JOTCS A
Journal of the Turkish Chemical Society
Section: A
Chemistry
TURKISH CHEMICAL SOCIETY

Synthesis, Characterization, and Use of Lanthanide Chelate of β-Diketonate Based Ligand as a Luminescent Biolabel

Semanur Sen Yuvayapan[1], Alper Tolga Colak[1]*, Onur Sahin[2], and Cumali Celik[3]

[1] Department of Chemistry, Faculty of Arts and Sciences, Dumlupinar University, Kutahya, Turkey

[2]Department of Occupational Health & Safety, Faculty of Health Sciences, Sinop University, Sinop, Turkey

[3]Department of Property Protection and Security, Yalova Vocational School, Yalova University, Yalova, Turkey

Abstract: In this study, we aimed to synthesize a β-diketonate-based ligand and its Eu(III) complex which are used for luminescent biolabel. For this purpose, we chose acetophenone as the starting material which contains methyl group at the alpha position of the ketone group. Firstly, we obtained 4,4,5,5,6,6,7,7-octafluoro-3,8-dihydroxy-1,10-diphenyldeca-2,8-diene-1,10-dione (H_2ODIT) ligand in a reaction between acetophenone and diethyloctafluoroadipate with Claisen condensation. This ligand was characterized by ^{1}H-NMR, ^{13}C-NMR, and mass spectral analyses. We obtained a single crystal H_2ODIT as characterized by X-ray analysis. At the second step, in order to bind H_2ODIT to the antibody, it is reacted with chlorosulfonic acid. As a result of this reaction, the functional group of -$ClSO_2$ was bound to the structure. The structure of H_2CODIT was characterized by NMR and mass spectral analysis. In the third step, to understand the usability of the ligand as a biolabel, the complex compound was synthesized with $EuCl_3$. The complex compound was excited with UV light at 306 nm wavelength, specific hypersensitive $^5D_0 \rightarrow {}^7F_2$ phosphorescence electronic transition of the Eu(III) was observed which proved that the luminescent H_2CODIT molecule can work as a biolabel.

Keywords: Beta-diketonate, Claisen condensation, europium(III) complex, keto-enol tautomerism, luminescence, biolabel.

Submitted: June 09, 2021. **Accepted:** July 18, 2021.

Cite this: Sen Yuvayapan S, Colak AT, Sahin O, Celik C. Synthesis, Characterization, and Use of Lanthanide Chelate of β-Diketonate Based Ligand as a Luminescent Biolabel. JOTCSA. 2021;8(3):835–50.

DOI: https://doi.org/10.18596/jotcsa.949970.

***Corresponding author. E-mail:** tolga.colak@dpu.edu.tr.

INTRODUCTION

The investigation of luminescence properties of complexes of rare earth elements was introduced upon the excitation of europium β-diketone complexes by ultraviolet light in 1942 (1). Biomedical and biolabeling of europium β-diketone complexes have been a subject of interest since then.

Lanthanides are known for their atomic emission properties. Europium is a versatile lanthanide; a myriad of luminescense applications of europium such as biomedical sensor, diagnosis, drug delivery, and optical screening exist (2-7). One of the most striking properties of the complexes that contain lanthanides is absorption of light with a specific wavelength followed by a reflection with a distinct wavelength. Either a ligand or a metal center are responsible for such absorption behavior. Energy is emitted through various ways,

which are florescence or phosphorescence, heat, and a photochemical product (8). In the Ln(III)-ligand bonding, 4f electrons are screened by the electrons of $5s^2$ and $5p^6$ so that they can rarely take place in covalent interactions with ligands. The covalency of a Ln(III)-ligand bond is around 5-7%. As a result of this screening, transitions are considered weak in respect to both absorption and emission. It is a difficult task to excite 4f electrons directly unless subjected to a laser, even for highly luminescent materials. Ln(III) ions cannot fluoresce through absorbing UV light (6,9). The lanthanide ion depends on fluorescence ligands in order to transfer to ^5D_j energy level through a viable mechanism, and the absorption of light is similar to an antenna. The long lifetime of the excited states of complex lanthanide ions (0.2-1.5 ms), large Stokes slip (over 200 nm), and emission at visible region makes them attractive ions in terms of possessing narrow and sharp emission bands (2). Visible emission spectrum of Eu^{3+} results from $^5D_0 \rightarrow {}^7F_J$ transition, and transitions at specific wavelength are: 594 nm ($^5D_0 \rightarrow {}^7F_1$); 617 nm ($^5D_0 \rightarrow {}^7F_2$); 659 nm ($^5D_0 \rightarrow {}^7F_3$), (10).

Eu^{3+} complexes usually provide higher sensitivity, which accounts for their very strong preference among lanthanides. Eu^{3+} complexes exhibit some distinct properties such as i) performing absorption pinnacle at UV region (about 330 nm) and fluorescence emission at 615 nm, ii) possessing a narrow emission band, iii) considerable lifetime of the triplet state of their complexes under excitation (longer than 100 μs), iv) indicating low background signal, v) demonstrating sharp emission peaks, and vi) not causing any radioactive contamination.

β-diketones or 1,3-diketones consist of two carbonyl groups separated by a carbon atom, which is an α-carbon. On the other hand, carbonyl substituents can be either alkyl, fluorinated alkyl, or aromatic/heteroaromatic groups. The selection of functional groups affect the properties of Eu(III) complexes. For instance, solubility and volatility are increased in organic solvents when branched alkyl chains such as tert-butyl group are introduced. Perfluorinated alkyl groups increase the Lewis acidity. Aromatic β-diketones better absorb light when compared to aliphatic ones. Furthermore, functional groups affect the energy state of the ligand (singlet and triplet), (11). Fluorescent intensity increases when an electron donor (R_1) and acceptor (R_2) are introduced in the same ligand for β-diketonato ($R_1COCHCOR_2$)-Eu complexes (12).

The selection of the ligands that will be constituted with europium(III) depends on the application. The ligands used as biolabeling in medical diagnostic kits can be divided into 4 main categories: i) ligands exhibiting no luminescent property, ii) polyamino carboxylate based luminescent ligands (PAC based), iii) β-Diketone based luminescent ligands, and iv) other luminescent ligands (13).

Luminescent lanthanide β-diketones have a wide range of applications, including electroluminescent device and sensors, lasers, and bioanalysis (14). Eu(III) complexes are established to be stable in aqueous solutions of β-diketonates, covalently bonded to the protein easily due to their tendency to chlorosulfonation, and possessing highly luminescent character. Their lesser side products in reactions, ease of their synthesis, and cost-effective synthesis can be counted as other advantages (15).

Ultrasensitive bioanalytical kits of analysis are based on the detection of the existence of analyte that bonds to the material of interest or measurement of the concentration (5). The applications regarding the use of luminescent lanthanide complexes in bioanalysis date back to 1983 (16). Most of them are designed for biochemical evaluation or qualitative measurement of specific antibodies or any antigen. Within the possibility of involving radioactive atoms (^{125}I, ^3H, and ^{14}C) that generate signals for some regions, many labels are available. In addition, enzymes, fluorescent probes, chemiluminescent compounds, metals, and metal chelates and liposomes, as non-radioactive labels, can be used due to their numerous advantages (17). This study aims to synthesize the non-radioactive label Eu(III) complex.

Employing ketone and fluorine compounds as precursors, a very limited number of compounds synthesized via Claisen condensation can be found in the literature. Most of them contain aromatic rings. Despite the vast number of synthesis of lanthanide-β-diketone complexes, very few of them have been utilized in biomolecular labeling (11) by reason of them being only bidentate that makes them have a limited stability. In order to go beyond this limit, tetradentate ligands comprise of two β-diketones in a molecule were synthesized. These ligands were then used in immunoanalysis for designation of various biomolecules (18,19). This study focuses on the synthesis of tetradentate β-diketonate based luminescent ligand with eight fluorine atoms and two aromatic rings and its availability on biomolecular labeling.

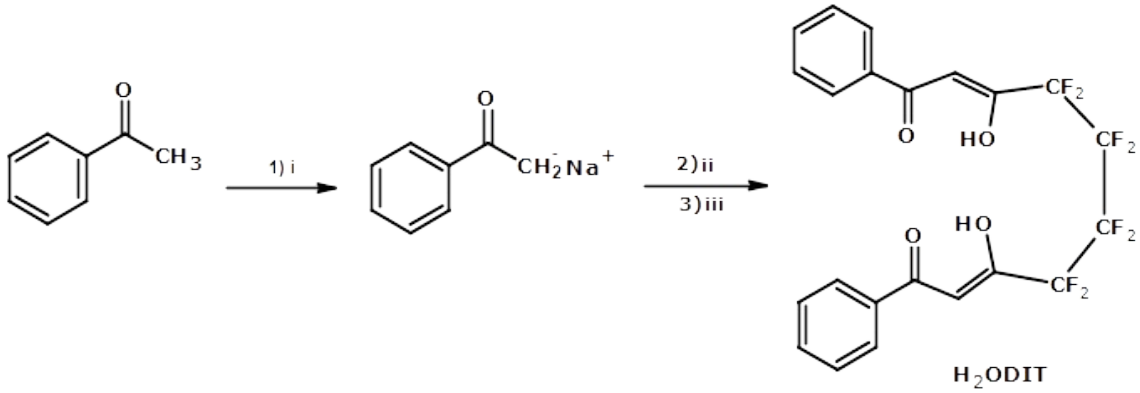
MATERIALS AND METHODS

$EuCl_3$, acetophenone, dimethyloctafluoroadipate (DEOFA), sodium methoxide ($NaOCH_3$), chlorosulfonic acid (HSO_3Cl), diethyl ether, ethanol, and 1,4-dioxane were all provided by Sigma-Aldrich and used without any purification. NMR

analysis were carried out by using a Bruker Biospin NMR Spectrometer with data acquisition by taking advantage of an Avance III 400 MHz instrument. FTIR studies were run by a Bruker Vertex 70 FT-IR. UV-visible electronic spectra were obtained by a Shimadzu UV-2450 spectrophotometer. Mass spectrometry of the complexes were performed by an Agilent 6530 Accurate Mass Q-TOF LC/MS. X-ray single crystallography of H_2ODIT ligand was studied by using D8-QUEST diffractometer with a graphite-monochromatic Mo-K_α light source at 296 K. The structure was examined by SHELX-2013 (20,21) through direct methods and least squares method was applied to refine the matrix at F^2 by taking advantage of WINGX software. Crystalline X-Ray Diffraction data was acquired from Bruker APEX-II (22) diffractometer. Molecular diagrams were generated by MERCURY software (23). Supramolecular analysis was run by WinGX software (24). Luminescence spectrum of the complex was obtained by Perkin Elmer LS55 florescence spectrophotometer.

Synthesis of (2Z,8Z)-4,4,5,5,6,6,7,7-octafluoro-3,8-dihydroxy-1,10-diphenyldeca-2,8-diene-1,10-dione (H_2ODIT)

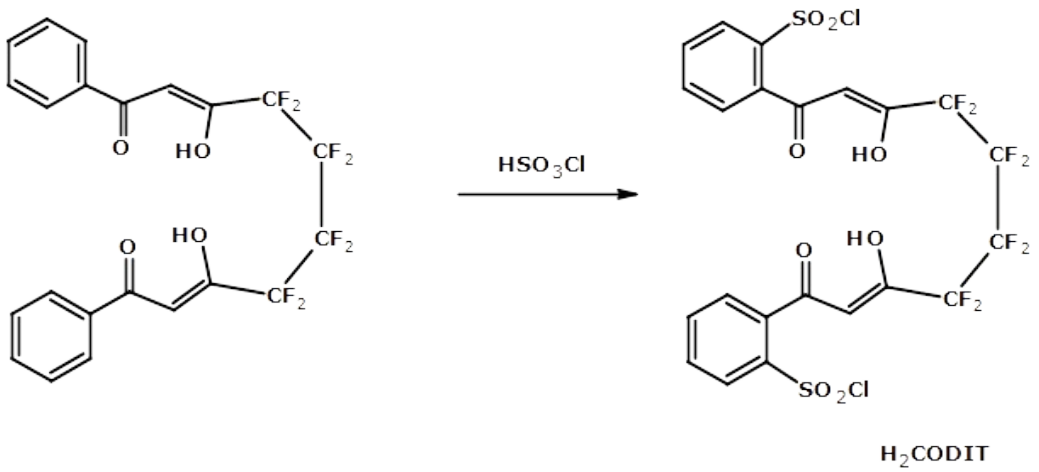
In order to form the acetophenone sodium salt, $NaOCH_3$ and 50 mL of diethyl ether in catalytic amount were placed in a two-necked round-bottom flask connected to a refrigeration apparatus. Acetophenone (2.50 g, 20 mmol) was slowly added into the flask, and the solution was stirred for 1 h. The observed color was pale light yellow. DEOFA (3.60 g, 10 mmol) was added in this solution drop-wise. A yellow color was observed. Thin layer chromotography was employed in order to determine the reaction time, amount of matter, and purity in a specific timespan. *n*-Hexane-chloroform (1:3, v:v) mixture was used for chromatographic analysis. The solution was stirred for 5 days at room temperature followed by the evaporation of the solvent. The residue was dissolved with water. pH adjustment (pH=6) was later carried out with 15% H_2SO_4. Precipitated substance at acidic medium was dried and crystallized from ethanol-1,4-dioxane (3:2, v:v) mixture (Scheme 1). Yield: 2.50 g (48.5 %).



Scheme 1: Reagents and conditions. (i) $NaOCH_3$, diethyl ether, rt, 1 h; (ii) DEOFA, rt, 5 days; (iii) H_2SO_4, H_2O.

Synthesis of (2Z,8Z)-1,10-bis(benzene-1-sulfonyl chloride)-4,4,5,5,6,6,7,7-octafluoro-3,8-dihydroxydeca-2,8-diene-1,10-dione (H_2CODIT)

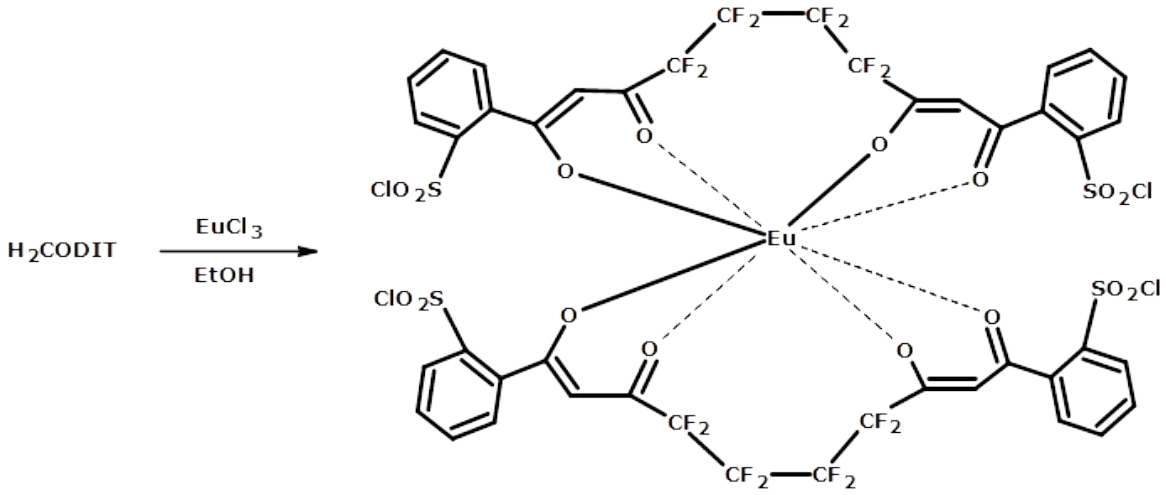
H_2ODIT (0.98 g, 2 mmol) compound was added slowly in HSO_3Cl (5 mL) that was placed in a balloon under refrigeration apparatus. The color of the solution turned to light green from yellow followed by a dark green color when the whole material was introduced into the solution. The solution was stirred for 5 days at room temperature. To abandon the excess chlorosulfonic acid, solution was dripped onto ice-water. A yellow solid was formed in ice-water. The solution was then filtered, rinsed with cold water, and dried (Scheme 2). Yield: 0,99 g (72%).



Scheme 2: Synthesis of H_2CODIT compound.

Synthesis of the Eu(III) complex

Chlorosulfonated ligand (H_2CODIT) (0.97 g, 1.4 mmol) was dissolved in ethanol. $EuCl_3$ (0.18 g, 0.70 mmol) was introduced in the stirring solution resulting that complexation occurs very rapidly. Subsequent to abandoning of ethanol, the obtained solid material was rinsed with water and diethyl ether followed by drying (Scheme 3). Yield: 0.87 g (81%).



Scheme 3. Synthesis of Eu(III) complex.

RESULTS AND DISCUSSION

Spectroscopic studies

While the the structure of the the synthesized H_2ODIT compound was illuminated via ^{1}H-NMR and ^{13}C-NMR spectroscopy, mass spectrometry, X-ray single crystal diffactometry and FT-IR techniques, ^{1}H-NMR and ^{13}C-NMR spectroscopy, mass spectrometry, and FT-IR were employed for the characterization of the H_2CODIT ligand. Structural properties of the complex was determined by FT-IR, mass, luminescence spectroscopy, and UV-Visible techniques.

FTIR spectra

ATR technique was employed in obtaining the FTIR spectra of the ligands and complexes in the range of 4000-550 cm^{-1} and characteristic vibrations were determined.

FTIR spectrum of DEOFA is given in Figure 1a. The absorption band at 1778 cm^{-1} results from C=O stretching (-CO-OR). Two other absorption bands at 1181 and 1142 cm^{-1} were observed and these bands were assigned to $-CF_2$ group vibrations. The stretching vibration absorption band at 2994 cm^{-1} could be assigned to the aliphatic C-H group.

In the FTIR spectrum of H_2ODIT compound (Figure 1b), the peaks observed at 1595 and 1565 cm^{-1} belong to tautomeric state keto and enol form carbonyls v(C=O), respectively. v(C=C) and v(C-O) vibration bands of enol form correspond to 1466 and 1228 cm^{-1}, respectively. Two sharp peaks at

1185 and 1156 cm^{-1} belong to $v(CF_2)$ vibrations. The intense peak observed at 751 cm^{-1} results from the vibration that belongs to the monosubstituted benzene ring (25).

$v(SO_2Cl)$ vibration peak is observed in FTIR spectrum of the H_2CODIT compound (Figure 1c) apart from H_2ODIT compound. Asymmetric vibration of sulfonyl chloride group gives a peak at 1343 cm^{-1}. On the other hand, two peaks at 842 and 806 cm^{-1} exhibit the disubstituted bonding to benzene ring (25).

FTIR spectrum of Eu(III) complex is given in Figure 1d. The peak observed at 1604 cm^{-1} relates to the v(C=O) vibration of keto form carbonyl that does not bond to the metal in H_2CODIT compound. On the other hand, peaks observed at 1564 cm^{-1} and 1404 cm^{-1} belong to v(C=O) and v(C=C) vibrations of enolic structure, respectively. Characteristic vibration bands shift to lower frequencies in β-diketone complex for the free ligand. This shift arises from the acidic proton loss of enolic structure from hydroxyl group and bonding of enolic hydroxyl oxygen and enolic carbonyl oxygen to the metal ion in the chelate mode (26). The peak that is coherent to the $v(CF_2)$ vibrations in the ligand is at 1240 cm^{-1} and $v(SO_2Cl)$ vibrations result in the peak at 1171 cm^{-1} (25). The emergent absorption band observed at 510 cm^{-1} in the complex suggests the existence of Eu-O coordination bond (27,28).

Mass Spectra

Mass spectrometric results obtained from H_2ODIT molecule in chloroform solution are presented in Figure 2. Investigating the mass spectrometry of H_2ODIT ligand, addition of one hydrogen to this ligand results in a strong molecular ion peak at m/z 495.08 that corresponds to the $[H_2ODIT + H]^+$ ion. The peak observed at m/z 517.07 corresponds to the $[H_2ODIT + Na]^+$ ion whereas the peak at m/z 518.07 belongs to the $[H_2ODIT + Na + H]$ ion. H_2ODIT structure is supported by the mass spectrometric data.

The mass spectra of ligand H_2CODIT was recorded in chloroform solution is presented in Figure 3a. The mass spectra of H_2CODIT showed accurate molecular ion peak at m/z 691.03, matched with the theoretical value. Two molecular ion peaks at 690.34 and 693.99 in the spectrum correspond to one hydrogen atom subtraction and two hydrogen atoms addition in the molecular ion peak, respectively.

Mass spectrum that belongs to Eu(III) complex in ethanol as solvent is given in Figure 3b. The product ion at m/z 1532.78 in the spectrum was observed through the introduction of protonated ion were resulted from the molecular ion peak of the ligand. The structure shown in the spectrum, which has the most bonding abundance, has a peak at m/z 1018.23.

NMR Spectroscopy

^{1}H-NMR and ^{13}C-NMR spectra of the ligands were recorded in benzene-d_6 as solvent. ^{1}H NMR and ^{13}C-NMR of H_2ODIT are presented in Figures 4 and 5, respectively. Due to the symmetry of the compounds in the solution, half of the molecules were taken into consideration when the spectra were being acquired.

For ^{1}H NMR spectrum of the H_2ODIT ligand, the triplet that belongs to the H_a proton bonded to aromatic ring was observed in the range of 6.92-6.89 ppm whereas the triplet that belongs to H_b protons in the range of 7.07-7.03 ppm. The doublet belonging to H_c protons were observed in the range of 7.45-7.43 ppm. The singlet at 6.42 ppm that belong to the active alpha proton (–CH) neighboring the carbonyl group suggests the formation of the (-HC=C) functional group, as a result of keto-enol tautomeric equilibrium in the structure, and a possible intramolecular hydrogen bond (O–H···O), (Figure 4a).

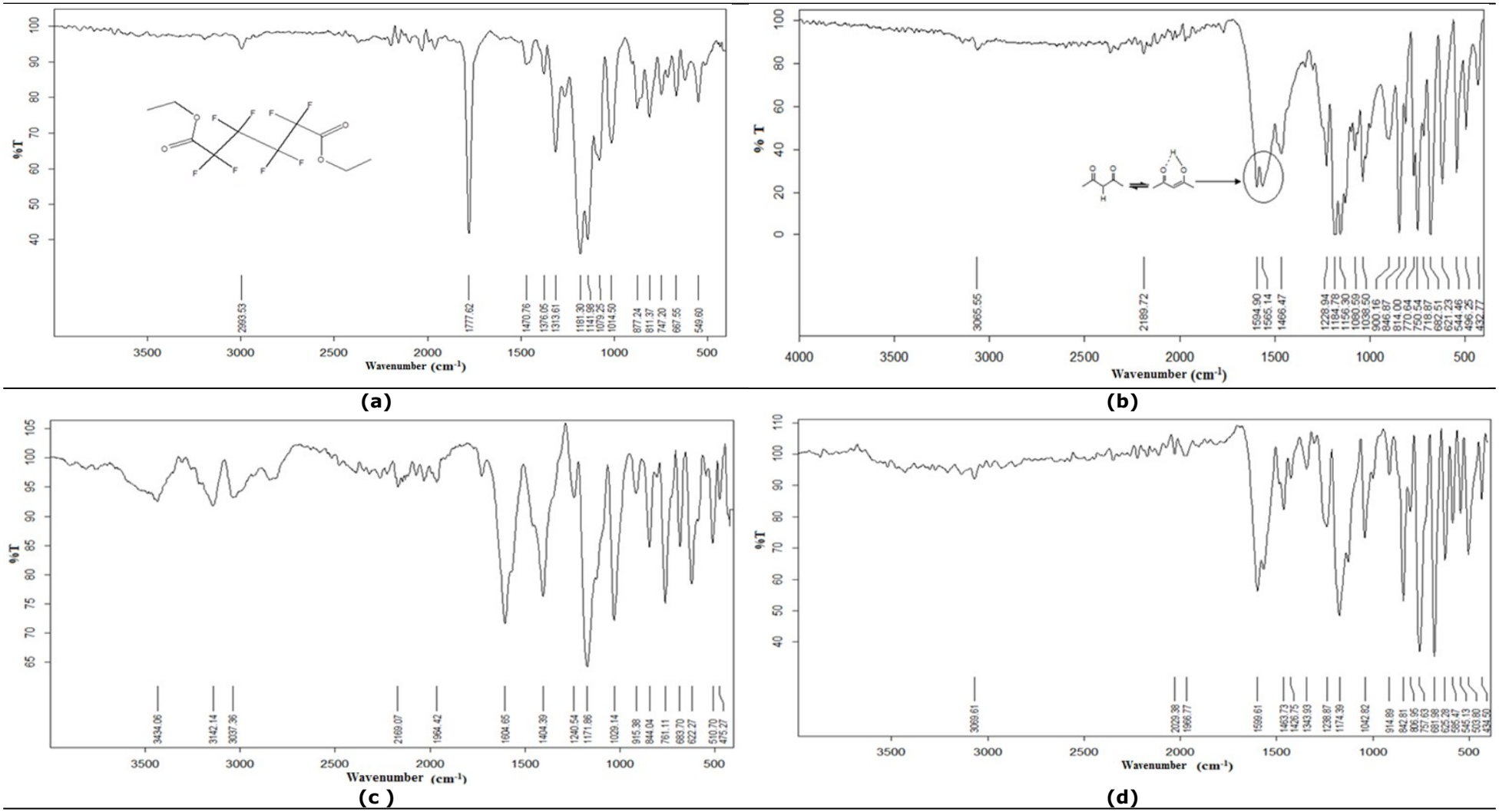


Figure 1: FTIR spectra of (a) DEOFA, (b) H_2ODIT, (c) H_2CODIT, and (d) Eu(III) compounds.

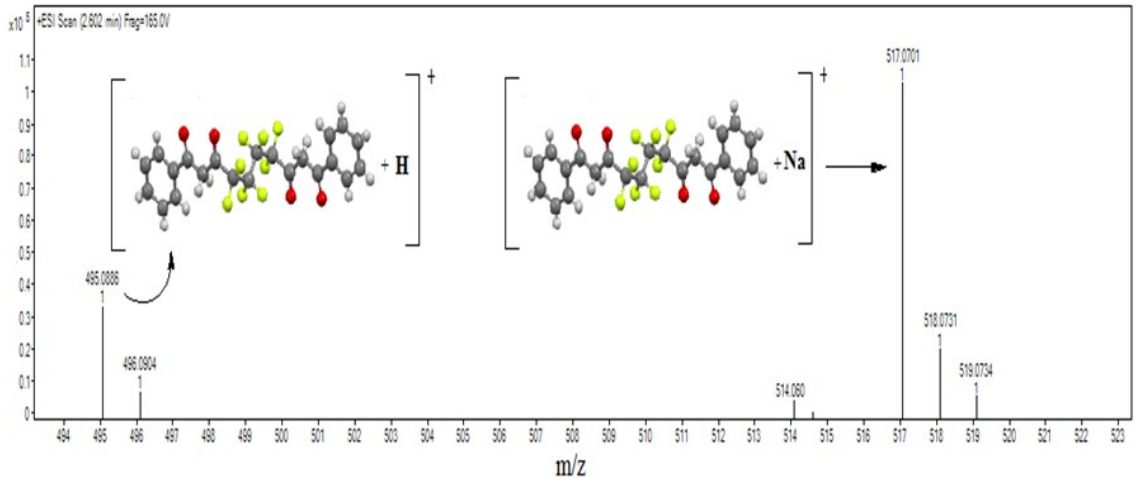
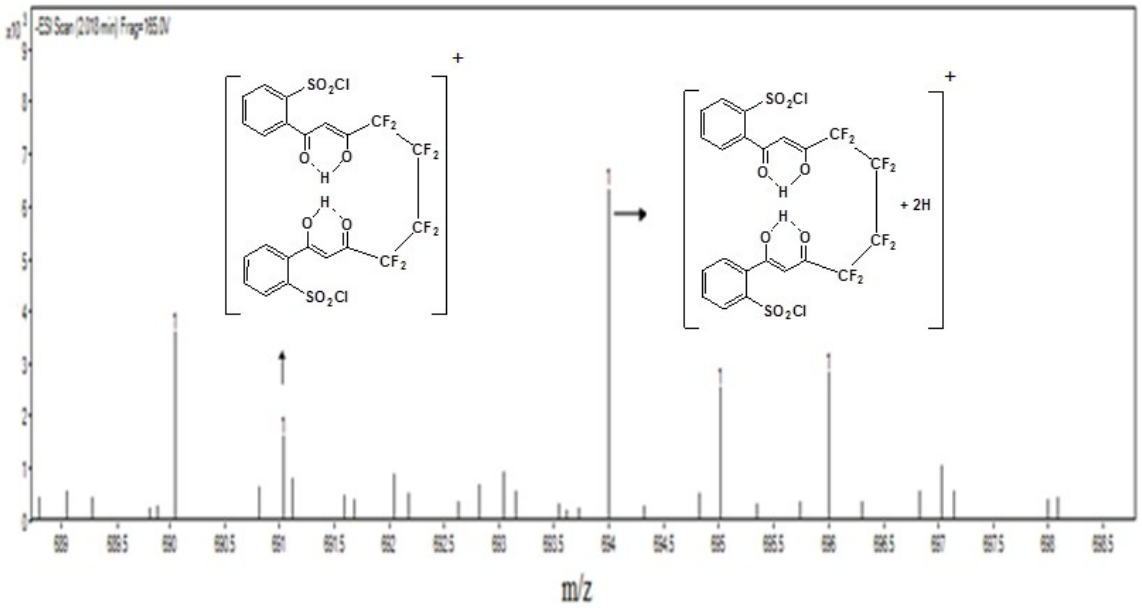
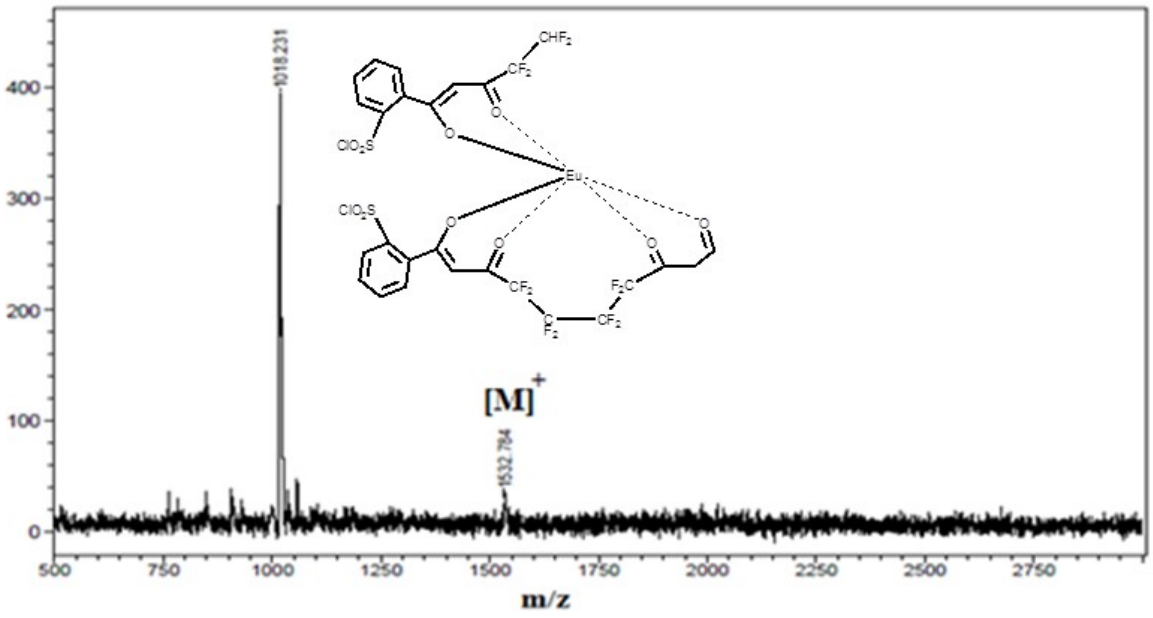


Figure 2: Mass spectrum of H_2ODIT ligand.



(a)

(b



(b)

Figure 3: Mass spectra of the compounds: (a) H_2CODIT, (b) Eu(III) chelate.

When the ^{1}H NMR spectrum of H_2CODIT ligand was investigated, H_a and H_b protons bonded to aromatic ring were observed in the range of 7.45-7.44 and 7.42-7.41 ppm as two doublets, respectively. On the other hand, H_c proton was detected in the range of 7.02-7.06 ppm as triplet, and H_d protons were detected in the range of 6.92-6.90 ppm also as triplet. Active alpha proton belonging to the H_2CODIT ligand was established at 6.41 ppm as singlet (Figure 4b).

When the ^{13}C-NMR spectrum of the H_2ODIT ligand was observed, the peaks that belong to aromatic ring carbons are surveyed in the range of 127.21-132.94 ppm. The carbon, which belongs to (–HC=) group, exhibiting keto-enol tautomer balance in the structure resonated at 93.27 ppm. Keto and enol carbons are characterized at 184.94 and 179.09 ppm, respectively. The peaks at 109.95 and 126.97 ppm belong to the carbon of (-CF_2) group (Figure 5a).

The carbons bonded to the aromatic ring in the ^{13}C-NMR spectrum of H_2CODIT ligand resonated in the range of 126.97-132.95 ppm. Keto-enol tautomeric equilibrium exhibits a peak at 93.28 ppm, belonging to the (–HC=) group. Keto tautomer carbonyl carbon and enol carbon resonated at 184.94 ppm and 178.83 ppm, respectively (Figure 5b).

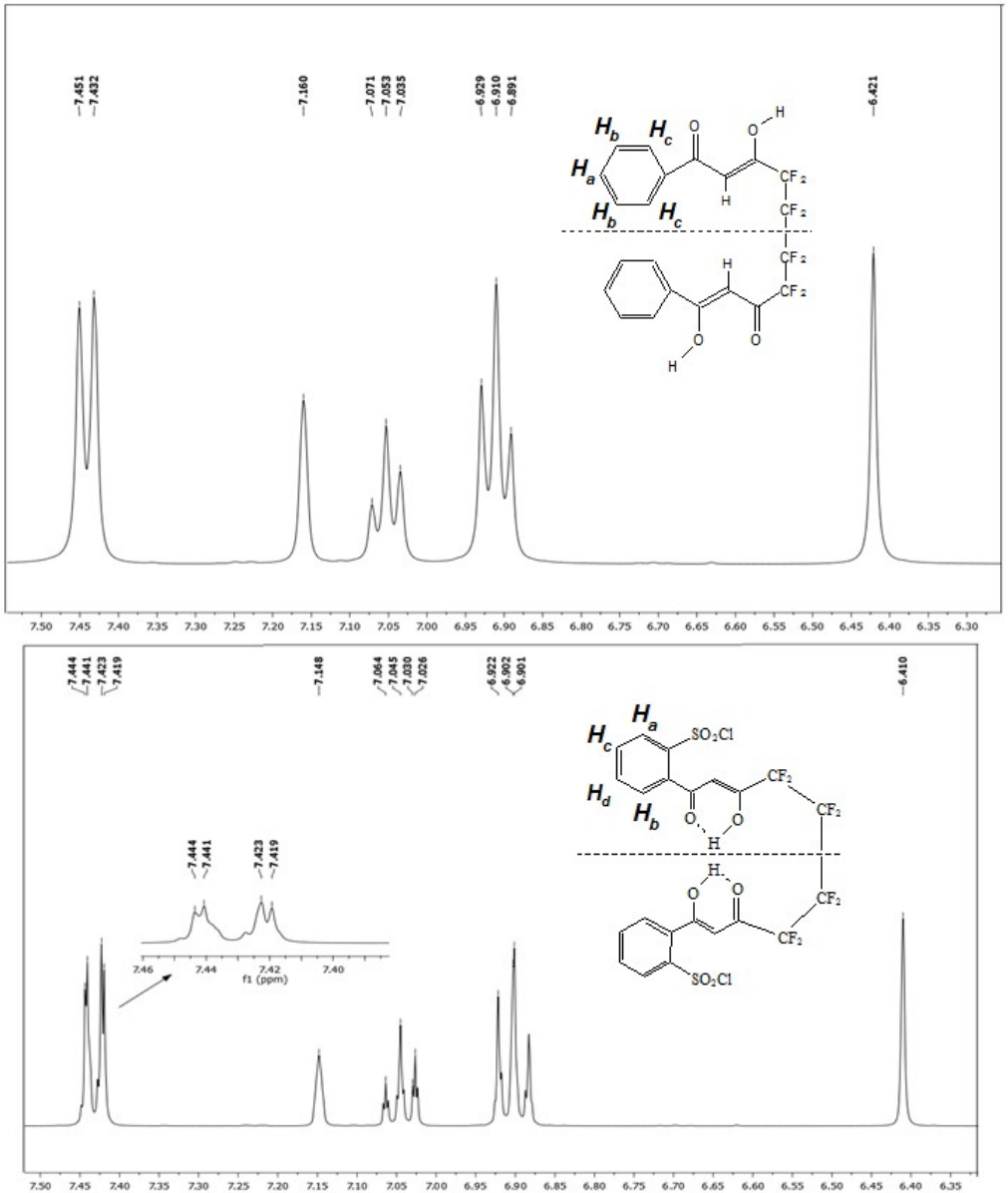


Figure 4: ^{1}H NMR spectra of the ligands: (top) H_2ODIT, (bottom) H_2CODIT.

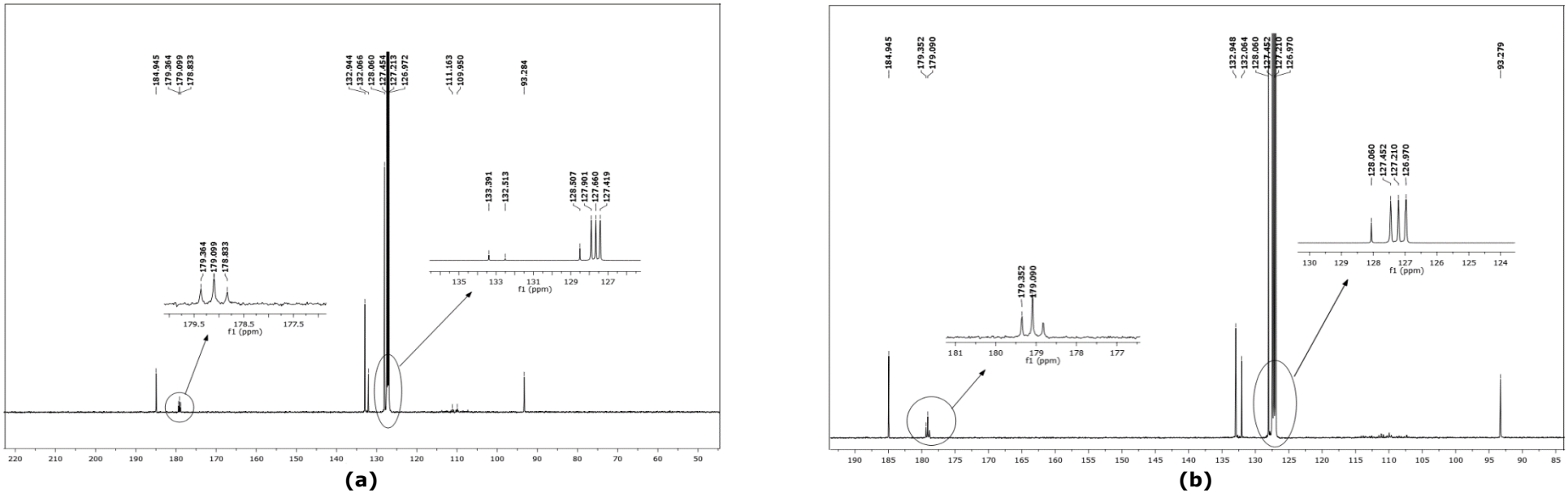
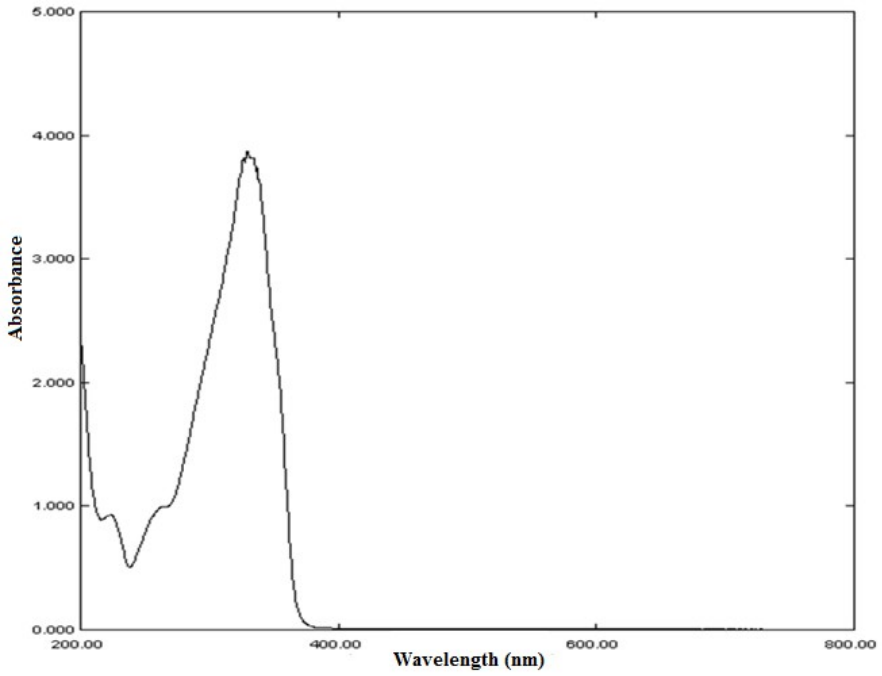


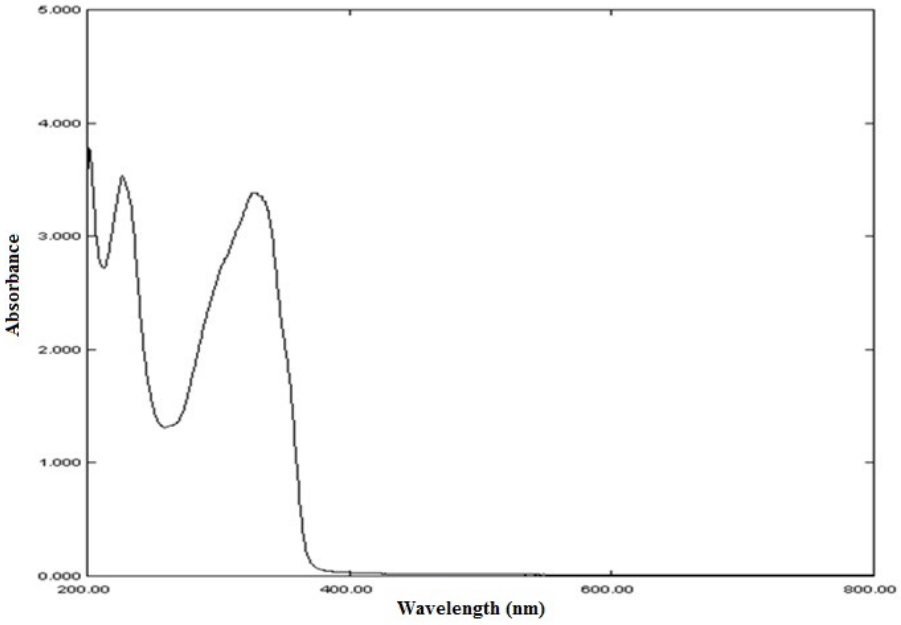
Figure 5: ^{13}C NMR spectra of the ligands: (a) H_2ODIT, (b) H_2CODIT.

UV–Visible Spectra

UV-visible absorption spectrum of H_2ODIT compound was observed in a 10^{-4} M ethanolic solution (Figure 6a). The band at 265 nm visible light short wavelength belongs to aromatic ring π→π* transition. The second band observed at 318 nm wavelength belongs to n→π* electronic transition of keto and enol carbonyl group of the tautomer. Lastly, the band observed at 329 nm (ε = 38660 L mol^{-1} cm^{-1}) corresponds to π→π* transition of conjugation and delocalization of the ligand in the electronic system (29,30).



(a)



(b)

Figure 6: UV-visible spectra of (EtOH, 1.0x10^{-4} M), (a) H_2ODIT, (b) H_2CODIT ligands.

H_2CODIT compound was characterized in 10^{-4} M ethanolic solution (Figure 6b). Three absorption bands at 268, 318 and 325 nm (ε = 33850 $Lmol^{-1}cm^{-1}$) resulting from π→π* and n→π* electronic transition were observed at UV-visible absorption spectrum.

UV-Visible spectrum of the complex in 10^{-4} M aqueous solution has an ε value of 91600 L mol^{-1} cm^{-1} at 268 nm, which belongs to π→π* and n→π* transitions whereas the band at 321.5 nm possessing a considerably high value of ε = 28980 L mol^{-1} cm^{-1} belongs to the charge transfer to the metal from the ligand (Figure 7).

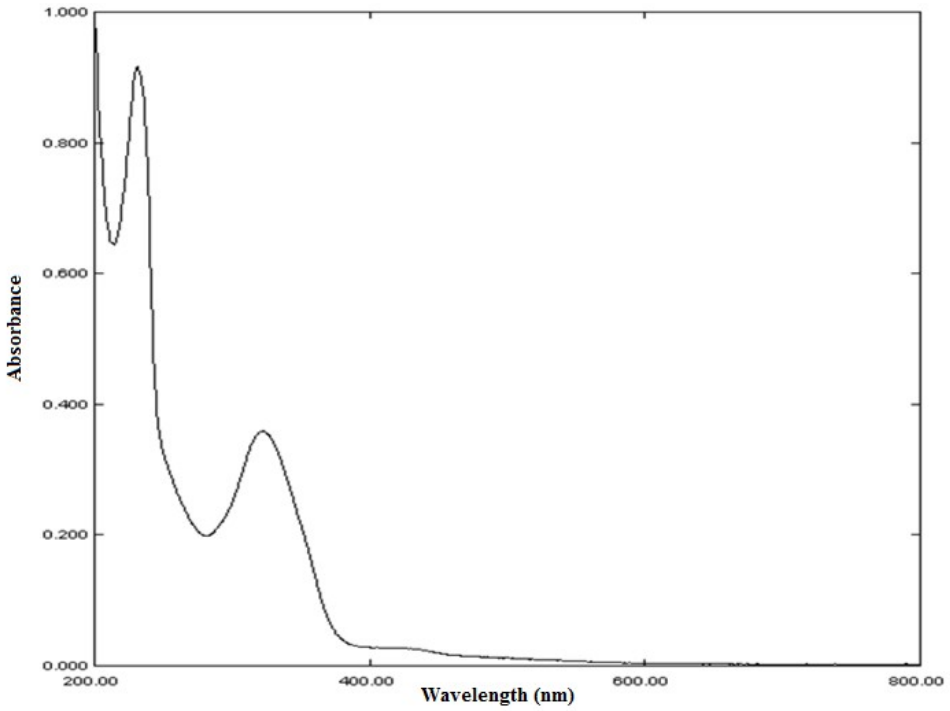


Figure 7: UV-Visible spectrum of the complex (H_2O, $1.0x10^{-5}$ M).

X-Ray Diffraction Analysis

Crystallographic data and refinement details of H_2ODIT are presented in Table 1; selected bond distances, angles and hydrogen bond geometries are listed in Table 2. H_2ODIT ligand belongs to the *$P2_1/c$* space group. As seen in Figure 8, a center of symmetry located on the mid position (1/2, 1/2, 0) of C–C [$C11–C11^i$] bond exists. The C9-O2 bond is typical double bond [1.245 (2) Å], while the C7-O1 bond is single bond [1.314 (2) Å]. The molecules of H_2ODIT are connected by π···π interactions. The π···π contact between the phenyl rings may stabilize the structure, with centroid-centroid distance of 3.992(3) Å. As seen in Figure 9, π···π interactions maintain the packing along [111] direction.

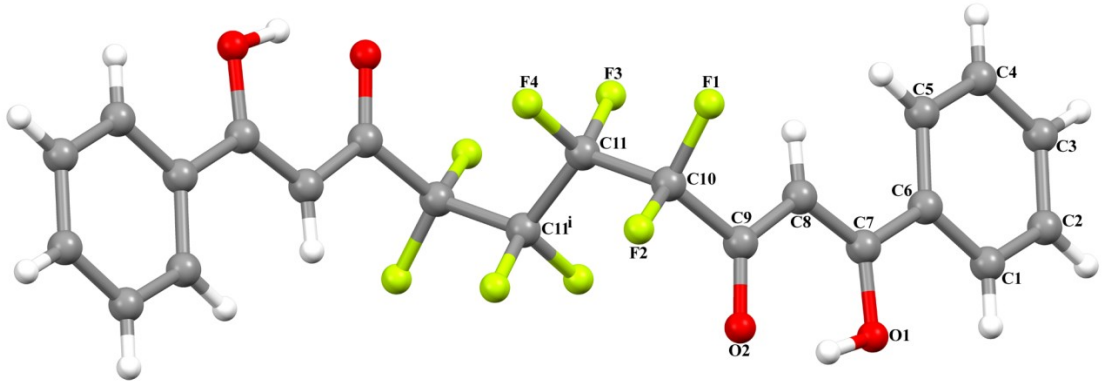


Figure 8: The molecular structure of H_2ODIT showing the atom numbering scheme. [(i) -x+1, -y+1, -z].

Table 1. Crystal data and structure refinement parameters for H_2ODIT.

Empirical formula	$C_{22}H_{14}F_8O_4$
Formula weight	494.33
Crystal system	Monoclinic
Space group	$P2_1/c$
a (Å)	8.168 (5)
b (Å)	11.385 (5)
c (Å)	11.425 (5)
β (º)	104.042 (5)
V (Å^3)	1030.7 (9)
Z	2
D_c (g cm^{-3})	1.593
μ (mm^{-1})	0.16
θ range (º)	3.1-28.3
Measured refls.	20454
Independent refls.	2552
R_{int}	0.027
S	1.06
R1/wR2	0.043/0.114
$\Delta\times_{max}/\Delta\times_{min}$ (eÅ^{-3})	0.28/-0.18

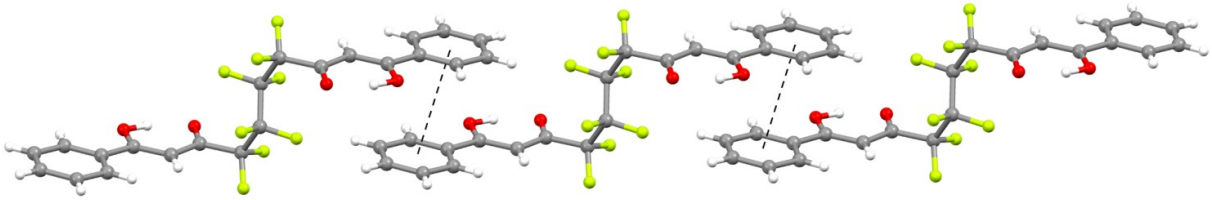


Figure 9. The π···π interactions in H_2ODIT.

Table 2: Selected bond distances and angles, hydrogen bond parameters for H_2ODIT (Å, °).

Bond distances (Å)				
C10-F1	1,3596 (19)		C10-F2	1.3478 (18)
C11-F3	1,3475 (18)		C11-F4	1,3519 (18)
C9-O2	1,245 (2)		C7-O1	1,314 (2)
Bond angles (º)				
C7—O1—H1A	105.0 (19)		O2—C9—C8	125.77 (15)
O1—C7—C8	120.67 (15)		O1—C7—C6	115.02 (14)
F2—C10—F1	107.33 (13)		F3—C11—F4	107.70 (13)
Hydrogen bond parameters (Å, °)				
D–H···A	D–H	H···A	D···A	D–H···A
O1—H1A···O2	0.95 (3)	1.67 (3)	2.552 (2)	153

Photoluminescent Properties of the Complex

Data acquisition in regards to excitation and radiation spectra for various modes such as luminescence, phosphorescence, and bioluminescence is available in modern photoluminescent spectroscopy. Acquiring the excitation spectrum, excitation chromator scans keeping a specific radiation wavelength constant while excitation wavelength is kept constant when radiation monochromator scans in obtaining a radiation spectrum.

Eu(III) radiation usually consists of stripes at red spectral region. This ion corresponds to transitions from excited $^5D_0$ of 4f configuration to ^7F_j (j = 0 to 6) level. The most intense red emission line results from $^5D_0 \rightarrow {}^7F_1$ magnetic dipole transitions, ranging between 610 and 630 nm wavelength.

Excitation and radiation spectra of the complex, in its solid state, was recorded at room temperature with a scan interval of λex:15 nm/λem:15.0 nm. Eu(III) complex was excited by UV light at 306 nm. The emission band observed at 615 nm corresponds to $^5D_0 \rightarrow {}^7F_2$ electrical dipole transition (Figure 10).

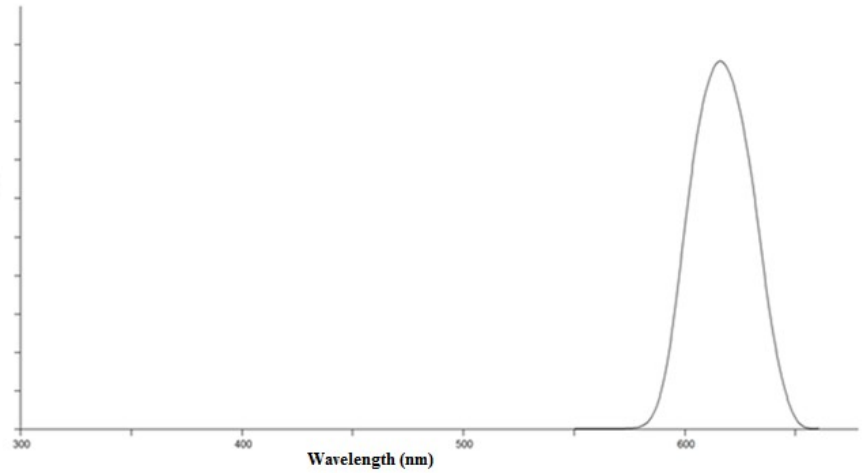


Figure 10: Emission spectrum of the complex (H_2O, $1.0x10^{-5}$ M).

CONCLUSION

A novel β-diketone based ligand and its Eu(III) complex has been synthesized in pursuit of advancing a biolabel in techniques for labeling biomolecules in this study. H_2ODIT and H_2CODIT ligands were elucidated by using ^{13}C-NMR, ^{1}H-NMR, mass spectrometry and FTIR spectroscopic techniques. Furthermore, X-ray analysis of single crystal synthesis of H_2ODIT ligand was carried out. As a result, this ligand prefers enolic structure in the equlibrium of keto-enol tautomer. H_2CODIT ligand was synthesized with $EuCl_3$ complex, and luminescent analysis was carried out in order to investigate the viability of its biolabeling.

SUPPLEMENTARY DATA

Crystallographic data for the structural analysis has been deposited with the Cambridge Crystallographic Data Centre, CCDC No. 1817092. Copies of this information may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk).

ACKNOWLEDGMENTS

This study has been supported by TÜBİTAK BİDEB 2210-C Master's Scholarship Program for Priority Fields.

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