İbrahim ŞAŞMAZ

M.S.Thesis in Chemistry

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SYNTHESIS, STRUCTURAL CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF DI AND TRI-ALKYNE CONTAINING MACROMOLECULE LIGANDS AND THEIR TRANSITION METAL COMPLEXES USING NMR TECHNIQUE

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in

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APPROVAL PAGE

I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science.

Prof. Dr. Cevdet NERGİZ
Head of Department

This is to certify that I have read this thesis and that in my opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

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It is approved that this thesis has been written in compliance with the formatting rules laid down by the Graduate Institute of Sciences and Engineering.

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ABSTRACT

Two new series of alkyne containing macromolecule ligands; di(prop-2-yn-1-yl) decanedioate **(L_1)**, di(but-3-yn-1yl) decanedioate **(L_2)**, di(pent-4-yn-1-yl) decanedioate **(L_3)**, di(hex-5-yn-1-yl) decanedioate **(L_4),** tri(prop-2-yn-1-yl) benzene-1,3,5-tricarboxylate **(L_5)**, tri(but-3-yn-1-yl) benzene-1,3,5-tricarboxylate **(L_6)** were synthesized.

The structural feature of these ligands were studied by elemental analysis, FT-IR, FT-Raman, ^{1}H, ^{13}C, (APT, DEPT 135, HETCOR, COESY, NOESY) NMR spectroscopy.

The antimicrobial and antifungal activities of the ligands were evaluated using disk diffusion and dilution method, against several bacteria and yeast cultures. The obtained results from disk diffuion method were compared with Amikacin, Cefotaxime, Ampicillin, Vancomycin, Ketaconazole, Clotrimazole, Nystatin. The results from dilution method were compared with Gentamicin and Nystatin.

Keywords: Macromolecule; Disk Diffusion; FT-Raman; Antimicrobial; long range coupling constant

İKİ VE ÜÇLÜ ALKİN İÇEREN MAKROMOLEKÜL LİGANTLAR VE GEÇİŞ METAL KOMPLEKSLERİNİN NMR TEKNİKLERİ İLE SENTEZİ, YAPISAL KARAKTERİZASYONU VE MİKROBİYAL AKTİVİTELERİ

İbrahim ŞAŞMAZ

Yüksek Lisans Tezi – Kimya
Ocak 2011

Tez Yöneticisi: Prof. Dr. Naz Mohammed AGHATABAY

ÖZ

Yeni di(prop-2-yn-1-yl) decanedioate **(L_1)**, di(but-3-yn-1yl) decanedioate **(L_2)**, di(pent-4-yn-1-yl) decanedioate **(L_3)**, di(hex-5-yn-1-yl) decanedioate **(L_4)**, tri(prop-2-yn-1-yl) benzene-1,3,5-tricarboxylate **(L_5)**, tri(but-3-yn-1-yl) benzene-1,3,5-tricarboxylate **(L_6)**, makromolekül ligandlar sentezlendi.

Bu ligandların yapısal özellikleri elementel analiz, FT-IR, FT-Raman, ^{1}H, ^{13}C (APT, DEPT 135, HETCOR, COESY, NOESY) NMR spektroskopileri ile incelendi.

Bu ligandların antimikrobiyal ve antifungal aktiviteleri bazı bakteri ve maya kültürlerine karşı disk difüzyon ve seyreltme metodu ile belirlendi. Disk difüzyonu metodu ile elde edilen sonuçlar Amikacin, Cefotaxime, Ampicillin, Vancomycin, Ketaconazole, Clotrimazole, Nystatin gibi standart antibakteriyel maddelere karşılaştırıldı. Seyreltme metodu ile elde edilen sonuçlar Gentamicin ve Nystatin standart antifungal maddelerle karşılaştırıldı.

Anahtar Kelimeler: Makromolekül; Disk Difüzyon; FT-Raman; Antimikrobiyal; long range coupling constant

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FT-Raman and Far FT-IR spectra were provided from Fatih Üniversitesi. I would like to thank Mehmet Karakız and Yakup Bakış for his help to get these spectra.

I would like to thank my family for their encouragement and my children Nilüfer and Muhammed Said Şaşmaz for their understanding.

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SYMBOL/ABBREVIATION

(NMR)	Nuclear Magnetic Resonance
(APT)	Attached Proton Test
(DEPT)	Distortionless Enhancement by Polarization Transfer
(HETCOR)	CH correlation
(COSY)	Correlated Spectroscopy
(NOESY)	Nuclear Overhause Effect Spectroscopy
(FT-IR)	Fourier Transform Infrared
(DMSO)	dimethylsulfoxide
(L1)	di (prop-2-yn-1-yl) decanedioate
(L2)	di (but-3-yn-1yl) decanedioate
(L3)	di (pent-4-yn-1-yl) decanedioate
(L4)	di (hex-5-yn-1-yl) decanedioate
(L5)	tri (prop-2-yn-1-yl) benzene-1, 3, 5-tricarboxylate
(L6)	tri (but-3-yn-1-yl) benzene-1, 3, 5-tricarboxylate
AK	Amikacin
CT	Cefotaxime
SM	Ampicillin
VA	Vancoymcin
NY	Nystatin
KT	Ketaconazole
CL	Clotrimazole
GEN	Gentamicin
δ	Bending
ν	Stretching
ov	overtone

ar	Aromatic
alp	aliphatic
s	Singlet
d	Doublet
dt	doublet of triplet
t	Triplet
as	asymmetric

CHAPTER I

INTRODUCTION

The alkyne function is a common structural motif in organic chemistry, linking linearily different moieties of the molecule's scaffold as well as allowing its transformation. The carbon–carbon triple bond is in this regard one of the most important functional groups in organic chemistry [1], because there are many reactions known, in which new compounds are formed via the trans- formation of acetylenic groups, such as the Sonogashira [2] Castro Stephens [2b] Glaser [3] and Cadiot-Chodkiewicz [4] couplings, the Pauson-Khand [5] and Nicholas [6] reactions, the Bergman, [7, 8] Moore [8] and Myers [8] cyclizations, the Rautenstrauch rearrangement [9] and the Huisgen 1,3-dipoar cycloaddition (click chemistry) [10]. The explosive growth of alkyne chemistry has particularly benefited from the recent development of new synthetic methodology based on transition metal catalysts and metal acetylides. Acetylenic functions are also readily found in a series of natural products and exhibit a broad distribution especially in plant species [11].

Reactions of alkynes with transitionmetal complexes frequently lead to alkyne di- , tri-, tetra- and polymerisation [12]. Their mechanisms have been widely studied over the last fourty years and in cases where cyclic oligomerisation product are observed the role of metallacyclic intermediates has been established[12-13]. However, if a reactive metal-ligand(usually metal-carbon or hydrogen) bond is present in the metal precursor, the incoming alkyne can undergo insertion leading to linear oligomerisation product instead[14].

The measurement of long range coupling constant by NMR spectroscopy, is very helpful for the structure elucidation and conformational analysis of organic compounds. [15-24].

The design and synthesis of macro hetero-multi-donor ligands have constituted one of the largest areas of research in organic and coordination chemistry for several decades[25-29]. In some cases, macromolecule derivatives are preferred in nature for many fundamental biological functions such as photosynthesis, storage and transport of oxygen in mammalian and other respiratory systems. Macromolecules which have various donor centers offer exciting possibilities to construct novel supramolecular assemblies that are capable of performing highly specific molecular functions. For example, the precise molecular recognition between these compounds and their guests, mostly transition metal ions or biomolecules (nucleic acids, proteins) provides a good opportunity to study key aspects of supramolecular chemistry, which also have a significance in a variety of disciplines including bioorganic chemistry, biocoordination chemistry, biology, medicine and related science and technology. Multi-donor ligands and particularly mixed donor atoms of these ligands are important in chemistry, because of great availability as ligands due to the presence of several potential donor centers and their flexibility to bind with biomolecules or to coordinate with various metal ions. Especially nitrogen and sulfur donor ligands have received special attention and theoretical interest, because they are capable of furnishing an enviromental of controlled geometry, their mixed hard-soft donor character, versatile coordination behaviour, and that may have numerous applications e.g. antibacterial, anticancerous, antiviral, antifungal, antifertile and other biochemical properties. In this area, researchers have synthesized new novel nitrogen-sulfur donor ligands through condensation that has played very important role in the development of synthetic macromolecule ligands. Due to intriguing observation that different ligands show different biological properties, the number of such type of compound synthesized continues to increase [30-40].

Currently considerable interest in the synthesis and investigation of polydentate ligands, owing to their interesting properties and particularly the ability to bind transition metals, which offers many possiblities for technology development and also modeling of biological metal-based systems. A variety of transition metals can be chelated, depending on the size and flexibility of the polydentate binding site. The ability of polydentate ligands to accommodate metals in two oxidation state opens possibilities for their uses as redox sensors, assuming that the change in metal oxidation state leads to changes in the structure or properties of the complex that can be

monitored, such as a change in spectrophotometric properties [41].

In addition; the study of metal complexes of macromolecule ligands appears to be interesting in view of the possibility of obtaining coordinating compound of unusual structure and stability. The formation of these macromolecule complexes depends on the size of the macromolecules; structural factors such as ligand rigidity, the type of donor atoms, and their disposition have been shown to play significant roles in determining the binding feature of macromolecule toward metal cation [53-55].

Therapeutical applications of platinum complex cisplatin as an antitumor drug and gold complex auranofin as an antirtheumatic drug, a large number of complexes with other metals have been studied, in several cases, subjected to clinical tests. The intense interest in synthetic chelating ligands and their metal complexes depends on the fact that they resemble the naturally occurring molecules in their structure and functional features and on their rich chemical behavior [56, 57]. The coordination chemistry of the square planar palladium(II) and platinum(II) complexes of nitrogen and sulfur/oxygen donor ligands have gained enormous importance because of their antitumour, anticancer, and catalytic activities. Antimicrobial aspects and antifertility activity of coordination compounds of palladium(II) and platinum(II) also reported in recent years [58]. In this expanding field, the interest towards transition metal complexes containing hetero-donor ligands has increased in order to obtain metal–based drugs either displaying a high biological activity with a reduced toxicity or reduced biological activity for metal ion detoxification. Metal ions are required for many critical functions in humans. Deficiency of some metal ions can lead to illness. Well-known examples include pernicious anemia resulting from scarcity of iron, growth retardation arising from insufficient dietary zinc and heart disease in infants owing to copper deficiency.

Metal ions can also induce toxicity in humans, classic examples being heavy metal poisons such as mercury, antimony and lead. Toxicity can arise from excessive quantities of either an essential metal, possibly the result of a metabolic deficiency, or a nonessential metal. Both acute and chronic exposure can be treated by chelation therapy, in which theoretical assumptions such as the Irving-Williams series of stability

and hard-soft acid-base principle of Pearson-Parr are useful in the choice of chelating agent [57-59]. The complexes of Palladium(II) with amino acids like glycine, serine, and glutamine have also been reported. Palladium(II) complexes of some Schiff base ligands derived from S-alkyl esters of dithiocarbazic acid have been found to exhibit striking cytotoxicity against leukemia and the human ovarian cancer cell lines. Out of the ordinary, for the first time, chemists have demonstrated that palladium complexes in the +3 oxidation state are intermediates in catalytic bond-forming processes [60].

In view of the above discussions and applications; in this thesis, the synthesis, the spectral characterizations and antimicrobial screeining of two series of di and tri-alkyne containing macromolecule ligands were reported. All the ligands evaluated for the same bacteria and pathogenic fungi. For such biological screeining disc diffusion technique and minimum inhibition concentration method (MIC) were used.

CHAPTER 2

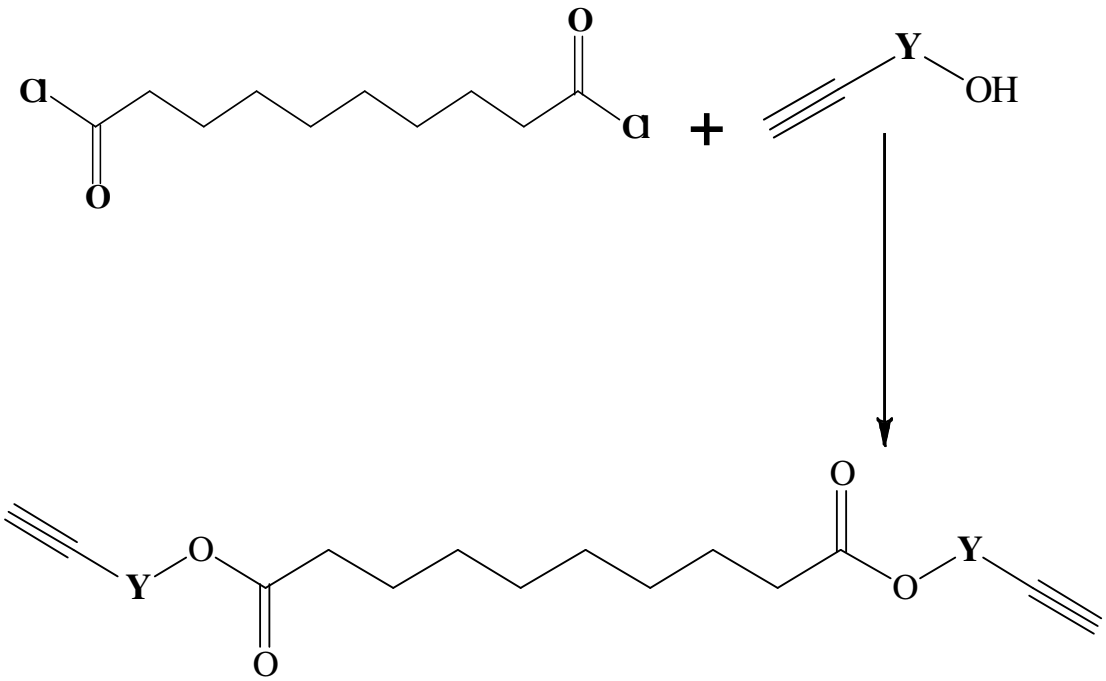
EXPERIMENTAL

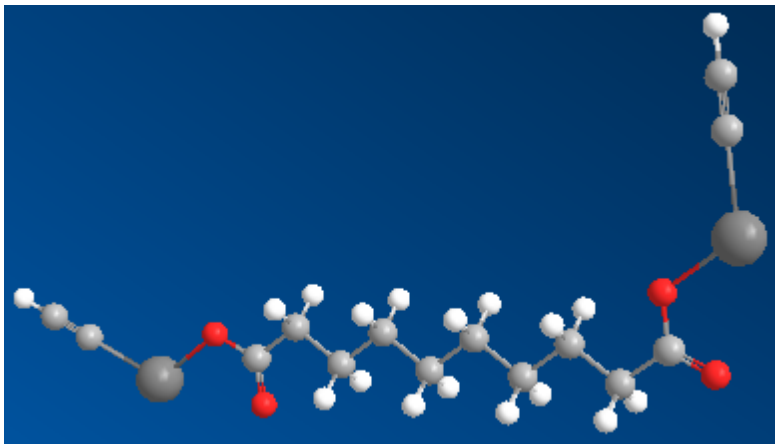
2.1. Chemistry

All chemicals and solvents were reagent grade and were used as purchased without further purification. Melting points were determined using a Electrothermal 9100 melting point apparatus. FT-IR spectra were recorded on the Perkin Elmer Spectrometer in the range of 4000-400 cm^{-1}. FT-Raman spectrum was obtained from powdered samples placed in a Pyrex tube using the Bruker RFS 100/S spectrometer in the range 3300-20 cm^{-1}. ^{1}H (400 MHz) and ^{13}C (100 MHz) spectra were recorded in $CDCl_3$ using the BRUKER Ultrashield Plus 400 MHz. Chemical shifts (δ) are expressed in units of parts per million relative to TMS. All compounds were optimized using ChemBioDraw Ultra 12.0 package program. The antimicrobial activities are evaluated against Gram-positive and Gram-negative bacteria, and yeast cultures using both the disk diffusion and dilution methods. The analytical and spectral data and physical properties were summerized for each experiment.

2.1.1. Synthesis of Ligands

2.1.1.1. Synthetic Pathways of L_1-L_4 Ligands





Y= (CH_2) , $(CH_2)_2$, $(CH_2)_3$, $(CH_2)_4$

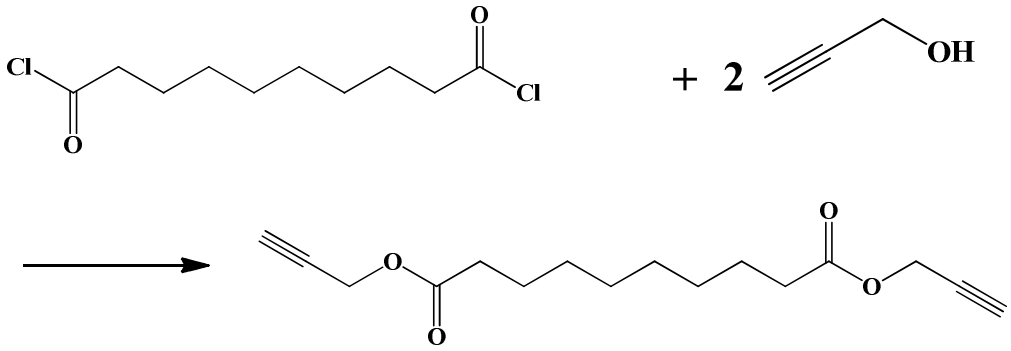
Scheme 2.1 Synthetic pathway for preparation of (L_1-L_4) ligands.

2.1.1.1.1 Synthesis of (di(prop-2-yn-1-yl) decanedioate) (L_1)

Di(prop-2-yn-1-yl) decanedioate was prepared from sebacoyl chloride and 2-propyn-1-ol as follows;

1.12 g (20 mmol) of 2-propyne-1-ol was dissolved in 20 mL pyridine and 2.39 g (10 mmol) of sebacoyl chloride was then added dropwise with constant stirring at room temperature. After 2 hours stirring the mixture was poured into cold sulphuric acid (100 mL, 1% H_2SO_4). The product was extracted with benzene and the solution was dried over $MgSO_4$. The mixture was filtered and the filtrate was evaporated and crude di(prop-2-yn-1-yl) decanedioate was obtained. Then it was purified by column chromatography using silica gel and benzene. **[$C_{16}H_{22}O_4$]** Found (calculated); C: 68.97 (69.04), H:8.08 (7.97).

The structure of the ligand is presented as shown below:



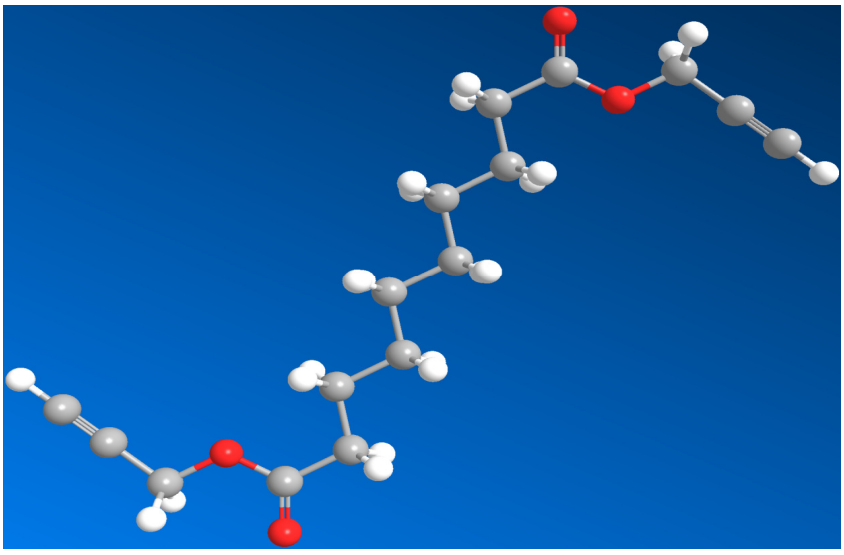
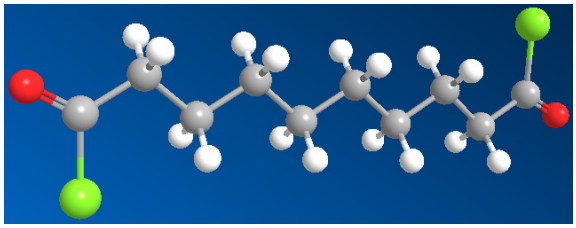
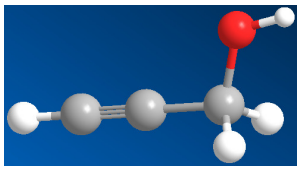


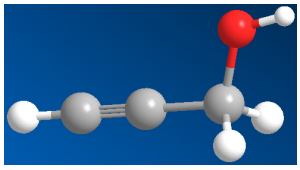
Fig.2.1 Optimized structure of L_1.



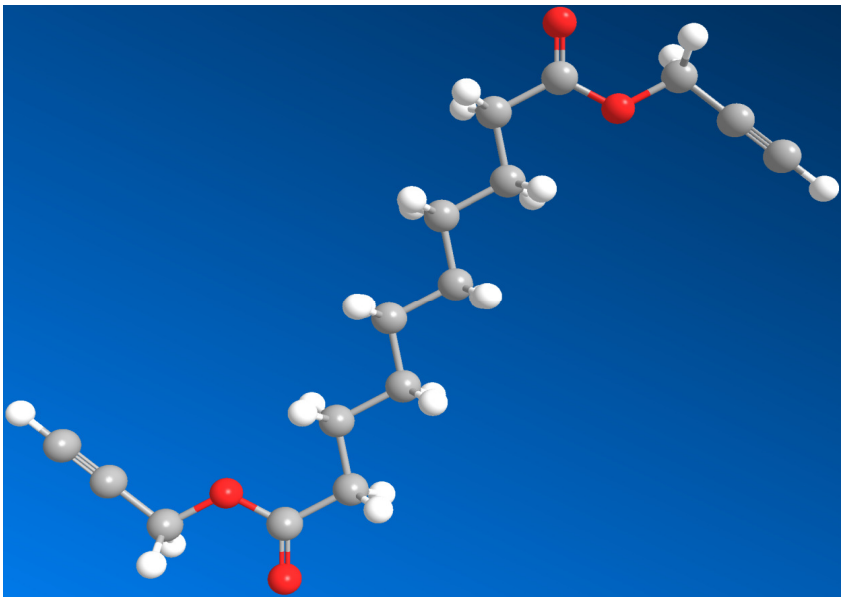
sebacoyl chloride

+





2-propyn-1-ol



di(prop-2-yn-1-yl) (L_1)

Scheme 2.1 Synthetic pathway for preparation of di(prop-2-yn-1-yl) decanedioate (L_1) ligand

2.1.1.1.2. Synthesis of di(but-3-yn-1yl) decanedioate (L_2)

Di(but-3-yn-1yl) decanedioate (L_2) was prepared from sebacoyl chloride and 3-butyn-1-ol as follows;

1.40 g (20 mmol) of 3-butyn-1-ol was dissolved in 20 mL pyridine and 2.39 g (10 mmol) of sebacoyl chloride was then added dropwise with constant stirring at room temperature. After 2 hours stirring the mixture was poured into cold sulphuric acid (100 mL, 1% H_2SO_4). The product was extracted with benzene and the solution was dried over $MgSO_4$. The mixture was filtered and the filtrate was evaporated and crude di(but-3-yn-1yl) decanedioate(L_2) was obtained. Then it was purified by column chromatography using silica gel and benzene. [$C_{18}H_{26}O_4$] Found (calculated); C: 70.58 (70.56), H:8.67 (8.55)

The structure of the ligand is presented as shown below:

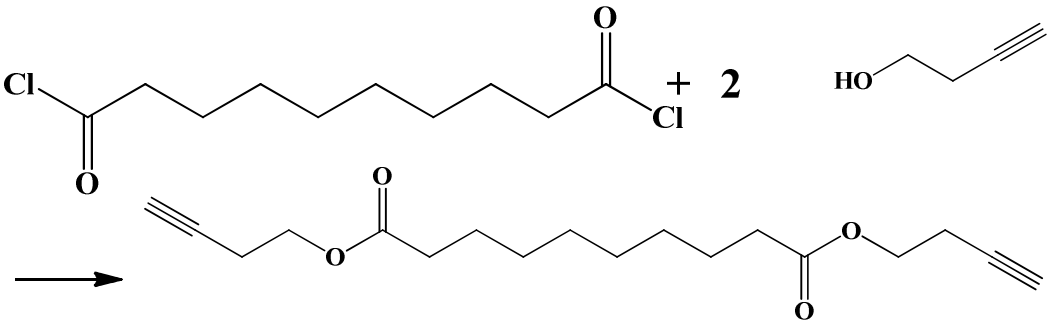


Fig.2.2 Optimized structure of di(but-3-yn-1yl) decanedioate (L_2)

2.1.1.1.3. Synthesis of di(pent-4-yn-1-yl) decanedioate (L_3)

Di(pent-4-yn-1-yl) decanedioate (L_3) was prepared from sebacoyl chloride and 4-pentyn-1-ol as follows;

1.68 g (20 mmol) of 4-pentyn-1-ol was dissolved in 20 mL pyridine and 2.39 g (10 mmol) of sebacoyl chloride was then added dropwise with constant stirring at room temperature. After 2 hours stirring the mixture was poured into cold sulphuric acid (100 mL, 1% H_2SO_4). The product was extracted with benzene and the solution was dried over $MgSO_4$. The mixture was filtered and the filtrate was evaporated and di(pent-4-yn-1-yl) decanedioate (L_3) was obtained. Then it was purified by column chromatography using silica gel and benzene. **[$C_{20}H_{30}O_4$]** Found (calculated); C: 71.53 (71.82), H:9.39 (9.04)

The structure of the ligand is presented as shown below:

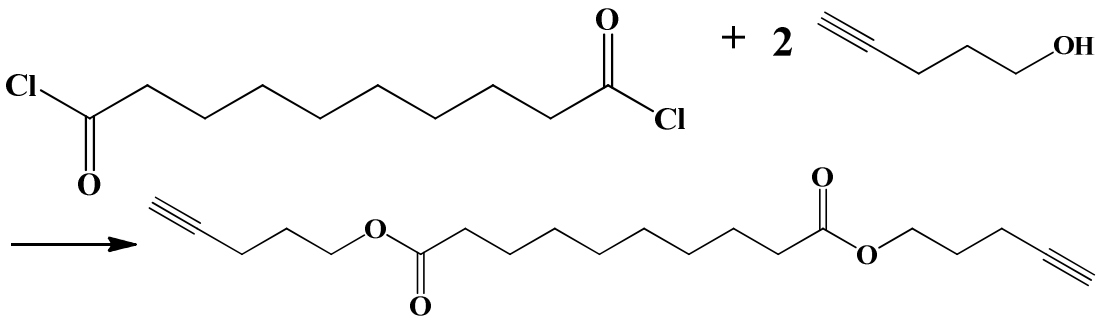


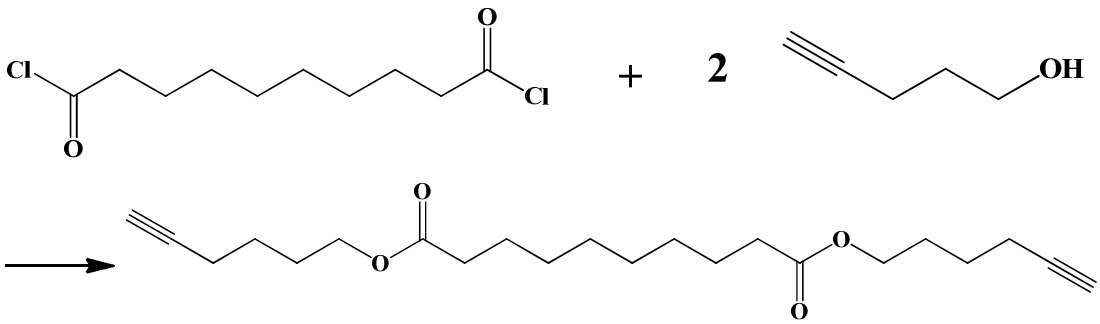
Fig.2.3 Optimized structure of di(pent-4-yn-1-yl)decanedioate L_3.

2.1.1.1.4. Synthesis of di(hex-5-yn-1-yl) decanedioate (L_4)

Di(hex-5-yn-1-yl) decanedioate (L_4) was prepared from sebacoyl chloride and 5-hexyn-1-ol as follows;

1.96 g (20 mmol) of 5-hexyn-1-ol was dissolved in 20 mL pyridine and 2.39 g (10 mmol) of sebacoyl chloride was then added dropwise with constant stirring at room temperature. After 2 hours stirring the mixture was poured into cold sulphuric acid (100 mL, 1% H_2SO_4). The product was extracted with benzene and the solution was dried over $MgSO_4$. The mixture was filtered and the filtrate was evaporated and di(hex-5-yn-1-yl) decanedioate (L_4) was obtained. Then it was purified by column chromatography using silica gel and benzene. **[$C_{22}H_{34}O_4$]** Found (calculated); C: 72.59 (72.89), H:9.57(9.45)

The structure of the ligand is presented as shown below:



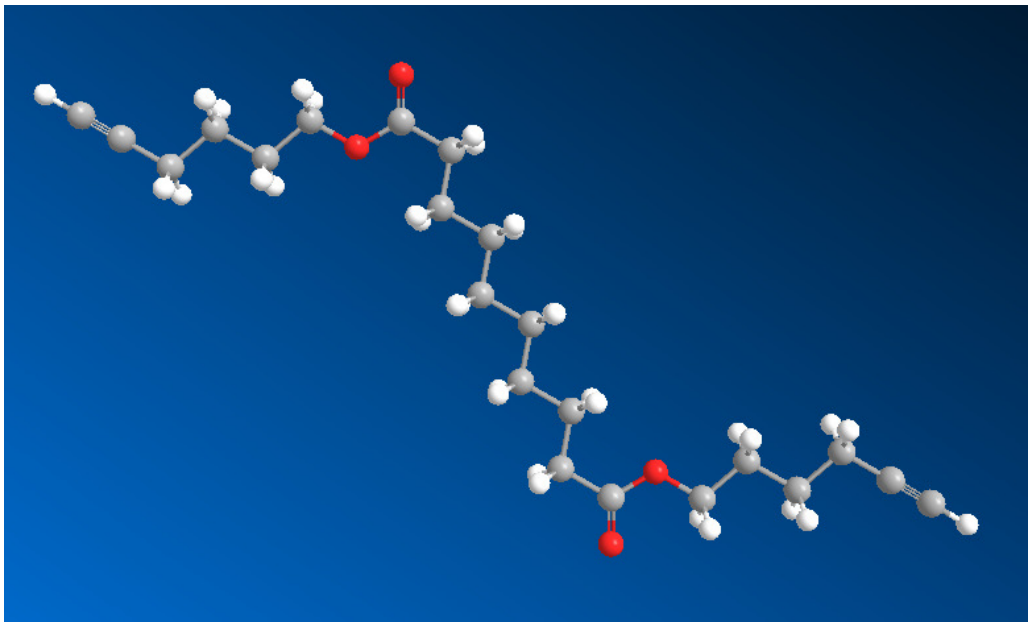
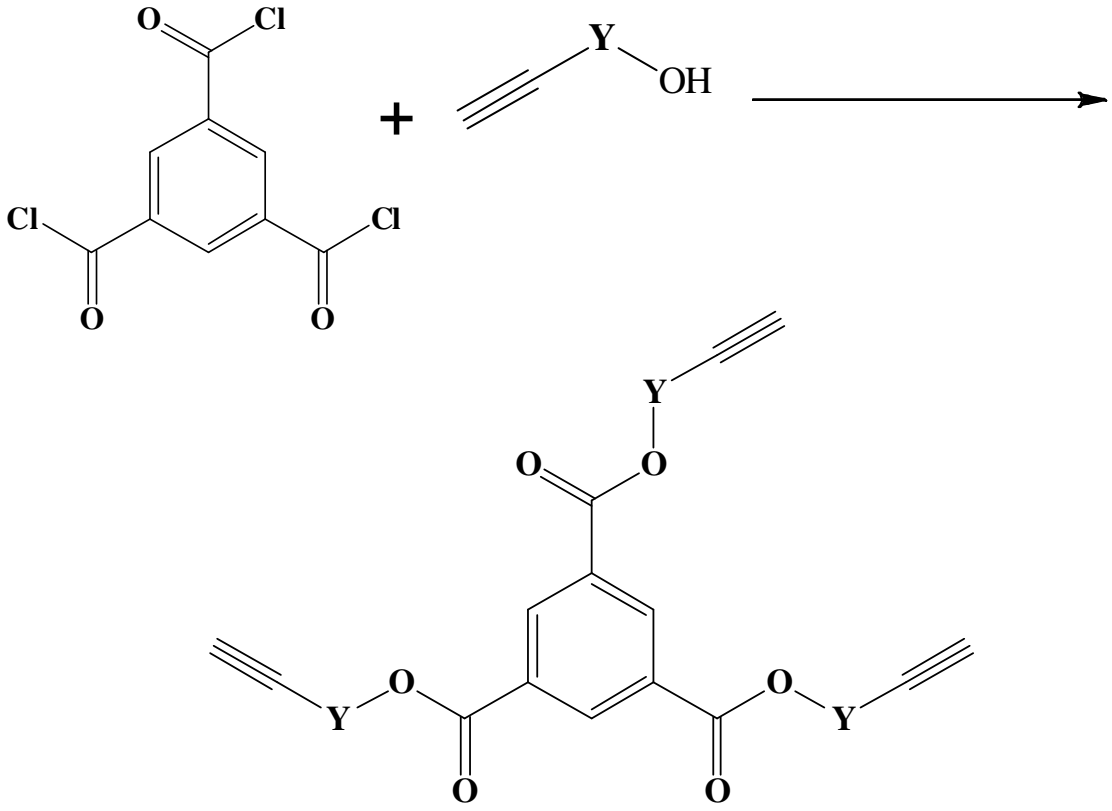
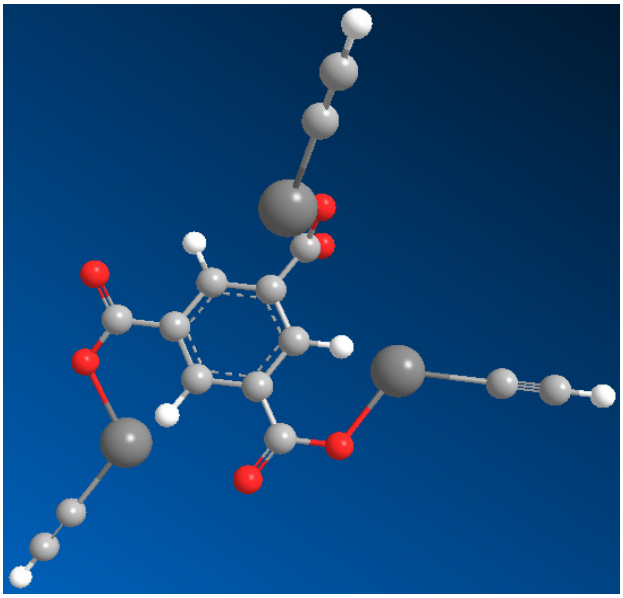


Fig.2.4 Optimized structure of di(hex-5-yn-1-yl) decanedioate (L_4)

2.1.1.2. Synthetic Pathways of L_5-L_6 Ligands





Y= (CH_2) , $(CH_2)_2$, $(CH_2)_3$, $(CH_2)_4$

Scheme 2.2 Synthetic pathway for preparation of (L_5-L_6) ligands.

2.1.1.2.1. Synthesis of tri(prop-2-yn-1-yl) benzene-1,3,5-tricarboxylate (L_5)

Trimesic acid tripropyn ester was prepared from trimesic acid trichloride and 2-propyn-1-ol as follows;

2 g of trimesic acid, 5 ml of oxalyl chloride and 50 ml of CH_2Cl_2 were refluxed overnight. The solvent and excess oxalyl chloride were rotoevaporated under vacuum and a 10% excess of 3-propyn-1-ol dissolved in 100 ml pyridine was added while cooling at 0^0C.The mixture was stirred for 3 hours at room temperature and poured into acidified water. The solid which is precipitated was filtered off and purified by column chromatography.

The structure of the ligand is presented as shown below:

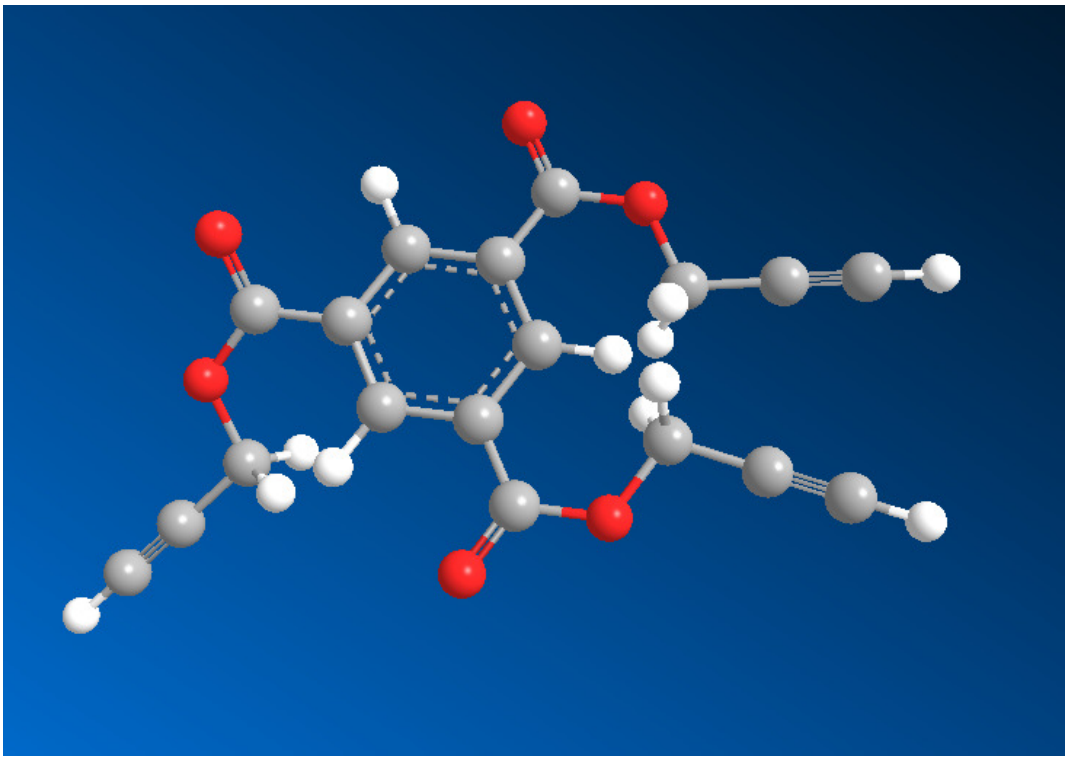


Fig.2.5 Optimized structure of tri(prop-2-yn-1-yl) benzene-1,3,5-tricarboxylate (L_5)

2.1.1.2.2. Synthesis of tri(but-3-yn-1-yl) benzene-1,3,5-tricarboxylate (L_6)

1.58 g of 3-butyn-1-ol was dissolved in 30 mL pyridine and 2 g of trimesic acid trichloride was added dropwise at room temperature. After 2 hours stirring at room temperature the mixture was poured into cold sulphuric acid (1% H_2SO_4) then dried over MgSO4. At this point, the color of compound was white like milk. Then the product was extracted with benzene. Benzene was rotoevaporated and crude trimesic acid tributyn ester was obtained. Then it was purified by column chromatography which was prepared by silica gel and benzene. After this step , the compound was recrystallized. The structure of the monomer and the synthesis of it is shown in below

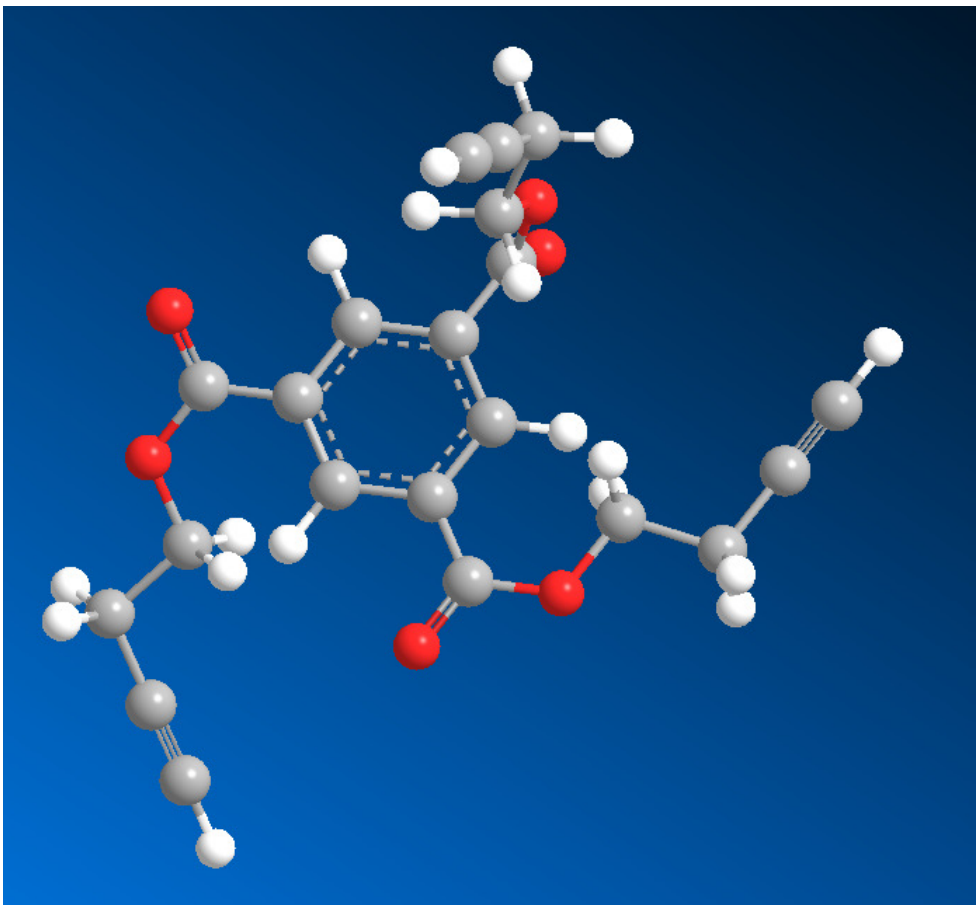


Fig.2.6 Optimized structure of tri(but-3-yn-1-yl) benzene-1,3,5-tricarboxylate (L_6)

2.3. Pharmacology

The antimicrobial activities are evaluated against Gram positive (*Escherichia coli, Enterobacter Aerogenes, Klebsiella Pneumoniae, Salmonella Typhi, Salmonella Typhimurium, Proteus Vulgaris, Pseudomonas Aeruginosa, Pseudomonas Fluorescens, Pseudomonas Extorquens*) and Gram negative (*Staphylococcus Aureus, Staphylococcus Epidermidis, Bacillus Cereus, Bacillus Subtilis, Bacillus Brevis, Bacillus Megaterium, Micrococcus Luteus, Micrococcus Flavus, Listeria Monocytogenes, Mycobacterium Smegmatis*) bacteria and yeast cultures (*Kluyveromyces Fragilis, Rhodotorula Rubra, Candida Albicans, Candida Parapsilosis, Candida Tropicalis, Cryptococcus Neoformans, Cryptococcus Laurentii, Hanseniaspora Guilliermondii, Debaryomyces Hansenii*) using both disk diffusion and dilution method.

2.3.1. Biological Data

Standardized samples are Amikacin (is most often used for treating severe, hospital-acquired infections with multidrug resistant Gram negative bacteria such as *Pseudomonas aeruginosa*, *Acinetobacter*, and *Enterobacter*), Cefotaxime (has broad spectrum activity against Gram positive and Gram negative bacteria), Ampicillin (is able to penetrate Gram-positive and some Gram-negative bacteria), Vancomycin (is a glycopeptide antibiotic used in the prophylaxis and treatment of infections caused by Gram-positive bacteria), Nystatin (is a polyene antifungal drug to which many molds and yeast infections are sensitive, including *Candida*), Ketoconazole (is a synthetic antifungal drug used to prevent and treat skin and fungal infections), Clotrimazole (is an antifungal medication commonly used in the treatment of fungal infections), and Gentamicin (is an aminoglycoside antibiotic, used to treat many types of bacterial infections, particularly those caused by Gram-negative bacteria) [60].

Amikacin: Amikacin has high resistance against bacterial inactivation. It resists attacks by most bacterial inactivating enzymes, this is accomplished by the L-hydroxyaminobuteroyl amide (L-HABA) moiety attached to N-3 which inhibits acetylation, phosphorylation and adenylation in the distant amino sugar ring (C-2,C-3,C-4) [60].

Cefotaxime: Inhibits bacterial cell wall synthesis by binding to one or more of the penicillin-binding proteins (PBPs) which in turn inhibits the final transpeptidation step of peptidoglycan synthesis in bacterial cell walls, thus inhibiting cell wall biosynthesis. Bacteria eventually lyse due to ongoing activity of cell wall autolytic enzymes (autolysins and murein hydrolases) while cell wall assembly is arrested [60].

Nystatin: Nystatin binds to ergosterol, a major component of the fungal cell membrane. When present in sufficient concentrations, it forms pores in the membrane that lead to K^+ leakage and death of the fungus. Ergosterol is fairly unique to fungi, so the drug does not have such catastrophic effects on animals [60].

Ketoconazole: As an antifungal, ketoconazole is structurally similar to imidazole and interferes with the fungal synthesis of ergosterol, a constituent of fungal cell membranes, as well as certain enzymes. As with all azole antifungal agents, ketoconazole works principally by inhibiting the enzyme cytochrome P450 14-alpha-demethylase (P45014DM) [60]

Ampicillin: Ampicillin is an orally active compounds and is commonly used a sa first line of defence against infection. This compounds is an acid resistant because of the presence of the electron-ithdrawing amino group [61,62].

Vancomycin: The fixed conformation of the hexapeptide cahin is important to vancomycin's unique mechanism of action, which involves targeting the cell wall's building blocks rather than a protein or a nucleic acid. Because vancomycin is a large molecule, it caps the tails and acts as a steric shield, blocking access to the transglycosidase enzymes [61,62].

Gentamicine: Binding to bacterial ribosomes takes place to inhibit protein synthesis. The binding is specially to the 30S ribosomal subunit and prevents the movement of the ribosome along mRNA so that the triplet code on mRNA can no longer be read. In some cases, protein synthesis is terminated and shortened proteins end up in the cell membrane. This can lead to a further increase in cell permeability

[61,62].

2.3.2. Methods

2.3.2.1. Disk Diffusion Method

Sterilised antibiotic discs (6 mm) were used following the literature procedure [62,63]. Fresh stock solutions of the macromolecule ligands were prepared in DMSO according to the needed concentrations for experiments. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO as the same procedures as used in the experiments. All the bacteria were incubated at 30^{O}C for 24 h in Nutrient Broth. The yeast were incubated in Malt Extract Broth for 48 h. The discs injected with solution were placed on the inoculated agar and incubated at 35 ^{O}C (24 h) and at 25^{O}C (72 h) for bacteria and yeast, respectively. In each case triplicate tests were performed and the average was taken as the final reading.

2.3.2.2. Dilution Method

Screenings for antibacterial and antifungal activites were carried out by preparing a broth micro dilution, following the procedure outlined in Manual of Clinical Microbial [64,65] All the bacteria were incubate and activated at 30^{O}C for 24 h inoculation into Nutrient Broth, and the yeasts were incubated in Malt Extract Broth 48 h. the compounds were dissolved in DMSO (2 mg mL^{-1}) and then diluted using adjusted Mueller Hinton Broth. Two–fold serial concentrations of the compounds were employed to determine the (MIC) ranging from 200 µg mL^{-1} to 1.56 µg mL $^{-1}$.

Cultures were grown at 37^{o}C (20 h) and the final inoculation (inoculums) was approximately 106 cfu mL^{-1}. Test cultures were incubated at 37^{o}C (24 h). The lowest concentrations of antimicrobial agents that result in complete inhibition of microorganismswere represented as (MIC) µg mL^{-1}. In each case triplicate tests were performed an the results are expressed as means.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Vibrational Spectroscopy Studies

3.1.1. Vibrational Spectroscopy Studies of L_1-L_4 Ligands

The present vibrational spectra can be discussed in terms of three characteristic wave regions: 3500-2800 cm^{-1} υ(C-H) characteristic alkynyl stretching modes, 1800-600 cm^{-1} belongs to υ(C-C). Appreance of two bands in the region 2925-2850 cm^{-1} in the IR spectra correspond to –methylene group. The streching vibration for υ C≡C group in IR and Raman spectra are observed in the region 2260-2190 cm^{-1}. In both Raman and IR spectra, the characteristic aliphatic groups are observed in the region 3000-2850 cm^{-1} and υ(C-H) characteristic stretching modes is observed 3100-3000 cm^{-1} and 1600-1500 cm^{-1}. The streching vibrations for C=O are observed in the region 1750 - 1735 cm^{-1}. The vibrations for C-O are observed in the region 1100-1300 cm-1

3.1.2. Vibrational Spectroscopy Studies of L_5-L_6 Ligands

The vibrational spectra can be discussed in terms of two characteristic wave regions: 3500-2800 cm^{-1} υ(C-H) characteristic stretching modes, 1800-1500 cm^{-1} belongs to υ (C=C)ar, υ (C-C); alkynyl stretching band is appeared in the region 3400-3100 cm-1. The C=O is observed in the region 1729 cm^{-1}; the C-O band is observed in the region 1226 cm^{-1}. In the region 1600-1550 cm-1 C=C aromatic ring stretching is observed.The streching vibrations for C=O are observed in the region 1750 - 1735 cm-1. The vibrations for C-O are observed in the region 1100-1300 cm^{-1}

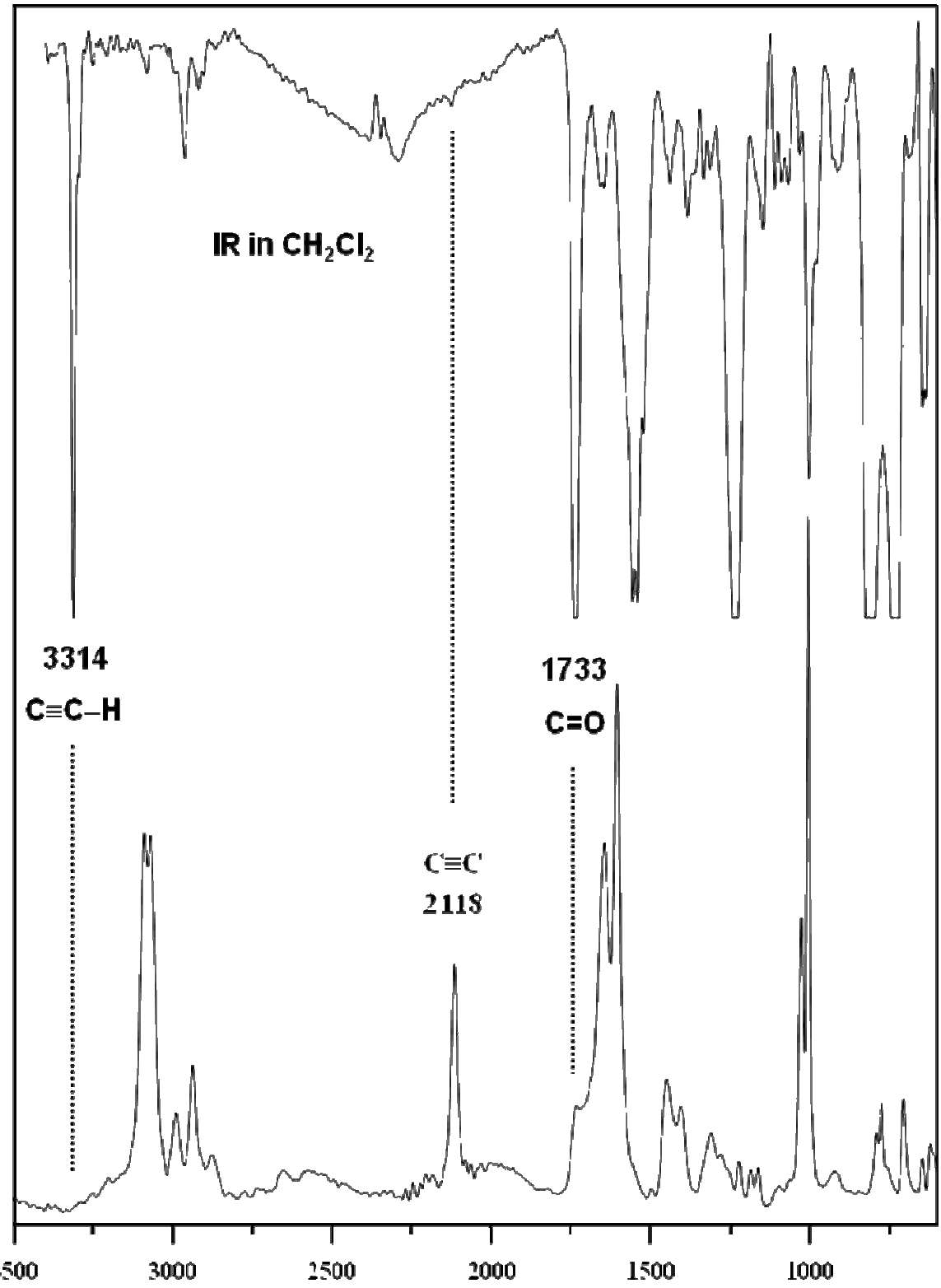


Fig.3.1 Vibrational Spectroscopy Studies of tri(but-3-yn-1-yl) benzene-1,3,5-tricarboxylate (L_6)

Table 3.1. Prominent IR and Raman bands for L_1-L_4 the compounds

Ligand	FT-IR (cm^{-1})	Raman (cm^{-1})	
di(prop-2-yn-1-yl) decanedioate (L_1)	3290	.....	C≡C—H
	2930	2930	
	2127	2127	C≡C
	1736	.....	C=O
	1155	.....	C—O
di(but-3-yn-1yl) decanedioate (L_2)	3280	.....	C≡C—H
	2918	2918	
	2117	2117	C≡C
	1735	1735	C=O
	1157	.....	C—O
di(pent-4-yn-1-yl) decanedioate (L_3)	3294	.....	C≡C—H
	2929	2929	
	2117	2117	C≡C
	1731	.....	C=O
	1168	.....	C—O
di(hex-5-yn-1-yl) decanedioate (L_4)	3295	.....	C≡C—H
	2930	2930	
	2121	2121	C≡C
	1729	.....	C=O
	1170	.....	C—O
tri(prop-2-yn-1-yl) benzene-1,3,5-tricarboxylate (L_5)	3279	.....	C≡C—H
	.....	2954	
	2121	2121	C≡C
	1729	1729	C=O
	1226	.....	C—O

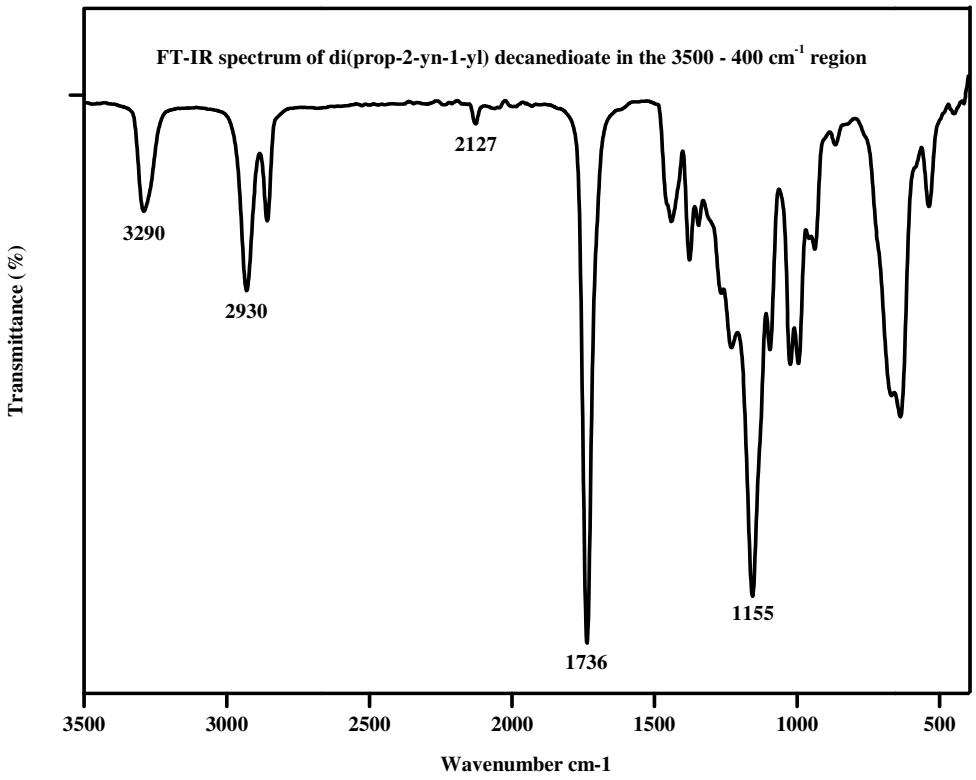


Fig. 3.1 FT-IR spectra of L_1 ligand in the 3500-400 cm^{-1} regions.

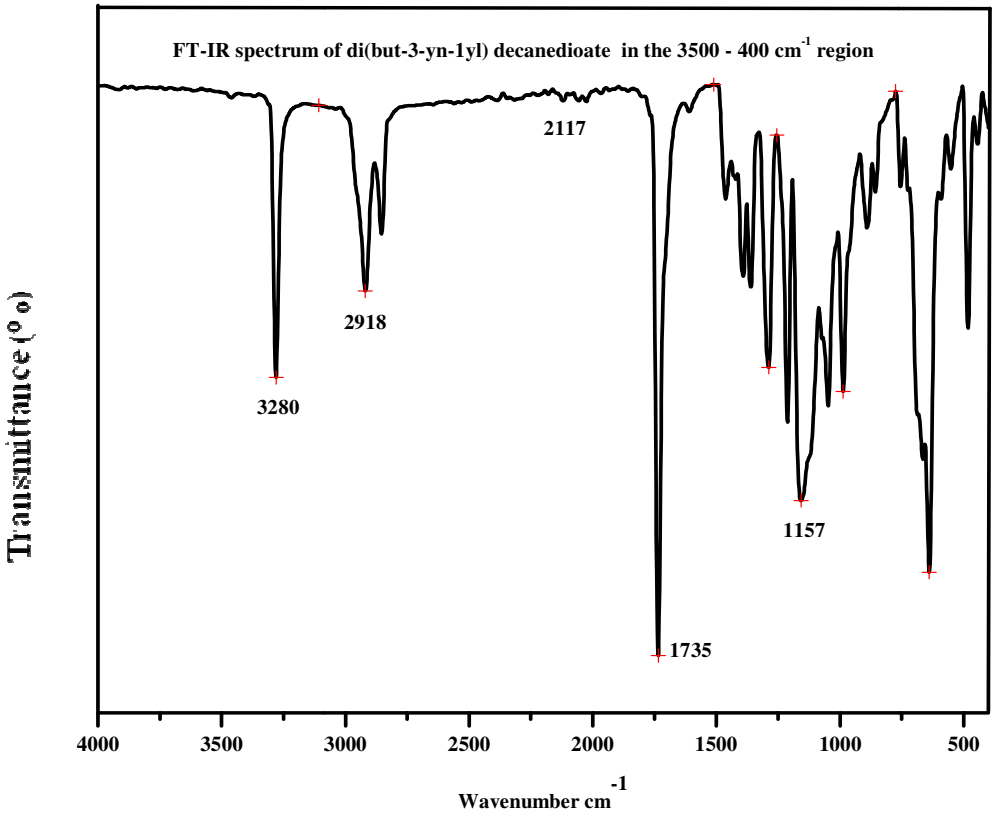


Fig. 3.2 FT-IR spectra of L_2 ligand in the 4000-400 cm^{-1} regions

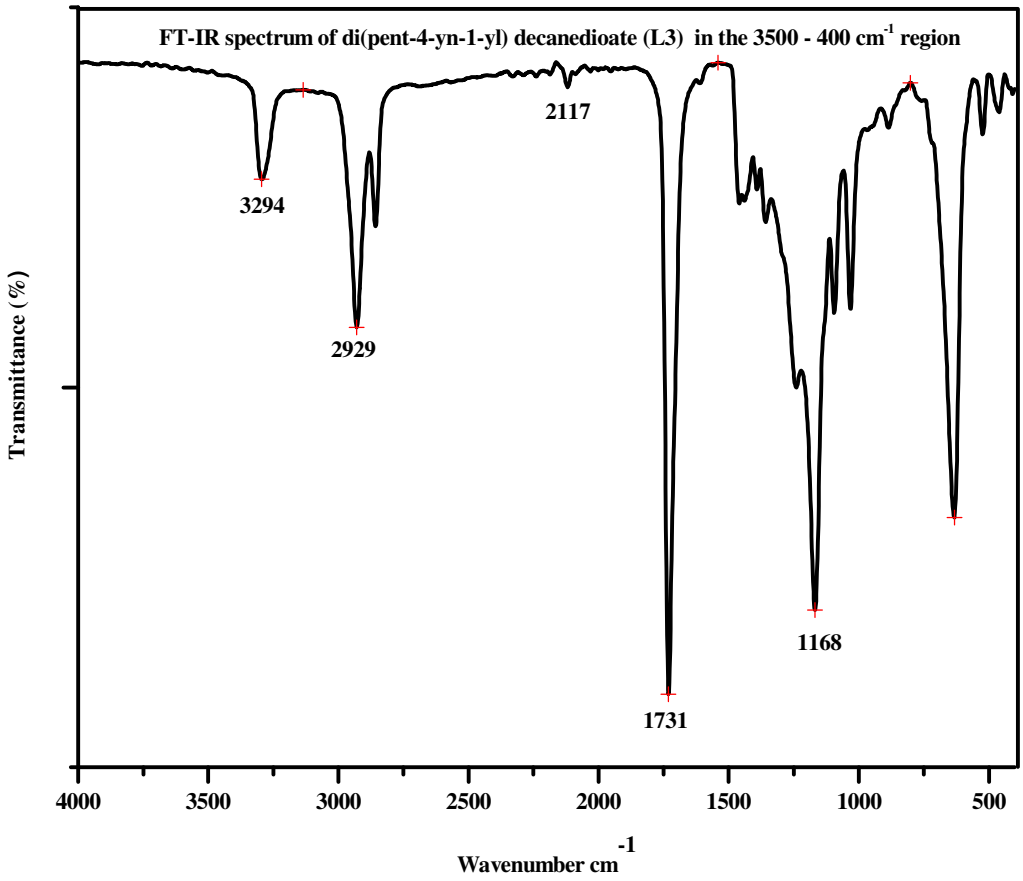


Fig. 3.3 FT-IR spectra of L_3 ligand in the 4000-400 cm^{-1} regions.

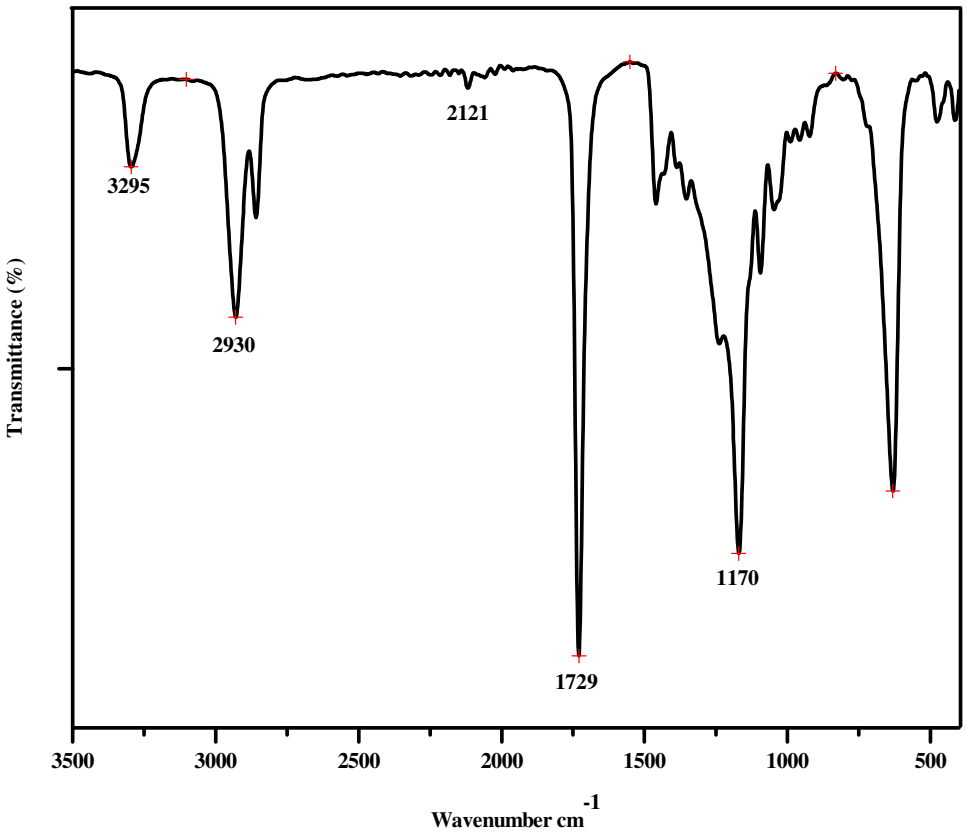


Fig. 3.4 FT-IR spectra of L_4 ligand in the3500-400 cm^{-1} regions

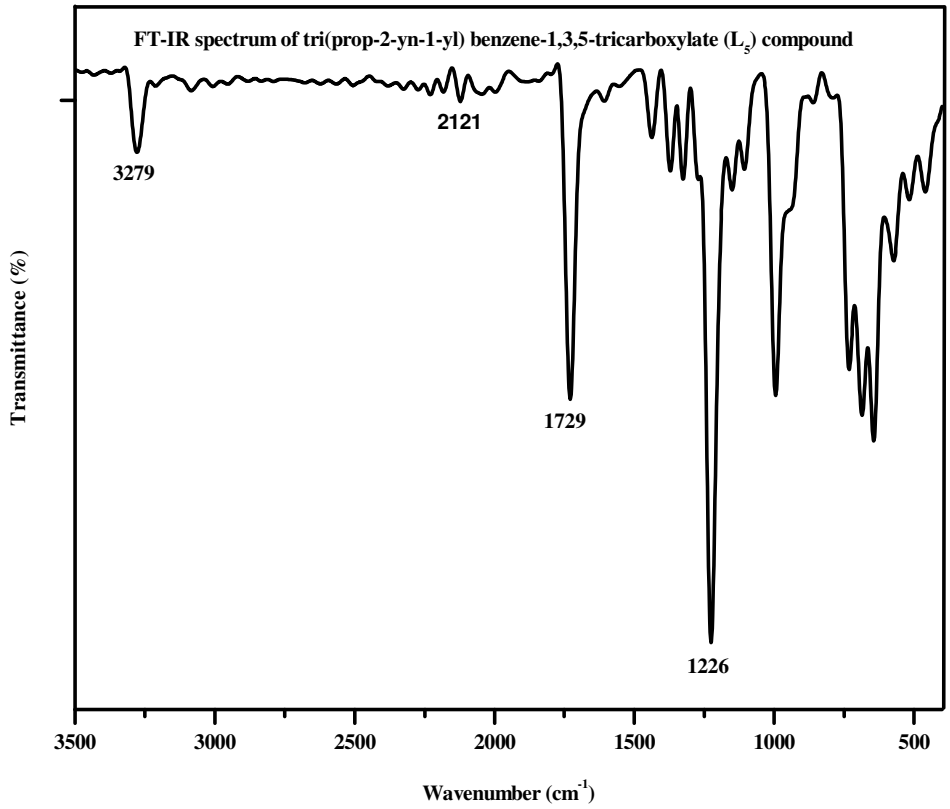


Fig. 3.5 FT-IR spectra of L_5 **ligand** in the 3500-400 cm^{-1} regions

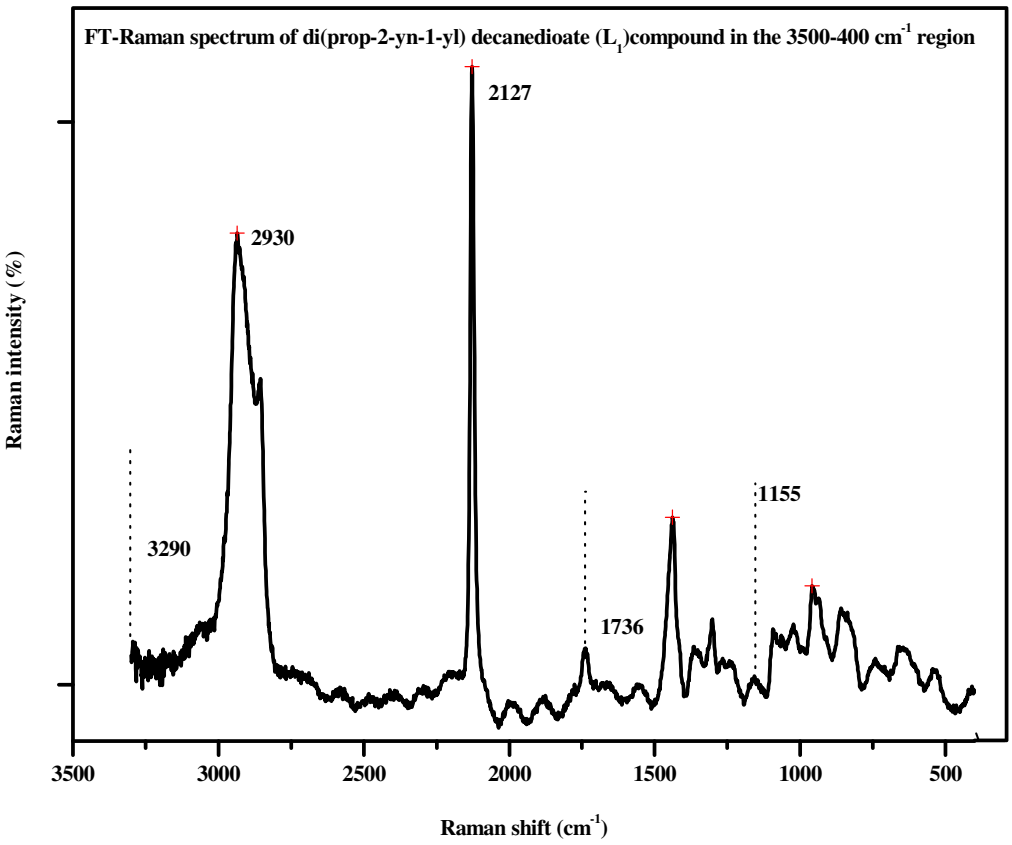


Fig. 3.6 FT-Raman spectrum of L_1 compound in the 3500-400 cm^{-1} region

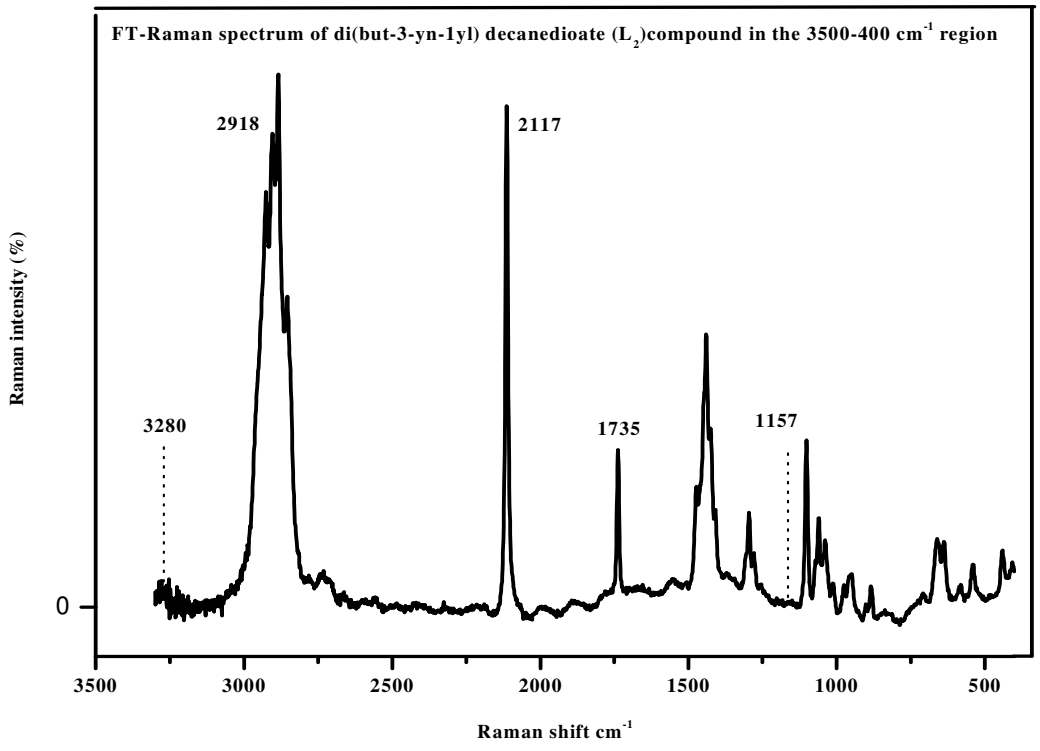


Fig. 3.7 FT-Raman spectrum of L_2 in the 3500-400 cm^{-1} region

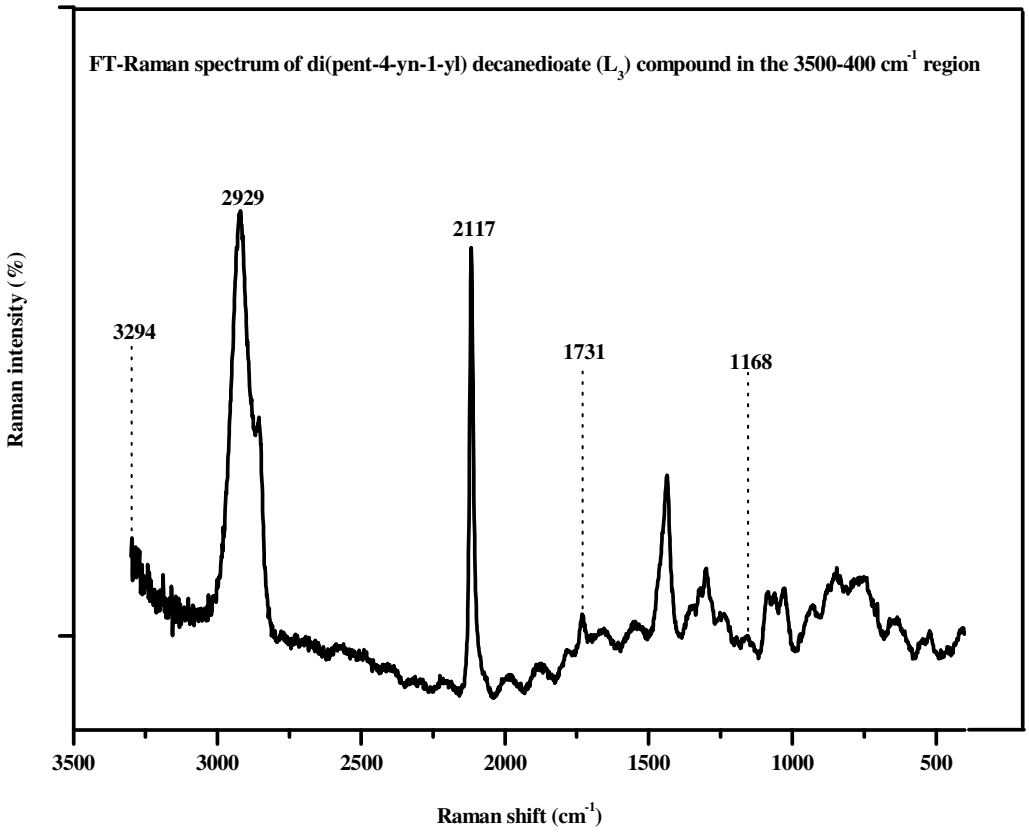


Fig. 3.8 FT-Raman spectrum of (L_3) in the 3500-400 cm^{-1} region

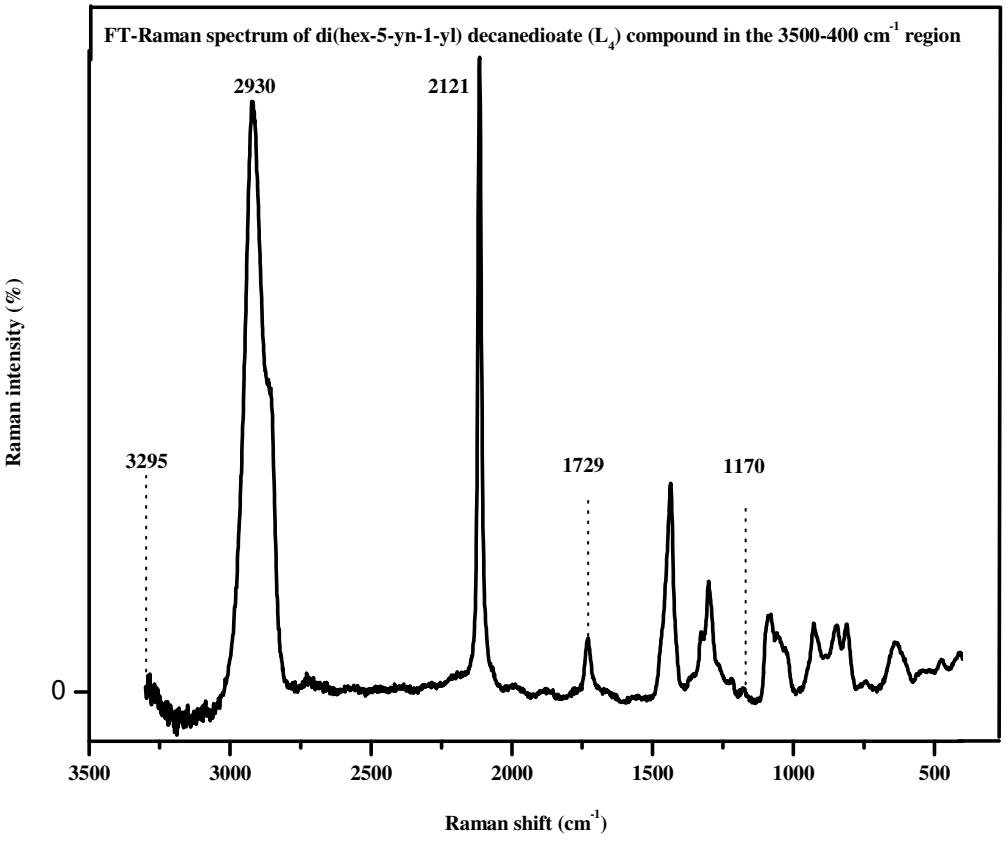


Fig. 3.9 FT-Raman spectrum of (L_4) compound in the 3500-400 cm^{-1} region

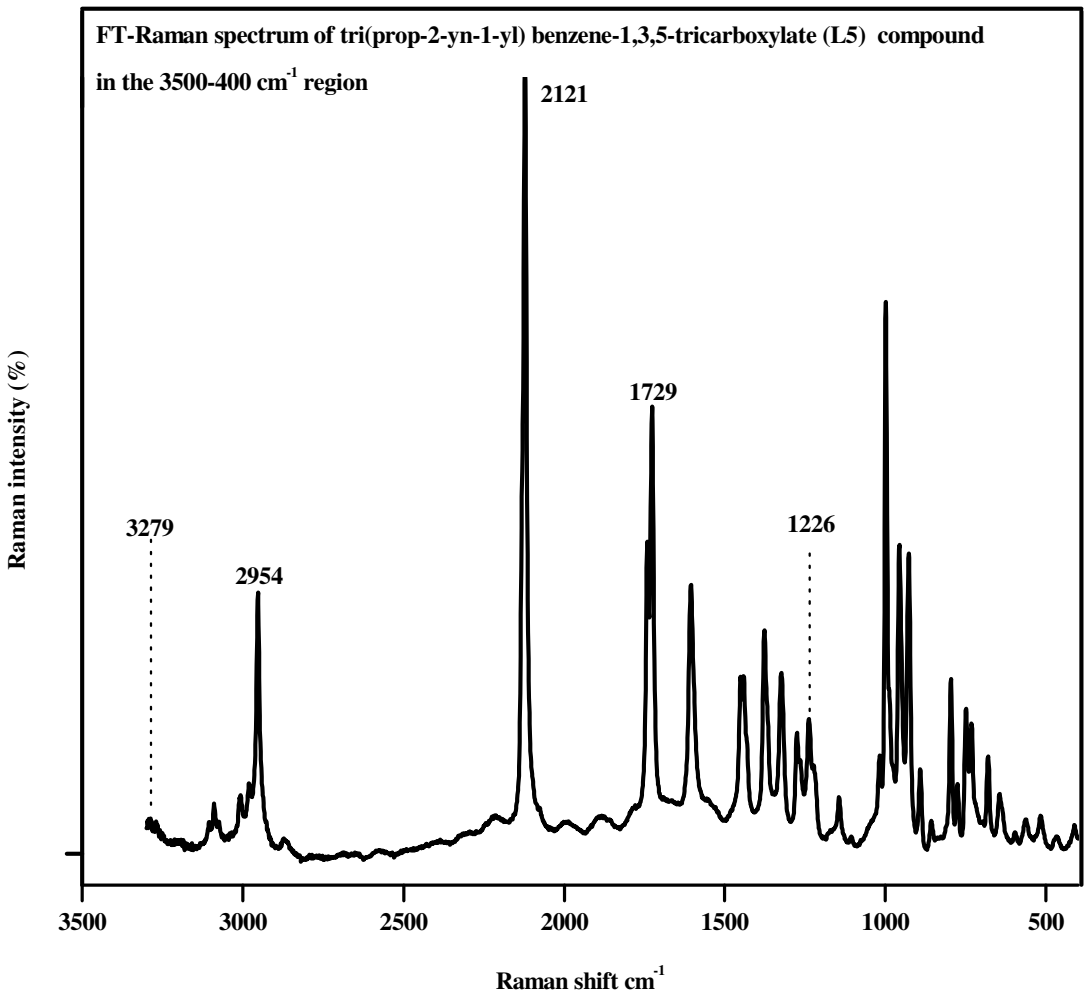


Fig. 3.10 FT-Raman spectrum of (L_5) in the 3500-400 cm^{-1} region

3.2. Nuclear Magnetic Resonance Studies

3.2.1. Nuclear Magnetic Resonance Studies of L_1-L_4 Ligands

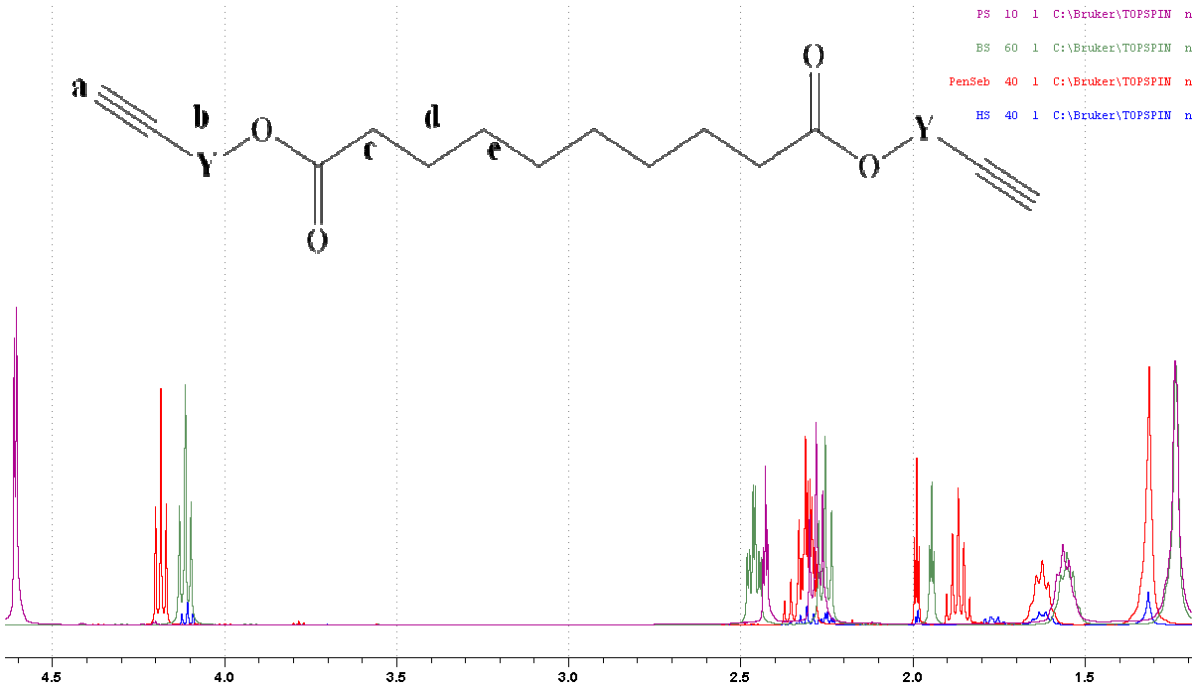
In the ^1H nmr spectra, -CH_2- protons of these ligands were observed in the region 2.5-1.1 ppm. All of these ligands have -CH_2- protons attached to-C≅C- bonds, these protons of L_1, L_2 L_3, L_4 and L_5 were observed in the region 4-4.70 ppm. Appearence of the peaks in the region 2.21-2.52 ppm corresponds to H-C≅C- protons.

In the ^{13}C nmr spectra, Methylene carbon atoms of these ligands were observed in the region 35-15 ppm. Carbon atoms (-CH_2-) attached to oxygen atoms were appeared in the region 50-52 ppm. Acetylenic carbon atoms attached to oxygen atoms were observed in the region 172-174 for L_1, L_2, L_3 and L_4.

3.2.2. Nuclear Magnetic Resonance Studies of L_5, L_6 Ligands

In the ^1H nmr spectra, H-C≅C- group protons were observed in the region 2.59-3.32 ppm. Methylene protons for L_5 was appeared in the region 2.5-3.00. Aromatic protons were observed in the region 9.00-8.50 ppm.

In the ^{13}C nmr spectra, carbonyl carbon atoms were observed in the region 164.5 ppm. Carbon atoms attached to oxygen atom in the ligand 5 were appeared in the region 50-55 ppm. The signals in the region 75-78 ppm are correspond to carbon atoms bonded to triple bond. Aromatic carbon atoms were observed in the region 129-138 ppm.



Y= (CH_2) , $(CH_2)_2$, $(CH_2)_3$, $(CH_2)_4$

Fig 3.11 Multiple display of all ligands L1,L2,L3,L4 in 1H NMR

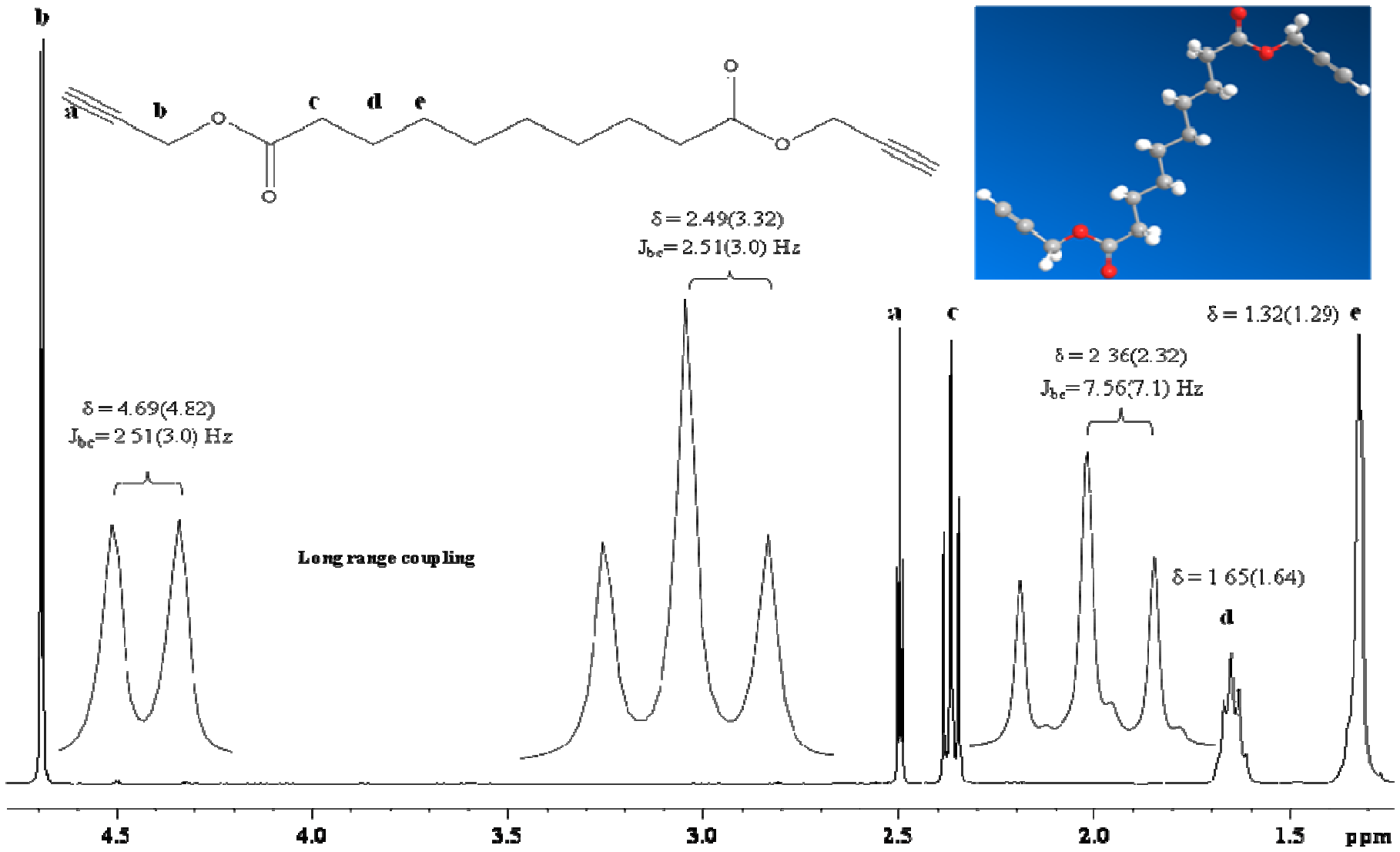


Fig.3.11 ^{1}H NMR ($CDCl_3$) spectra of di(prop-2-yn-1-yl) decanedioate

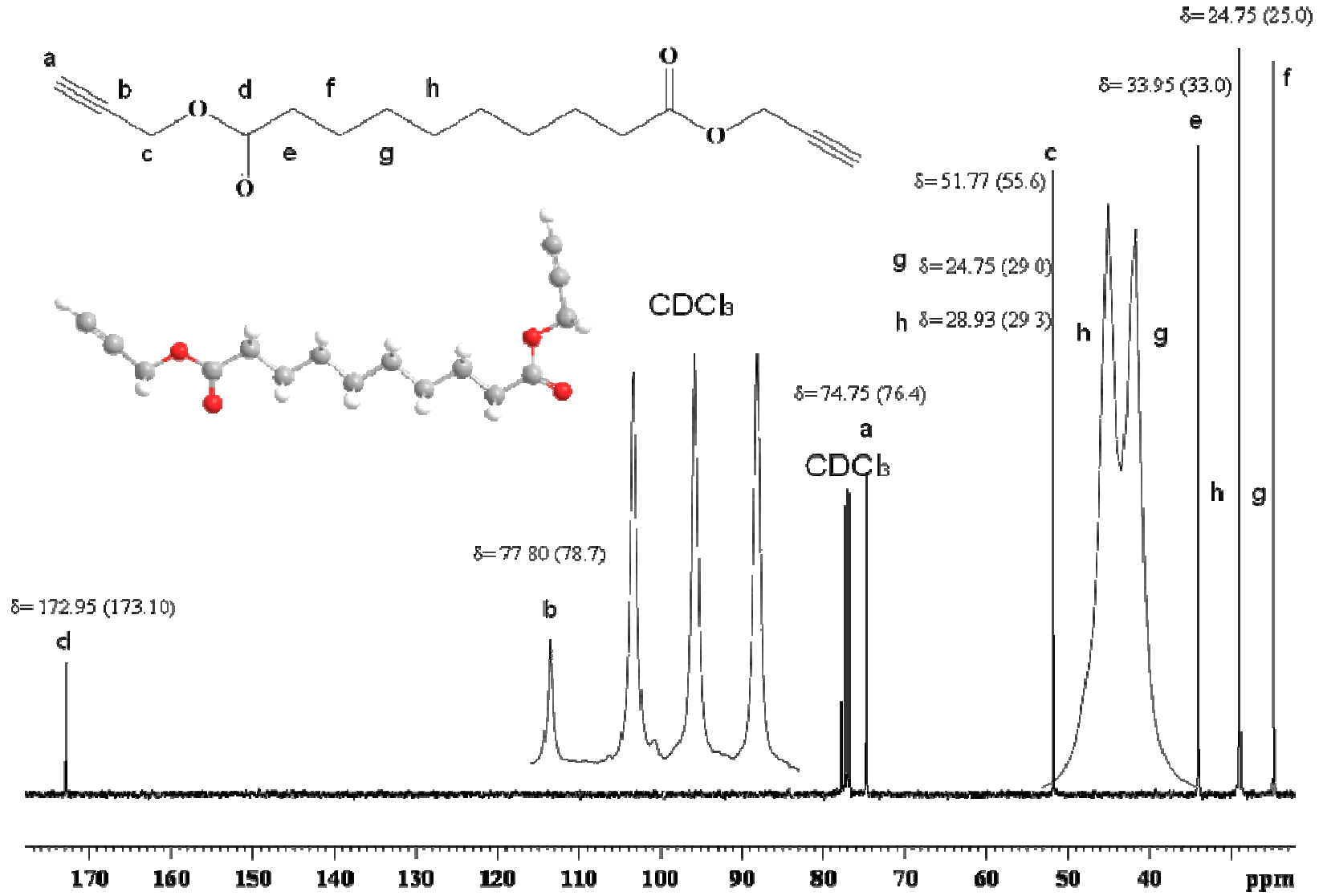


Fig.3.12 ^{13}C NMR ($CDCl_3$) spectra of di(prop-2-yn-1-yl) decanedioate

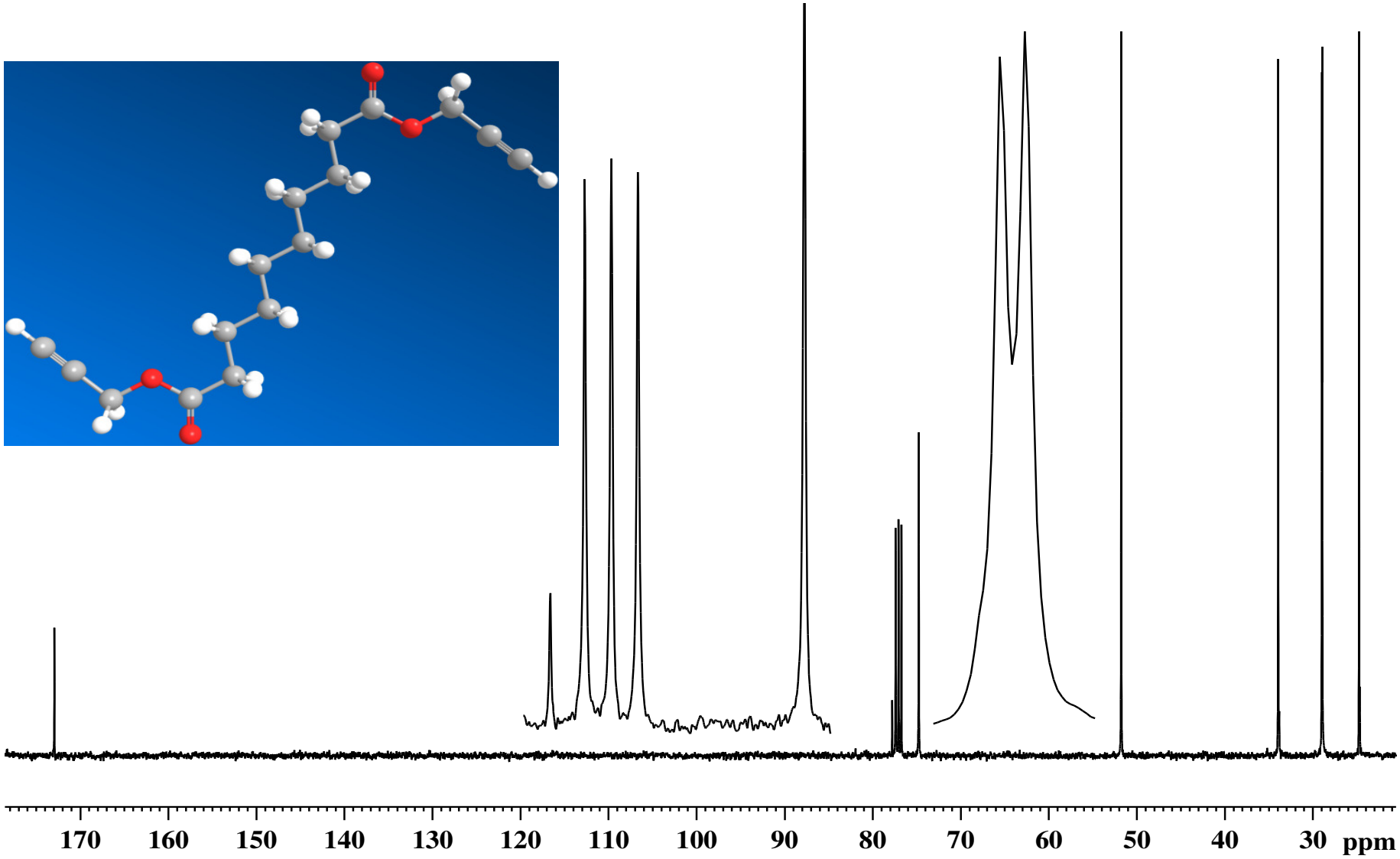


Fig.3.13 C13 APT NMR spectra of di(prop-2-yn-1-yl) decanedioate

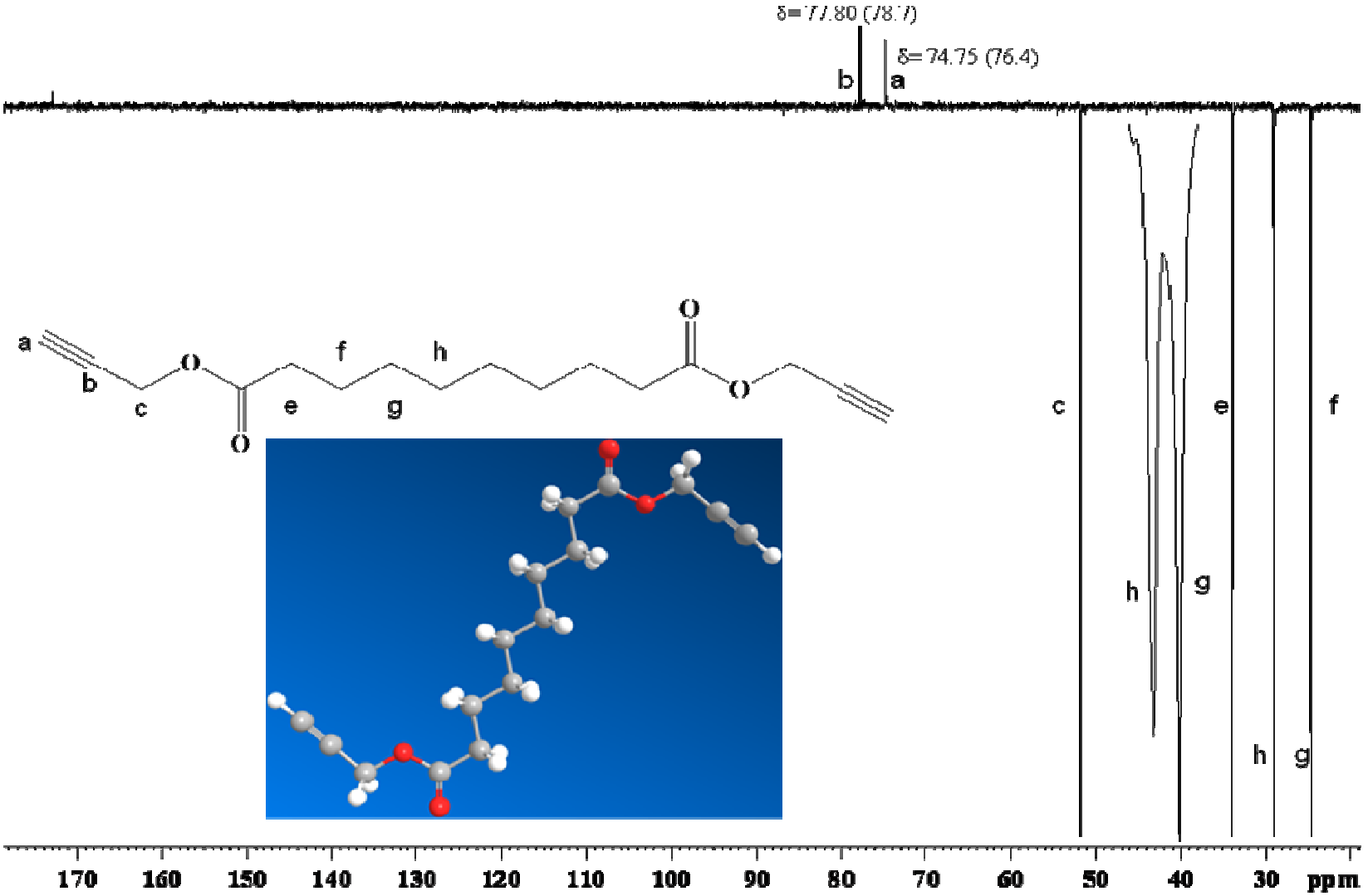


Fig.3.14 C13 DEPT 135 NMR spectra of di(prop-2-yn-1-yl) decanedioate , (CH2 neg.)

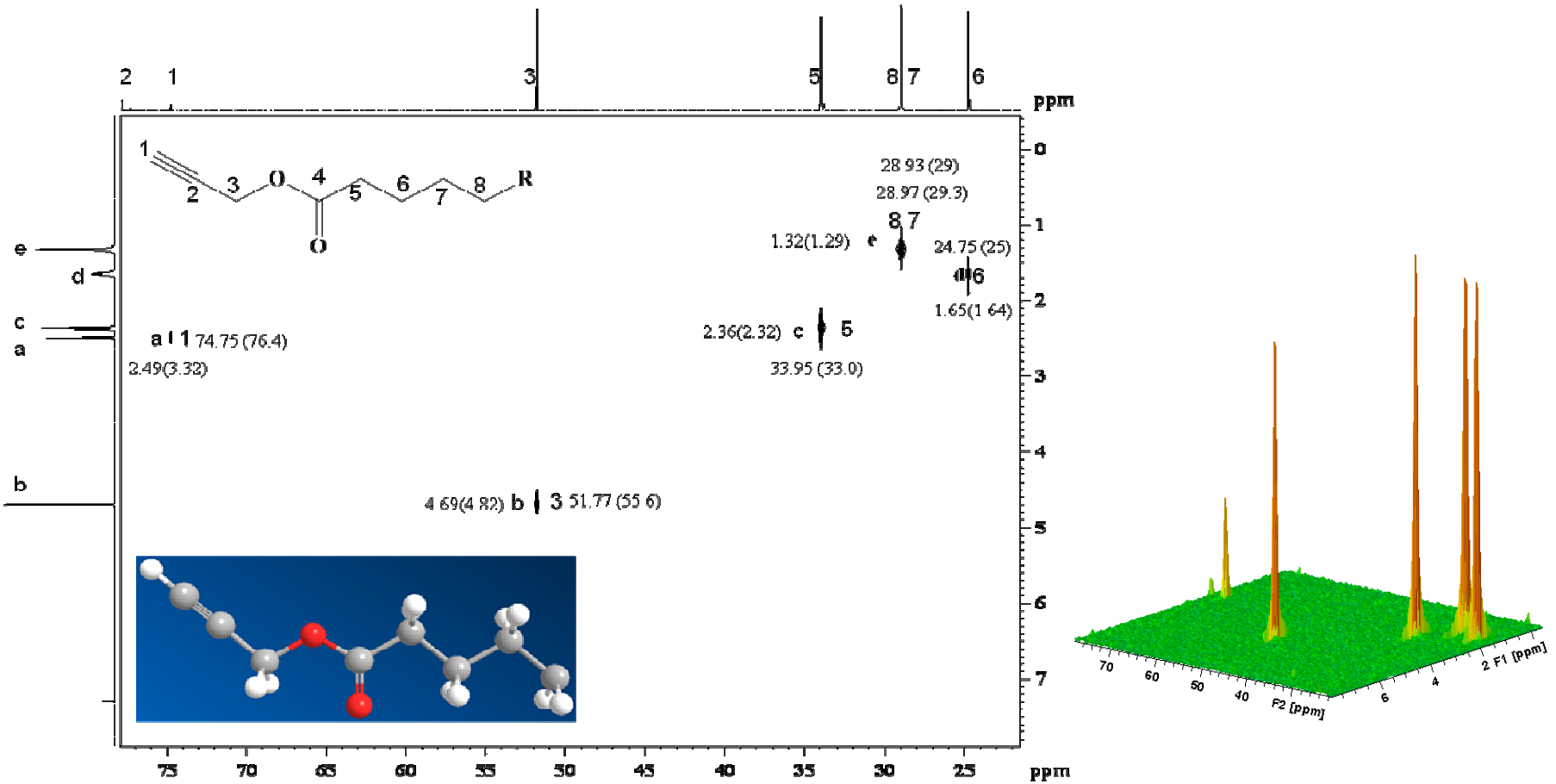


Fig.3.15 HETCOR – 2D spectra of di(prop-2-yn-1-yl) decanedioate

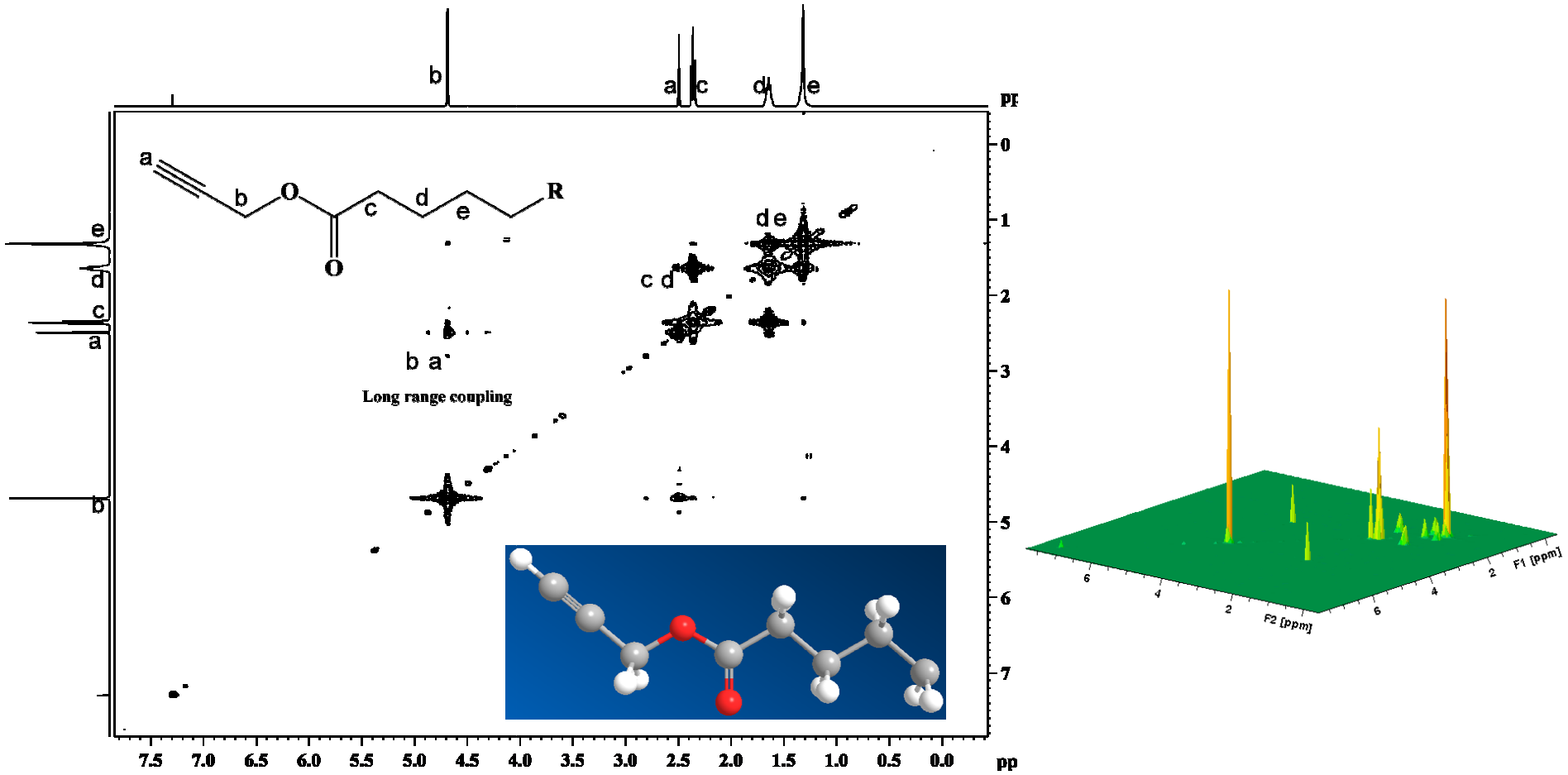
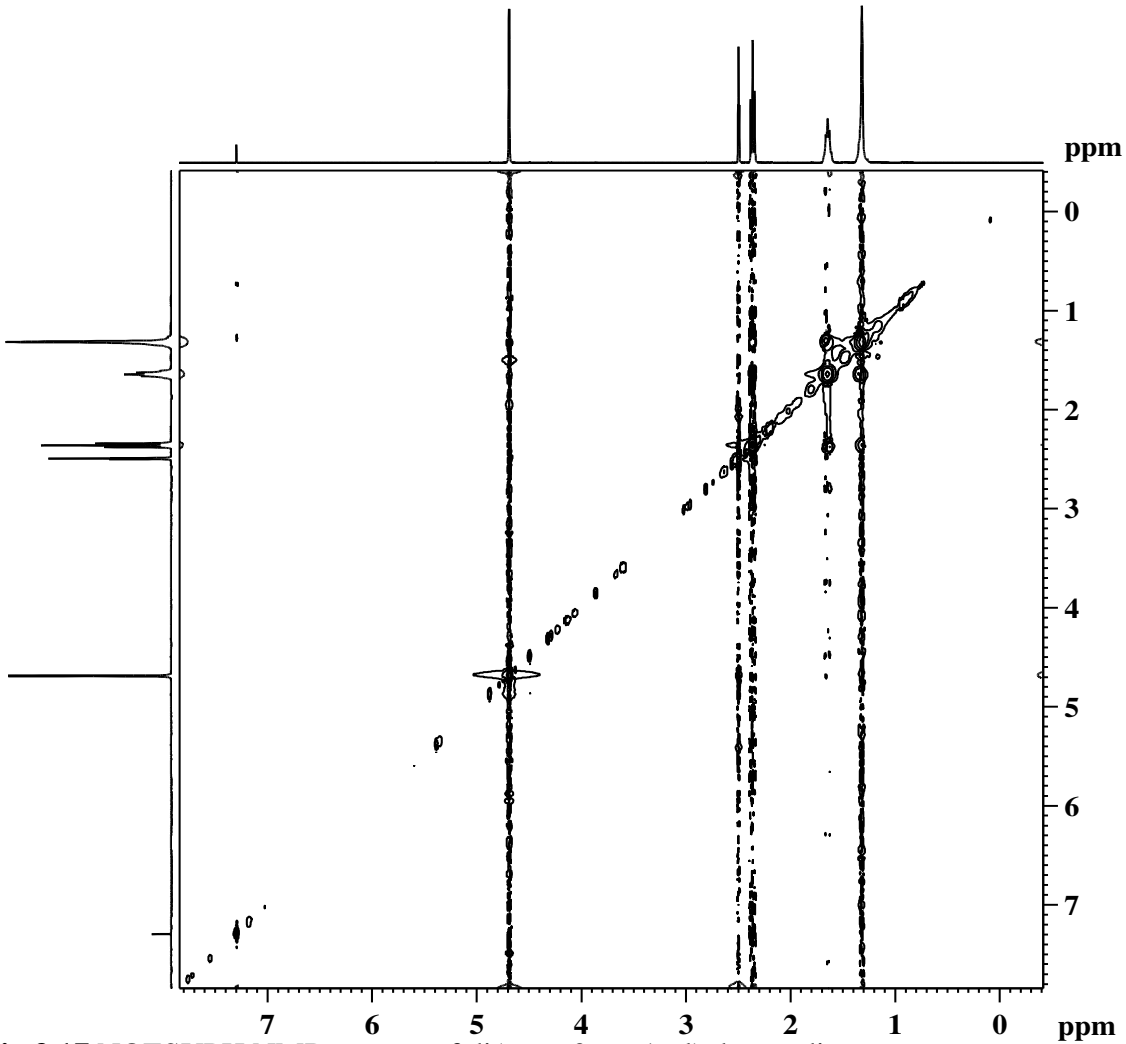


Fig.3.16 COESY NMR spectra of di(prop-2-yn-1-yl) decanedioate



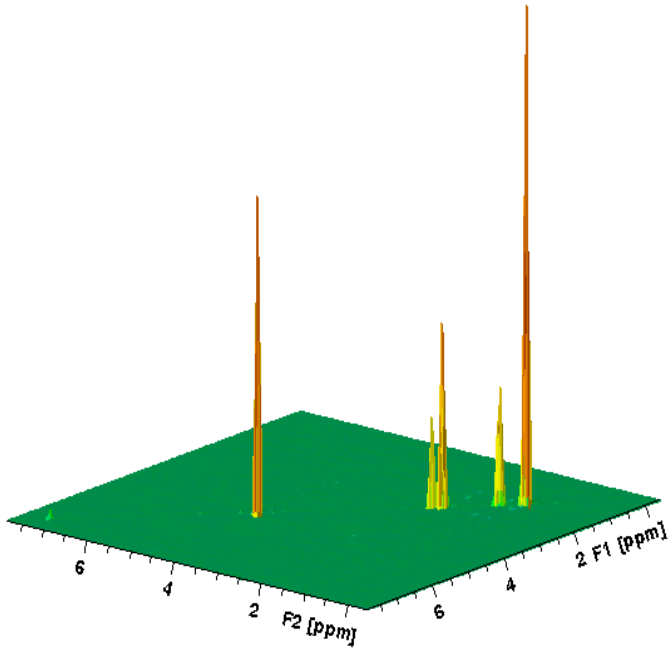


Fig.3.17 NOESYPH NMR spectra of di(prop-2-yn-1-yl) decanedioate

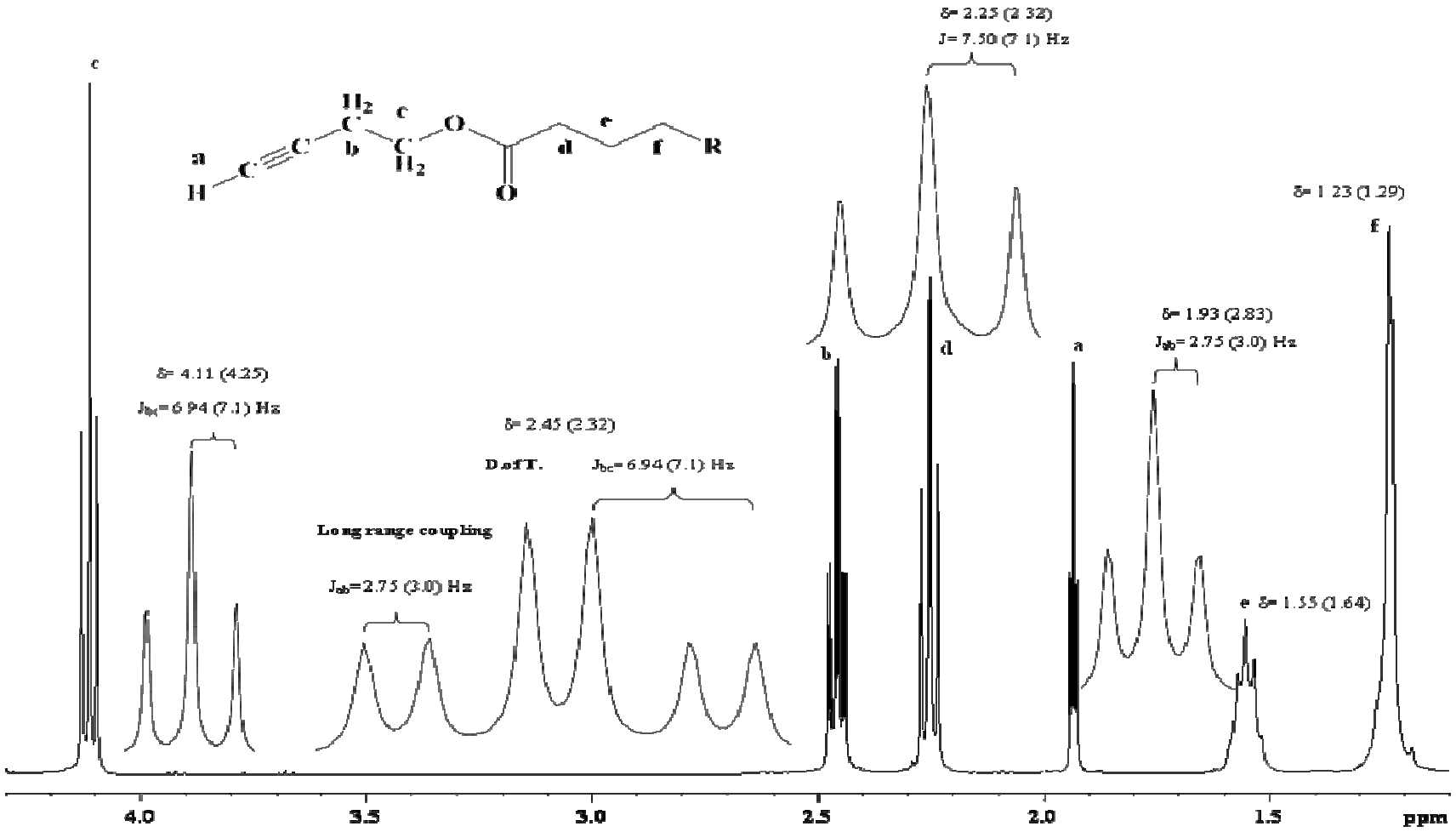


Fig.3.18 ^{1}H NMR ($CDCl_3$) spectra of di (but-3-yn-1-yl) decanedioate

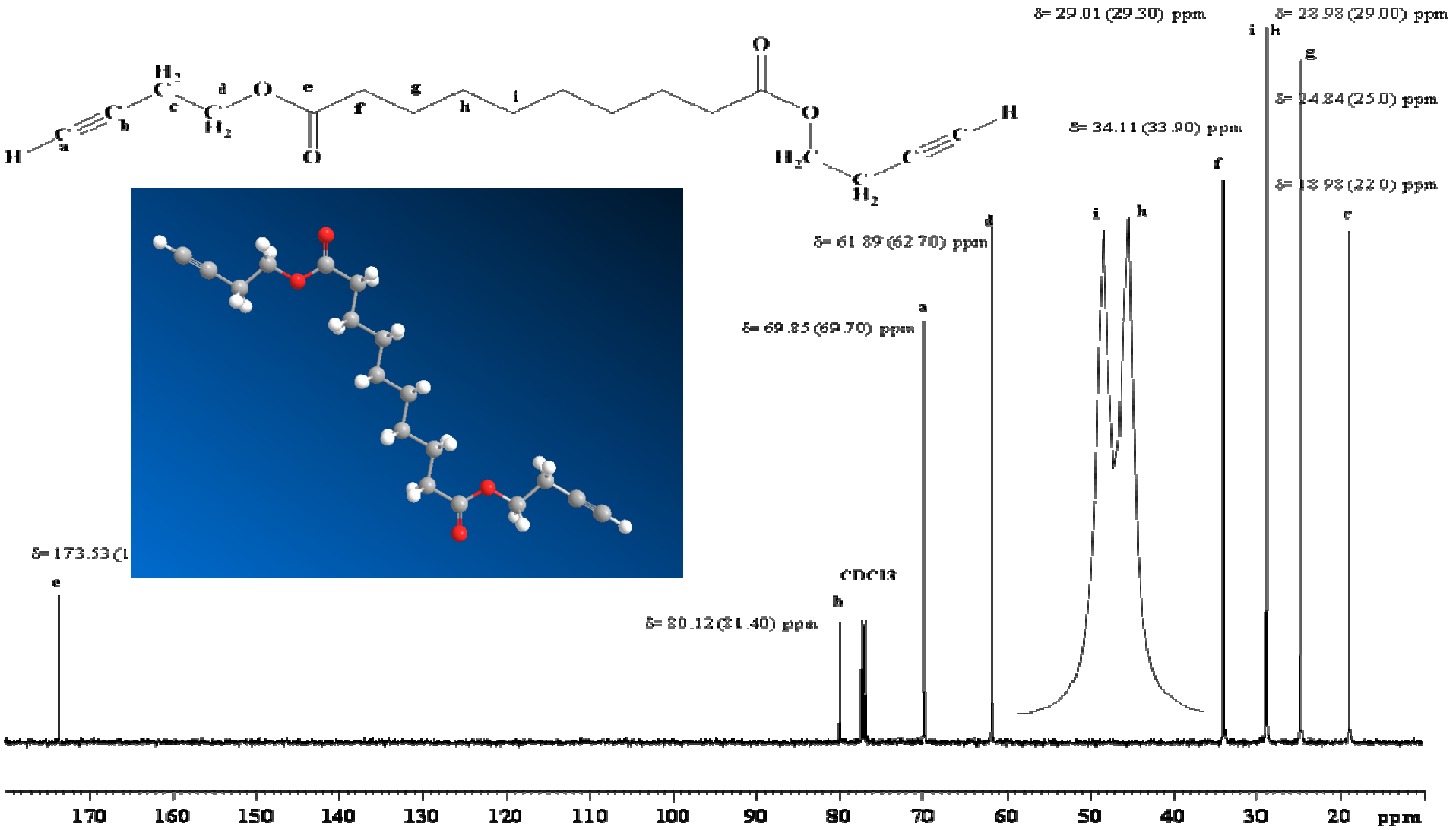


Fig.3.19 13 C NMR (CDCl3) spectra of di (but-3-yn-1-yl) decanedioate

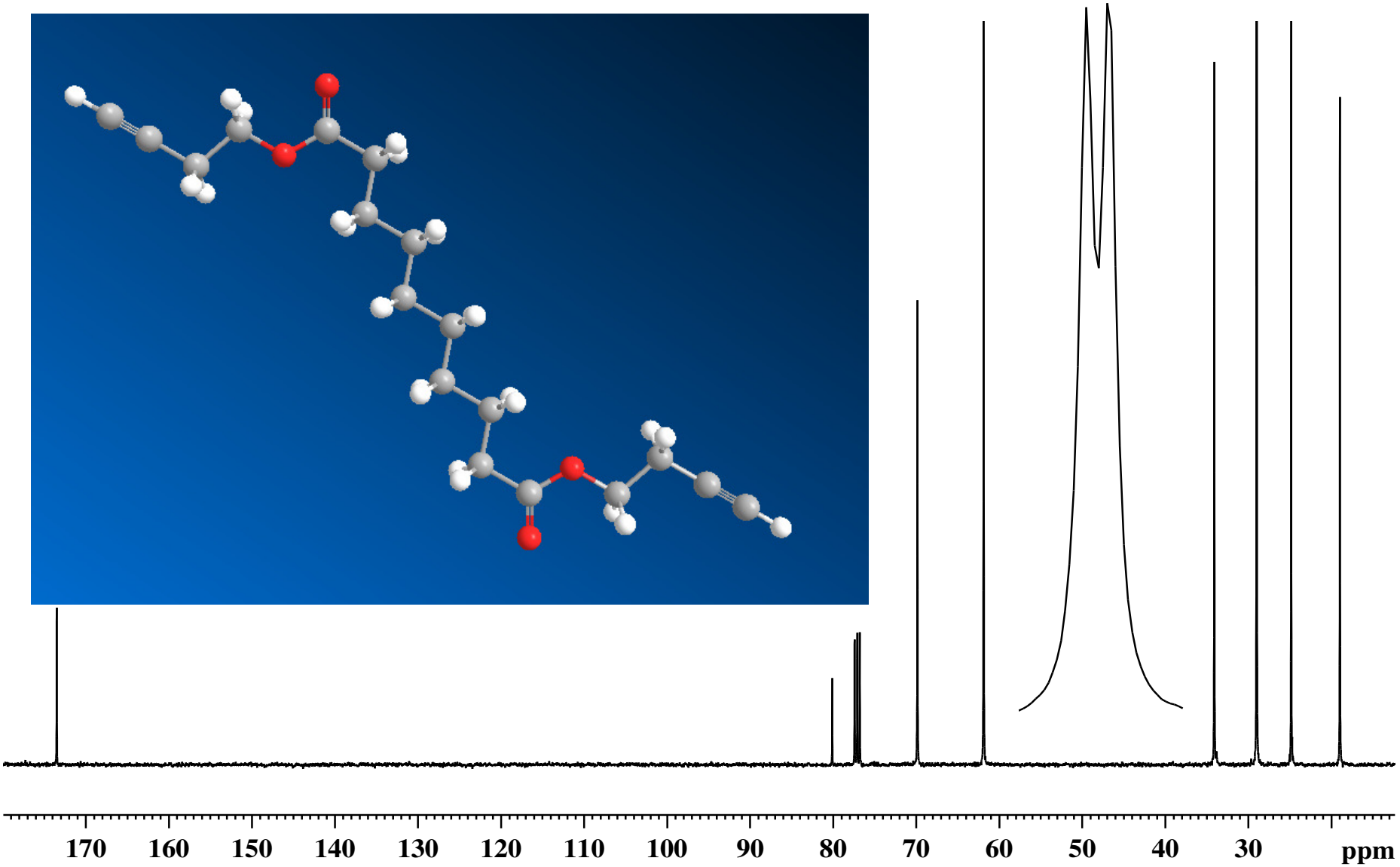


Fig.3.20 C13 APT spectra of di (but-3-yn-1-yl) decanedioate

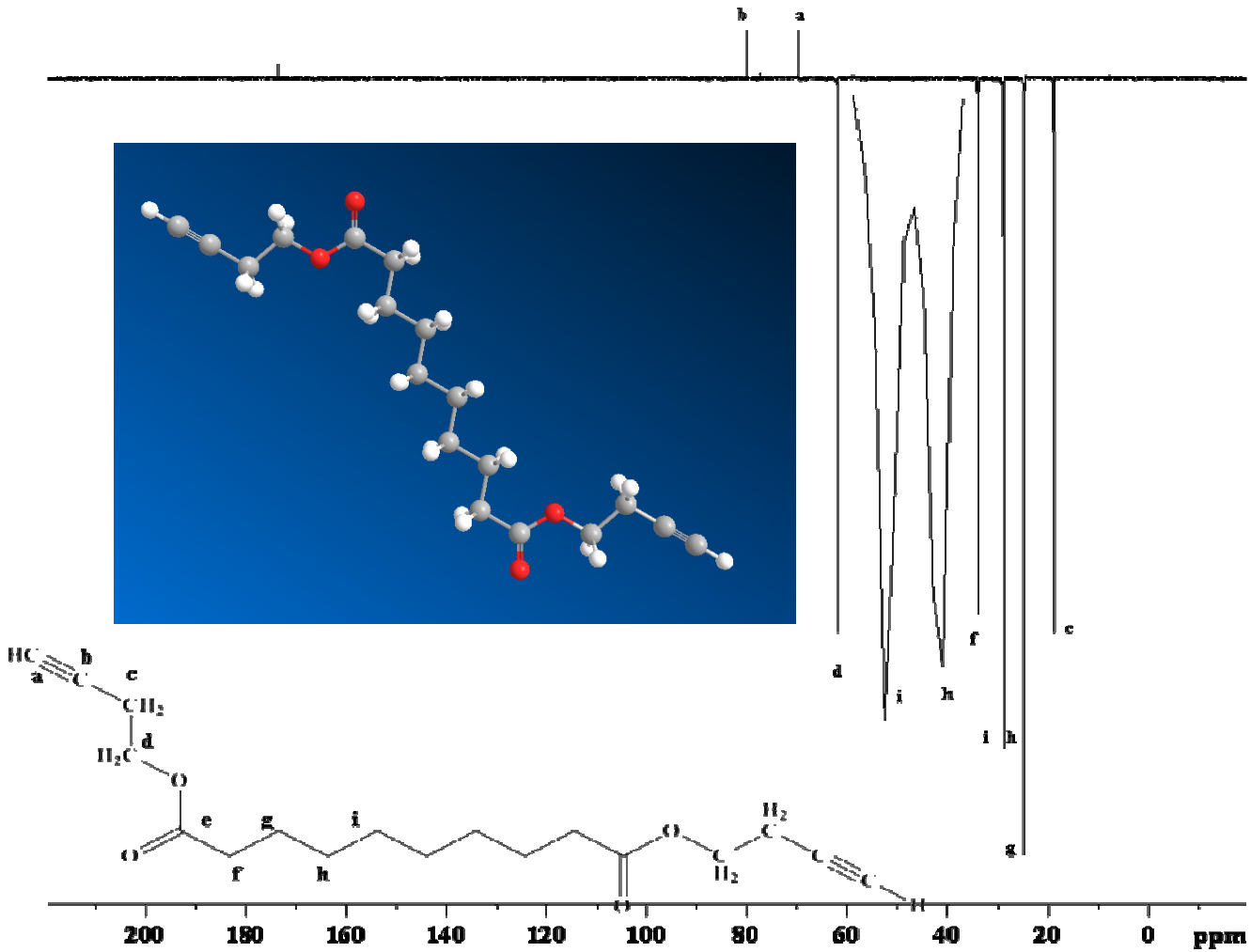


Fig.3.21 C13 DEPT 135 NMR spectra of di(but-3-yn-1-yl) decanedioate

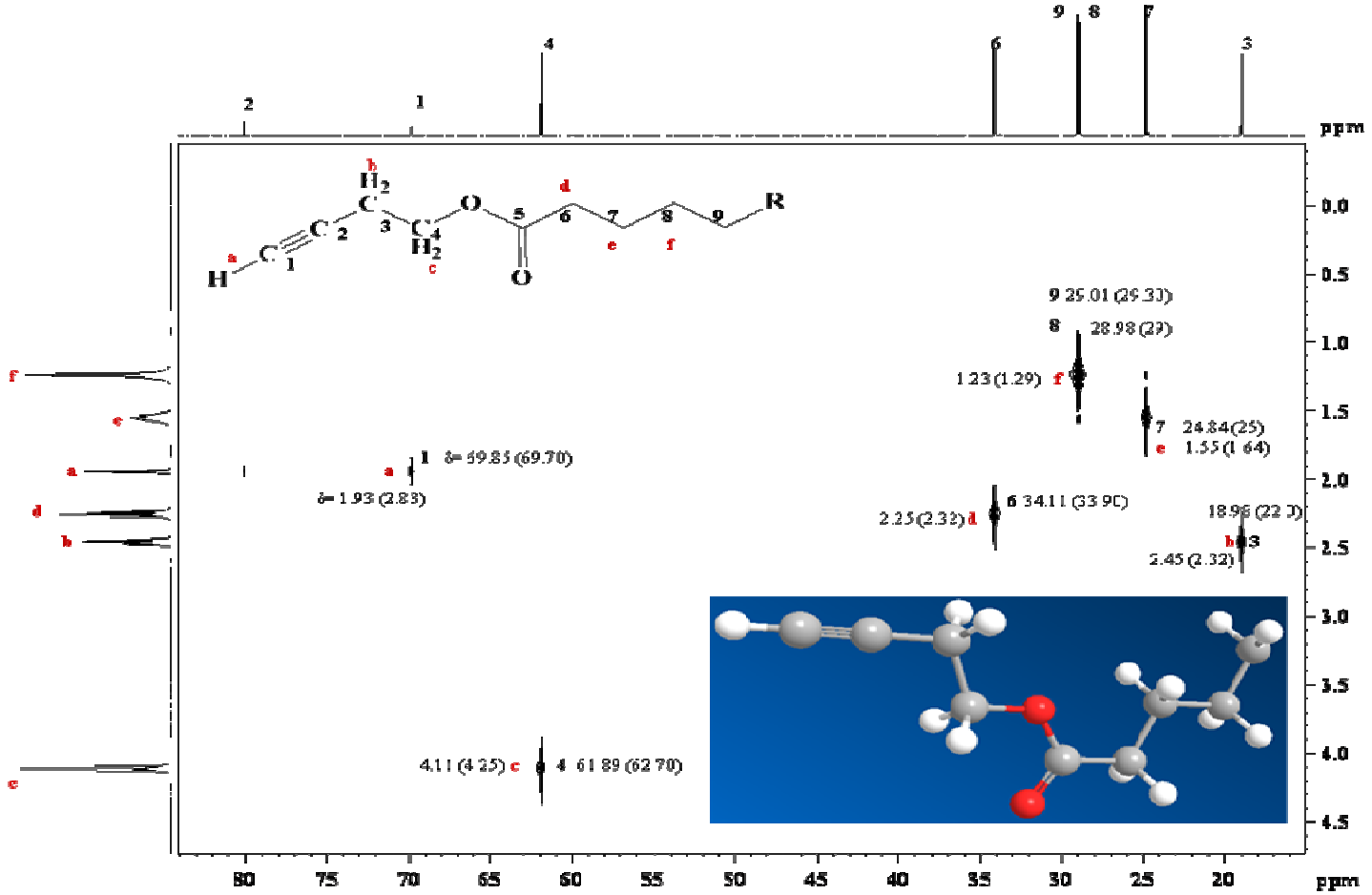


Fig.3.22 HETCOR NMR spectra of di(but-3-yn-1-yl) decanedioate

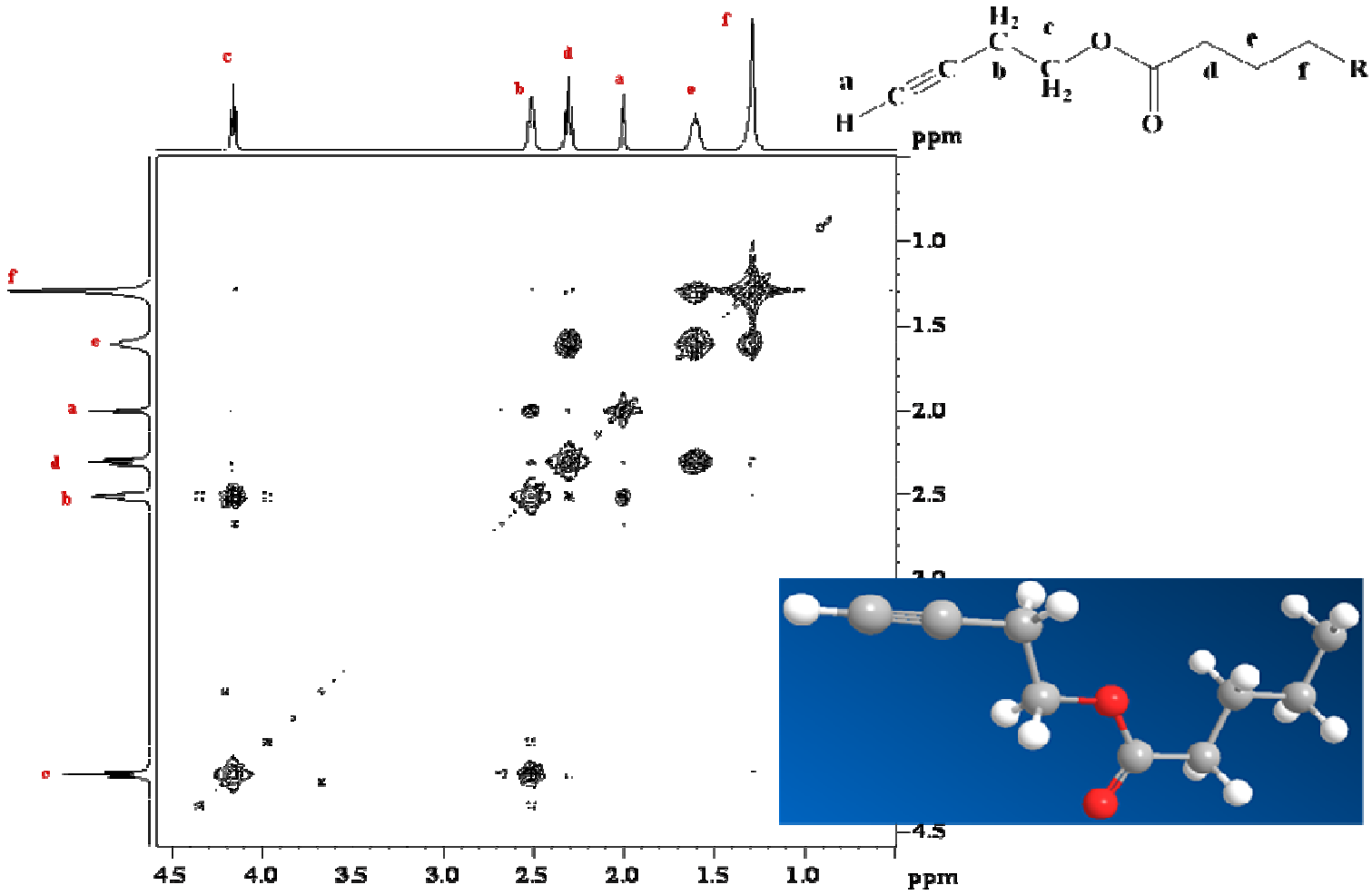


Fig.3.23 COESY NMR spectra of di(but-3-yn-1-yl) decanedioate

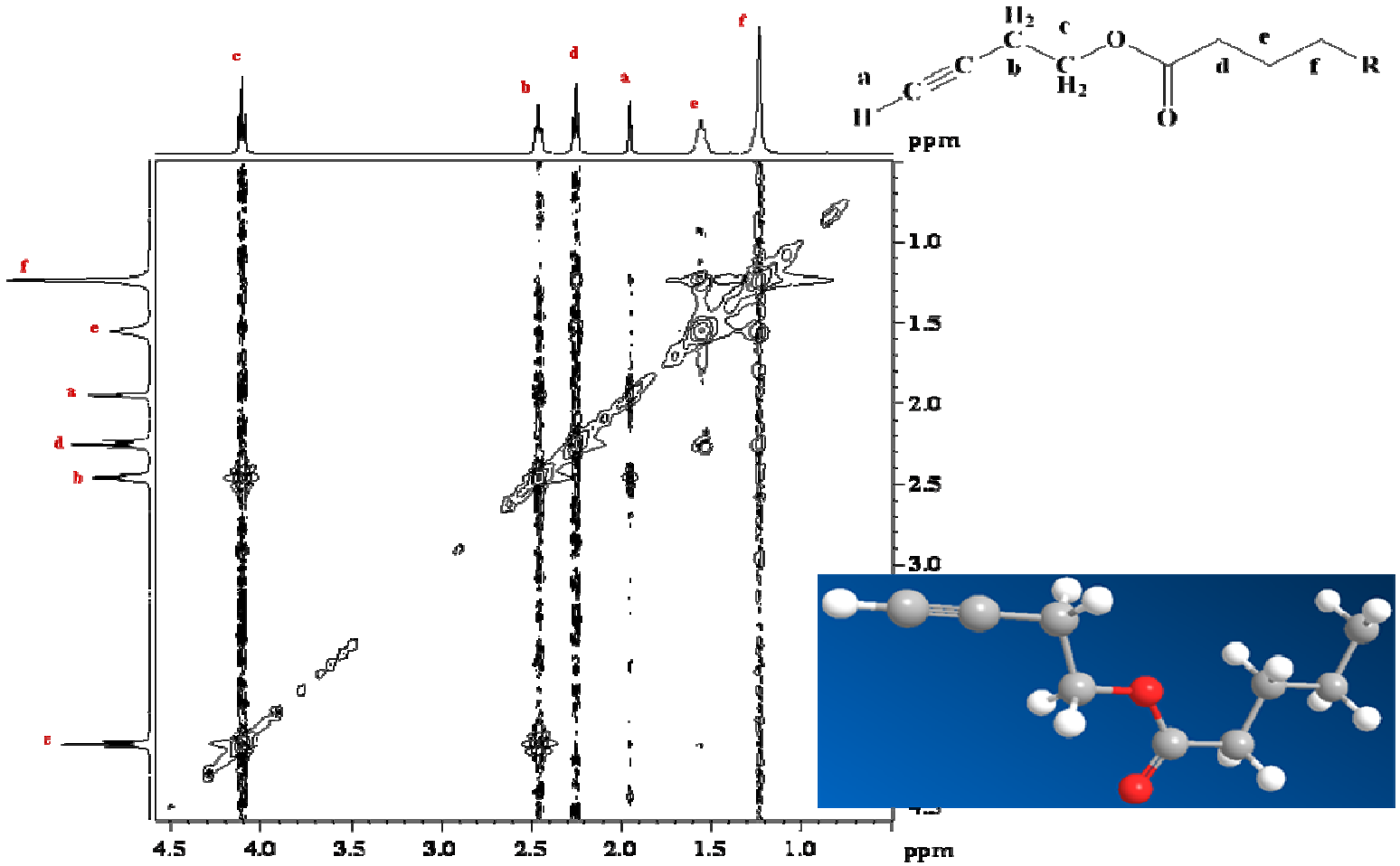


Fig.3.24 NOESYPH spectra of di(but-3-yn-1-yl) decanedioate

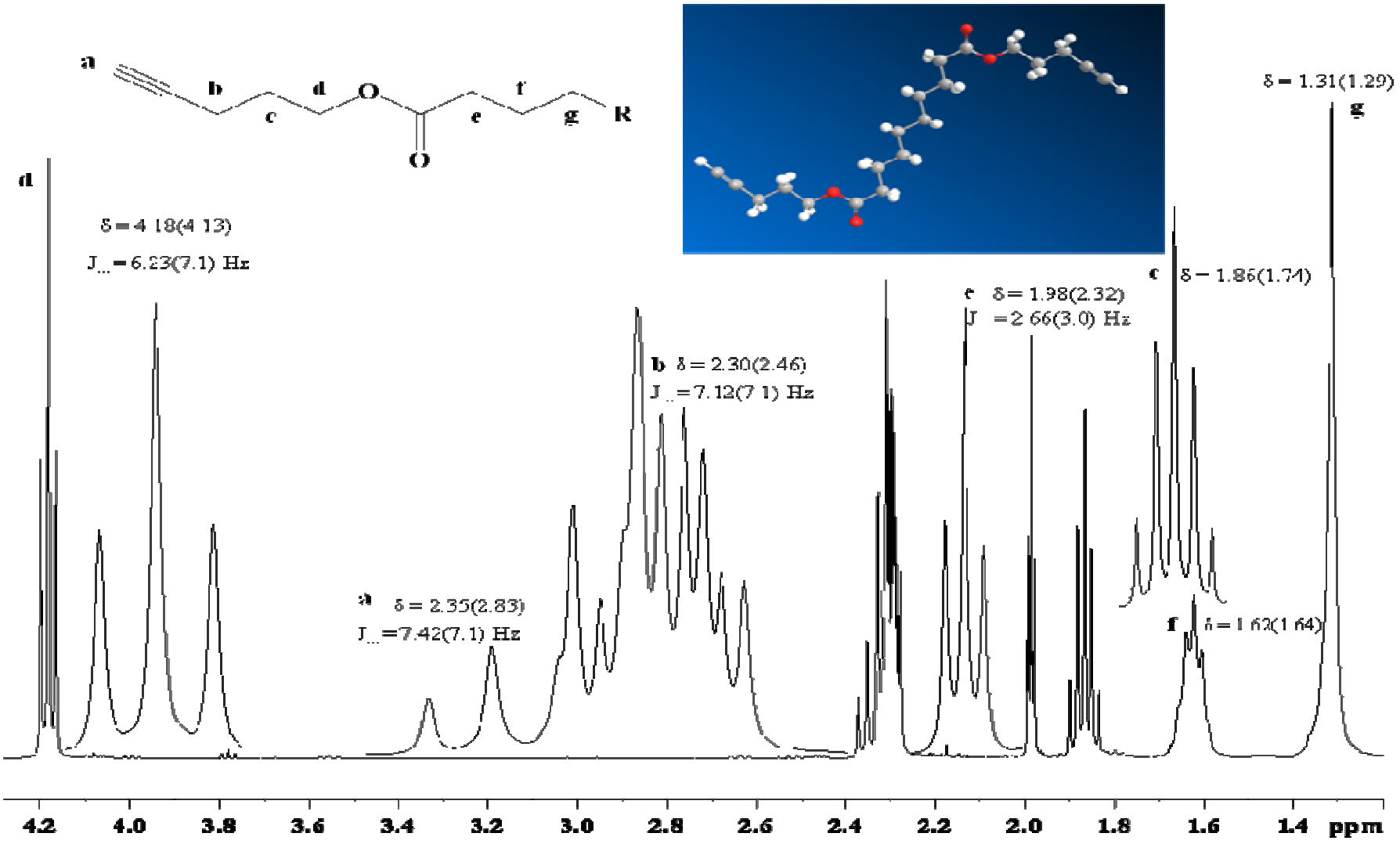


Fig 3.25 ^{1}H NMR ($CDCl_3$) spectra of di(pent-4-yn-1-yl) decanedioate

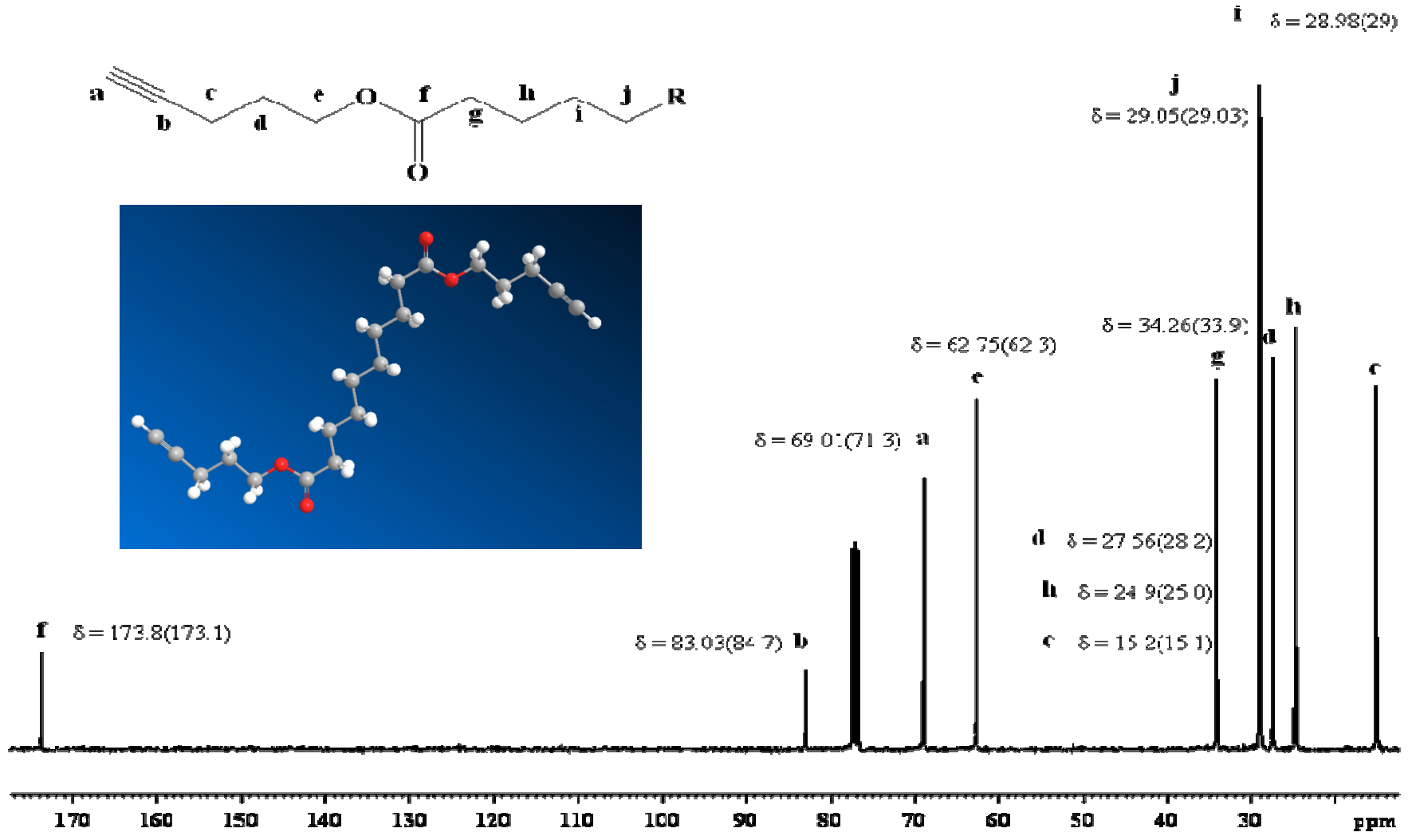


Fig.3.26 ^{13}C NMR ($CDCl_3$) spectra of di (pent-4-yn-1-yl) decanedioate

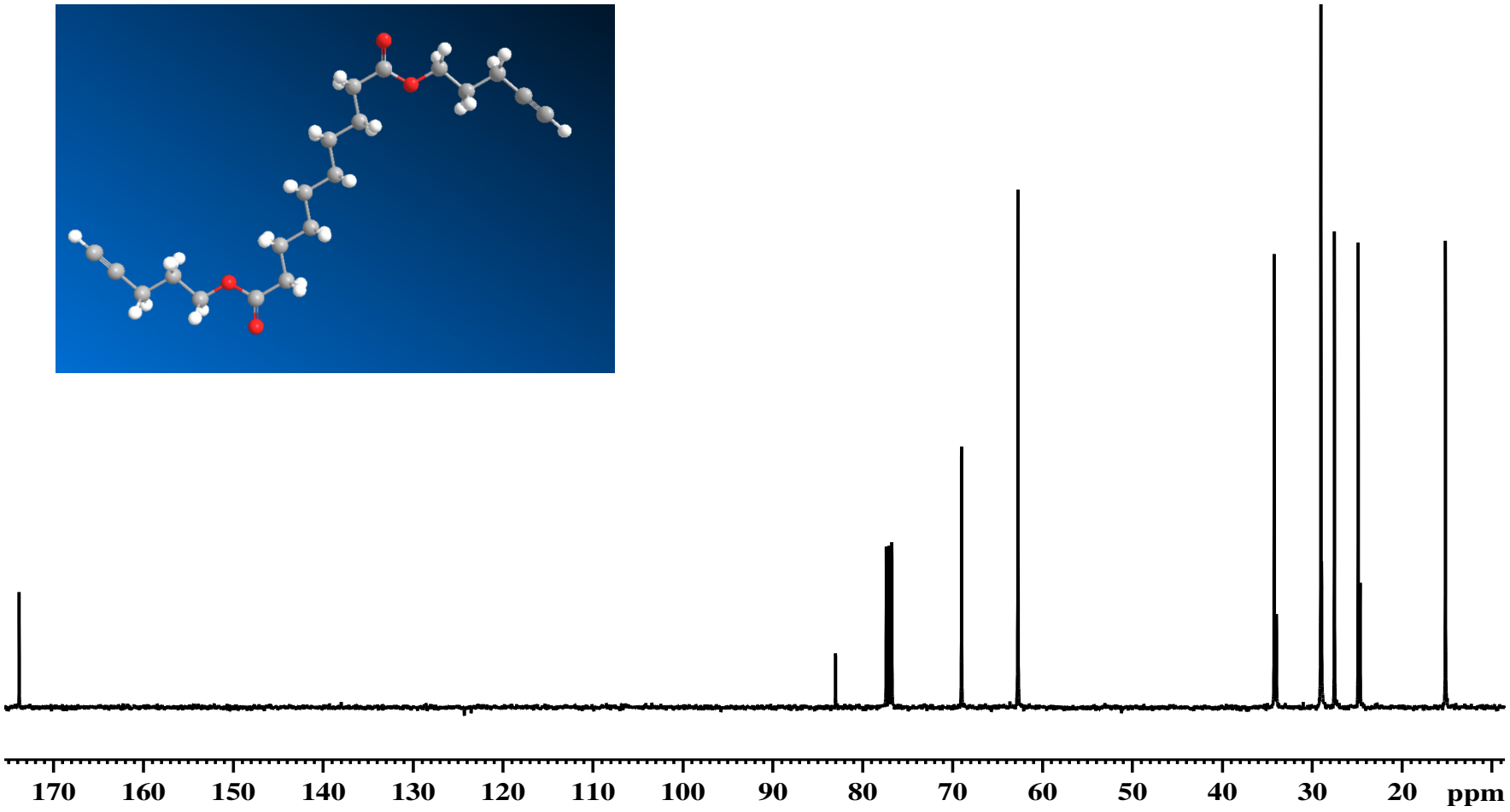


Fig.3.27 C13 APT NMR spectra of di (pent-4-yn-1-yl) decanedioate

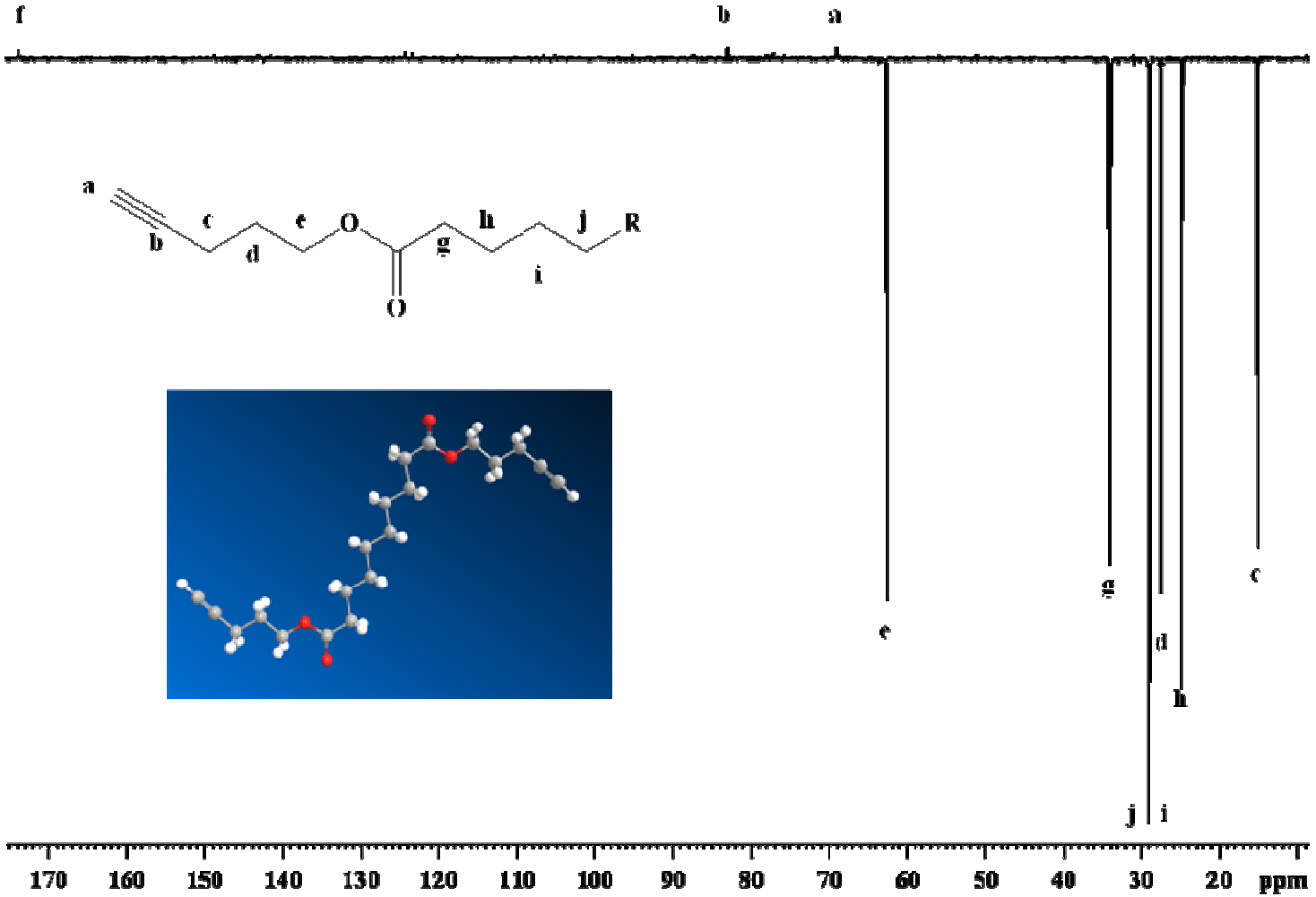


Fig.3.28 C13 DEPT 135 NMR spectra of di (pent-4-yn-1-yl) decanedioate

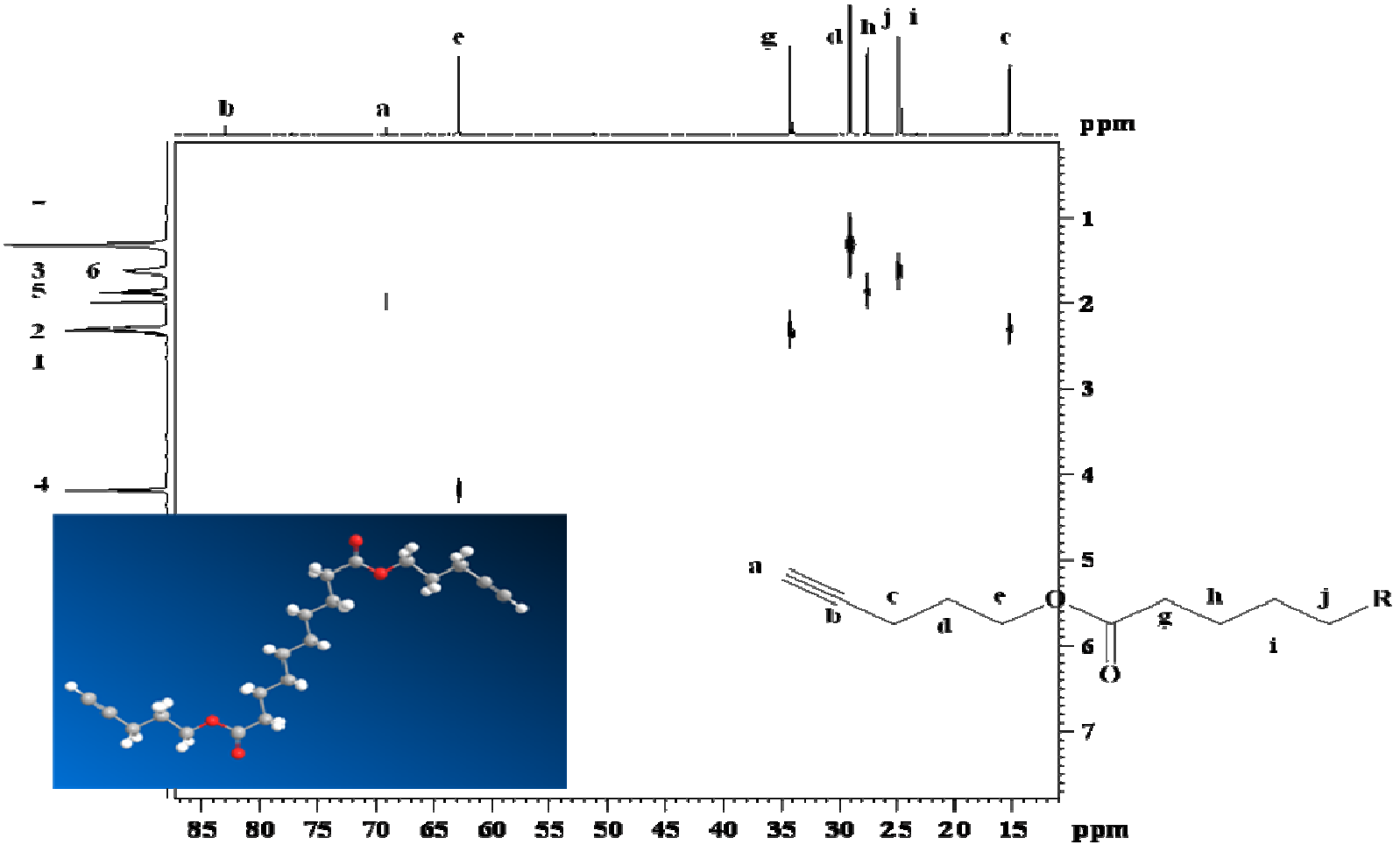


Fig.3.29 HCCOSW sw opt. CH correlation spectra of di (pent-4-yn-1-yl) decanedioate

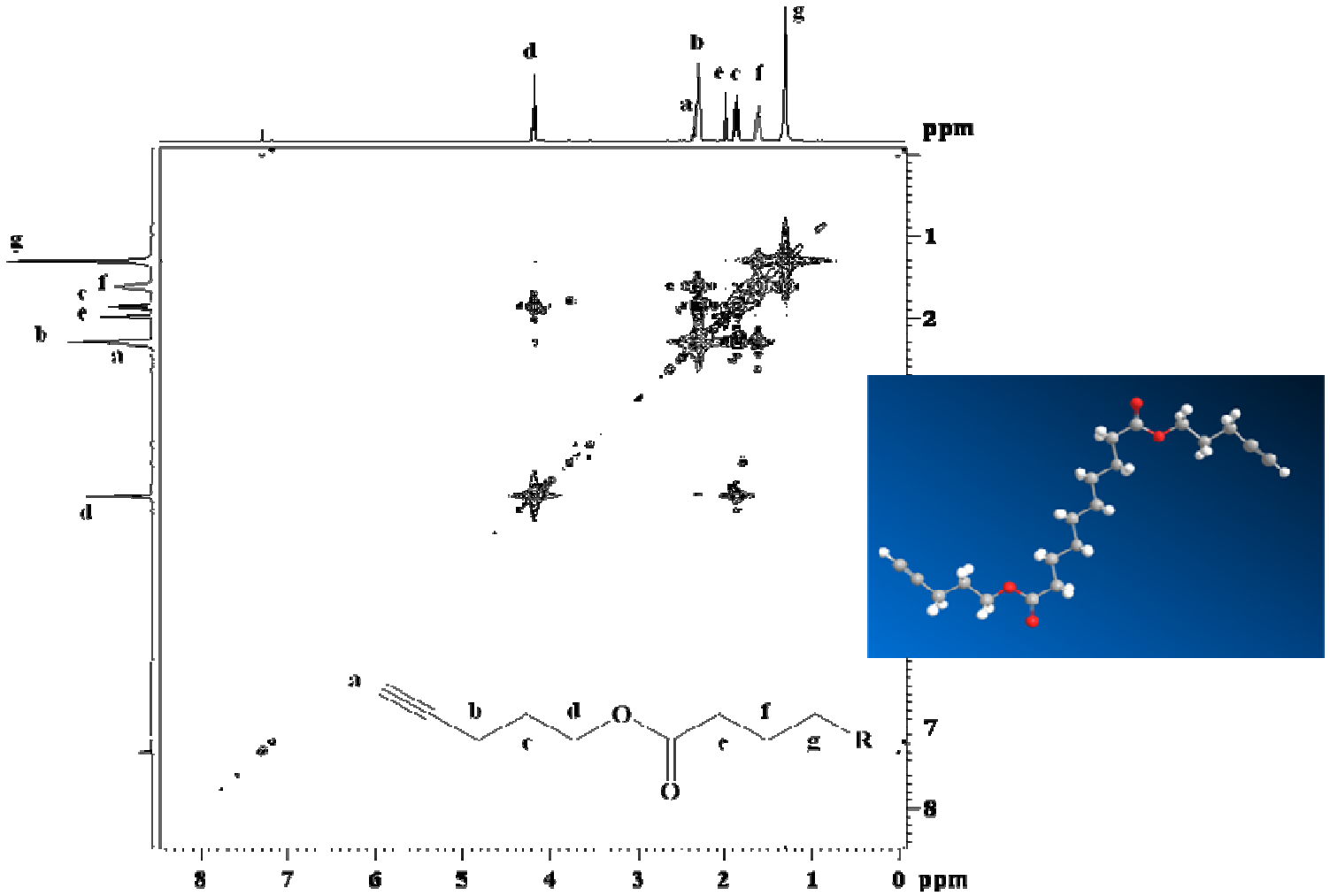


Fig.3.30 COESY NMR spectra of di(pent-4-yn-1-yl) decanedioate

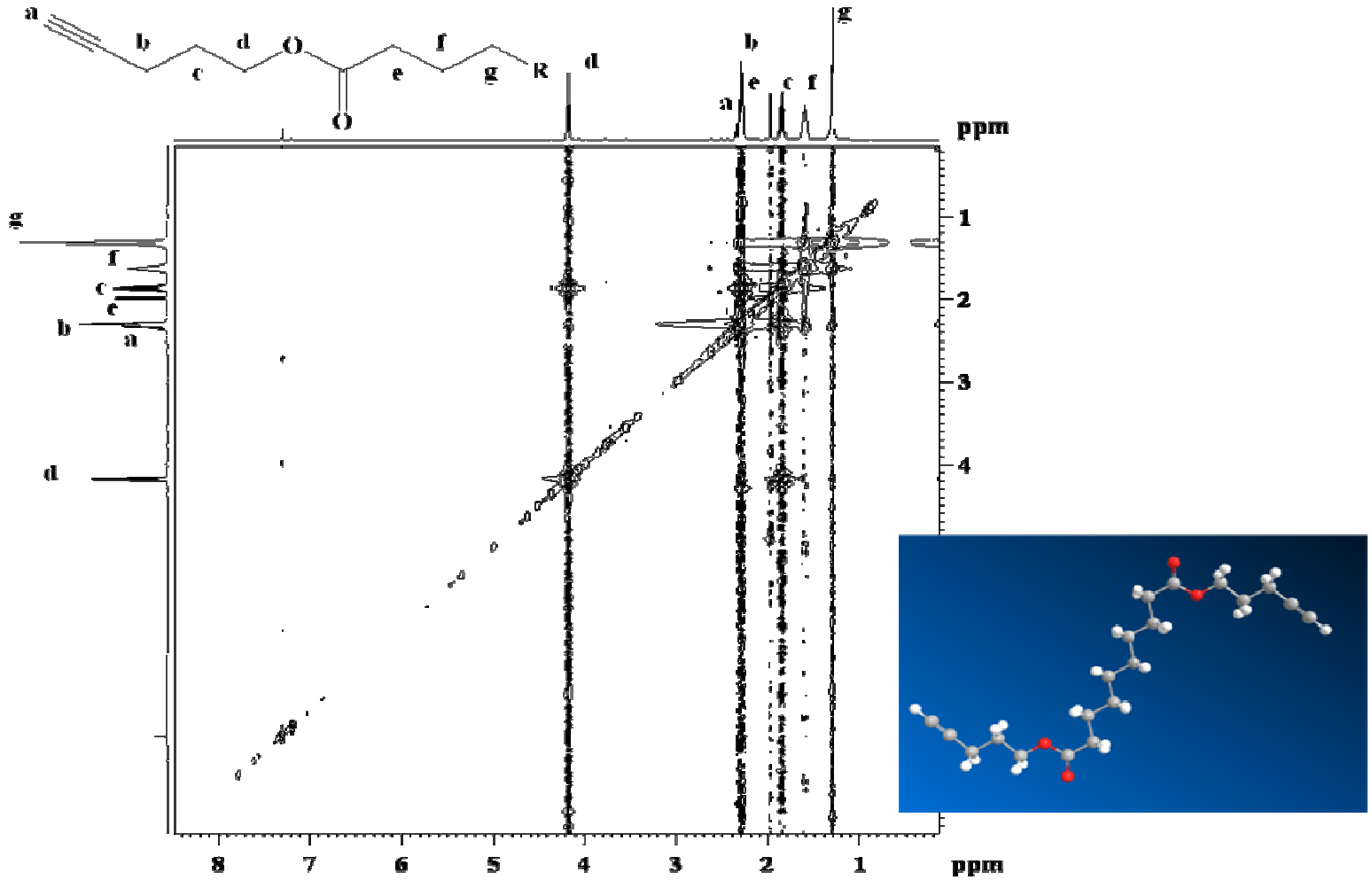


Fig.3.31 NOESYPH NMR spectra of di (pent-4-yn-1-yl) decanedioate

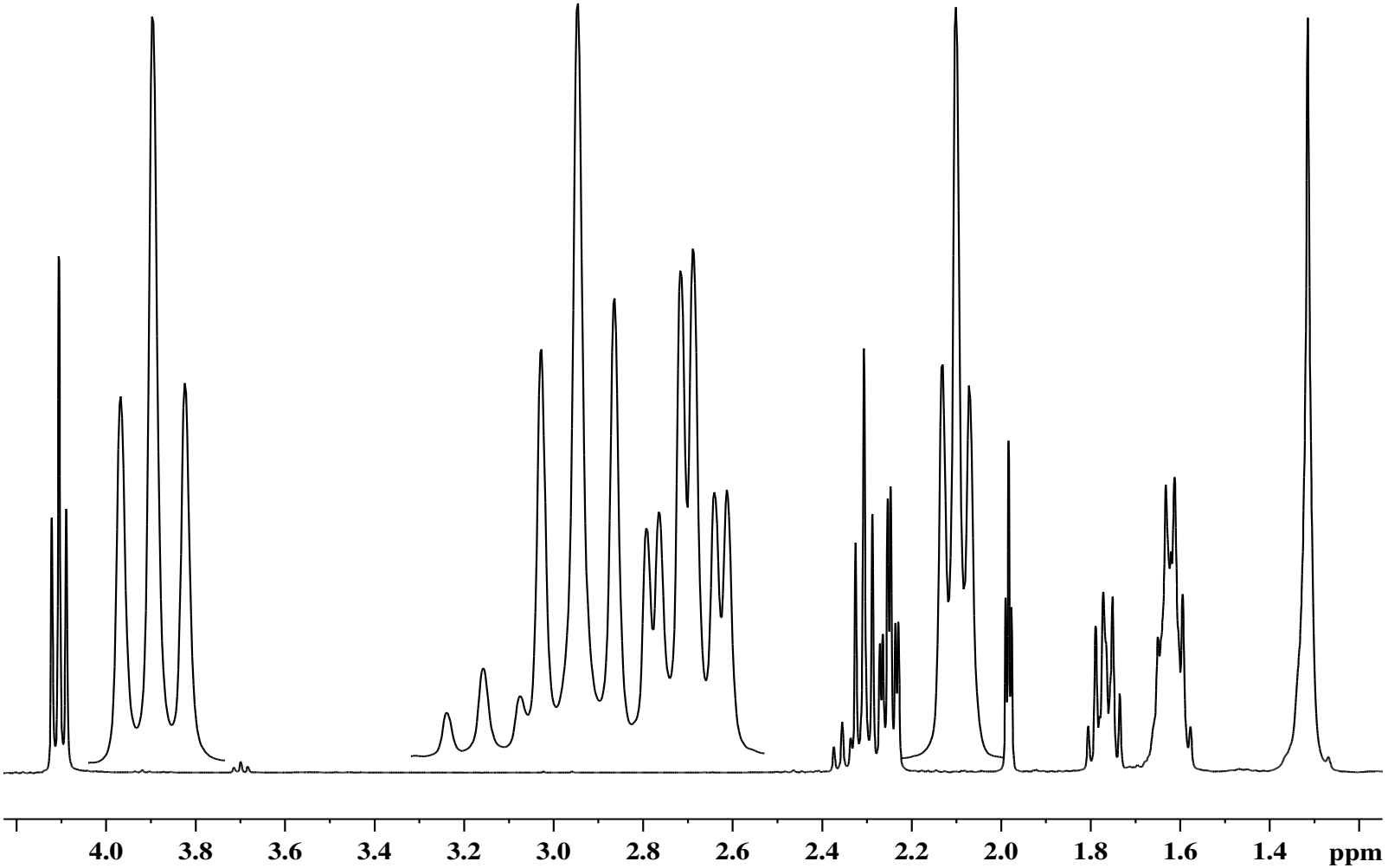


Fig.3.32 ^{1}H NMR ($CDCl_3$) spectra of di(hex-5-yn-1-yl) decanedioate

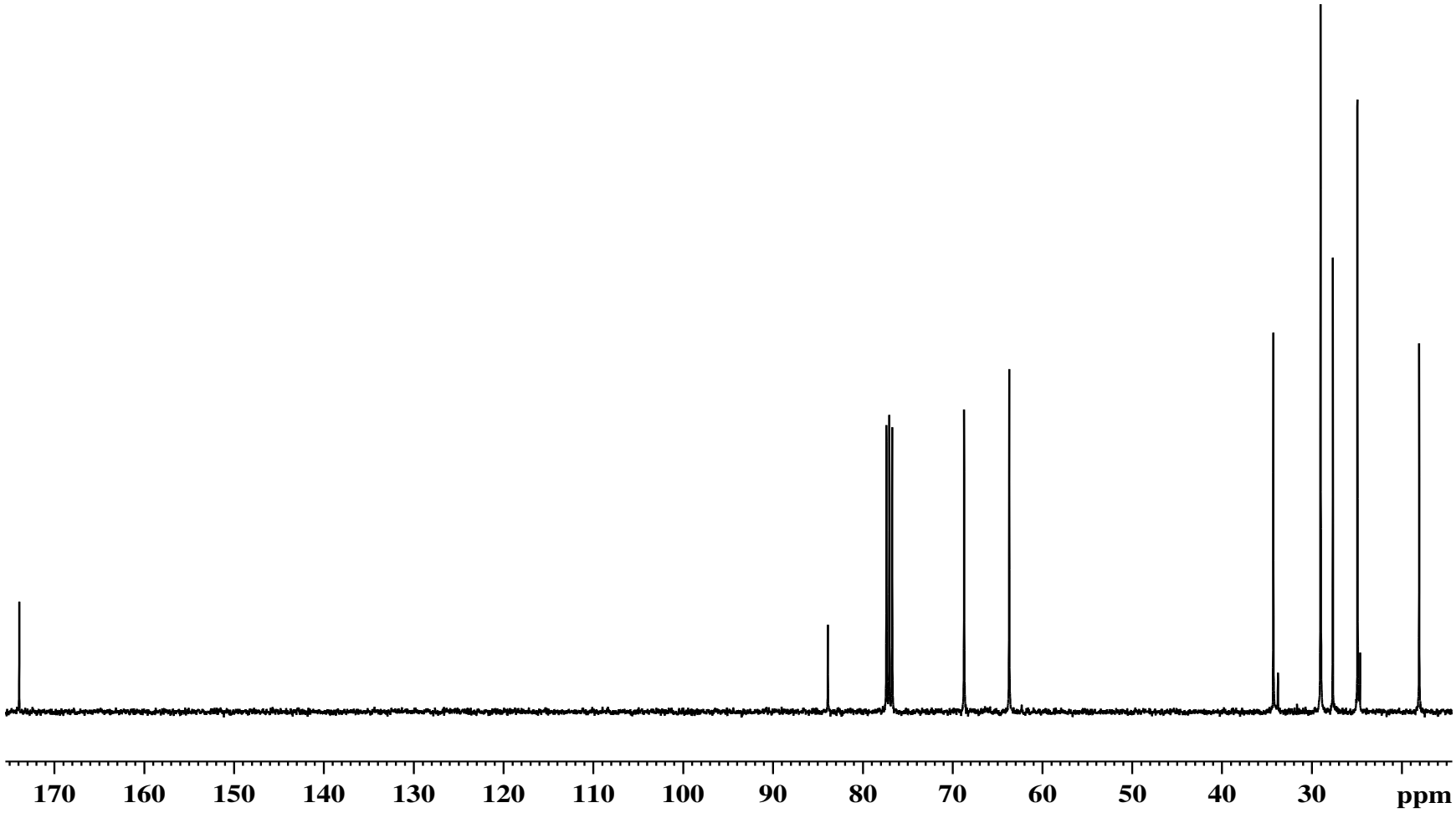


Fig.3.33 ^{13}C NMR ($CDCl_3$) spectra of di(hex-5-yn-1-yl) decanedioate

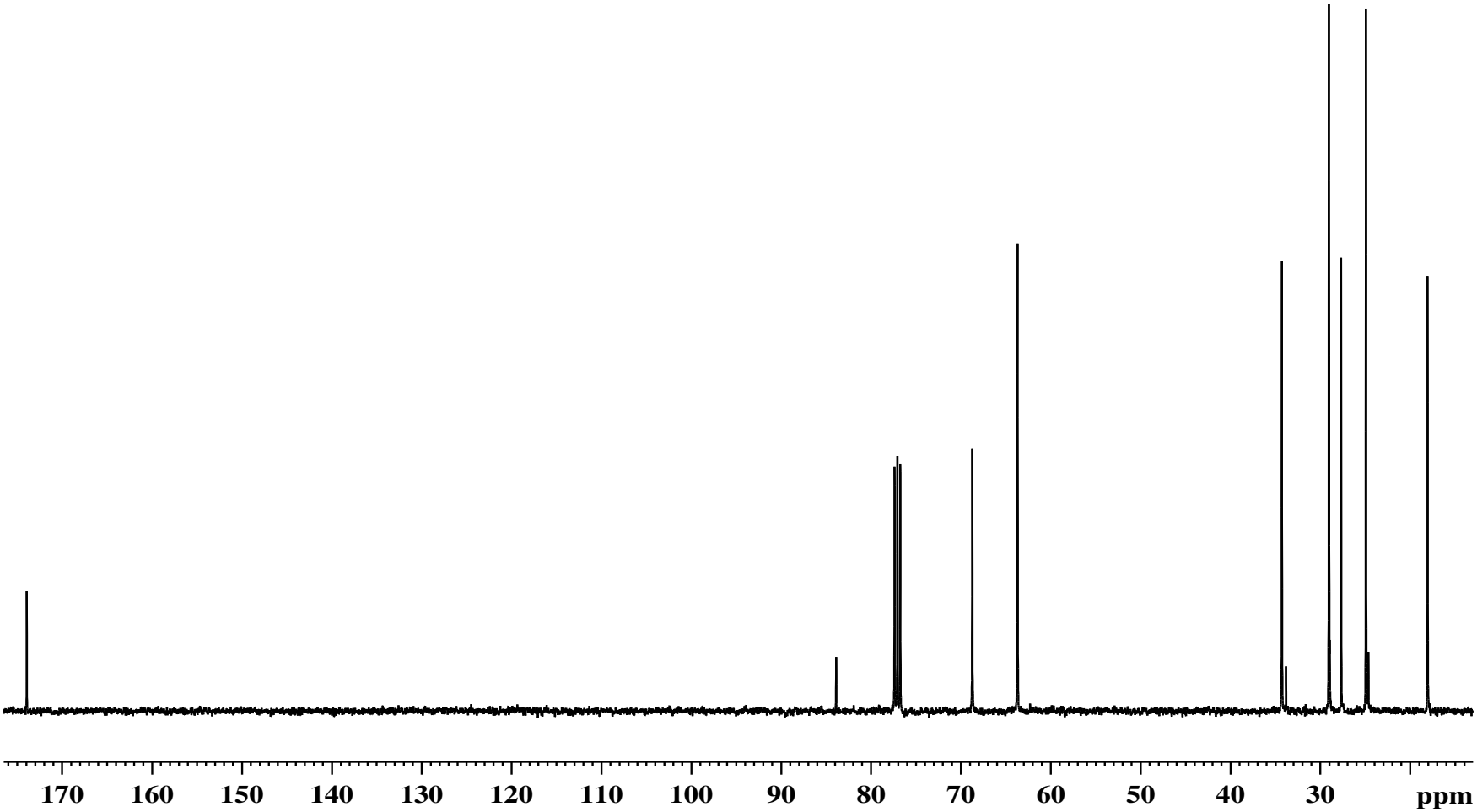


Fig.3.34 C13 APT Attached Proton Test of di(hex-5-yn-1-yl) decanedioate

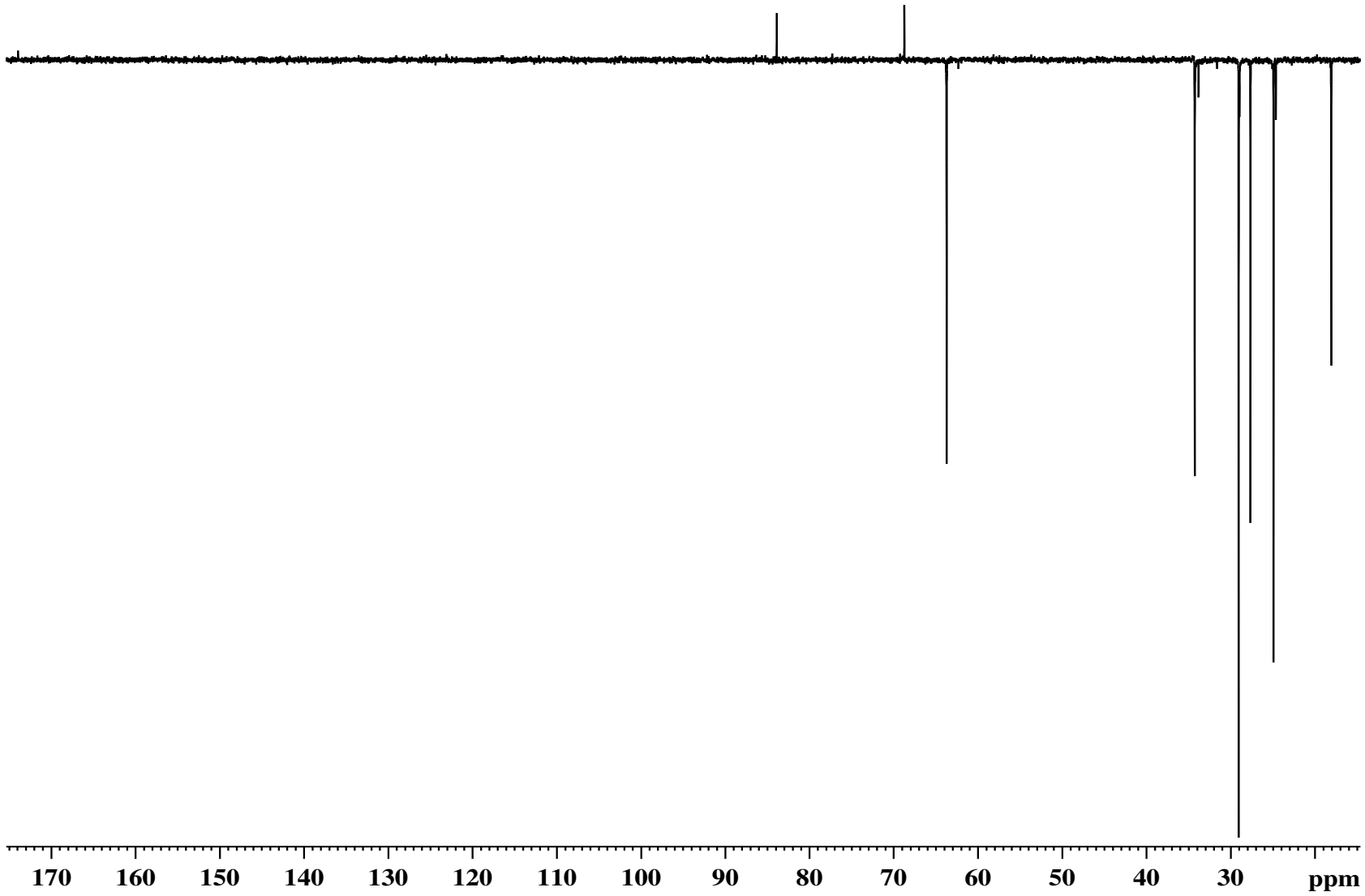


Fig.3.35 C13 DEPT 135 of di(hex-5-yn-1-yl) decanedioate

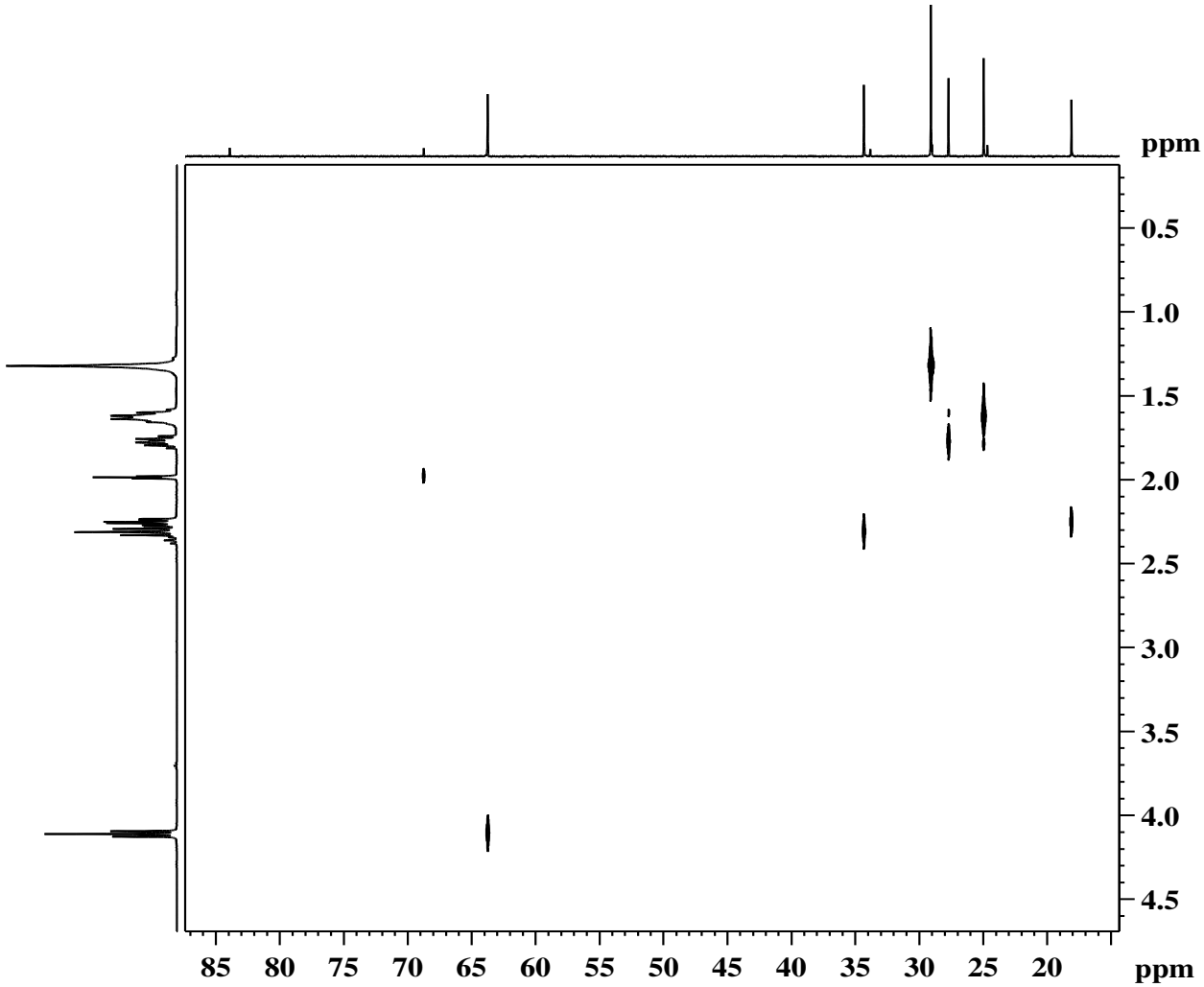


Fig.3.36 HCCOSW sw opt. CH correlation of di(hex-5-yn-1-yl) decanedioate

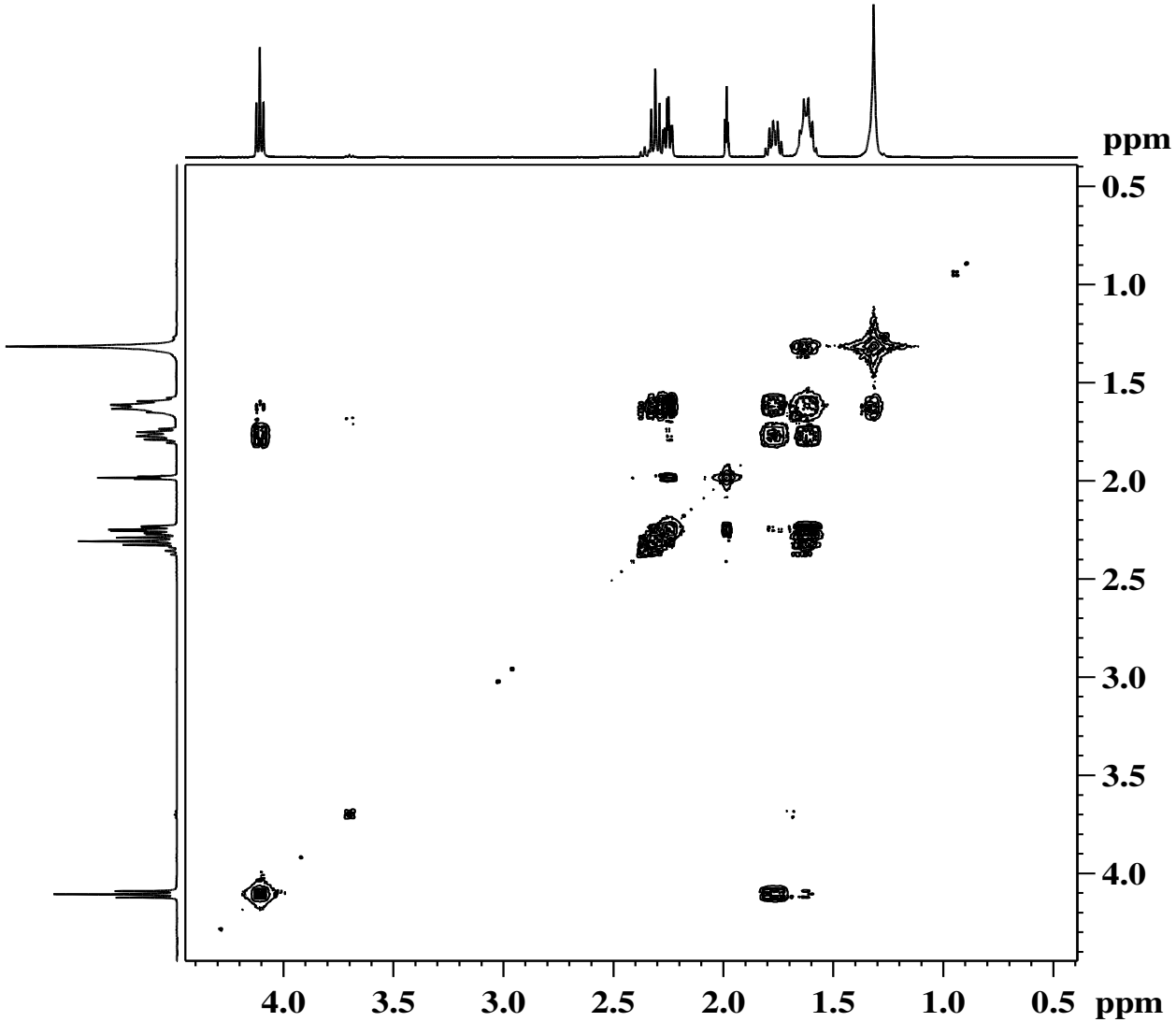


Fig.3.37 COESY (Correlated Spectroscopy) of di(hex-5-yn-1-yl) decanedioate

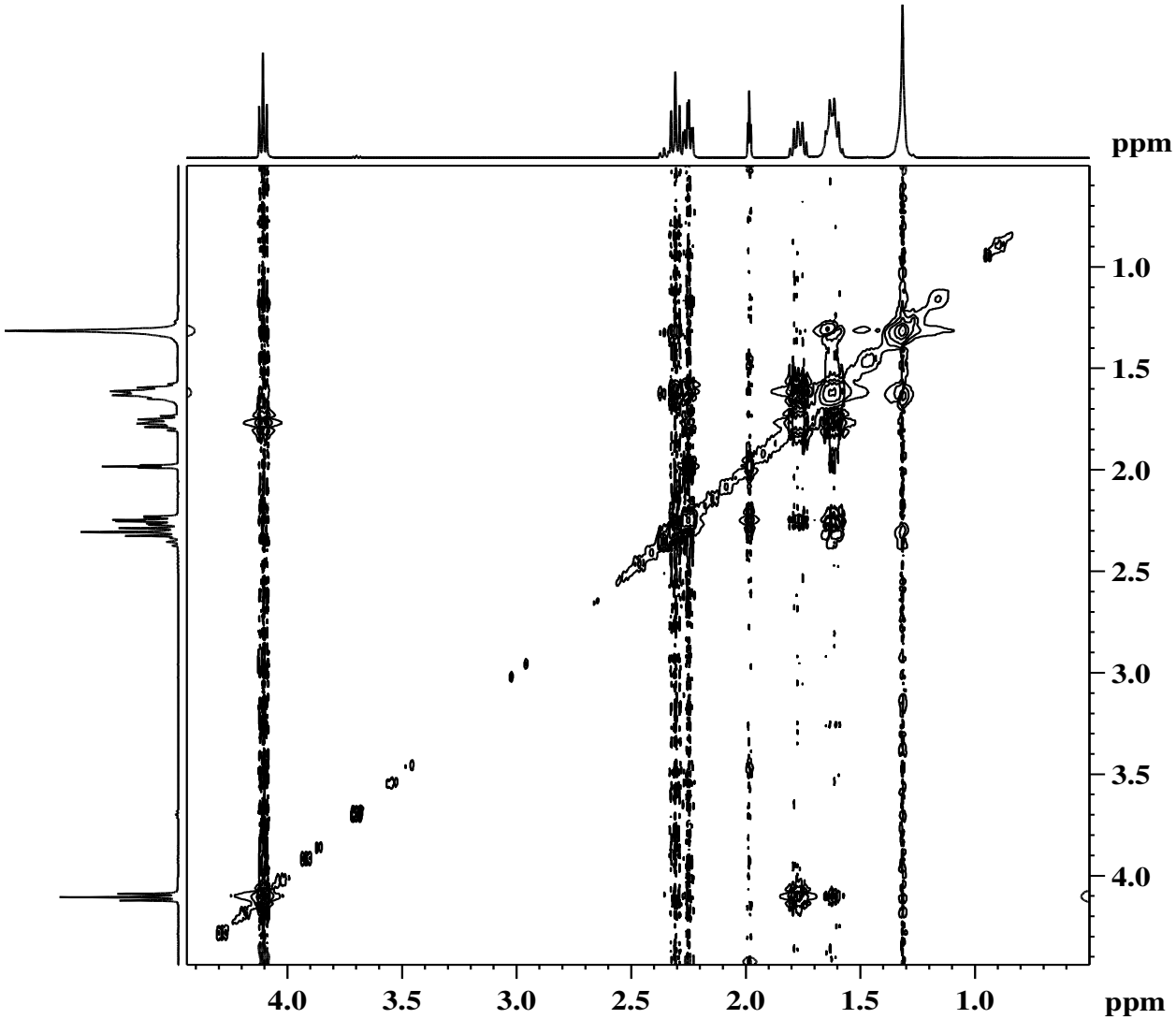


Fig.3.38 NOESY of di(hex-5-yn-1-yl) decanedioate

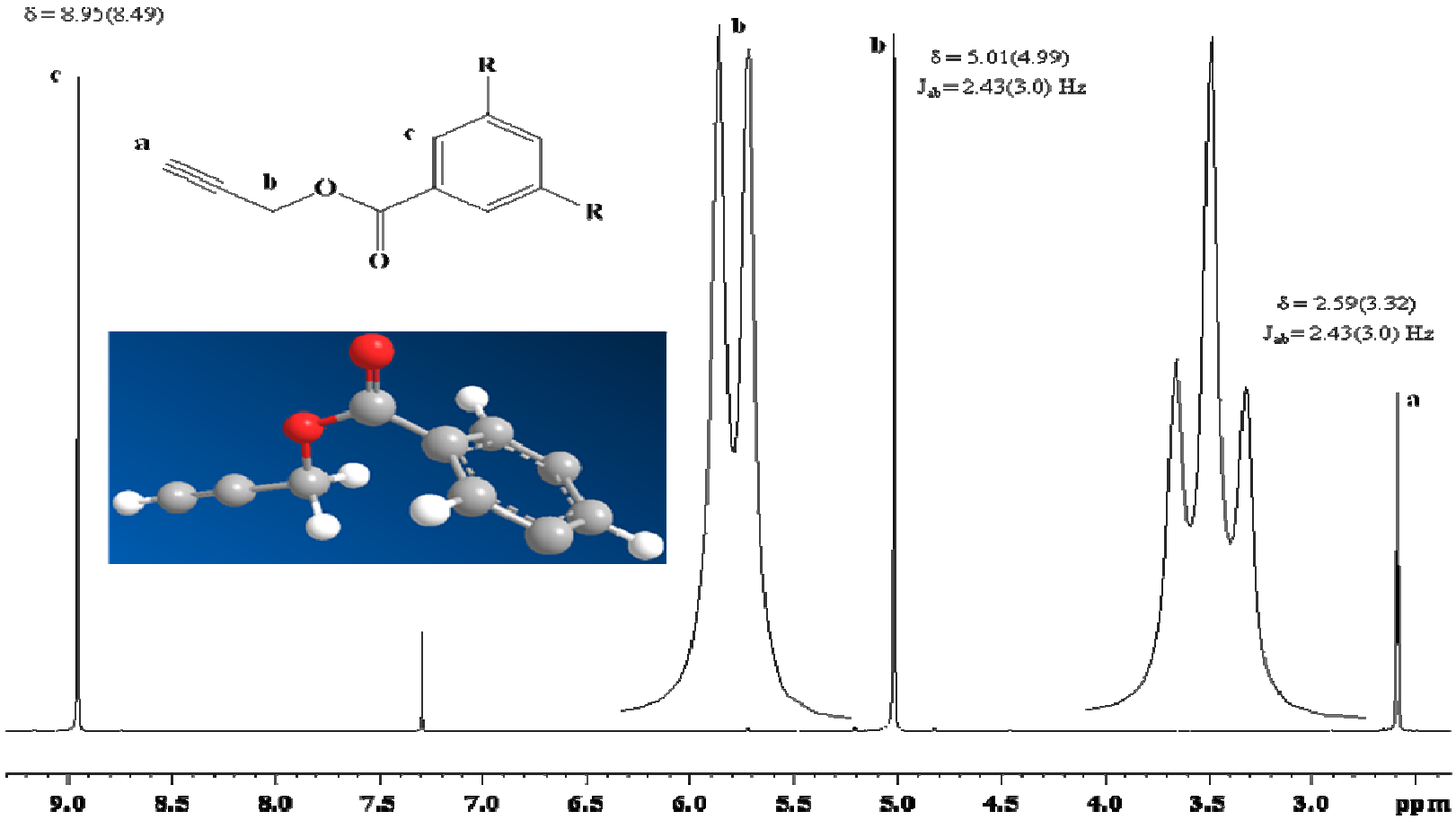


Fig.3.39 ^{1}H NMR ($CDCl_3$) spectra of tri(prop-2-yn-1-yl) benzene-1,3,5-tricarboxylate

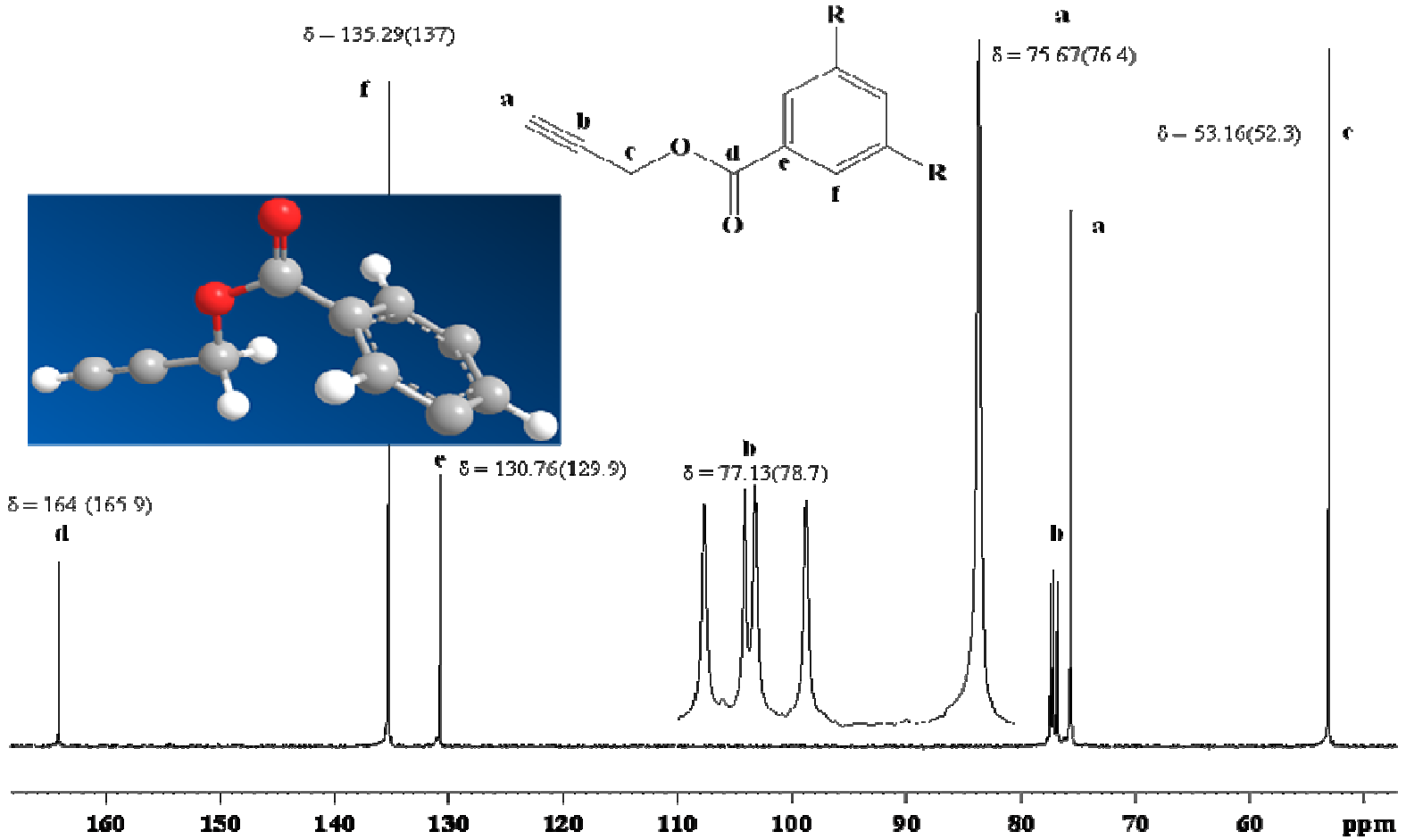


Fig.3.40 ^{13}C NMR ($CDCl_3$) spectra of tri(prop-2-yn-1-yl) benzene-1,3,5-tricarboxylate

3.3. Antimicrobial Activity Studies

The results concerning *in vitro* antimicrobial activities of the multidentate ligands together with the inhibition zone(mm) and MIC values of compared standard antibiotic and antifungal reagents are listed in Table 3.1 and Table 3.2

Inhibition zone is the clear region that is an indication of the absence, or the effective inhibition of microbial growth by the antimicrobial agent. If the bacteria are sensitive to the antibiotic, they can not grow near the disk. The size of the zone is proportional to how sensitive the organism is. If the organism is resistant to the antibiotic, it will grow right up to the disk. It could be said that larger inhibition zone corresponds to high antimicrobial activity.

MIC values is the lowest concentration of antimicrobial agents which inhibits growth of the microorganism. Antimicrobial activity of the antibiotics increases with decreasing MIC values.

3.3.1. Antibacterial Activity Studies of L_1-L_5 Ligands

All of the compounds showed moderate or slightly higher antibacterial activity against Gram negative and Gram positive bacteria compared with standard antibacterial reagents. For instance these six ligands have higher antibacterial avtivity against E.coli compared with Cefotaxime and Ampicillin. Especially L3, and L4 have slightly higher antibacterial activity against *Escherichia coli, Proteus Vulgaris.* The data indicate that L4 have higher activity against some bacteria such as *Enterobacter Aerogenes, Proteus Vulgaris* compared with. AK, SM, CT, and VA.

Generally, these ligands have lower antibacterial activity compared with Gentamicin. But some ligands have same or higher antibacterial acvtivity against some Gram negative and Gram positive bacteria such as *Escherichia coli, Enterobacter Aerogenes, Staphylococcus Aureus, Staphylococcus Epidermidis, Micrococcus Luteus, Micrococcus Flavus.* Among Gram negative-Gram positive bacteria, *Escherichia coli, Enterobacter Aerogenes* have more sensitive. compared with Gentamicin.

3.3.2. Antifungal Activity Studies of L_1-L_5 Ligands

These ligands showed moderate or slightly higher antifungal activity against some yeast culture compared with NY, KT, and Cl standard antifungal reagents. All these ligands have higher antifungal activity against *Kluyveromyces Fragilis, Rhodotorula Rubra, Candida Tropicalis,* and *Debaryomyces Hansenii* compared with Standard reagents. Especially L4 showed higher antifungal activity against *Debaryomyces Hansenii* compared with NY, KT, and Cl.

According to Table 3.1 all these ligands usually show same or lower antifungal activity against some yeast culutures compared with standard antifungal reagent NY. Among these ligands, L4 and L3 showed higher activity against *Kluyveromyces Fragilis* compared with NY and L4 have also higher activity against *Debaryomyces Hansenii*

Table 3.1 Antimicrobial activity of the ligands

	Inhibition zone (mm)				
Microorganisms/Compounds	L1	L2	L3	L4	L5
Escherichia coli ATCC	15.0	18.0	19.0	21.0	16.0
Enterobacter aerogenes ATCC	17.0	22.0	17.0	20.0	14.0
Staphylococcus aureus ATCC	14.0	18.0	21.0	17.0	16.0
Staphylococcusepidermidis NRRL	18.0	17.0	17.0	24.0	15.0
Klebsiella pneumoniae UC57	18.0	20.0	17.0	19.0	20.0
Bacillus cereus ATCC	12.0	12.0	12.0	14.0	14.0
Bacillus subtilis ATCC	13.0	12.0	12.0	15.0	14.0
Bacillus brevis ATCC	12.0	12.0	10.0	15.0	12.0
Bacillus megaterium ATCC	12.0	13.0	14.0	16.0	14.0
Micrococcus luteus LA	12.0	15.0	13.0	17.0	14.0
Micrococcus flavus ATCC	15.0	12.0	12.0	22.0	15.0
Salmonella typhi ATCC 1	17.0	20.0	16.0	18.0	15.0
Salmonella typhimurium CCM	14.0	16.0	12.0	15.0	15.0
Proteus vulgaris ATCC	20.0	17.0	13.0	13.0	15.0
Mycobacterium smegmatis CCM	14.0	15.0	12.0	11.0	12.0
Listeria monocytogenes ATCC	16.0	13.0	14.0	12.0	13.0
Pseudomonas aeruginosa ATCC	17.0	14.0	14.0	15.0	12.0
Pseudomonas extorquens ATCC	15.0	15.0	12.0	16.0	16.0
Pseudomonas fluorescens ATCC	14.0	16.0	16.0	14.0	14.0
Kluyveromyces fragilis NRRL	26.0	18.0	16.0	22.0	18.0
Rhodotorula rubra DSM	22.0	19.0	20.0	25.0	17.0
Candida albicans ATCC	22.0	21.0	20.0	24.0	17.0
Candida parapsilosis ATCC	18.0	16.0	17.0	22.0	16.0
Candida tropicalis ATCC	24.0	18.0	22.0	20.0	18.0
Cryptococcus neoformans ATCC	16.0	15.0	16.0	18.0	16.0
Cryptococcus laurentii ATCC	18.0	16.0	16.0	20.0	16.0
HanseniasporaguilliermondiiDSM	20.0	16.0	21.0	22.0	15.0
Debaryomyces hansenii DSM	16.0	18.0	15.0	18.0	15.0

Table 3.2 Antimicrobial activities of some standard antibiotics

Microorganisms / Antibiotics	Inhibition zone (mm)						
	AK30	SAM20	CTX30	VA30	N100	KETO20	CLT10
Escherichia coli	17	12	10	22	-	-	-
Enterobacter aerogenes	18	15	14	18	-	-	-
Staphylococcus epidermidis	23	18	15	15	-	-	-
Staphylococcus aureus	24	16	12	13	-	-	-
Klebsiella pneumoniae	20	14	13	22	-	-	-
Pseudomonas aeruginosa	19	10	54	10	-	-	-
Pseudomonas extorquens	20	14	40	14	-	-	-
Pseudomonas fluorescens	18	16	36	16	-	-	-
Proteus vulgaris	18	16	18	20	-	-	-
Bacillus cereus	16	12	14	18	-	-	-
Bacillus subtilis	20	14	16	20	-	-	-
Bacillus brevis	18	16	15	17	-	-	-
Bacillus megaterium	17	14	16	18	-	-	-
Mycobacterium smegmatis	18	21	11	20	-	-	-
Listeria monocytogenes	20	12	16	26	-	-	-
Micrococcus luteus	24	32	32	34	-	-	-
Micrococcus flavus	22	36	34	36	-	-	-
Salmonella typhi	19	18	18	18	-	-	-
Salmonella typhimurium	20	20	18	16	-	-	-
Candida tropicalis	-	-	-	-	18	18	16
Candida parapsilosis	-	-	-	-	22	20	16
Candida albicans	-	-	-	-	20	21	15
Kluyveromyces fragilis	-	-	-	-	18	16	18
Rhodotorula rubra	-	-	-	-	18	22	16
Cryptococcus laurentii	-	-	-	-	20	20	18
Cryptococcus neoformans	-	-	-	-	22	18	20
H. guilliermondii	-	-	-	-	21	24	22
Debaryomyces hansenii	-	-	-	-	16	14	18

AK30 : Amikacin 30 µg, SAM20 : Ampicillin 10 µg, CTX30 : Cefotaxime 30 µg, V30 : Vancomycin 30 µg, N100 : Nystatin 100 µg, KETO20 : Ketaconazole 20 µg, CLT10 : Clotrimazole 10 µg

Table 3.3 MIC values of the compounds

	MIC Values (µg/mL)						
Microorganisms Compounds	L1	L2	L3	L4	L5	Gent	Nyst
Escherichia coli	6.25	6.25	6.25	3.125	6.25	6,25	ND
Enterobacter aerogenes	6.25	3.125	6.25	6.25	6.25	6.25	ND
Staphylococcus aureus	12.5	12.5	6.25	12.5	12.5	25	ND
Staphylococcus epidermidis	12.5	12.5	12.5	6.25	12.5	12.5	ND
Klebsiella pneumoniae	12.5	12.5	25	12.5	12.5	6,25	ND
Bacillus cereus	50	50	50	50	50	6,25	ND
Bacillus subtilis	50	50	50	50	50	6.25	ND
Bacillus brevis	50	50	50	50	50	6.25	ND
Bacillus megaterium	50	50	50	25	50	6.25	ND
Micrococcus luteus	50	50	50	25	50	25	ND
Micrococcus flavus	50	50	50	25	50	12.5	ND
Salmonella typhi	50	25	50	25	50	6.25	ND
Salmonella typhimurium	50	50	50	50	50	6.25	ND
Proteus vulgaris	12.5	25	50	50	50	6,25	ND
Mycobacterium smegmatis	50	50	50	50	50	12,5	ND
Listeria monocytogenes	50	50	50	50	50	12,5	ND
Pseudomonas aeruginosa	25	50	50	25	50	6,25	ND
Pseudomonas extorquens	25	50	50	25	25	6.25	ND
Pseudomonas fluorescens	50	50	25	50	50	6.25	ND
Kluyveromyces fragilis	3.125	12.5	25	3.125	12.5	ND	6,25
Rhodotorula rubra	6.25	12.5	12.5	3.125	25	ND	6,25
Candida albicans	6.25	12.5	6.25	3.125	25	ND	3,125
Candida parapsilosis	12.5	25	25	6.25	25	ND	6.25
Candida tropicalis	6.25	25	12.5	12.5	25	ND	3.125
Cyrptococcus neoformans	50	50	50	25	50	ND	3.125
Cryptococcus laurentii	50	50	25	12.5	50	ND	3.125
Hanseniaspora guilliermondii	12.5	25	12.5	6.25	50	ND	3,125
Debaryomyces hansenii	50	12.5	50	12.5	50	ND	12,5

ND : Not done

CHAPTER 4

CONCLUSION

In this thesis; the synthesis and characterization of new macromolecule ligands and complexes of were described.

These macromolecule ligands and complexes have been characterized using FT-IR, FT-Raman, ^{1}H-NMR, ^{13}C-NMR, and elemental analysis. Vibrational and NMR spectroscopies provided confirming the structures of the compounds. Especially Far FT-IR was very important for the characterization of alkyne containing componds

The measurement of long range coupling constant by NMR spectroscopy, is very helpful for the structure elucidation and conformational analysis of organic compounds.

The synthesized ligands were evaluated for their antibacterial and antifungal actions. Both antibacterial and antifungal tests were carried out by using disk diffusion and minimum inhibitory concentration method. According to Table 3.1 and 3.3, compouds showed higher antibacterial and antifungal activity against some bacteria and yeast cultures compared with the standards, but the some ligands have lower antimicrobial activity compared with the standard antimicrobial reagents.

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