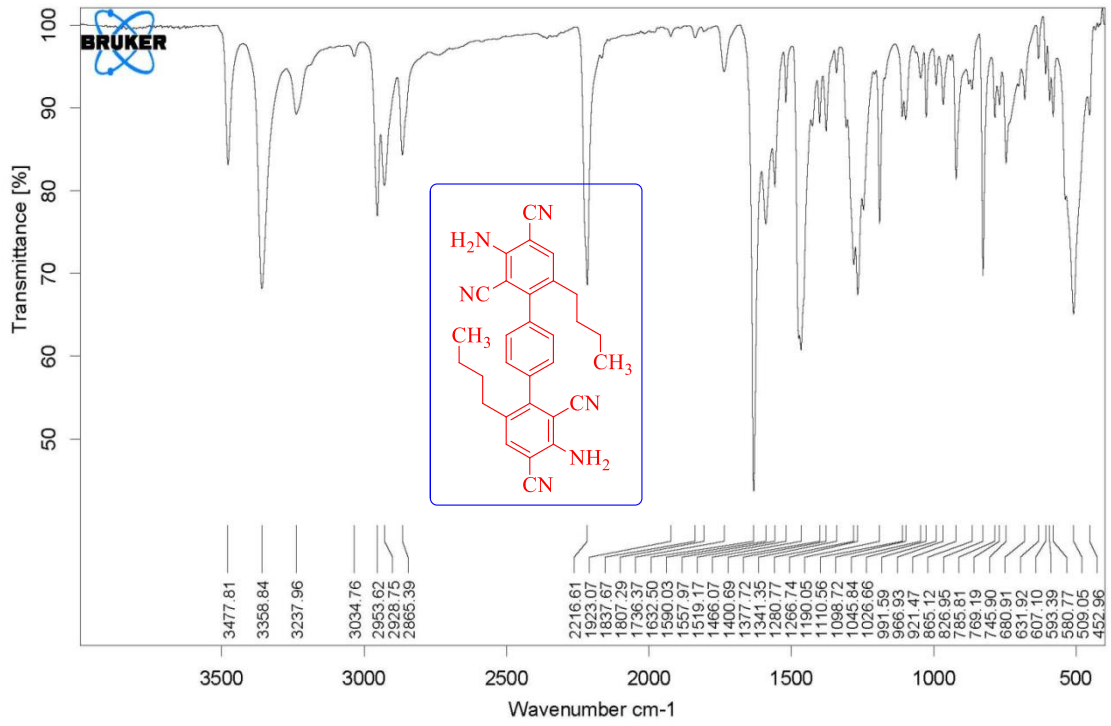


Fig. 23. FT-IR spectrum of compound 2b (Table 1, entry 5)

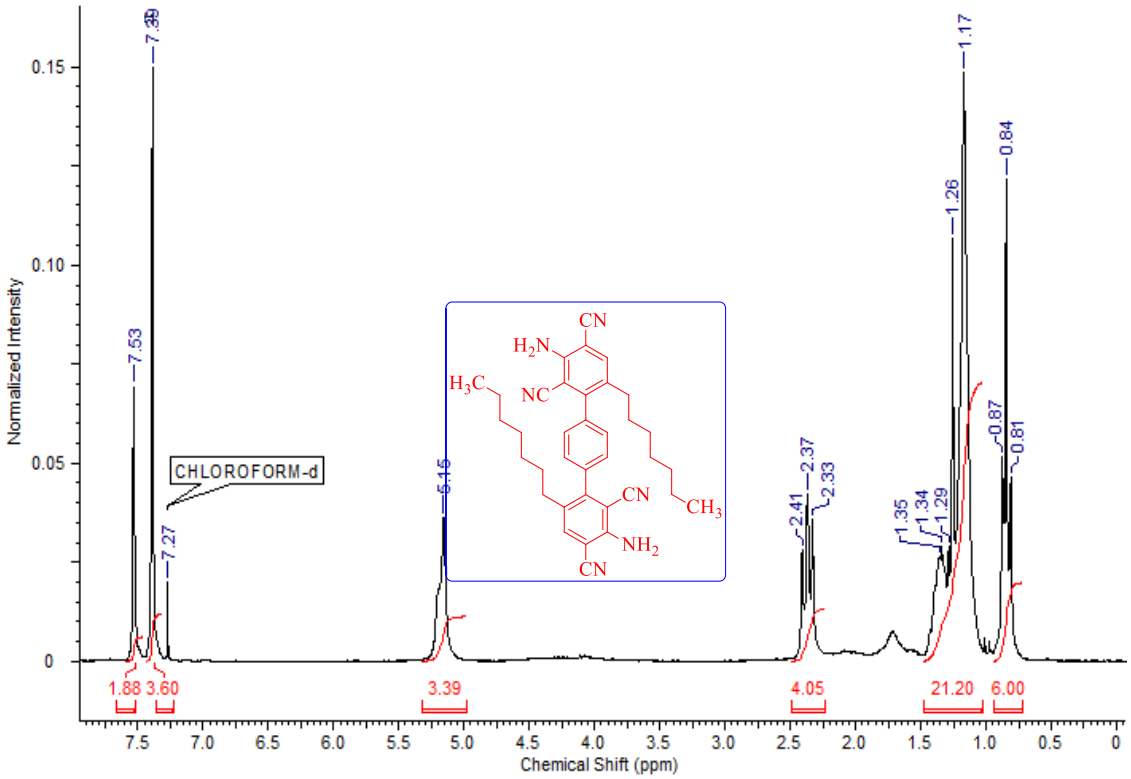


Fig. 24.^{1}H NMR spectrum of compound 2c (Table 1, entry 6)

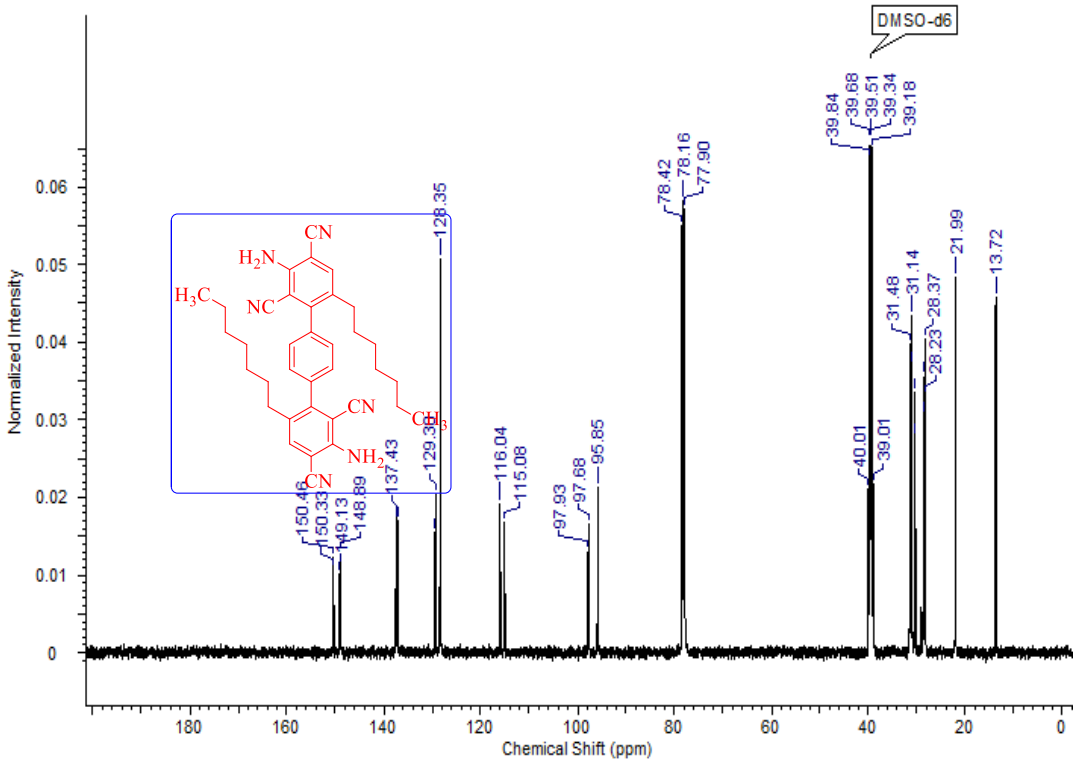


Fig. 25.^{13}C NMR spectrum of compound 2c (Table 1, entry 6)

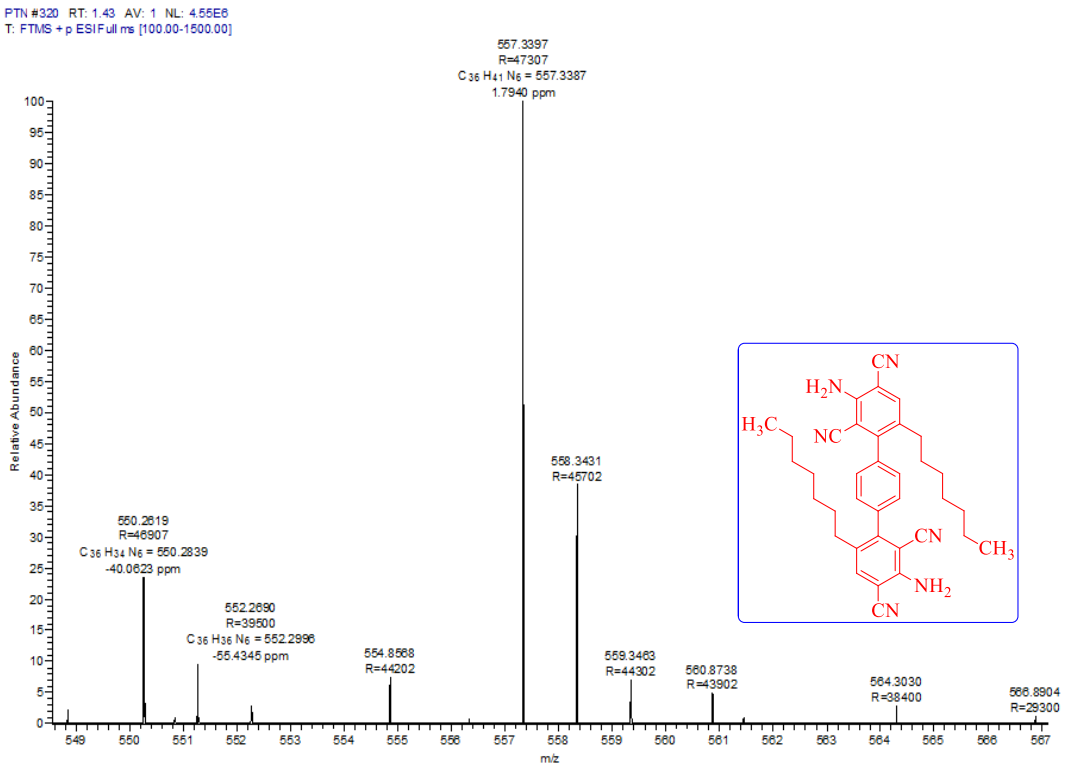


Fig. 26. HR-MS spectrum of compound 2c (Table 1, entry 6)

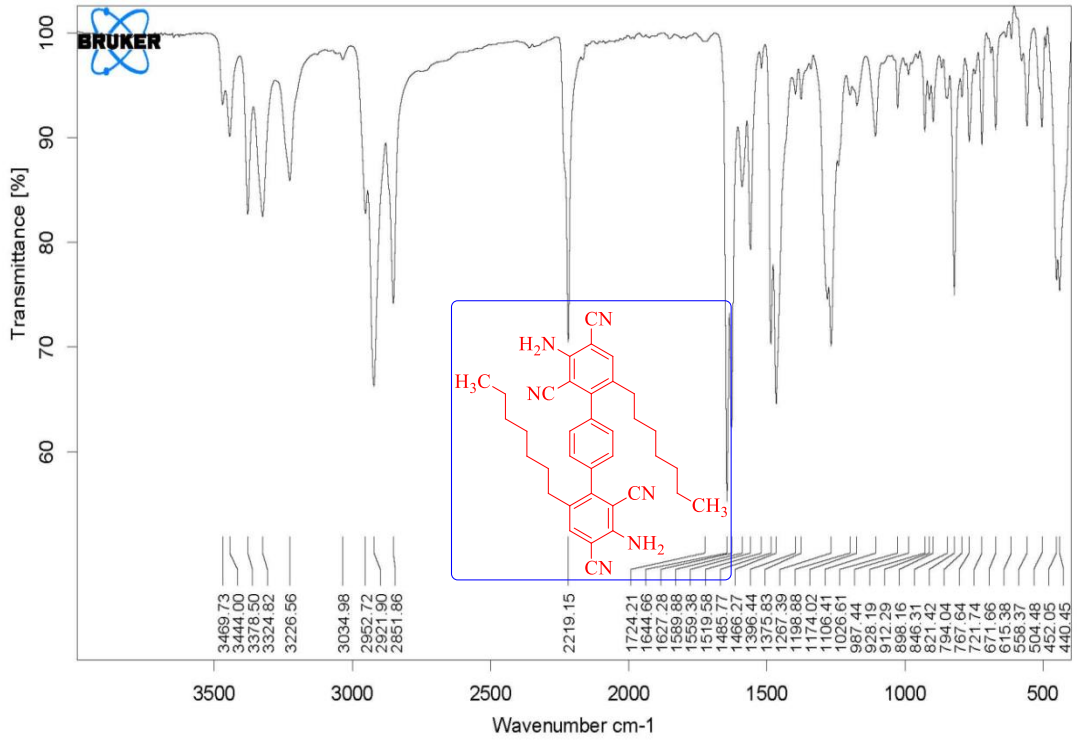


Fig. 27. FT-IR spectrum of compound 2c (Table 1, entry 6)

6. CONCLUSION

i) This work describes the preliminary results about synthesis of novel and interesting 4-alkyl-3-aryl-2,6-dicyanoanilines obtained from aromatic dialdehydes such as isophthalaldehyde and terephthalaldehyde.
ii) All the compounds were characterized by spectroscopic techniques like ^{1}H NMR, ^{13}C NMR, IR and High-Resolution Mass Spectrometry.
iii) Fluorescence properties of newly synthesized compounds were studied. The compound **1b** shows exciting quantum yield (67 %) in comparison with standard quinine sulphate (54 %)
iv) The compounds in the present study have remarkable cell imaging potential.
v) Presence of amino and cyano groups as structural modification sites, excellent quantum yield, stoke shifts etc these molecules can be modified and screened for various other applications.

CONSENT

As per international standards or university standards, respondents' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

Ethical approval for fluorescence imaging study on human oral mucosa cells was obtained from the D. Y. Patil Medical College, Kasba Bawada, Kolhapur, MS, India (Ref. No. DYPMCK/166/2018; Dated 21/08/2018).

ACKNOWLEDGEMENTS

Ananada S. Kudale thanks Government of Maharashtra, Home department for granting permission for Ph. D. research work and Director, Forensic science laboratories, Mumbai for the necessary support. We acknowledges to Department of Biotechnology Governemnt of India for Interdisciplinary Programme of Life Sciences for Advanced Research and Education (IPLS) (Referece no. BT/PR4572/INF/22/147/2012). We greatly acknowledge the help of Dr. Rakesh K. Sharma, M. D., Dean, D. Y. Patil Medical College, Kolhapur for help in presenting proposal for institutional ethical approval and Mr. Amrut Bhosale, Department of Zoology, Shivaji University, Kolhapur for help in cell imaging experiments.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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