Highly Lipophilic Terpyridine Ligand as an Efficient Fluorescent Probe for the Selective Detection of Zinc(II) Ions Under Biological Conditions

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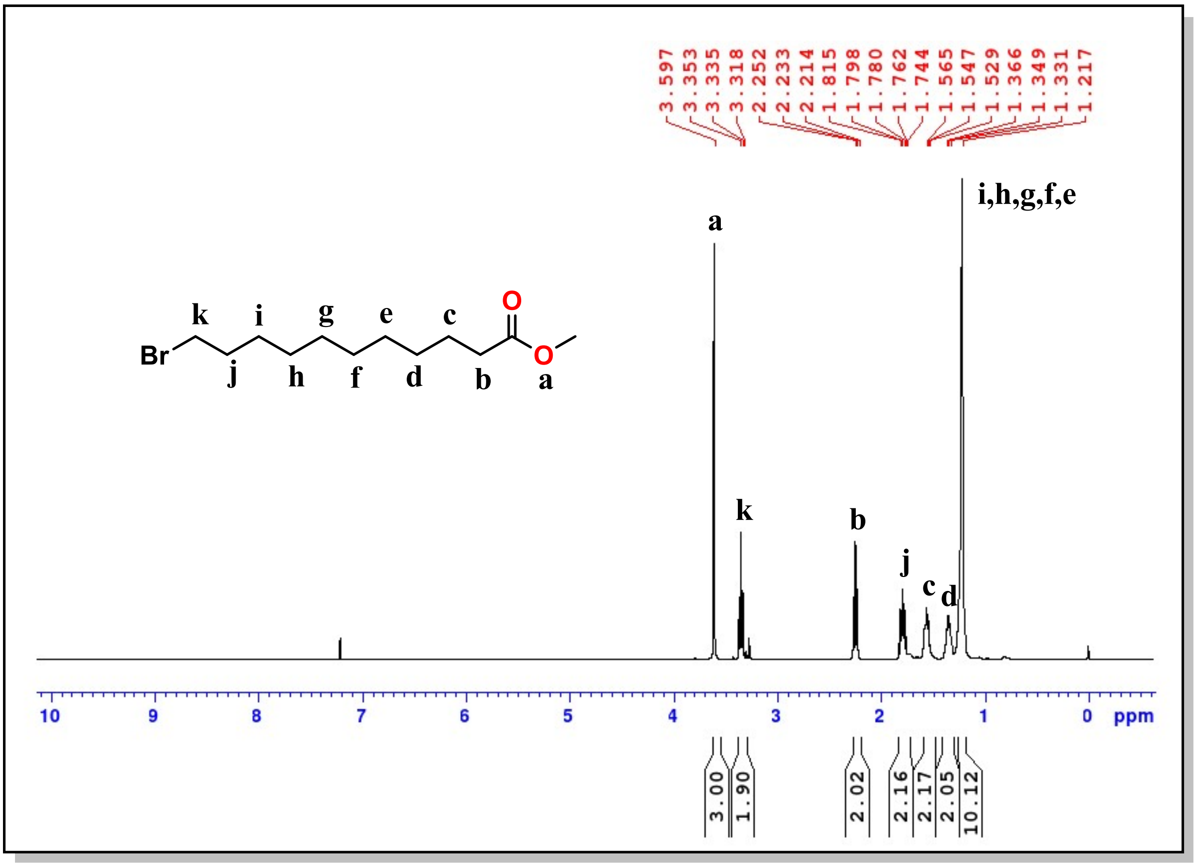


Fig. S1 ^{1}H NMR Spectrum of compound of **2**

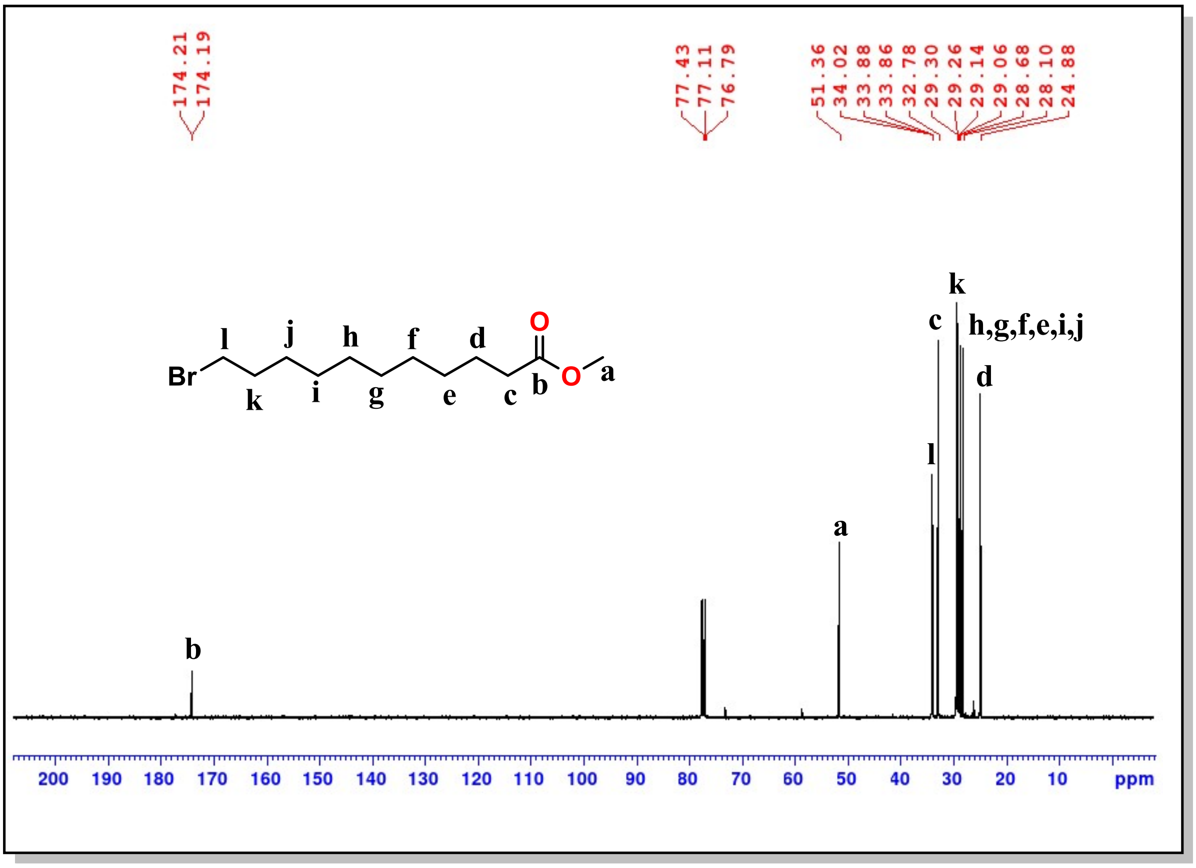


Fig. S2 ^{13}C NMR spectrum of compound **2**

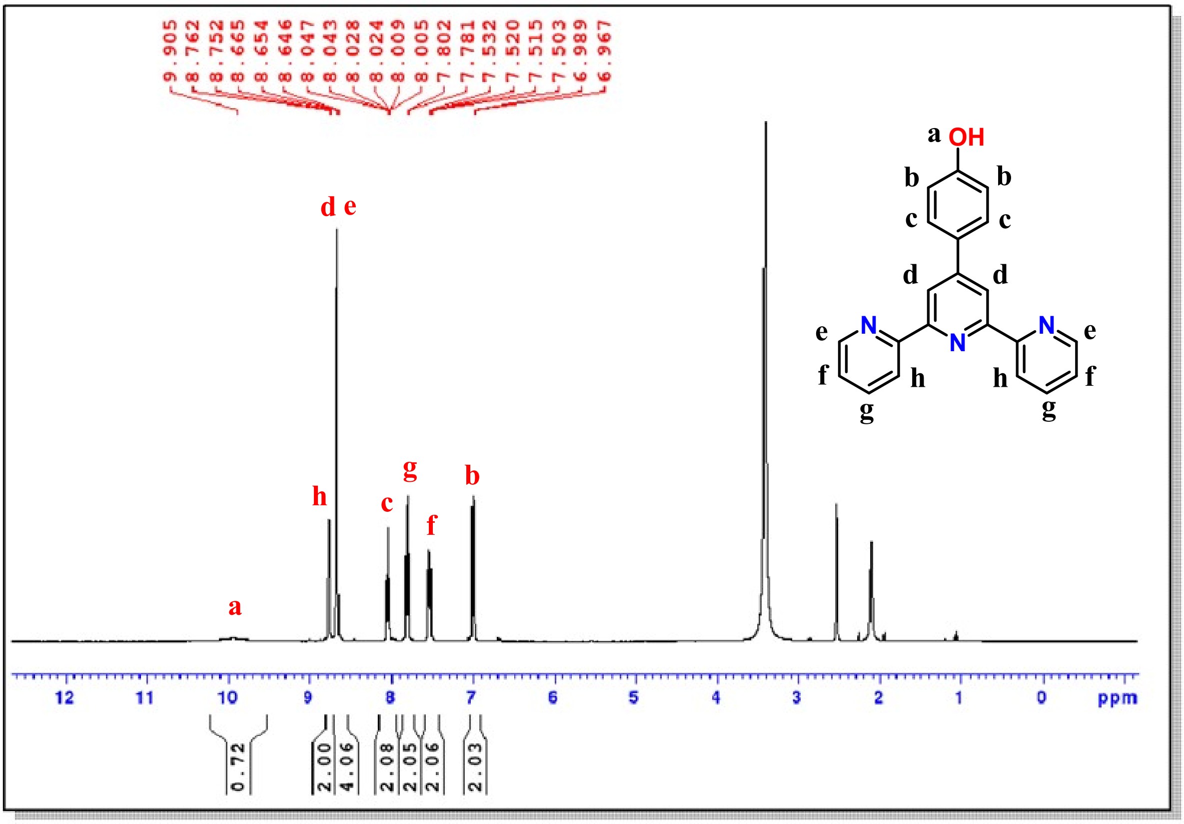


Fig. S3 ^{1}H NMR spectrum of compound **tpy-OH**

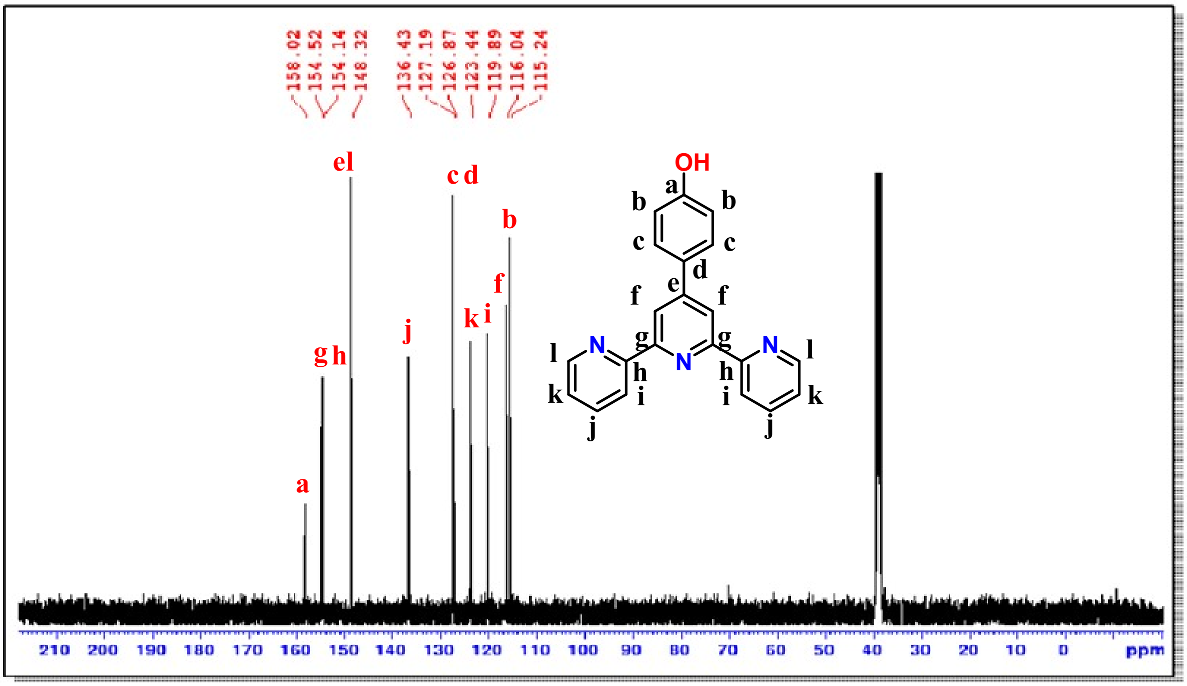


Fig. S4 ^{13}C NMR spectrum spectrum of compound **tpy-OH**

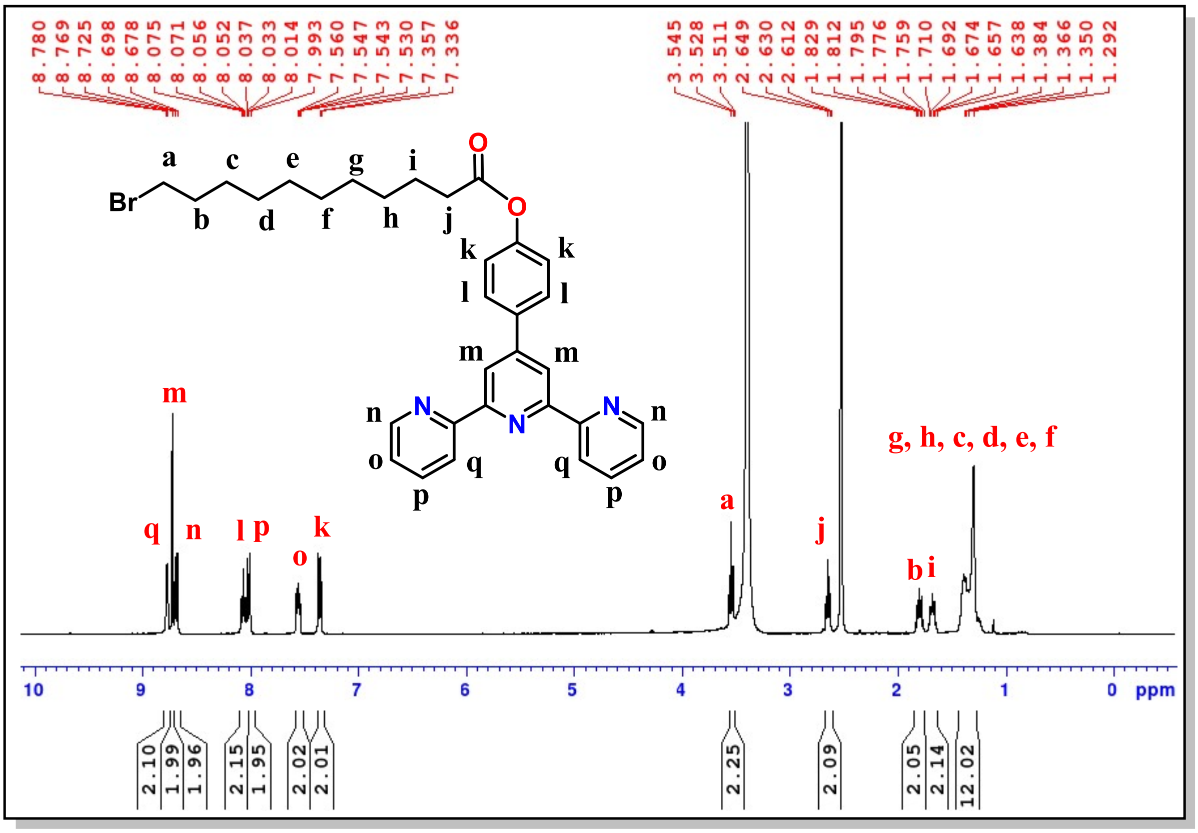


Fig. S5 ^{1}H NMR spectrum of compound **L1**

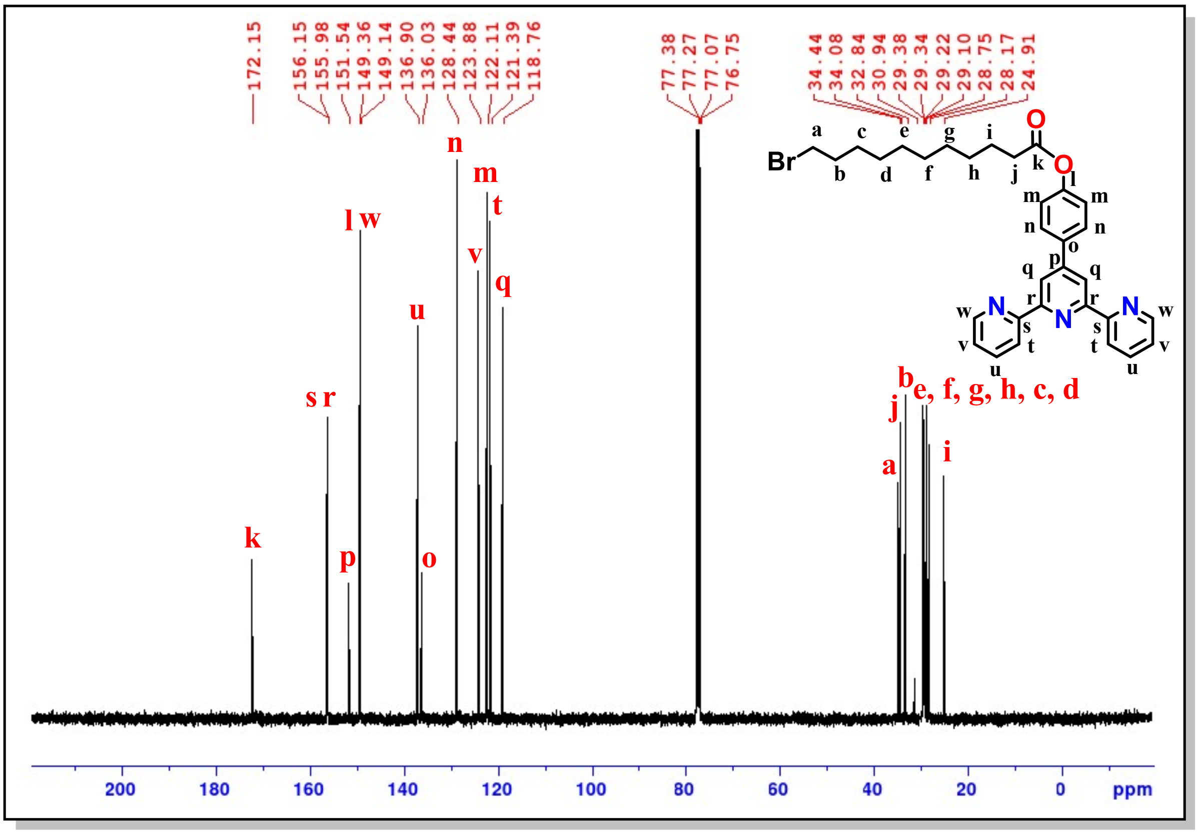


Fig. S6 ^{13}C NMR spectrum spectrum of compound **L1**

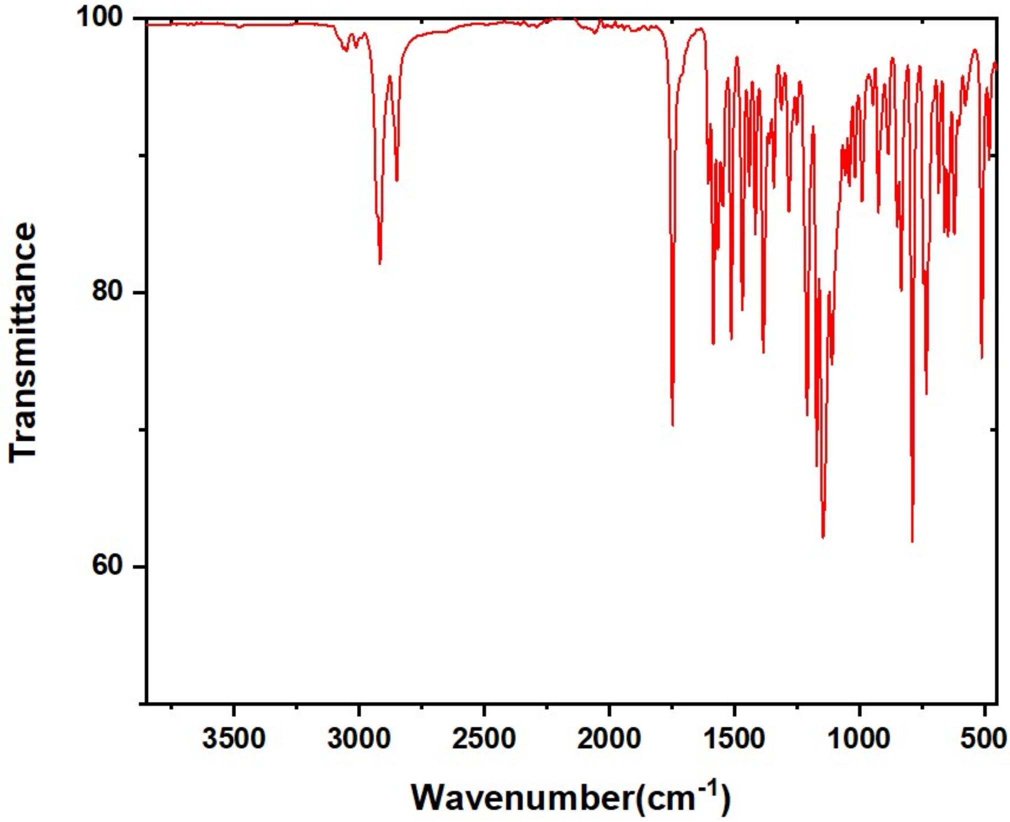


Fig. S7 FT-IR spectrum of compound **L1**

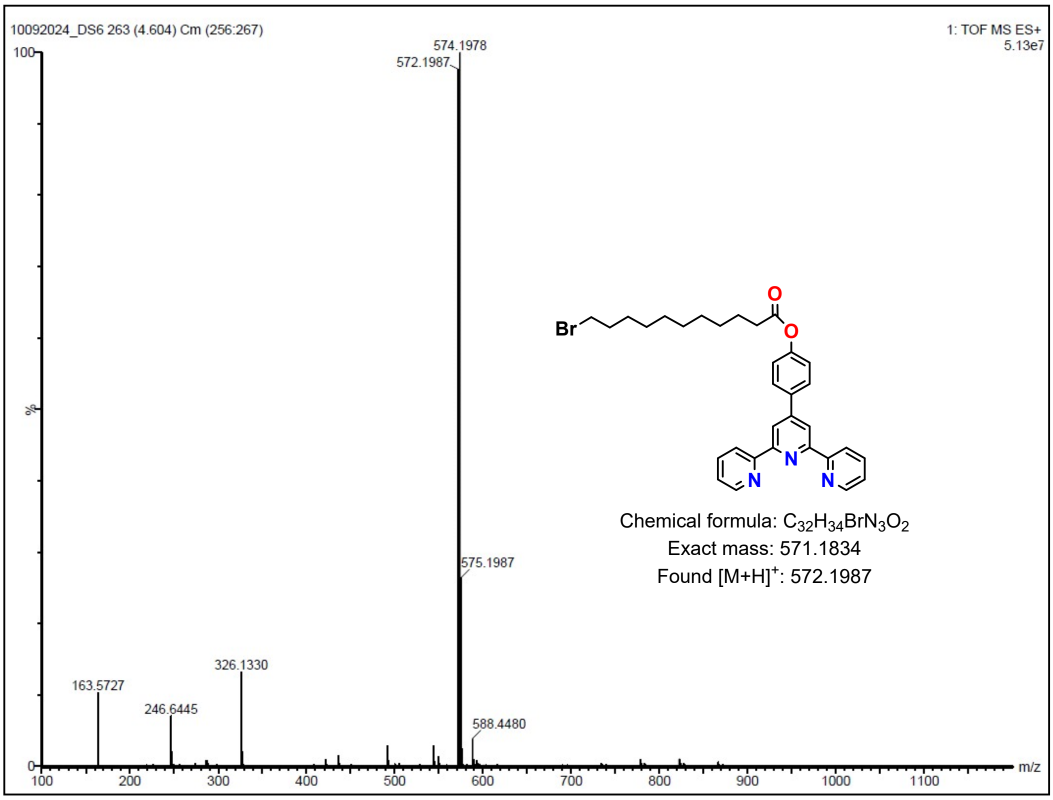


Fig. S8 ESI-MS Spectrum of compound **L1**

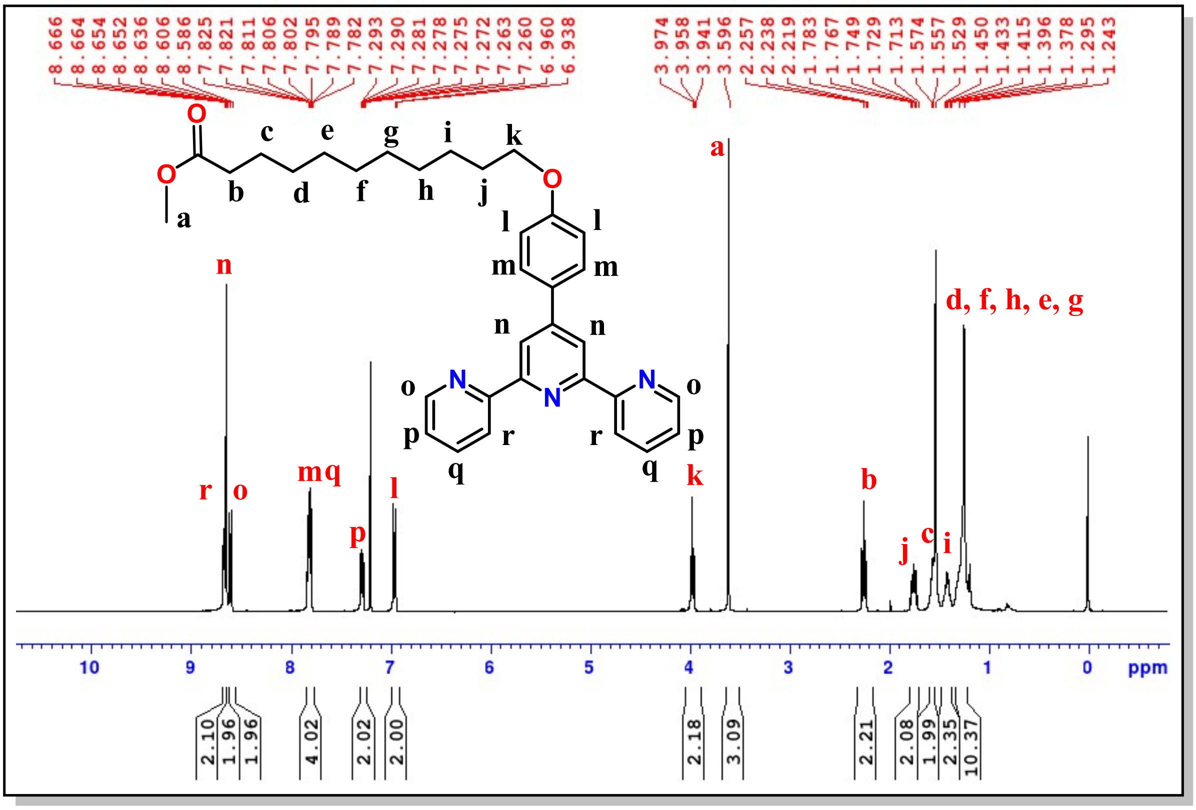


Fig. S9 ^{1}H NMR spectrum of compound **L2**

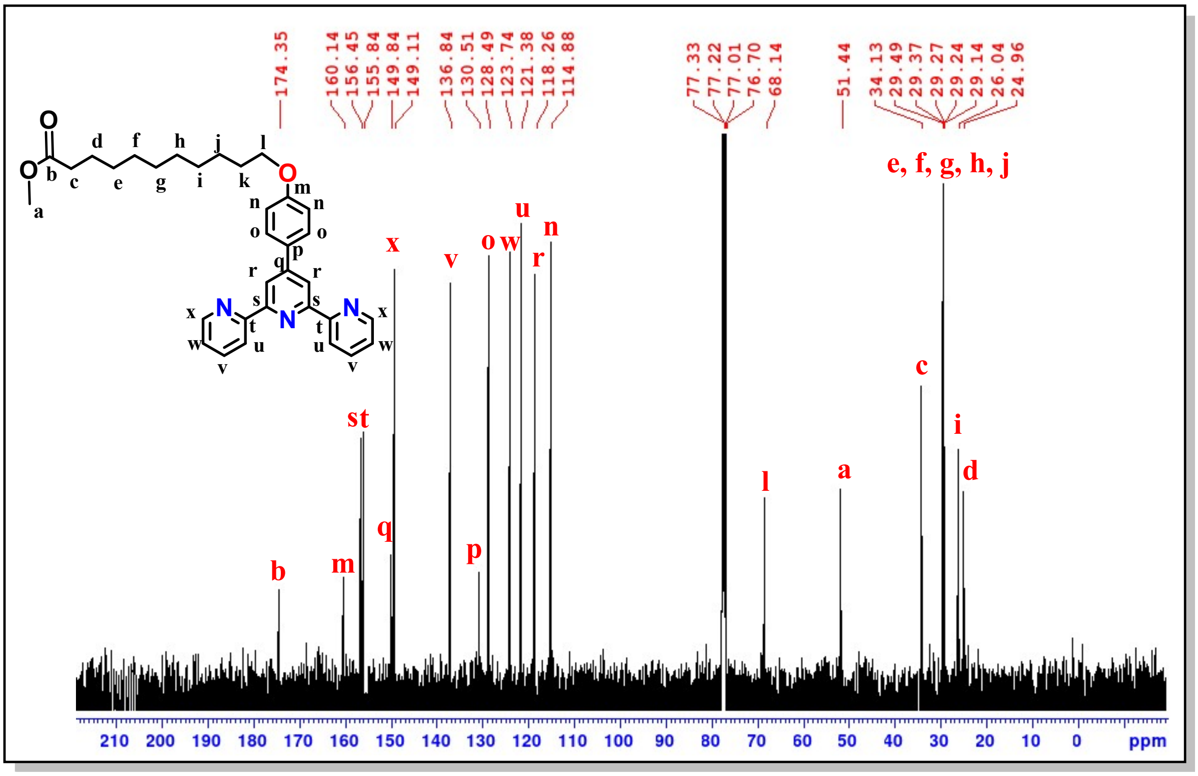


Fig. S10 ^{13}C NMR spectrum of compound **L2**

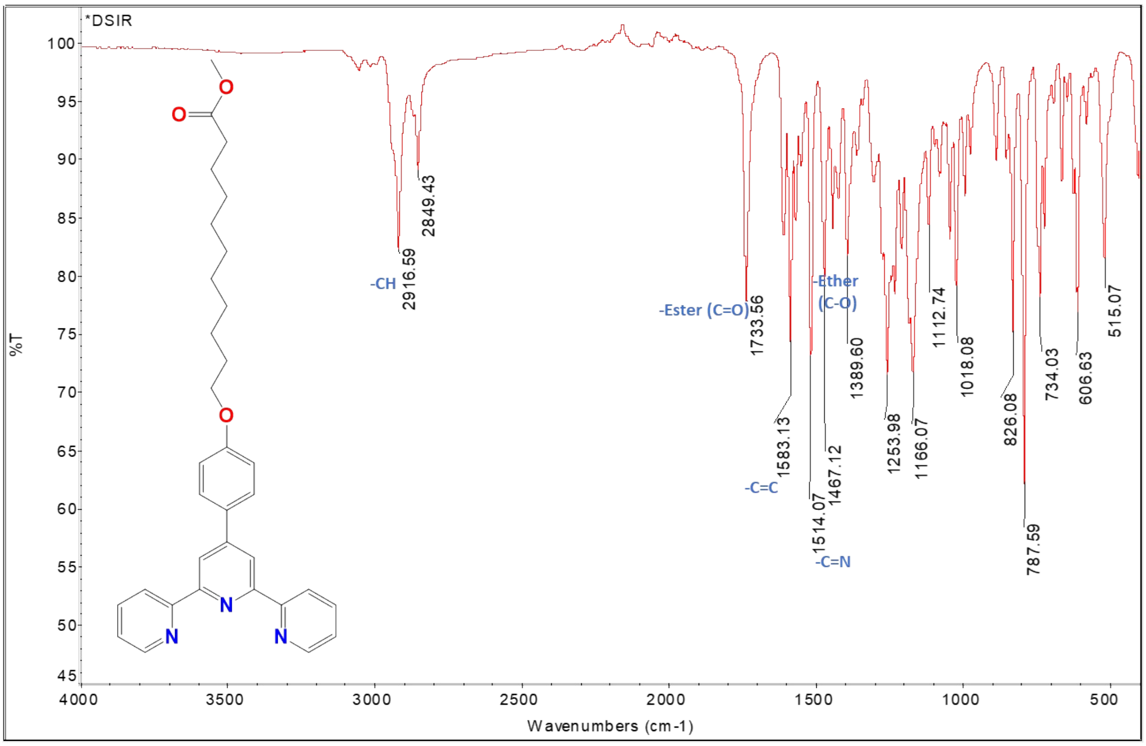


Fig. S11 FT-IR spectrum of compound **L2**

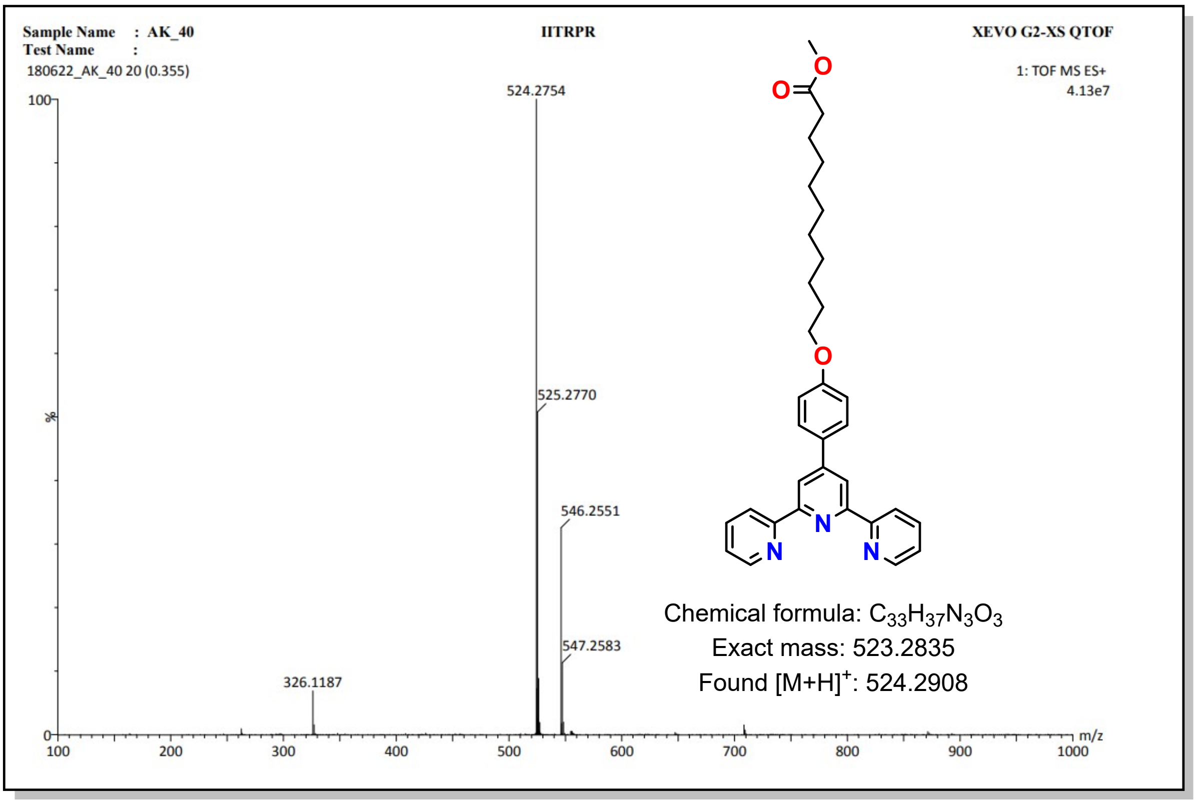


Fig. S12 ESI-MS Spectrum of compound **L2**

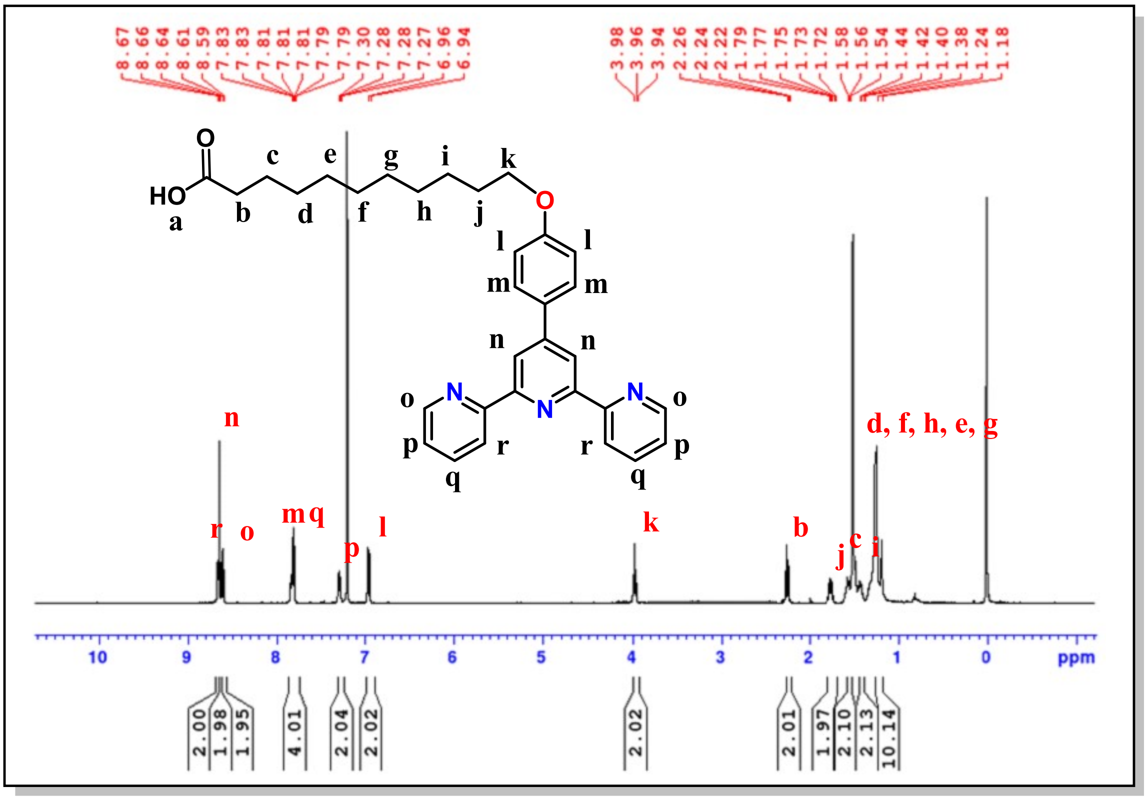


Fig. S13 ^{1}H NMR spectrum of compound **L3**

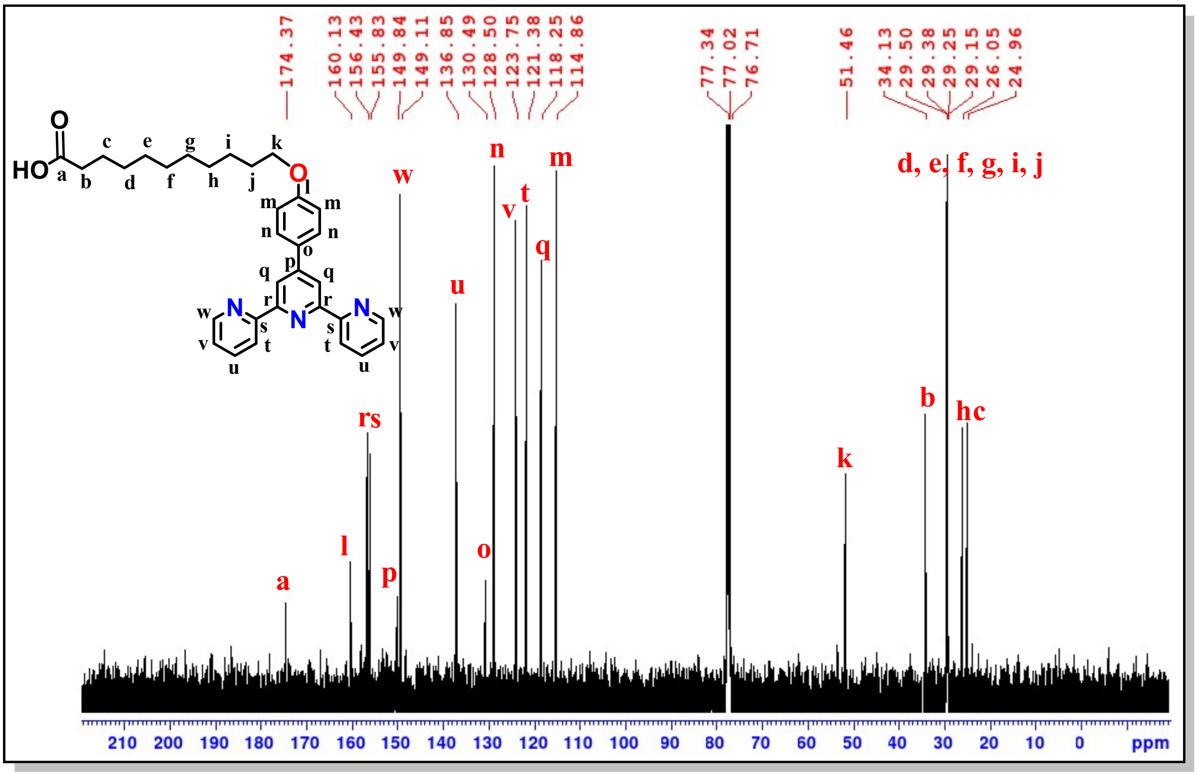


Fig. S14 ^{13}C NMR spectrum of compound **L3**

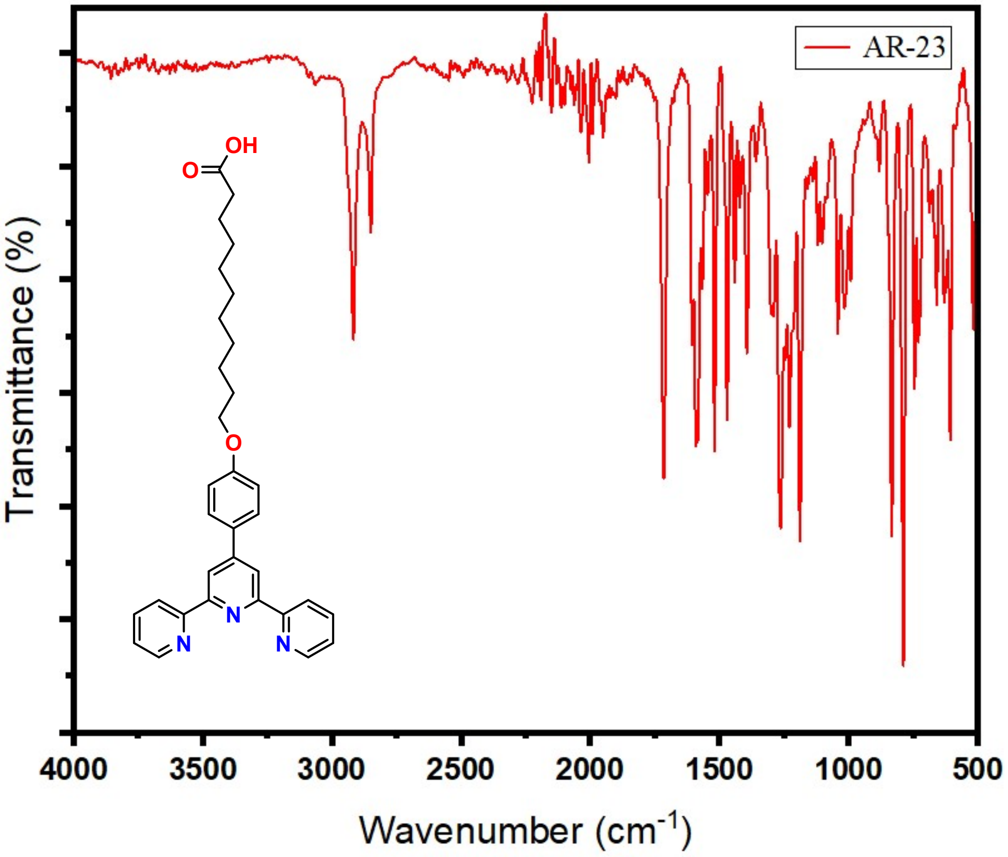
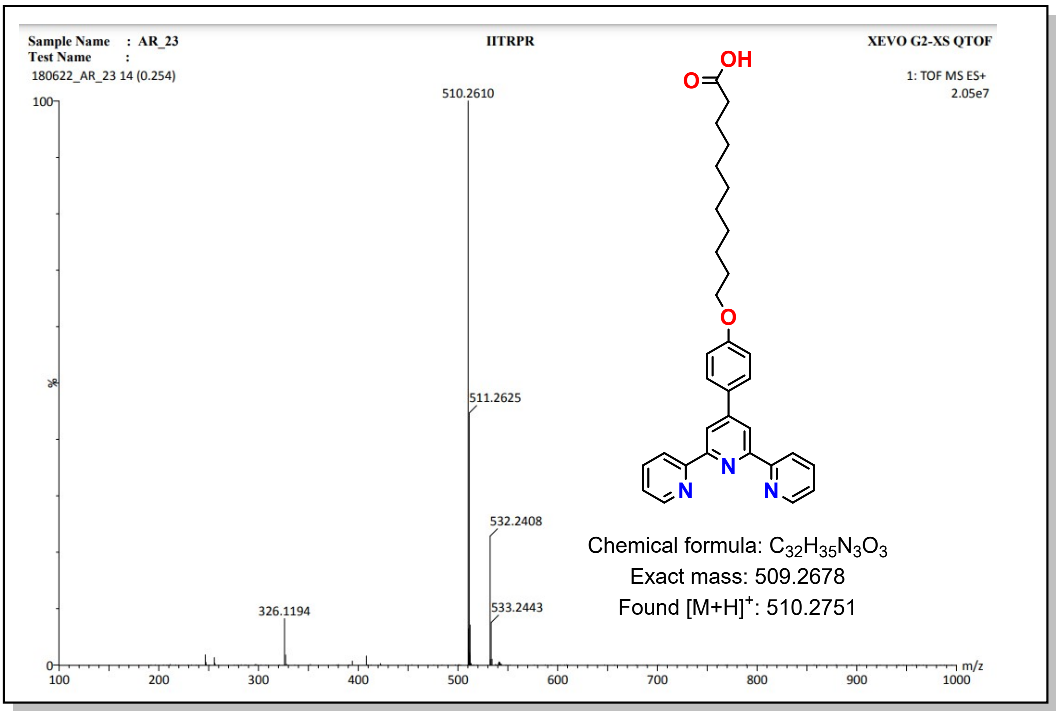


Fig. S15 IR Spectrum of compound **L3**



Chemical formula: $C_{32}H_{35}N_3O_3$
Exact mass: 509.2678
Found $[M+H]^+$: 510.2751

Fig. S16 ESI-MS Spectrum of compound **L3**

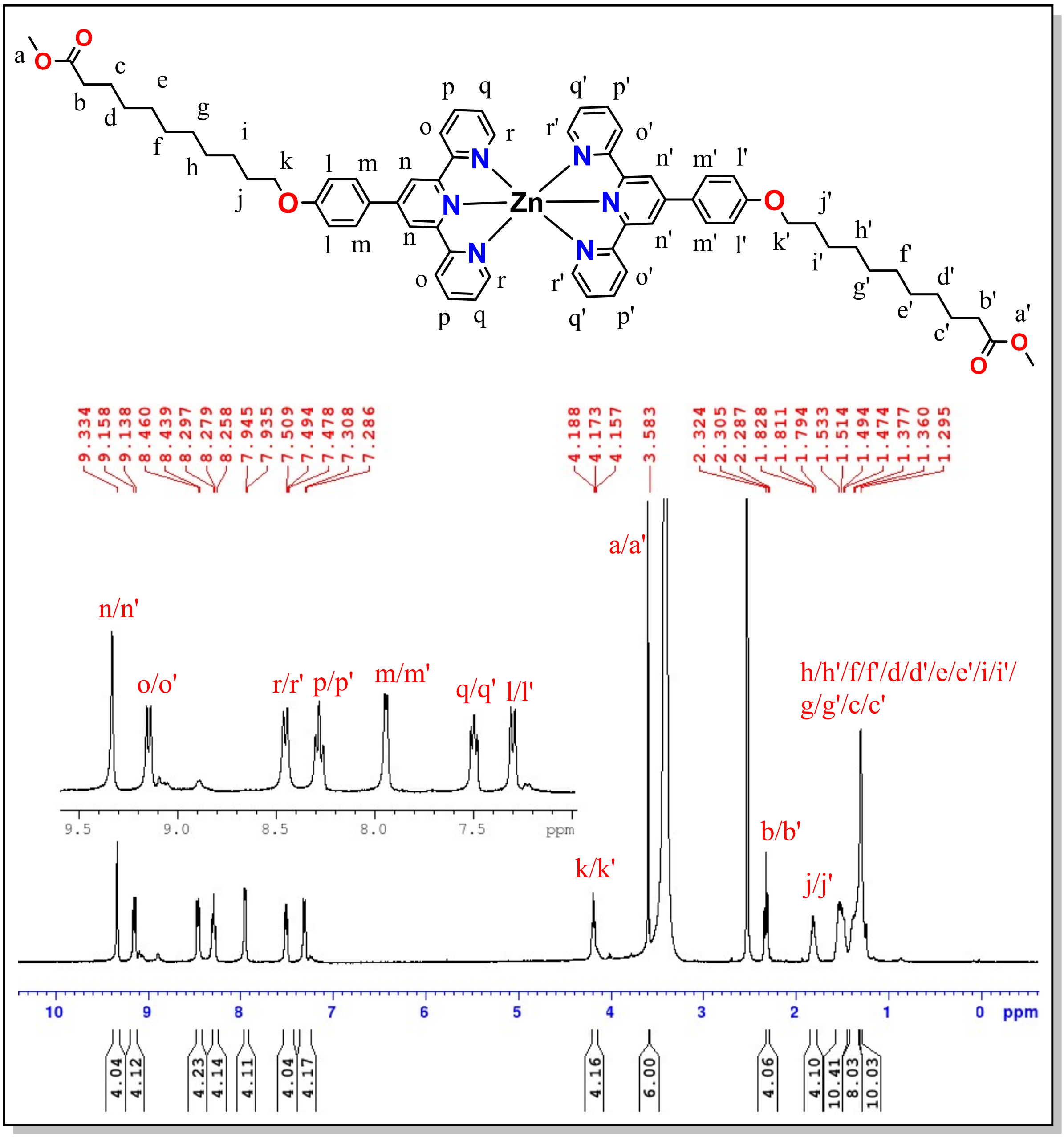


Fig. S17 ^{1}H NMR spectrum of Zn(II) complex **L_2-Zn**

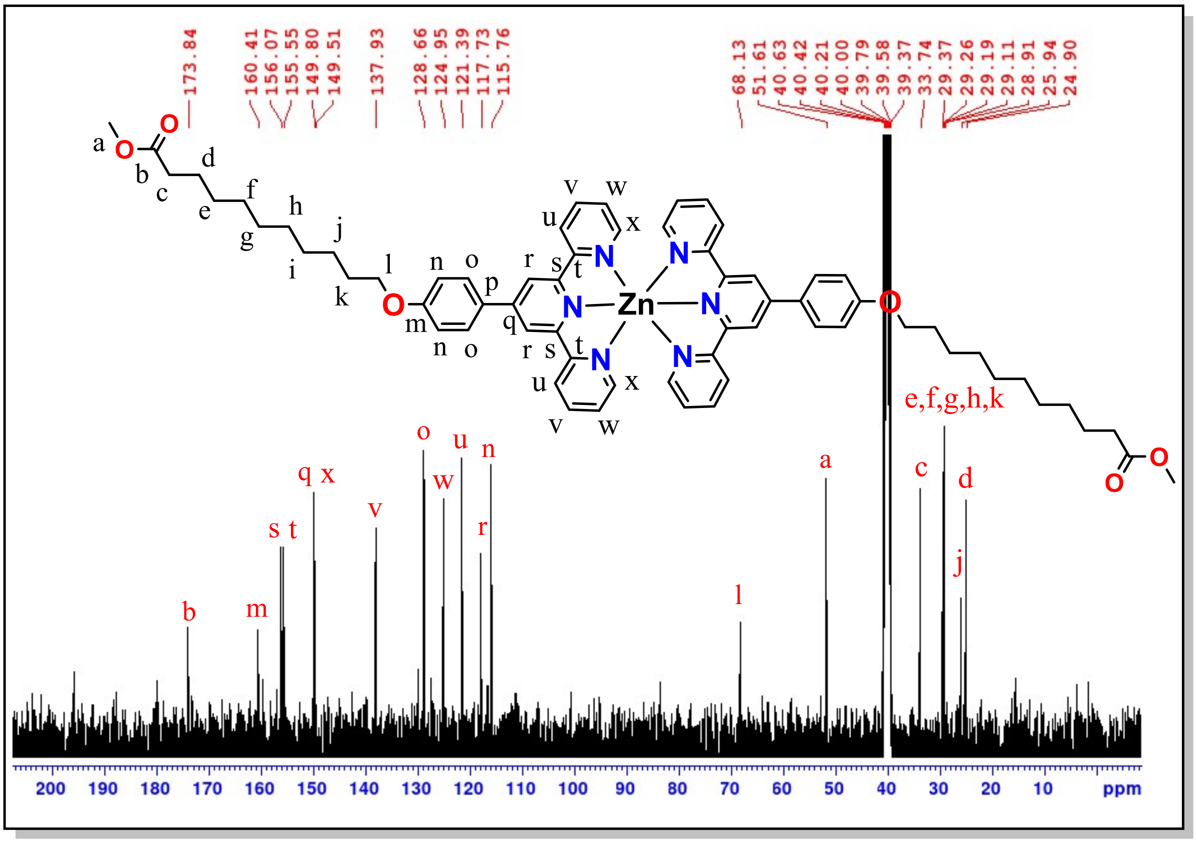


Fig. S18 ^{13}C NMR spectrum of Zn(II) complex **L$_2$-Zn**

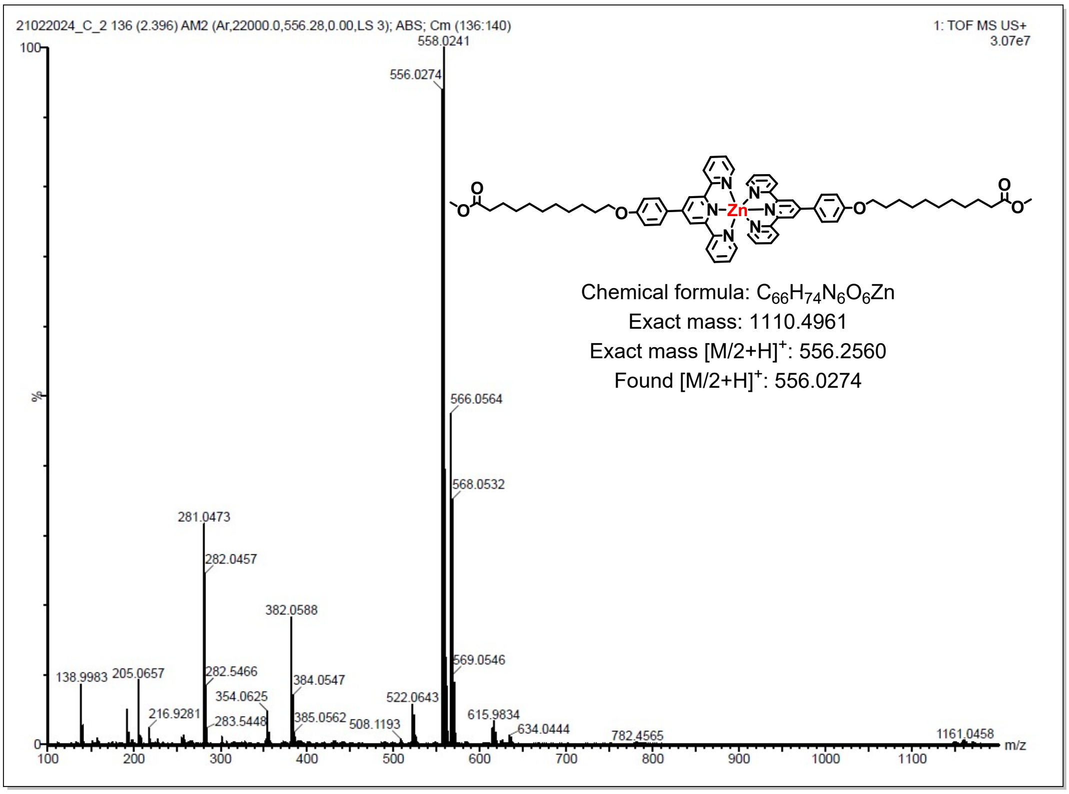


Fig. S19 ESI-MS Spectrum of complex **L$_2$-Zn**

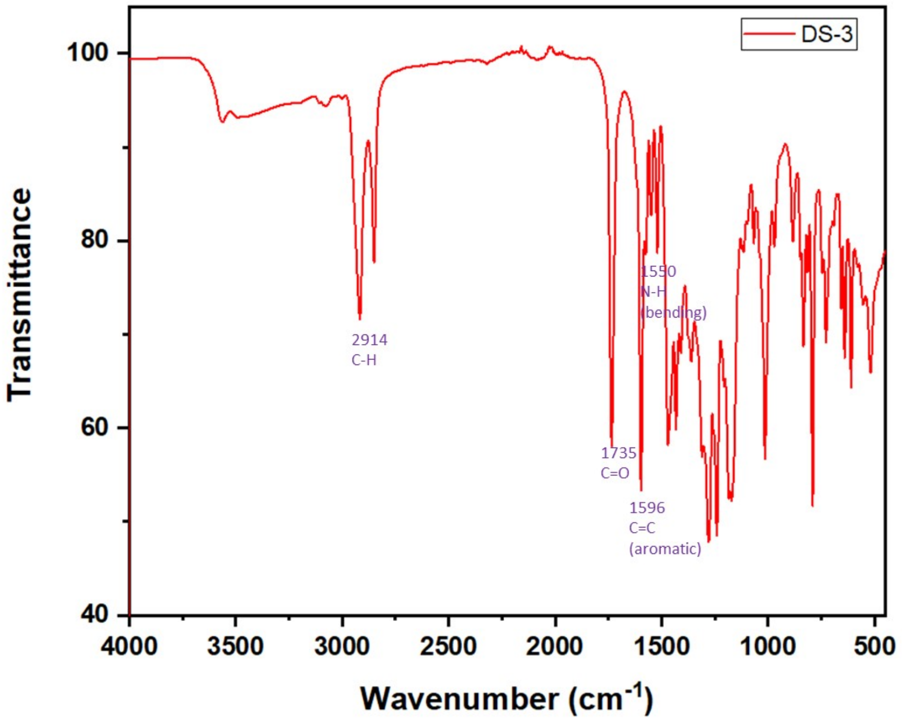


Fig. S20 IR Spectrum of complex **L_2-Zn**

Interpretation of the spectroscopic data of the ligands and tpy-Zn(II) complex (L_2-Zn)

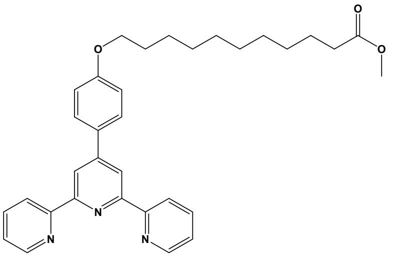
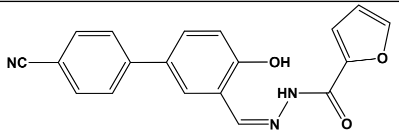
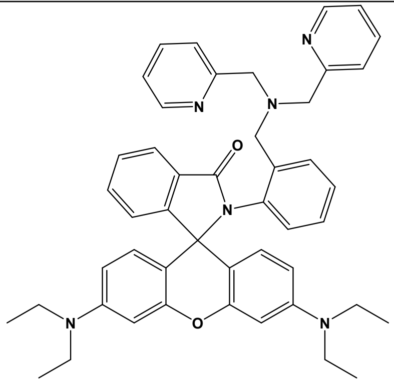
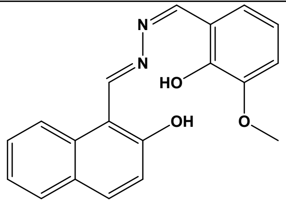
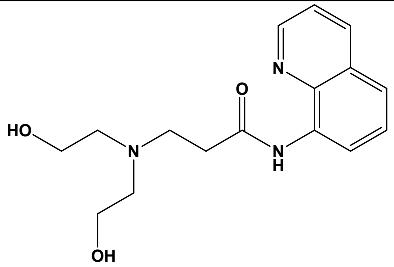
In ligand **L_2,** protons corresponding to the -OCH_3 group conformed with the singlet signal at δ 3.59 ppm. The 2 protons of the middle pyridine ring were seen as an intense singlet at δ 8.63 ppm. Similarly, protons attributed to the ether part (O-CH_2) resonated as a triplet at 3.95 ppm. Likewise, every aromatic proton of compound **L_3** resonated between 6.94 ppm and 8.67 ppm. The 2 protons of the middle pyridine ring were exhibited as a sharp singlet at δ 8.64 ppm and protons attributed to the O-CH_2 group resonated as a triplet at 3.96 ppm.

In the ligand **L_2**, the C=O carbon resonated at δ 174.35 ppm. The signals at 68.14 ppm and 51.44 ppm were attributed to the O-CH_2 and O-CH_3 carbons, respectively. Similarly. In **L_3**, the C=O was resonated at 174.37 ppm and all other aromatic and aliphatic carbons were resonated in the range of 114.86-160.13 ppm and 24.96-51-46 ppm, accordingly.

The spectral conformation of the synthesized tpy molecules **L_1-L_3** was verified with the spectral data from ESI-MS. In the mass spectrum of ligand **L_2**, the molecular ion peak at *m/z* 524.29 [M (523.29) +H]$^+$ and the fragment signal at 546.25 [M+Na]$^+$ were observed. The signal appeared at an *m/z* 326.11 [M+H$^+$] corresponds to the tpy precursor molecule (**tpy-OH)**. The signal with *m/z* 510.27 [M (509.26) +H]$^+$ confirmed the presence of **L_3** and showed a fragment peak position of [M+Na]$^+$ at *m/z* 532.24.

As seen in Fig. S17–S20 (ESI), the purity of the Zn(II) complex associated with the ligand **L_2** was investigated by ^{1}H NMR and ^{13}C NMR in DMSO-d$_6$ at room temperature, IR, Elemental analysis and ESI-MS analysis. In the ^{1}H NMR spectrum of the complex **L_2-Zn**, a multiplet with a chemical shift of δ 1.29-1.37 ppm represented the protons bonded to the strongly shielded aliphatic groups. Moreover, the 6 protons corresponding to the -OCH$_3$ groups conformed with the singlet signal at δ 3.58 ppm. The 4 protons of the middle pyridine ring were seen as an intense singlet at δ 9.33 which is shifted towards the de-shielded region in comparison to the associated ^{1}H spectrum of the ligand. Similarly, the 4 protons attributed to the ether part (O-CH$_2$) resonated as a triplet at 4.17 ppm and all other aromatic protons resonated between 7.28 ppm and 9.15 ppm. Similarly, in ^{13}C NMR, the C=O carbon resonated at δ 173.84 ppm. The signals at 68.13 ppm and 51.61 ppm were attributed to the O-CH$_2$ and O-CH$_3$ carbons, accordingly. The spectral conformation of the synthesized complex (**L_2:Zn^{2+}**) were verified with the spectral data from ESI-MS. The exact molecular mass and [M/2+H]$^+$ of the Zn(II) complex having molecular formula $C_{66}H_{74}N_6O_6Zn$ is calculated as 1110.4961 and 556.2560, accordingly. In the ESI-MS spectrum, the molecular ion peak at *m/z* 556.0274 was attributed to the [M/2+H]$^+$ mass value, which is matching with the theoretical mass and hence confirms the product **L_2-Zn**. The FT-IR spectrum of **L_2-Zn** is displayed in Figure S19. The stretching frequencies of the terpyridyl ν(C=N) in the complex **L_2-Zn** are visible at 1596 cm^{-1} in the spectrum. Likewise, the ester ν(C=O) and ν(OCH$_3$) stretching is observed at 1735 cm^{-1} and 2914 cm^{-1}, accordingly. The frequency at 1469 cm^{-1} depicts the aromatic rings ν(C-C) stretching. Additionally, the ether ν(C=O) in the terpyridine ring exhibited the stretching frequency at 1392 cm^{-1}. Moreover, the infrared frequency at 586 cm^{-1} attributed to ν(Zn-N).

Table S1. Comparison of L_2 with previously reported fluorescent probes for the selective detection of Zn^{2+} ions.

Probe Structure	**Target**	**λ_{em} (nm)**	**Limit of Detection**	**RGB Integration**	**Application**	**Reference No. in Manuscript**
	Zn	480	84 nM	Yes	Test paper strip, Live cell imaging	Current Work
	Zn and Al	512	1.02 nM	NO	Live cell imaging – Human colon cancer cells	37a
	Zn	550	--	NO	Live-cell imaging of HeLa cells	37b
	Zn	498	1.1×10^{-7} M	NO	Test paper strip	37c
	Zn	536	4.48 µM	NO	Detection in pure water	37f

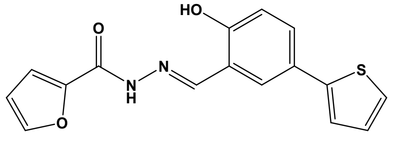
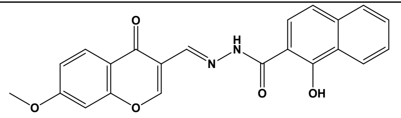
	Zn	567	77 nmol L^{-1}	NO	Detection in aqueous solutions	37d
	Zn	498	1.73 x 10^{-7} M	NO	--	37e

Table S2. Determination of Zn^{2+} in ambient water samples (samples are analyzed in triplicate).

Sample	**Zn^{2+} added (μM)**	**Zn^{2+} found (μM)**		**Recovery %**	
		Spectrophotometry	**Solution phase-RGB**	**Spectrophotometry**	**Solution phase-RGB**
Drinking water	5.00	4.98 ± 0.13	4.91 ± 0.19	99.6	98.2
Tap water	5.00	4.96 ± 0.14	4.89 ± 0.21	99.2	97.8

[a]Conditions: $[L_2]$ = 10 μM in DMSO: H_2O (7: 3, v/v) medium

Table S3. Theoretically, predicted excited state electronic properties of L_2 and L_2-Zn^{2+} using TD-DFT.

Code	**λ_{abs} (nm)**	**Mol. Abs. Coefficient (L m^{-1} cm^{-1})**	**Oscillator strength (f)**	**Transition**	**Orbital Contributions**
L_2	294	32823	0.3608	$S_0 \rightarrow S_2$	H→L (17 %), H→L+1 (82 %)
L_2-Zn^{2+}	351	12047	0.4435	$S_0 \rightarrow S_8$	H→L+5 (37 %), H→L+6 (20 %), H→L+7 (33 %)

*H - HOMO, L - LUMO orbitals