Jotyar Faris MOHAMMED

SYNTHESIS, STRUCTURAL, CHARACTERIZATION, ANTIMICROBIAL PROPERTIES INVESTIGATION OF NOVEL MACROCYCLIC AMIDE AND AMINE CONTAINING COMPOUNDS

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Jotyar Faris MOHAMMED

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in

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APPROVAL PAGE

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

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M.S. Thesis – Chemistry
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Thesis Supervisor: Prof. Naz Mohammed ATABAY

ABSTRACT

Series of new multi dentate macromolecular compounds having aza, oxo and oxa donor centre featuring di-amide, di-amine, and macrocyclic with ether units are synthesized. Generic synthetic pathways for the new starting materials such as di-amide, di-amine and the macrocyclic compounds were presented in this thesis. The compounds were described by elemental analyses, FT-IR, ^{1}H and ^{13}C NMR spectral data. Minimal inhibitory concentration (MIC) dilution method and disk diffusion method in dimethyl sulfoxide (DMSO-d_6) were used for evaluating the antimicrobial activities of the compounds against several bacteria and yeast cultures. The results were compared with commercial antibiotic and antifungal agents. Structure activity relationships were also discussed.

Keywords: Antimicrobial, Di-amide, Di-amine, Dilution method, Macrocyclic.

YENİ AMİD VE AMİN İÇERİKLİ MAKROSİKLİK BİLEŞİKLERİN SENTEZİ, YAPISAL KARAKTARİZASYONU VE ANTİMİKROBİYAL ÖZELLİKLERİNİN İNCELENMESİ

Jotyar Faris MOHAMMED

Yüksek Lisans Tezi – Kimya
Haziran 2015

Tez Danışmanı: Prof. Naz Mohammed ATABAY

ÖZET

Aza, okso ve oksa verici merkezi içeren di-amit, di-amin ve makrosiklik eter bileşikleri olmak üzere yeni multi dentat makromoleküller sentezlenmiştir. Genel sentetik yollarla di-amit, di-amin ve makrosiklik bileşikler gibi başlangıç malzemeler de sentezlenmiştir. Tüm bu bileşiklerin yapısal özellikleri, elemental analiz, FT-IR, ^{1}H ve ^{13}C NMR spektroskopi yöntemleri ile karakterize edilmiştir. Bazı makrosiklik bileşiklerin antimikrobiyal aktiviteleri (DMSO-d_6 içinde) disk difüzyon metodu ve minimum inhibitor konsantrasyonu seyreltme yöntemiyle de birçok bakteri ve maya kültürüne karşı uygulanarak incelendi. Sonuçlar ticari antibiyotik ve antifungi ajanlarla karşılaştırılarak yapı-aktivite ilişkileri tartışıldı.

Anahtar Kelimeler: Antimikrobiyel, Di-amin, Di-amid, Difüzyon Metodu, Makrosiklik.

Dedicated to my beloved mother, loving fiancée, little brother
and all members of my family

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The deepest appreciation to my mother and all member of my family for their encouragement, motivation and patience. I would like to express my special thanks to my fiancée Maryam for her understanding and supporting me during this thesis.

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LIST OF SYMBOLS AND ABBREVIATIONS

SYMBOL/ABBREVIATION

c_1	2-acetamidophenol
c_2	3-acetamidophenol
c_3	4-acetamidophenol
e_1	1,3-Bis(2-acetamidophenoxymethyl)mesitylene
e_2	1,3-Bis (3-acetamidophenoxymethyl) mesitylene
e_3	1,3-Bis(4-acetamidophenoxymethyl)mesitylene
f_1	1,3-Bis(2-aminophenoxymethyl)mesitylene
f_2	1,3-Bis(3-aminophenoxymethyl)mesitylene
f_3	1,3-Bis(4-aminophenoxymethyl)mesitylene
m_1	4,10(1,3)-dibenzina-1(1,3)-mesitylene-7(1.3)-pyridine-5,9-diaza-6,8-dioxo- 3,11-dioxa-2,12-dimethylene-dodecacyclophane
m_2	4,7,10(1,3)-tribenzina-1(1,3)-mesitylene-5,9-diaza-6,8-dioxo-3,11-dioxa-2,12-dimethylene-dodecacyclophane
m_3	4,10(1,4)-dibenzina-1(1,3)-mesitylene-7(1.3)-pyridine-5,9-diaza-6,8-dioxo-3,11-dioxa-2,12-dimethylene-dodecacyclophane
m_4	4,10(1,4)1(1,3)-tribenzina-1(1,3)-mesitylene-5,9-diaza-6,8-dioxo-3,11-dioxa-2,12-dimethylene-dodecacyclophane
^{13}C NMR	Carbon (13) NMR Spectroscopy
^{1}H NMR	Proton NMR Spectroscoy
DMSO-d_6	Deuterated Dimethyl sulphoxide
FT-IR	Fourier Transform Infrared Spectroscopy
MP	Melting Point
NMR	Nuclear Magnetic Reasonance Spectroscopy
ppm	Parts per million
GEN	Gentamycin

NY	Nystatin
AM	Ampicillin
CT	Cefotaxime
VA	Vancomycin
OF	Ofloxacin
TE	Tetracycline
KE	Ketaconazole
CL	Clotrimazole
δ	Bending vibration
δ_C	Carbon chemical shift
δ_H	Proton chemical shift
υ	Streching vibration
s	Singlet
d	Doublet
t	Triplet
as	Asymmetric
s	Symmetric

CHAPTER 1

INTRODUCTION

It is common that antibiotics are normally the core prevents or killers of the development and growth of microorganism. Nowadays, these cores regard as achievements that participate in production of drugs that known as antibiotic drugs as well as antimicrobals are falsely formed multiply [1]. Antimicrobial has varying effects upon human and animal cells and bacteria, fungi, or protozoa in which these effects regard as its advantage. Moreover, the purposes of antimicrobial drugs are to reply and toxicity toward the microbes. Interestingly, it has no toxicity or a minimum on animals and human. The activities of antimicrobial goals are divided into five categories that can be showed as Inhibition of Cell Wall Synthesis, Inhibition of Protein Synthesis, Inhibition of Nucleic Acid Synthesis, cell membrane sterols (antifungal agents) affection and inhibition of unique metabolic steps [2].

According to the productions of antimicrobial (within all kingdoms) there are great importance to the usage of the extract component from original mixture [3,4], as well as different components and structural to be more active to inhibit and kill microorganisms especially for those which resisted [5-8]. These lead the study and explore the elements that cause macrocyclic compound to become considered in a wide range, and using as drugs against bacteria, fungi and viruses [9,10]. In addition, the Marine cyanobacteria, green algae, Sponges marine, tropical phoma that all are the natural causes of making macrocycles [11,12].

As for the preparation and design of macrocyclic mixtures, it is noticed that have hetero-multi-donor center. Since the role of mixed multi-function materials in: as ligands in coordination chemistry and organic chemistry, as antimicrobial and antitumor in medicinal chemistry and as photography and dyes in material applications, provides a result that is widely suggested for researching. Due to the structural influence, certain macrocyclic products have fundamental biological roles in their nature, such as storage

storage of oxygen and conveyance in mammalian and other biological respiratory systems, photosynthesis and enzymatic activity [13]. It's worth mentioning that the macrocycle is an important structural pattern among bioactive natural products, and informs advantageous physicochemical and pharmacological aspects. A subtle balance between flexibility and inflexibility is preserved by macrocyclic geometry that is widely assumed the binding, soluble, and permeability to biological aims [14].

There are wide ranges of biological properties in macrocyclic ligands and their metal complexes, for example, many works have been done on transition metal complexes with macrocyclic ligands such as antifungal, anti- bacterial, anticancer, antiviral, anti-HIV activity [15]. In addition, the use of the chemistry of macrocycles, in metalloproeins as models for metal binding sites of protein, in chelate therapy as therapeutic agents for the treatment, catalysis, and in the cancer treatment, have provoked a noticeable attention in recent years [16]. Moreover, macrocyclic compounds have contrast-enhancing agents i.e. medical applications for magnetic resonance imaging (MRI), in nuclear medicine for radioimmunoscintigraphy or radioimmunotherapy [17].

It can be mentioned that macrocyclic compounds and their derivatives are suitable landlord for metal anions, organic cation invitee and neutral molecules. The combinations of host–guest chemistry and metal-ion of macrocyclic is very beneficial in basic studies such as biological and phase transfer catalysis studies [18]. Multi-donor ligands are the macrocyclic that work through its ligand or ligands. So, the complex or ligand mixing donor atoms are so important in chemistry and organic chemistry. These ligands also combine with numerous metals and produce constant complexes [19]. Besides that, the accommodate apposite groups in the aliphatic or aromatic chains of the amino precursors and formyl- or keto- can be regarded as macrocyclic systems' function [20]. Another intermediary in encouraging the curiosity in macrocycles is having the heteroatoms or functional group of heteroatomic in the macrocyclic complexes. Oxygen, sulfur and nitrogen donor atoms in the structure of acyclic ligands act as worthy chelating agents [21]. Some ligands like the thiazole and its derivatives with potential nitrogen and sultur bands have clear attention in all the structural chemistry of their rank in pharmaceutical and medicinal field and their multifunctional coordination approach. They also display biological happenings involving antibacterial, anticancer, antitumor, antifungal, antiproliferative, herbicidal [22]. It can be said that

the variability and of many donor site presences leads to the characterization and property of ligands.

Distinct ions or biomolecules may have the ability to react and bind, theories related to the theoretical expectations such as Irving Williams' sequence of constancy and hard soft acid center values of Pearson Parr are depended by the different values that govern the selectivity and specificity of macromolecules [23]. Blended donor atoms and the multi-donor ligands of these ligands are also significant since ligands admits a great accessibility in having several possible contributor center and their adaptability to tie with biomolecules [24].

There is a worth mentioning fact that N atom has a main role in the organizing of metals at the active sites of several metallobiomolecules [25]. Obviously, during the last few years, the Synthetic improvement of novel macrocyclic peptide antibiotics against bacteria has gone through affected changes involving biphenomycin B , vancomycine type glycopeptide antibiotics. Cyclic peptides with open pores are helpful and useful since they are means of transport for biologically important ions and neutral molecules. In addition, the Supramolecular amides are also used as molecular receptors and in molecular identification of biologically cooperating substrates including anti-HIV active macrocyclic amides [26]. The remarkable field of chemistry is the study of well-ordered metal including macrocycles. It has also concerned the attention of both bioinorganic and inorganic chemists in recent years [27]. Many macrocyclic combinations are produced by the developments with the help of chemistry; including the proper coordination geometry organometallic composite and several organic ligands. Additionally, hydrogen bonds, van der Waals forces, non-bonded contacts and C–Hp interactions i.e. non-covalent weak molecular forces able to combine these metallic subunits into easier and more interesting supramolecular bases, that have been extensively researched in structural chemistry and biology and the pharmaceutical sciences [28].

It is the fact that synthesis of macrocycles and their metal complexes are mimic and macrocyclic molecules naturally appear in their functional and structural aspect due to wealthy chemical prosperities. Furthermore, the increased kinetic and thermochemical solidity of the complexes in respect to their breakdown is regarded as one of these properties, which is due to lesser responsibility and larger connotation measurements than the complexes with homologous open-chain chelating ligands [29]. The creations of macrocyclic complexes rely on the size of the macrocycles, nature of

its presenter atoms and in the difficult conduct of the anions took part in coordination [30]. Usually the coordination sites, ring size and electronic effect of the ligand framwork in the macrocyclic framworks influence the redox and magnetic exchange properties of the metal centers. Thus, the synthesis and studies of model systems are critical and give additionally comprehension concerning the agreeable phenomena, electron exchange and attractive cooperation between the metal focuses [31]. Numbers of factors involve in the stability of macrocyclic complexes such as the numbers and sorts of contributor molecules existing in the ligand and their relative positions inside of the macrocyclic skeleton, additionally the size and number of the chelate ring molded on complexation [32]. It is illuminated that 2,6-Pyridinedicarboxylic acid can act as an intriguing ligand because of its capacity to shape powerful covalent bonds. Additionally, there is a possible impact of nitrogen particle on the coordination mode and the spatial departure of the two carboxylate groups, enclosed to the same aromatic ring that manufactures either a cyclooligomeric ring or a polymeric chain structure [33].

Three diamides, three di-amines and four macrocyclic compounds were synthesized and characterized by FT-IR, H^1 NMR, ^{13}C NMR, and their melting and decomposition temperatures were also reported in this thesis. The three diamides were synthesized by the reaction of ortho, meta and para acetamidophenol with 2,4-Bis(chloromethyl)mesitylene. The three diamines were synthesiszed by reduction of diamides by using sodium hydroxide. For synthesis four macrocyclic compounds meta and para diamides were reacted with pyridine-2,6-dicarbonyl dichloride and isophthaloyl chloride. Other workups such as purification and some intermediary synthesis were also reported. Purification, filtaration, some intermediary synthesis and other workups were also reported.

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 Electro-thermal 9100 melting point apparatus. FT-IR spectra were recorded on the Bruker Alpha-P in the range of 4000-400 cm^{-1}. ^{1}H (400 MHz) and ^{13}C (100 MHz) spectra were recorded in DMSO-d_6 at ambient temperature on a Bruker Ultrashield Plus 400MHz instrument. The antimicrobial activities are evaluated against Gram-positive and Gram-negative bacteria and yeast culters unsing both disk diffucion and dilution methods. The analytical and spectral data and physical properties were summarized for each experiment.

2.1.1 General Synthesis Pathway

Ortho, meta and para-aminophenol were added to distill water with continuous stirring at room temperature. Then acetic anhydride was slowly added to the solution for three hours. Three acetamidophenols were produced. The resulting products were washed, filtered and dried (c_1-c_3). The di-amides (e_1-e_3) were synthesized by dissolving potassium hydroxide in ethanol, then acetamidophenols (c_1-c_3) were added to the solution to produce potassium salts of acetamidophenols. 2,4-Bis(chloromethyl) mesitylene was added slowly to the potassium salts of acetamidophenols. The resulting di-amides were washed, filtered, and dried (e_1-e_3). In the third step of reaction sodium hydroxide was added to ethanol until it was completely dissolved, di-amides (e_1-e_3) were added to the solution with constant heating and stirring for several days. The obtained di-amines (f_1-f_3) were washed, filtered and dried under vacuum. Last step of reaction pyridine in CH_2Cl_2, pyridine-2,6-dicarbonyl dichloride in CH_2Cl_2,

isophthaloyl chloride in CH_2Cl_2 and diamines (f_2 and f_3) in CH_2Cl_2, were reacted to produce four macrocyclic compounds (m_1-m_4). The reactions were schematically presented as follows:

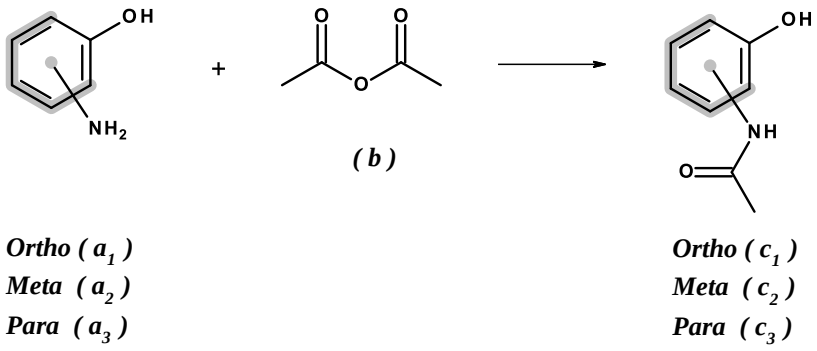

Figure 2.1 Synthesis of acetamidophenols.

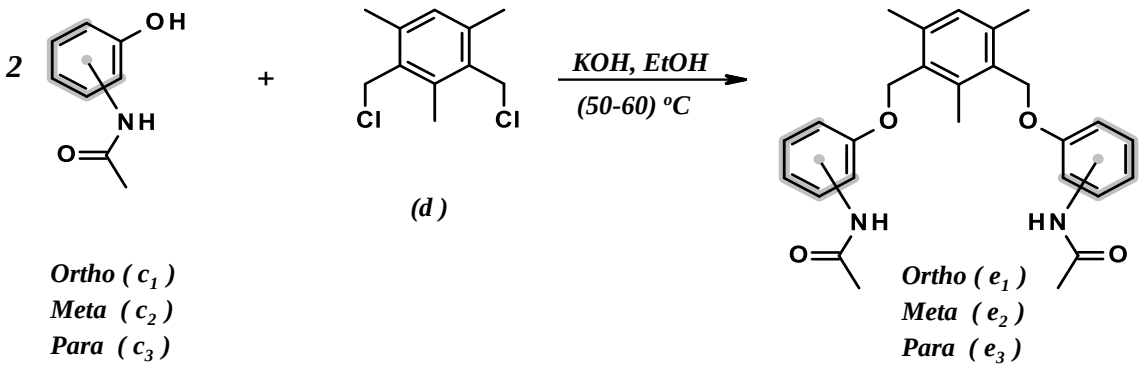

Figure 2.2 Synthesis of di-amides.

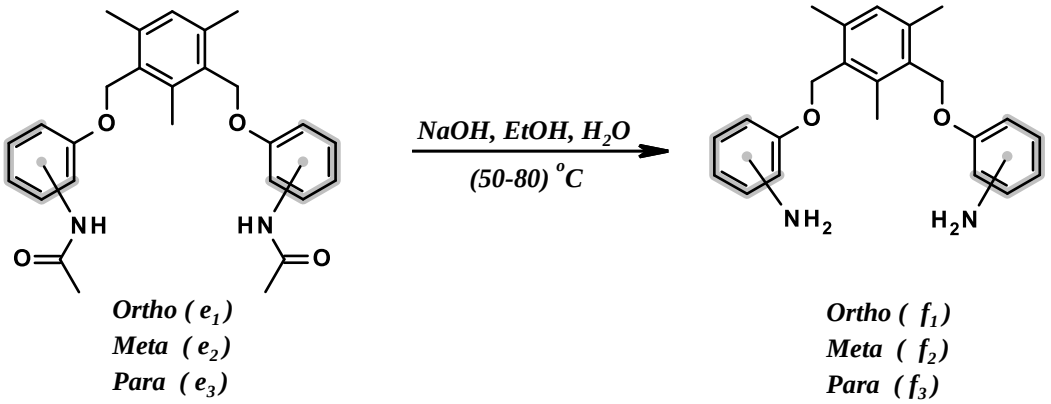

Figure 2.3 Synthesis of di-amines.

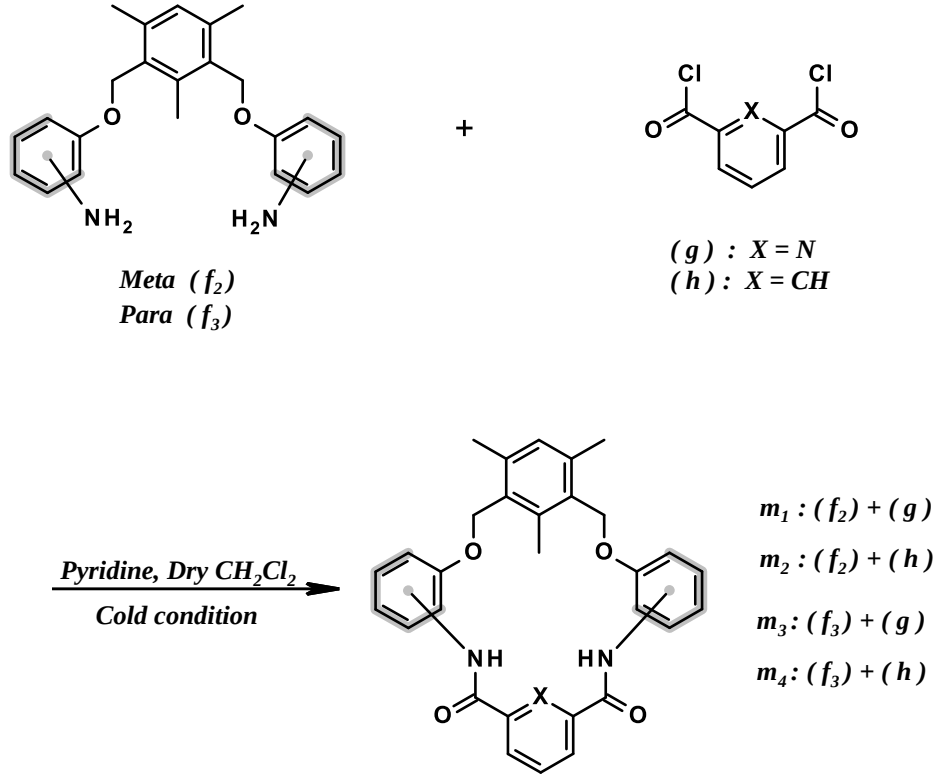

Figure 2.4 Synthesis of macrocyclic compounds.

2.2 SYNTHESIS OF ACETAMIDOPHENOLS

2.2.1 2-acetamidophenol (c_1)

For synthesis of ortho-acetamidophenol, 2-aminophenol (1.63 g, 15 mmol) was added to distill water (25 ml) and stirred for (15 min) in room temperature. Then acetic anhydride (1.63 g, 16 mmol) was added drop by drop to the solution and stirred for (3 h) in room temperature. The resulting product was washed by distill water 3 times, filtered and dried. Pale yellow precipitate was obtained. 1.88 g, yield 83%, MP: 209-215^{o}C. FT-IR (solid, cm^{-1}): 3403 ν(NH), 3085–2981 ν(C═H), 2972–2612 $\nu_s(CH_3)$, $\nu_{as}(CH_3)$, 1659 ν(C=O), 1596 ν(C═C), 1285 ν(C–O), 768 δ(C═H).

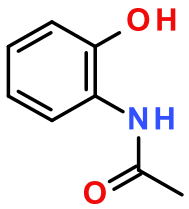

Figure 2.5 Structure of acetamidophenol (c_1).

2.2.2 3-acetamidophenol (c_2)

This compound was prepared by a method similar to that described for **(a_1)** by using 3-aminophenol (1.63 g, 15 mmol), acetic anhydride (1.63 g, 16 mmol).white product was formed. 1.99g, yield 88%, MP: 146-151^{o}C. FT-IR (solid, cm^{-1}): 3325 ν(NH), 3060–3000 ν(C═H), 2970–2720 $\nu_s(CH_3)$, $\nu_{as}(CH_3)$, 1667 ν(C=O), 1515 ν(C═C), 1265 ν(C–O), 771 δ(C═H).

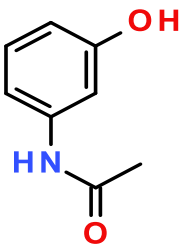

Figure 2.6 Structure of acetamidophenol (c_2).

2.2.3 4-acetamidophenol (c_3)

This compound was prepared by a method similar to that described for **(a_1)** by using 3-aminophenol (1.36 g, 15 mmol), acetic anhydride (1.36 g, 16 mmol).white precipitate was formed. 1.92 g, yield 85%, MP: 170-173ºC. FT-IR (solid, cm^{-1}): 3325 ν(NH), 3170–3050 ν(C═H), 2960–2720 $\nu_s(CH_3)$, $\nu_{as}(CH_3)$, 1666 ν(C=O), 1565 ν(C═C), 1261 ν(C–O), 797 δ(C═H). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H ppm: 2.0 (s, 3H, CH_3), 6.71 (d, 2H), 7.37 (d, 2H), 9.15 (s, 1H, OH), 9.66 (S, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C ppm: 23.68 (C_a), 114.99 ($2C_b$), 120.89 ($2C_c$), 130.96 (C_d), 153.11 (C_e), 167.56 (C_f).

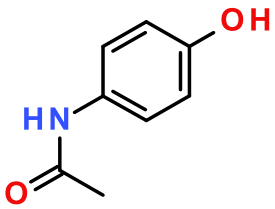

Figure 2.7 Structure of acetamidophenol (c_3).

2.3 SYNTHESIS OF DI-AMIDES

2.3.1 1,3-Bis(2-acetamidophenoxymethyl)mesitylene (e_1)

After the addition of (600 mg , 10.69 mmol) of potassium hydroxide KOH to (35 mL) of ethanol, the solution was stirred and heated in 60°C until the potassium hydroxide was completely dissolved in ethanol. Then, ortho-acetamidophenol (1.51 g, 10 mmol) was added to the solution and stirred for 1 hour. Later, (1.085 g, 5 mmol) of 2,4-Bis(chloromethyl)mesitylene was slowly added to the resulting potassium salt of ortho-acetamidophenol for 30 minutes. The mixture was stirred overnight at the same temperature. Yellow precipitate was formed. The product was filtered, washed with cold distilled water for 3 times and dried under vacuum. Weight 1.93 g, yield 86%, MP: 79-85ºC. FT-IR (solid, cm^{-1}): 3422 υ(NH), 3064-3007 ν(C═H), 2965-2917 $\nu_{as}(CH_3)$, $\nu_s(CH_3)$, $\nu_{as}(CH_2)$, $\nu_s(CH_2)$, 1676 υ(C=O), 1595 ν(C═C), 1443 δ(C-H), 1241 υ(C–O), 745 δ(C═H). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H ppm, J_{Hz}: 2.0 (s, 6H, $2CH_3$), 2.37 (s, 6H, $2CH_3$), 2.39 (s, 3H, CH_3), 5.11 (s, 4H, CH_2), 6.97 (m, 2H), 7.03 (s, 1H), 7.14 (t,

J=7.3, 2H), 7.31(d, J=7.8, 2H), 7.8(d, J=7.3, 2H), 8.87 (br, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C ppm: 15.9 (C_a), 19.36 ($2C_b$), 23.7 ($2C_c$), 65.96 ($2C_d$), 113.54 ($2C_e$), 120.73 ($2C_f$), 123.41 ($2C_g$), 124.79 ($2C_h$), 127.78 ($2C_i$), 129.91 (C_j), 130.94 ($2C_k$), 138.44 ($2C_l$), 138.95 (C_m), 149.93 ($2C_n$), 168.31 ($2C_o$).

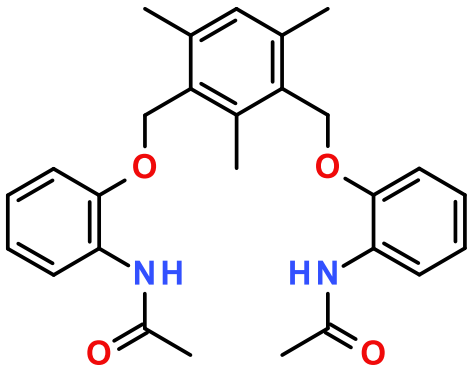

Figure 2.8 Structure of di-amide (e_1).

2.3.2 1,3-Bis (3-acetamidophenoxymethyl) mesitylene (e_2)

This compound was synthesized by following the previous procedure by using (600 mg , 10.69 mmol) of potassium hydroxide KOH, meta-acetamidophenol (1.51 g, 10 mmol) and (1.085 g, 5 mmol) of 2,4-Bis(chloromethyl)mesitylene. White precipitate was obtained. Weight 2.03 g, yield 91%, MP: 100-107^{o}C. FT-IR (solid, cm^1): 3283 υ(NH), 3085-3030 υ(C=H), 2970-2890 $\nu_{as}(CH_3)$, $\nu_s(CH_3)$, $\nu_{as}(CH_2)$, $\nu_s(CH_2)$, 1664 υ(C=O), 1599 υ(C=C), 1480 δ(C-H), 1259 υ(C–O), 770 δ(C=H). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H ppm, J_{Hz}: 2.05 (s, 6H, $2CH_3$), 2.1 (s, 6H, $2CH_3$), 2.32 (s, H, CH_3), 5.02 (s, 4H, CH_2), 6.77 (d, J=7.3, 2H), 7.01 (s, 1H), 7.16 (m, 2H), 7.23 (m, 2H), 7.38 (br, 2H), 9.95 (s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C ppm: 15.14 (C_a), 19.26 ($2C_b$), 24.03 ($2C_c$), 64.43 ($2C_d$), 105.66 ($2C_e$), 108.92 ($2C_f$), 111.62 ($2C_g$), 129.48 ($2C_h$), 131.1 ($2C_i$), 132.76 (C_j), 137.86 ($2C_k$), 138.29 (C_l), 140.51 ($2C_m$), 159.15 ($2C_n$), 168.34 ($2C_o$).

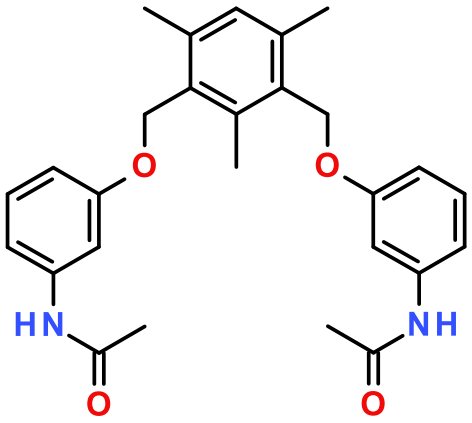

Figure 2.9 Structure of di-amide (e_2).

2.3.3 1,3-Bis(4-acetamidophenoxymethyl)mesitylene (e_3)

This compound was prepared by following the procedure of (e_1) via using (600 mg, 10.69 mmol) of potassium hydroxide KOH, para-acetamidophenol (1.51 g, 10 mmol) and (1.085 g, 5 mmol) of 2,4-Bis(chloromethyl)mesitylene. A white precipitate was obtained. Weight 1.9 g, yield 85%, MP: 205-207^{o}C. FT-IR (solid, cm^{-1}): 3246 υ(NH), 3070-3023 υ(C═H), 2970-2886 $\nu_{as}(CH_3)$, $\nu_s(CH_3)$, $\nu_{as}(CH_2)$, $\nu_s(CH_2)$, 1660 υ(C=O), 1607 υ(C═H), 1500 δ(C-H), 1228 υ(C–O), 819 δ(C═H). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H ppm, J_{Hz}: 2.03 (s, 6H, $2CH_3$), 2.1 (s, 6H, $2CH_3$), 2.32 (s, 3H, CH_3), 5.01 (s, 4H, CH_2), 6.99 (m, 5H), 7.52 (d, J=8.5, 4H), 9.83 (s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C ppm: 15.15 (C_a), 19.30 ($2C_b$), 23.78 ($2C_c$), 64.75 ($2C_d$), 114.61 ($2C_e$), 120.53 ($2C_f$), 129.74 (C_g), 131.21 ($2C_h$), 132.78 ($2C_i$), 137.78 ($2C_j$), 138.29 (C_k), 154.7 ($2C_l$), 167.72 ($2C_m$).

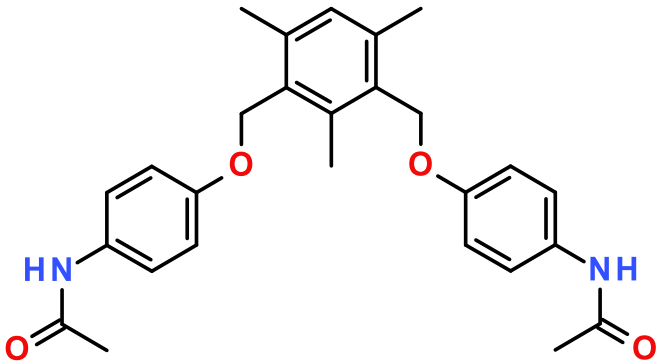

Figure 2.10 Structure of di-amide (e_3).

2.4 SYNTHESIS OF DI-AMINE

2.4.1 1,3-Bis(2-aminophenoxymethyl)mesitylene (f_1)

Addition of (1.4 g, 35 mmol) of sodium hydroxide (NaOH) and (10 ml) distills water to (30 ml) of ethanol. The solution was stirred and heated in 60°C until NaoOH was completely dissolved in ethanol. Di-amide (e_1) (2.3 g, 5.15 mmol) was added to the solution with continuous stirring for four days. The obtained product was washed three times with cold distillable water, then filtered and dried under vacuum. A light brown precipitate was formed. Weight 1.42 g, yield 76%, MP: 132-136°C. FT-IR (solid, cm^{-1}): 3462,3370 $\upsilon(NH_2)$, 30888-3018 $\upsilon(C{=}H)$, 2951-2908 $\nu_{as}(CH_3)$, $\nu_s(CH_3)$, $\nu_{as}(CH_2)$, $\nu_s(CH_2)$, 1606 $\upsilon(C{=}C)$, 1500 δ (C-H), 1201 $\upsilon(C–O)$, 739 $\delta(C{=}H)$. ^1H NMR (400 MHz, DMSO-d_6) δ_H ppm, J_{Hz}: 2.36 (s, 6H, $2CH_3$), 2.38 (s, 3H, CH_3), 4.55 (s, 4H, $2NH_2$), 5.04 (s, 4H, CH_2), 6.58 (td, J=7.5, 2H), 6.66 (m, 2H), 6.72 (d, J=7.3, 2H), 7.02 (d, J=3.8 , 2H), 7.04 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C ppm: 14.86 (C_a), 19.29 ($2C_b$), 64.93 ($2C_c$), 112.27 ($2C_d$), 114.05 ($2C_e$), 116.31 ($2C_f$), 121.21 ($2C_g$), 129.77 (C_h), 131.41 ($2C_i$), 137.89 ($2C_j$), 137.94 ($2C_k$), 138.67 (C_l), 145.88 ($2C_m$).

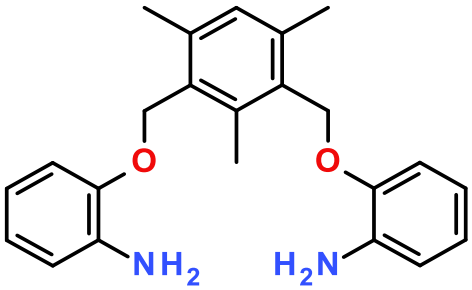

Figure 2.11 Structure of di-amine (f_1).

2.4.2 1,3-Bis(3-aminophenoxymethyl)mesitylene (f_2)

This compound was synthesized by following the above procedure by using (1.4 g, 35 mmol) of sodium hydroxide (NaOH), Di-amide (e_2) (2.3 g, 5.15 mmol) and the reaction was heated under 70°C. A pale yellow precipitate was obtained. Weight 1.5 g, yield 80.5%, MP: 122-127°C. FT-IR (solid, cm^{-1}): 3459,3367 $\upsilon(NH_2)$, 3060-3017 $\upsilon(C{=}H)$, 2919-2860 $\nu_{as}(CH_3)$, $\nu_s(CH_3)$, $\nu_{as}(CH_2)$, $\nu_s(CH_2)$, 1588 $\upsilon(C{=}C)$, 1490 δ(C-H), 1181 $\upsilon(C–O)$, 736 $\delta(C{=}H)$. ^1H NMR (400 MHz, DMSO-d_6) δ_H ppm, J_{Hz}: 2.30 (s, 3H,

CH_3), 2.32 (s, 6H, $2CH_3$), 4.94 (s, 4H, $2NH_2$), 5.08 (s, 4H, CH_2), 6.21 (m, 4H), 6.25 (d, J=2, 2H), 6.94 (m, 2H), 6.99 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ_C ppm: 14.83 (C_a), 19.25 ($2C_b$), 64.04 ($2C_c$), 100.18 ($2C_d$),102.08 ($2C_e$), 107.1 ($2C_f$), 129.56 ($2C_g$), 131.38 ($2C_h$), 132.72 (C_i), 137.62 ($2C_j$), 138.19 (C_k), 149.98 ($2C_l$), 159.97 ($2C_m$).

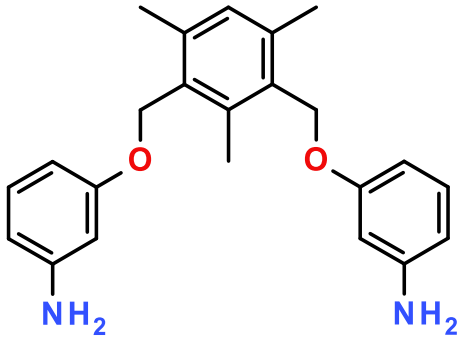

Figure 2.12 Structure of di-amine (f_2).

2.4.3 1,3-Bis(4-aminophenoxymethyl)mesitylene (f_3)

This compound was prepared by the same procedure of (c_1) by using (1.4 g ,35 mmol) of sodium hydroxide (NaOH) , Di-amide (e_3) (2.3 g, 5.15 mmol) and the reaction was heated under 80°C . A light-brown precipitate was obtained.Weight 1.33 g, yield 71%, MP: 204-208ºC. FT-IR (solid, cm^{-1}): 3453,3367 υ(NH_2), 3050-3003 υ(C═H), 2984-2906 $\nu_{as}(CH_3)$, $\nu_s(CH_3)$, $\nu_{as}(CH_2)$, $\nu_s(CH_2)$, 1611 (C═C), 1503 δ(C-H), 1213 υ(C–O), 823 δ(C═H). ^1H NMR (400 MHz, DMSO-d_6) δ_H ppm, J_{Hz}: 2.31 (s, 6H, $2CH_3$), 2.32 (s, 3H, CH_3), 4.66 (s, 4H, $2NH_2$), 4.87 (s, 4H, CH_2), 6.55 (d, J=8.5, 4H), 6.78 (d, J=8.5, 4H), 6.95 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C ppm: 14.86 (C_a), 19.30 ($2C_b$), 65.31 ($2C_c$), 114.86 ($2C_d$), 115.75 ($2C_e$), 129.60 (C_f), 131.64 ($2C_g$), 137.44 ($2C_h$), 138.13 (C_i), 142.7 ($2C_j$), 150.28 ($2C_k$).

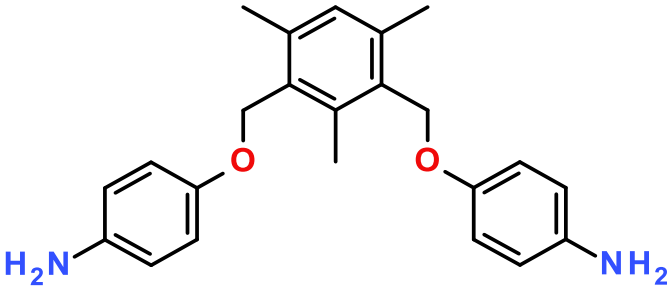

Figure 2.13 Structure of di-amine (f_3).

2.5 SYNTHESIS OF MACROCYCLICS

2.5.1 4,10(1,3)-dibenzina-1(1,3)-mesitylene-7(1.3)-pyridine-5,9-diaza-6,8-dioxo-3,11-dioxa-2,12-dimethylene-dodecacyclophane (m_1).

Three-head round bottom flask (THRBF), two dropping flask and argon gas were used for synthesis this macrocyclic compound. (50 ml) of dichloromethane and (5 ml) of pyridine were added to the (THRBF) . Di-amin (f_2) (362 mg,1mmol) was dissolved in (50 ml) of dichloromethane then it was added to one of the dropping flasks. On the other hand, dissolving 2,6-pyridinedicarbonyle dichloride (204 mg, 1mmol) in (50 ml) dichloromethane then added to other dropping flask. The solution was stirred in 0^oC under argon gas. Di-amine (f_2) and 2,6-pyridinedicarbonyle dichloride were released to (THRBF) drop by drop for five hours. After that the reaction was stirred for 24 hours in room temperature. the resulting product was washed with cold distill water and acetone .then it was filtered and dried. Pale yellow precipitate was obtained. Weight 271 mg, yield 55%, decompose at 208^0C. FT-IR (solid, cm^{-1}): 3375 υ(NH), 3098-3040 υ(C≕H), 2960-2930 $\nu_{as}(CH_3)$, $\nu_s(CH_3)$, $\nu_{as}(CH_2)$, $\nu_s(CH_2)$, 1670 υ(C=O), 1600 υ(C≕C), 1442 δ(C-H), 1292 υ(C–O), 807 δ(C≕H). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H ppm: 2.15 (s, 6H, $2CH_3$), 2.34 (s, 3H, CH_3), 5.09 (s, 4H, CH_2), 6.91 (m, 4H), 7.39 (m, 2H), 7.76 (m, 3H), 8.34 (m, 3H), 11.13 (s, 2H, 2NH).

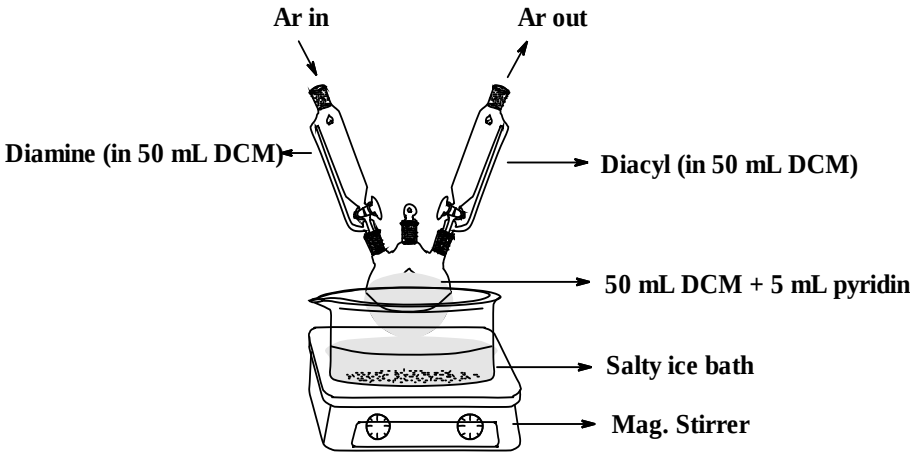

Figure 2.14 Experimental reaction's system for synthesis of macrocyclics.

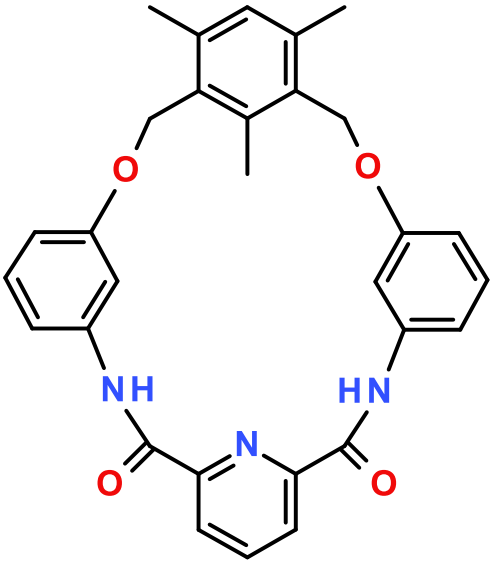

Figure 2.15 Structure of macrocyclic (m_1).

2.5.2 4,7,10(1,3)-tribenzina-1(1,3)-mesitylene-5,9-diaza-6,8-dioxo-3,11-dioxa-2,12-dimethylene-dodecacyclophane (m_2).

The same procedure for preparation of previous macrocyclic was repeated for synthesis of this compound by using di-amine (f_2) (362 mg,1 mmol) and isophthaloyl chloride (203 mg, 1 mmol). Yellow precipitate was formed. Weight: 300 mg, yield 61%, decompose at 215°C. FT-IR (solid, cm^{-1}): 3298 υ(NH), 3070-3015 υ(C=H), 2959-2910 $\nu_{as}(CH_3)$, $\nu_s(CH_3)$, $\nu_{as}(CH_2)$, $\nu_s(CH_2)$, 1667 υ(C=O), 1599 υ(C=C), 1490 δ(C-H), 1243 υ(C–O), 769 δ(C=H). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H ppm, J_{Hz}: 2.1 (s, 3H, CH_3), 2.35 (s, 6H, $2CH_3$), 5.07 (s, 4H, CH_2), 6.83 (m, 2H), 7.02 (s, 1H), 7.3 (m, 2H), 7.46 (d, J=7.8, 2H), 7.6 (m, 2H), 7.7 (s, 1H), 8.15 (d, J=6.8, 2H), 8.56 (m, 1H), 10.45 (s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C ppm: 14.92 (C_a), 19.30 ($2C_b$), 64.55 ($2C_c$), 106.86 ($2C_d$), 110.0 ($2C_e$), 112.92 ($2C_f$), 126.94 (C_g), 128.62 (C_h), 129.51 ($2C_i$), 130.66 (C_j), 131.09($2C_k$), 135.12 ($2C_l$), 137.92 ($2C_m$), 138.35 ($2C_n$), 140.24 (C_o),159.11 ($2C_p$), 165.06 ($2C_q$).

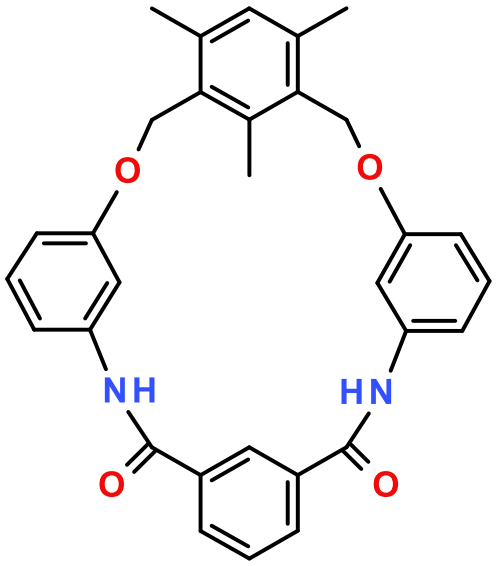

Figure 2.16 Structure of macrocyclic (m_2).

2.5.3 4,10(1,4)-dibenzina-1(1,3)-mesitylene-7(1.3)-pyridine-5,9-diaza-6,8-dioxo-3,11-dioxa-2,12-dimethylene-dodecacyclophane (m_3).

The same procedure for preparation of (m_1) macrocyclic was repeated by using diamine (f_3) (362 mg,1mmol) and pyridine-2,6-dicarbonyl dichloride (204 mg, 1 mmol). Brown precipitate was formed. Weight 286 mg, yield 58%, MP: 257-262^{o}C. FT-IR (solid, cm^{-1}): 3292 υ(NH), 3060-3010 υ(C=H), 2957-2906 $\nu_{as}(CH_3)$, $\nu_s(CH_3)$, $\nu_{as}(CH_2)$, $\nu_s(CH_2)$, 1664 υ(C=O), 1508 υ(C=C), 1220 υ(C–O), 816 δ(C=H). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H ppm: 2.13 (s, 3H, CH_3), 2.38 (s, 6H, $2CH_3$), 5.1 (s, 4H, CH_2), 7.1 (m, 5H), 7.85 (m, 4H), 8.37 (m, 3H), 11.02 (s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C ppm: 14.95 (C_a), 19.35 ($2C_b$), 64.81 ($2C_c$), 114.69 ($2C_d$), 122.23 (C_e), 122.81 ($2C_f$), 124.99 ($2C_g$), 129.77 (C_h), 131.209 (C_i), 131.28 ($2C_j$), 137.86 ($2C_k$), 138.32 ($2C_l$), 148.89 ($2C_m$), 155.78 ($2C_n$), 161.32 ($2C_o$).

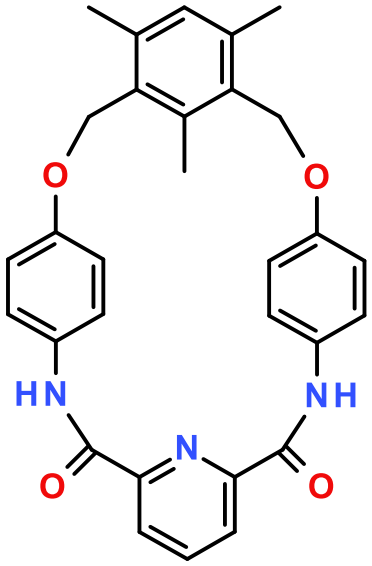

Figure 2.17 Structure of macrocyclic (m_3).

2.5.4 4,10(1,4)1(1,3)-tribenzina-1(1,3)-mesitylene-5,9-diaza-6,8-dioxo-3,11-dioxa-2,12-dimethylene-dodecacyclophane (m_4).

The same procedure for preparation of (m_1) macrocyclic was repeated by using diamine (f_3) (362 mg,1mmol) and isophthaloyl chloride (203 mg, 1 mmol). Light Brown precipitate was formed. Weight 256 mg, yield 52%, MP: 280-285^{o}C). FT-IR (solid, cm^{-1}): 3288 υ(NH), 3090-3029 υ(C═H), 2954-2932 $\nu_{as}(CH_3)$, $\nu_s(CH_3)$, $\nu_{as}(CH_2)$, $\nu_s(CH_2)$, 1649 υ(C=O), 1501 υ(C═C), 1216 υ(C–O), 816 δ(C═H). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H ppm: 2.42 (m, 9H, CH_3), 5.1 (s, 4H, CH_2), 7.1 (m, 5H), 7.74 (m, 6H), 8.23 (m, 1H), 8.59 (s, 1H), 10.4 (s, 2H, 2NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C ppm: 14.95 (C_a), 19.32 (2C_b), 64.81 (2C_c), 114.62 (2C_d), 122.02 (2C_e), 126.82 (C_f), 128.99 (C_g), 129.82 (2C_h), 130.41 (C_i), 131.19 (2C_j), 132.37 (2C_k), 135.22 (2C_l), 137.66 (2C_m), 138.34 (C_n), 155.37 (2C_o), 164.65 (2C_p).

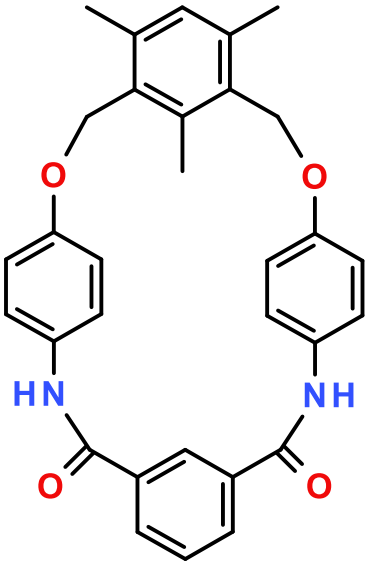

Figure 2.18 Structure of macrocyclic (m_4).

2.6 Pharmacology

2.6.1 Microorganisms

The antimicrobial activities were assessed against yeast cultures (Rhodotorula rubra DSM 70403,Candida albicans ATCC 10231, Debaryomyces hansenii DSM 70238, Kluyveromyces fragilis NRRL 2415 and Hanseniaspora guilliermondii DSM 3432), Gram-positive bacteria (Mycobacterium smegmatis CCM 2067, Listeria monocytogenes ATCC 15313, Staphylococcus aureus ATCC 6538, Micrococcus luteus La 2971, Bacillus cereus ATCC 7064,) and Gram-negative bacteria (Klebsiella pneumoniae UC57, Escherichia coli ATCC 11230, Pseudomonas aeruginosa ATCC 27853, Proteus vulgaris ATCC 8427) using both disc diffusion and dilution methods.

2.6.2 Antimicrobial screening

2.6.2.1 Disc diffusion method

The antimicrobial activity of the macrocyclic compound was assessed utilizing a somewhat adjusted disc diffusion method .The compounds were broken up in DMSO (2 mg mL-1). Disinfected antibiotic plates (6 mm, Schleicher & Schull No. 2668, Germany) were each impregnated with 20 µL of solution. To guarantee that the solvent had no impact on bacterial development, a control test was performed with test medium supplemented with DMSO in the same techniques used in the experiments. At 30 ± 0.1ºC for 24 h all the bacteria were incubated by inoculation into Nutrient Broth (Difco) and in Malt Extract Broth (Difco) for 48 h the yeasts were incubated. on Mueller-Minton Agar (Oxoid) plates (1 mL inoculum/plate) An inoculum containing 10^8 yeast cells or 10^6 bacterial cells/mL were spread. The discs injected with solutions were placed on the inoculated agar by pressing slightly and incubated at 35ºC (24 h) for the bacteria and at 25ºC (72 h) for the yeasts. On each plate an appropriate reference antibiotic disc was applied depending on the test microorganisms. In each case three times was repeated and the average was taken as final reading.

2.6.2.2 Dilution method

Antifungal and antibacterial activity tests were performed by preparing a broth micro dilution, pursuing the method outlined in Manual of Clinical Microbiology. In Nutrient Broth for 24 hours at 30^{o}C All the bacteria were activated and incubated and in Malt Extract Broth the yeasts were incubated for 48 hours. In DMSO (2 mg mL^{-1}) all the compound were dissolved and by using caution adjusted Mueller Hinton Broth (Oxoid) were diluted. From 200 μg mL^{-1} to 1.56 μg mL^{-1} Two fold serial concentrations of the compounds were employed to determine the (MIC) ranging. At 37°C (20 hours) cultures were grown and the final inoculation was approximately 10^{6} cfu mL^{-1}. At 37°C (24 hours) test cultures were incubated. The least concentration of antimicrobial agents that outcome in complete restraint of microorganisms were represented as (MIC) μg mL^{-1} . In each case three test were performed and the average was recorded.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 VIBRATIONAL SPECTROSCOPY

In most cases, secondary amides usually have their NH and C=O groups turns with each other. Cis-configured secondary amides are found practically only in forms of lactams, where the *trans*-configuration is difficult because of the high ring tension. However, the bands lie essentially in the same regions as for the *trans*-configured derivatives. The vibrational spectra of the cyclic compounds can be considered in terms of two specific wave regions: 3350–2900 cm^{-1} corresponding to the ν_{as}(N–H), ν_{s}(N–H), ν(C–H) aromatic, ν(C–H) aliphatic characteristic modes, and 1700–1500 cm^{-1} belonging to ν(CO), δ(N–H), ν(C=C) vibration modes. The bands corresponding to free aniline-NH_2 ν(N–H) (≈ 3420 cm^{-1}) and acid chloride COCl *ν*(CO) (1750 cm^{-1}) groups are not observed in the IR spectra of the products, which suggests complete condensation of the reactions and possible formation of amide linkages. The medium bands observed in the regions 3318-3308 and 3280-3245 cm^{-1} are assignable to ν_{as}(N–H) and ν_{s}(N–H) vibration modes, respectively. These modes of vibration are not dependent on the backbone conformation, but are very sensitive to the strength of a hydrogen bonding, which may cause broadening and as well as causing multiplicity in certain cases. Stretching vibration C=O is considerably lower than corresponding acyl ν(CO) vibration frequencies. The characteristic ν(CH) modes of aromatic and aliphatic groups are observed in the wave region 3100-3000 and 2990–2870 cm^{-1} respectively.

3.1.1 VIBRATIONAL SPECTROSCOPIC STUDIES OF DI-AMIDE, DI-AMINE AND MACROCYCLIC COMPOUNDS

Di-amides ($e_1 – e_3$)

Vibrational spectroscopy of di-amides are studied in terms of the following important peaks 3500-3100 cm^{-1} medium for ν(NH) always one peak, 1690-1640 cm^{-1} strong for ν(C=O), 1600-1400 cm^{-1} strong for ν(C=C), 1300-1000 cm^{-1} for ν(C–O) and finally strong peak around 700 cm^{-1} for δ(C=H).

Di-amines ($f_1 – f_3$)

In the di-amines spectra, the medium peak for ν(NH) between 3500-3100 cm^{-1} was converted to two peaks and strong peak for ν(C=O) between 1690-1640 cm^{-1} of di-amides were completely disappeared indicating that the reaction was take placed and convert diamide to diamine.

macrocyclics ($m_1 – m_4$)

In the macrocyclic spectra, two medium peaks of diamines (NH_2) between 3500-3100 cm^{-1}, were changed to the single peak of diamide for ν(NH) and at 1690-1640 cm^{-1} the new strong peak of ν(C=O) have been observed.

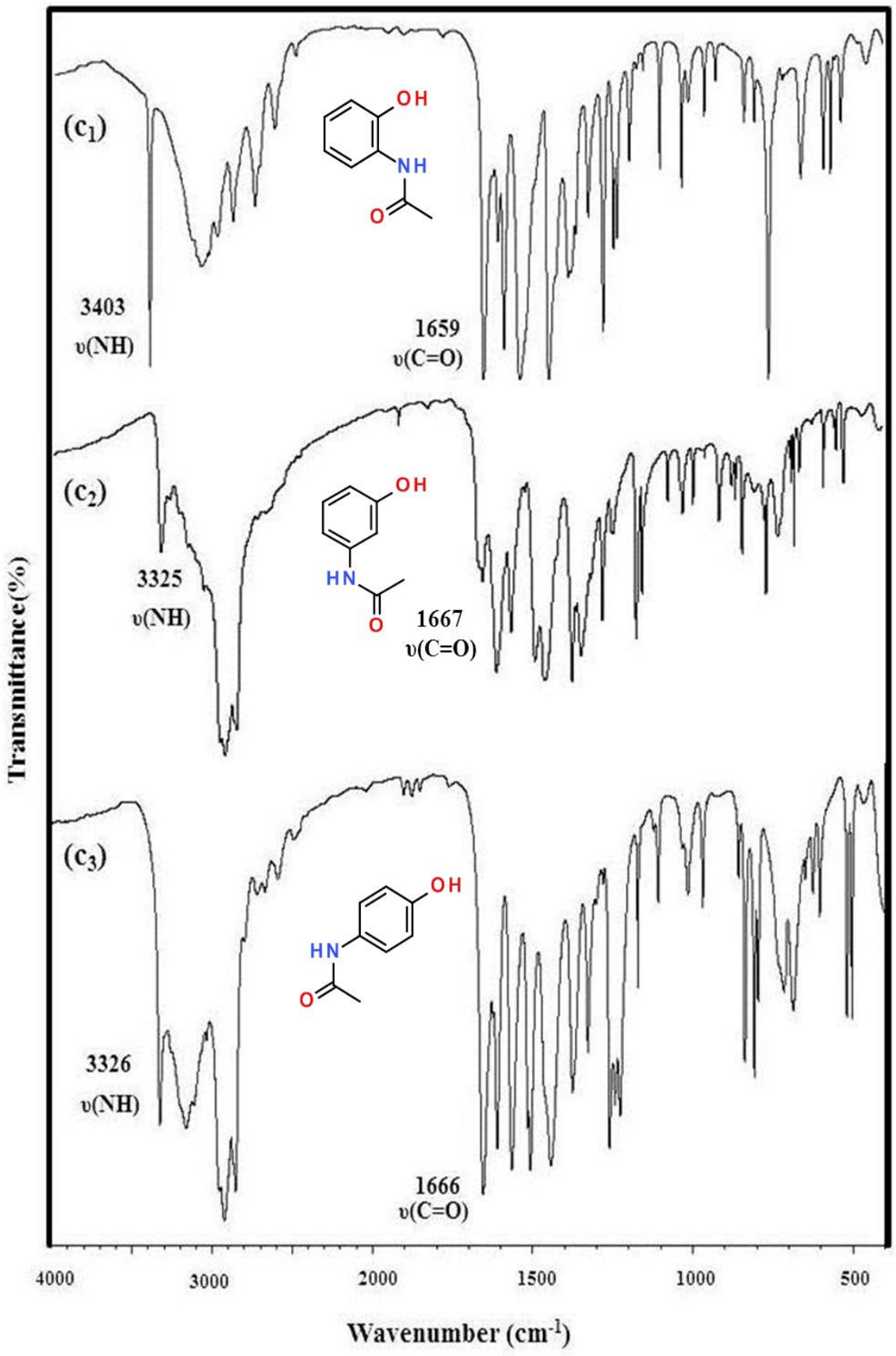

Figure 3.1 Comparative FT-IR spectra of acetamidophenols (c_1), (c_2) and (c_3).

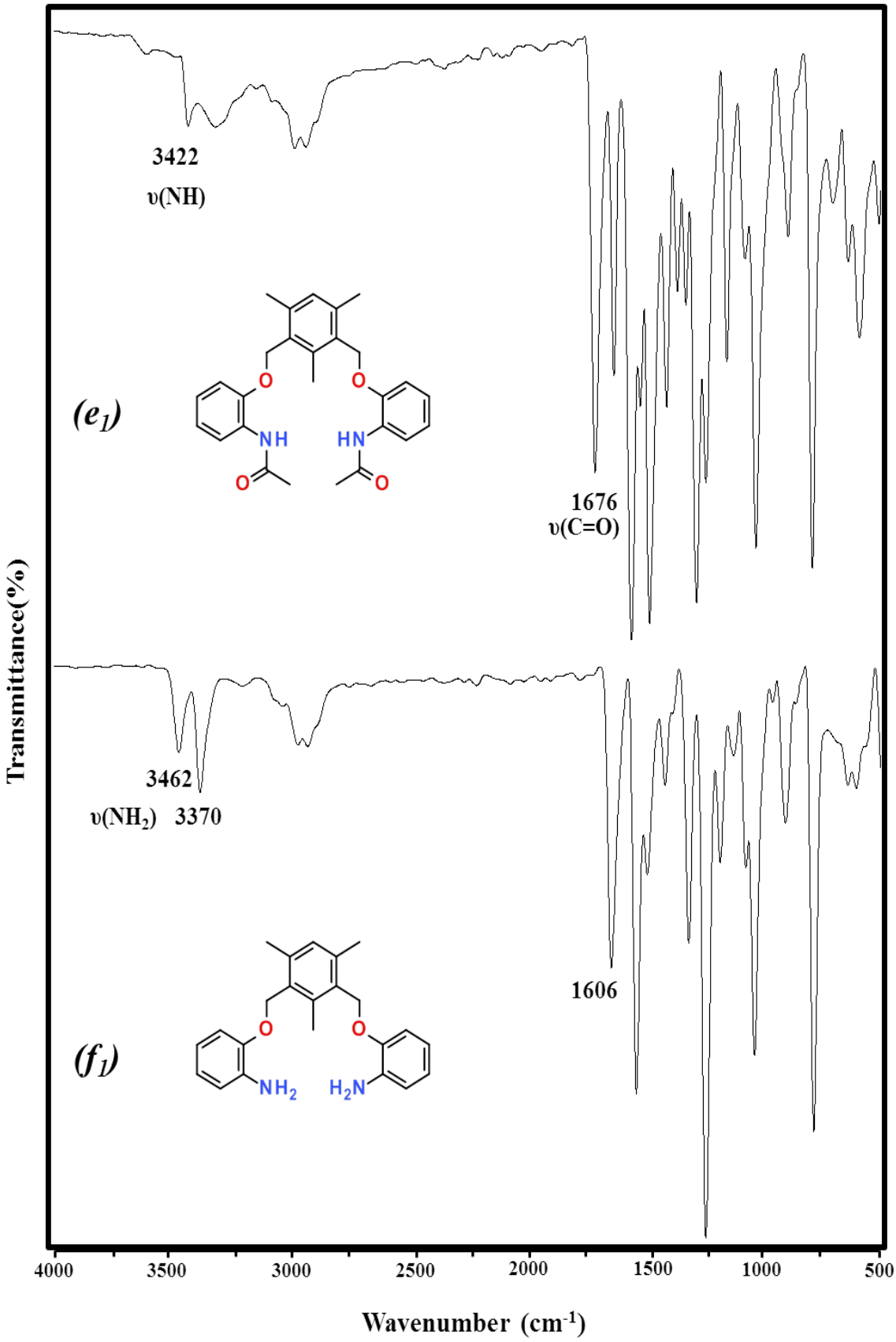

Figure 3.2 Comparative FT-IR spectra of diamide (e_1) and diamine (f_1).

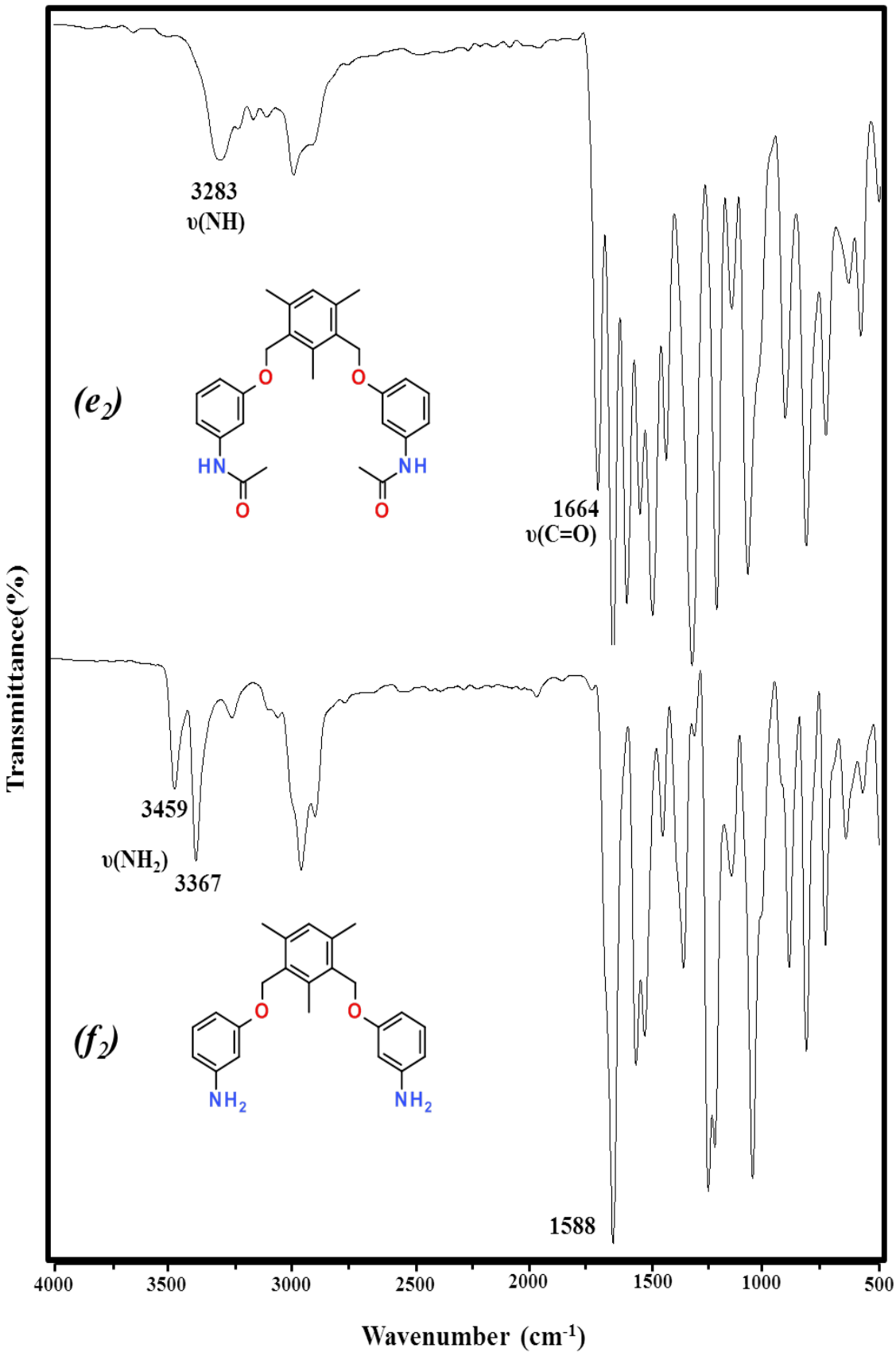

Figure 3.3 Comparative FT-IR spectra of diamide (e_2) and diamine (f_2).

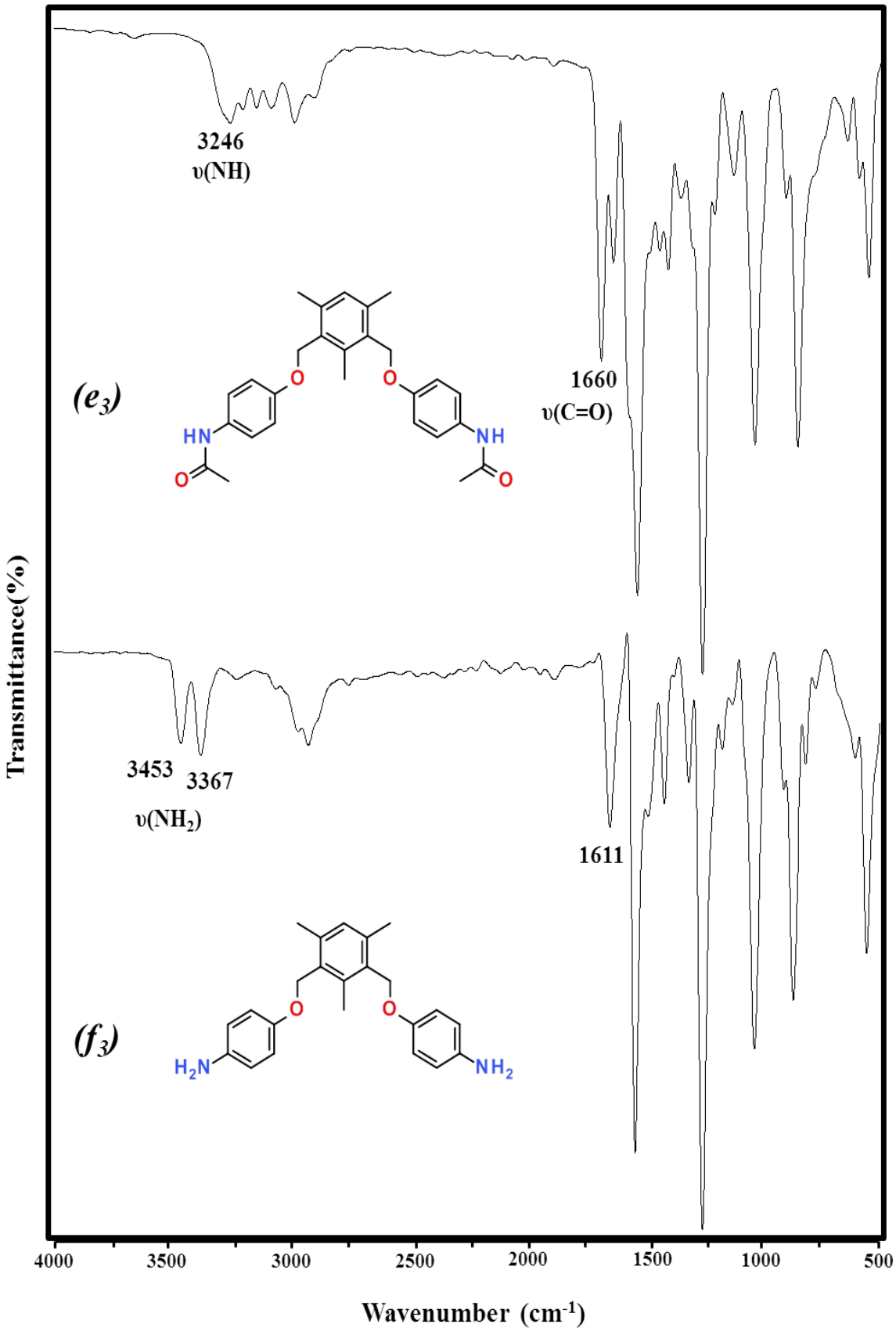

Figure 3.4 Comparative FT-IR spectra of diamide (e_3) and diamine (f_3).

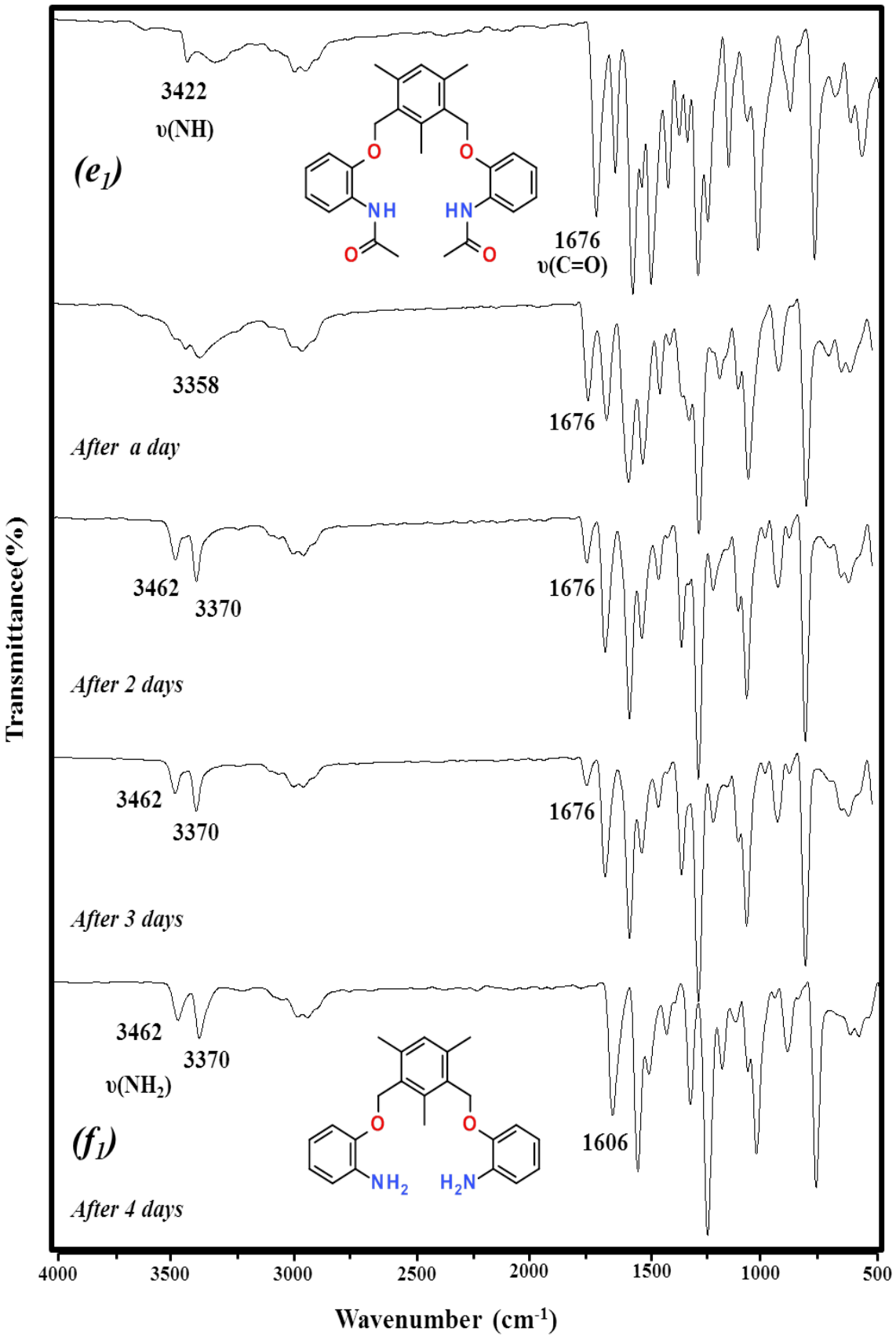

Figure 3.5 Comparative FT-IR spectra of diamide (e_1) to diamine (f_1).

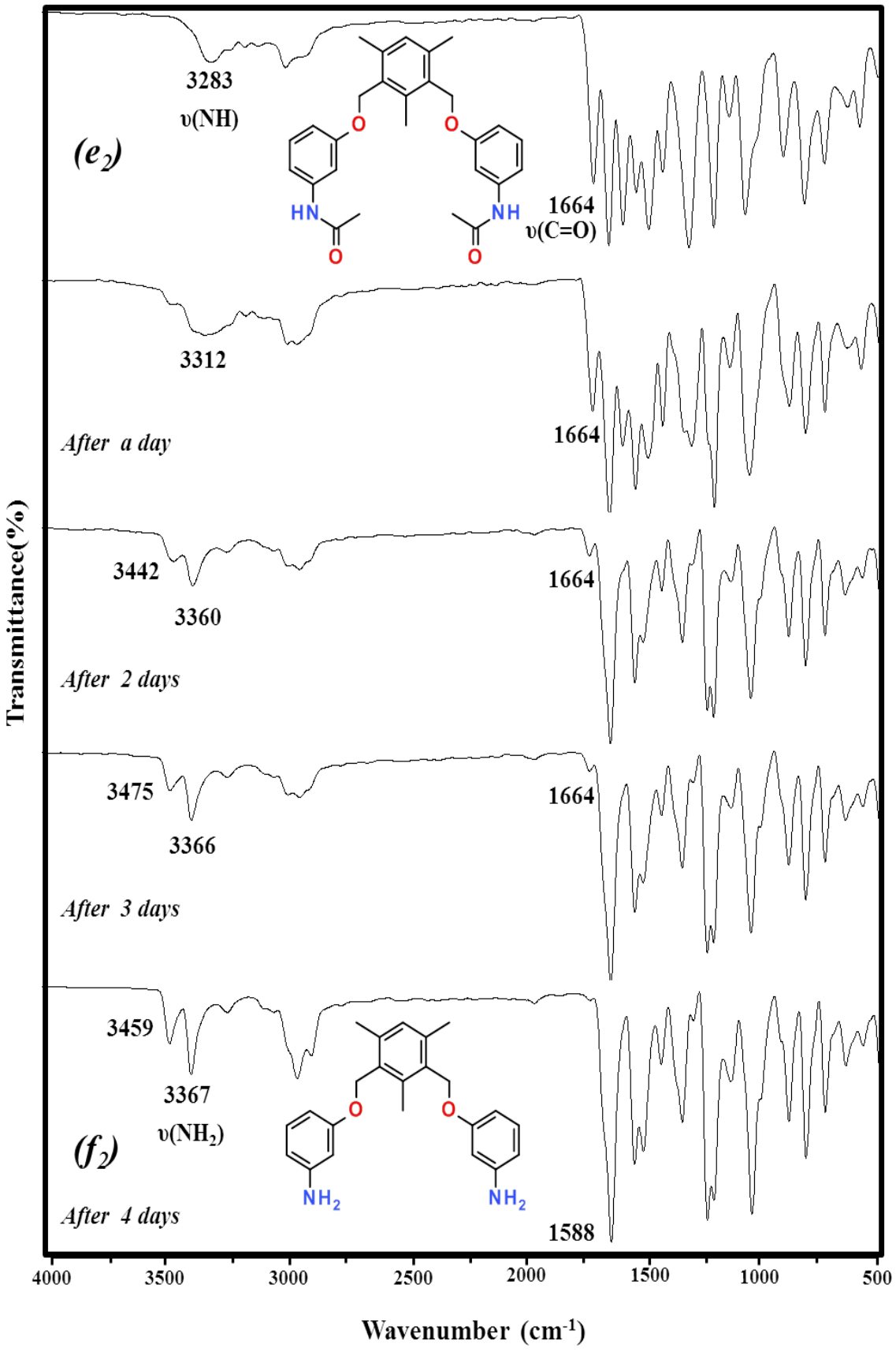

Figure 3.6 Comparative FT-IR spectra of diamide (e_2) to diamine (f_2).

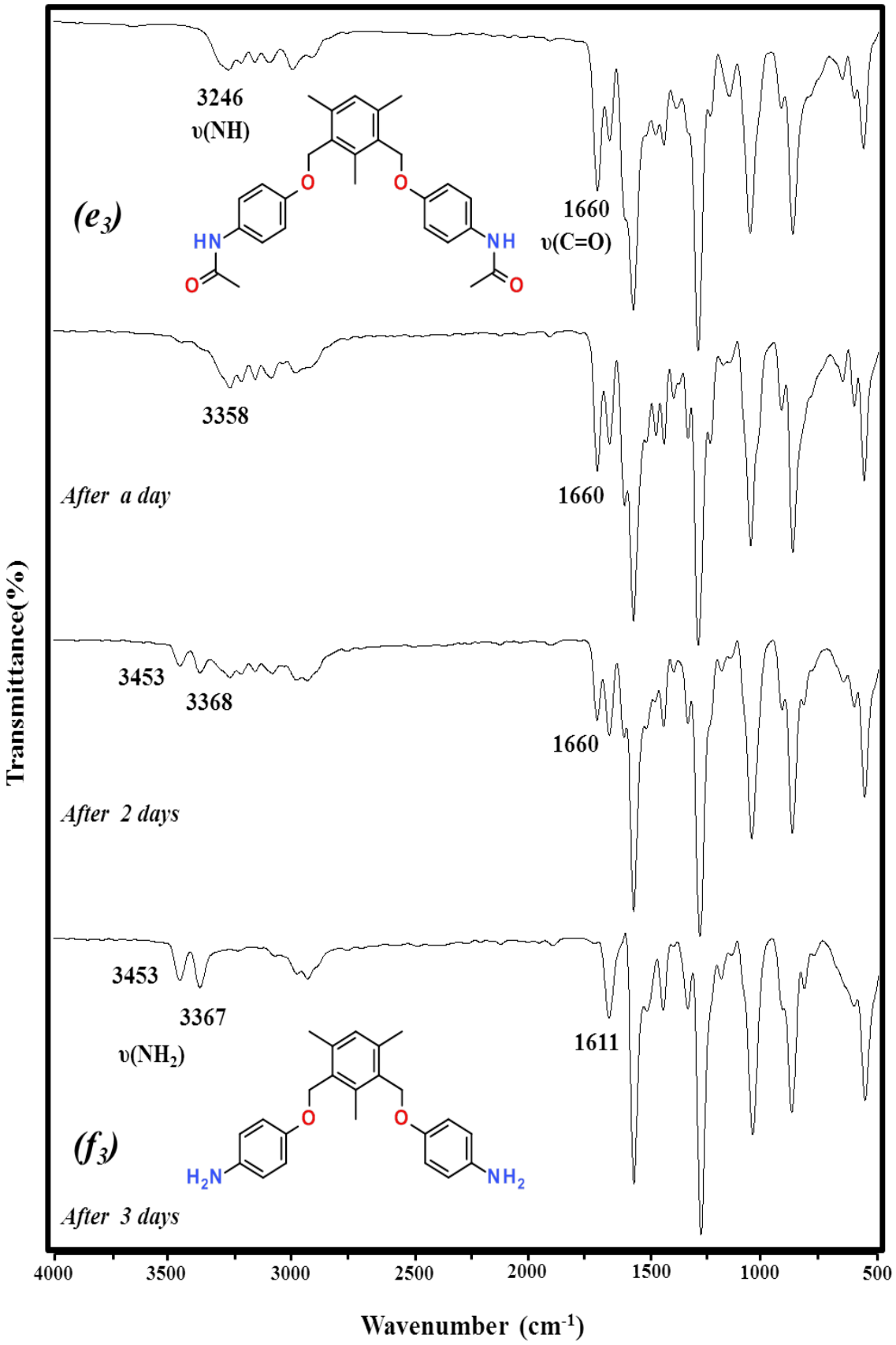

Figure 3.7 Comparative FT-IR spectra of diamide (e_3) to diamine (f_3).

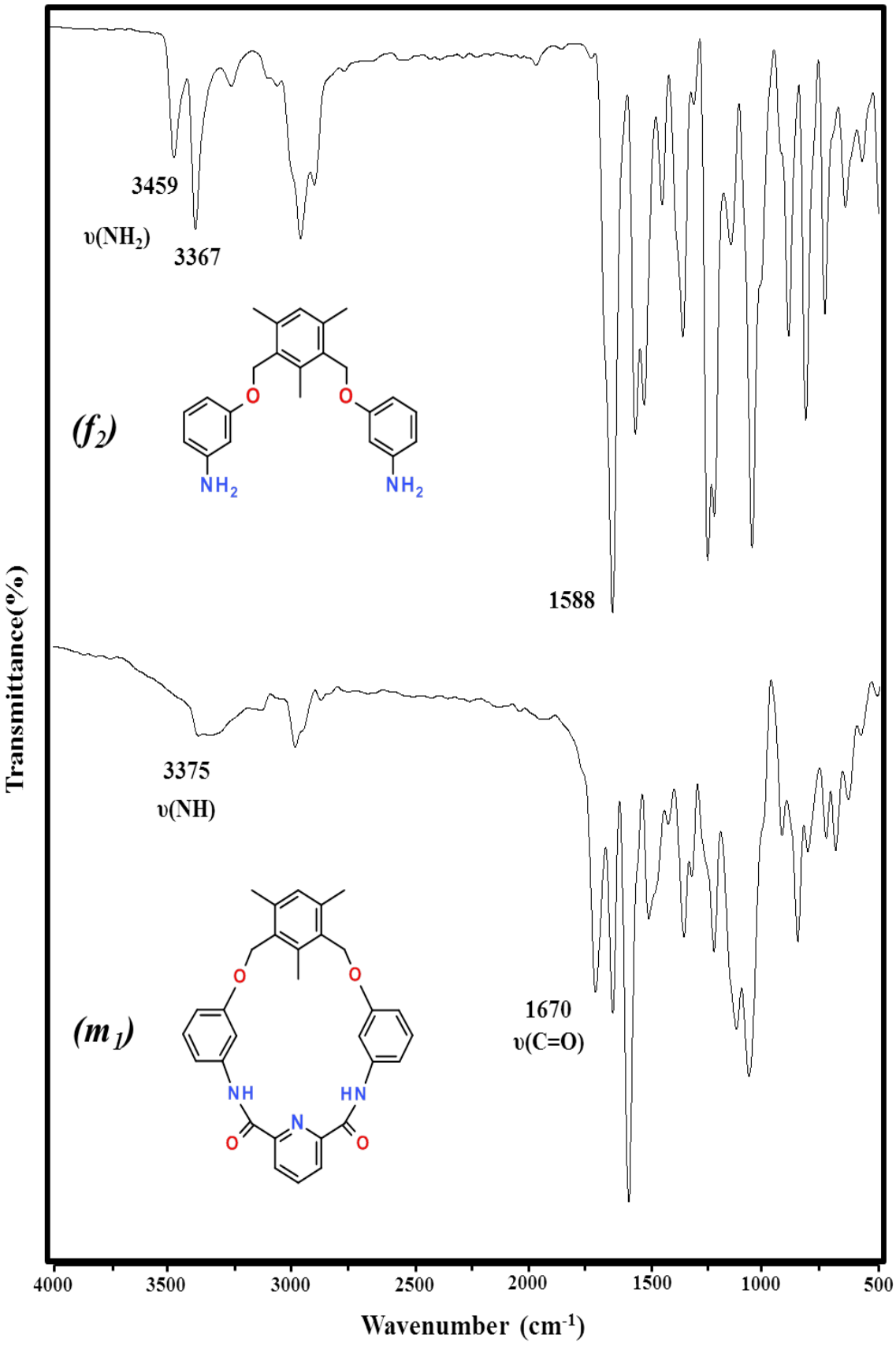

Figure 3.8 Comparative FT-IR spectra of diamine (f_2) and macrocyclic (m_1).

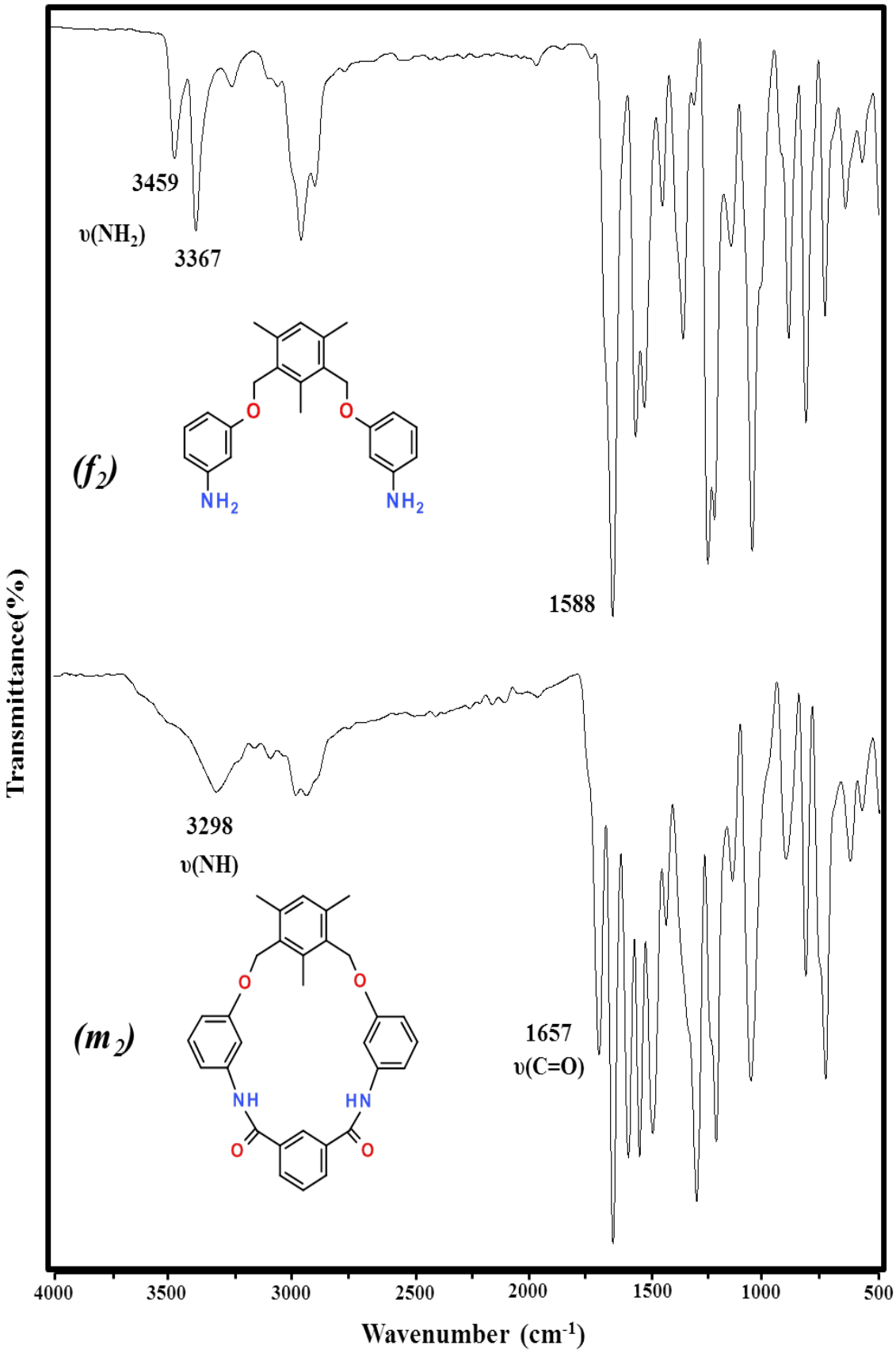

Figure 3.9 Comparative FT-IR spectra of diamine (f_2) and macrocyclic (m_2).

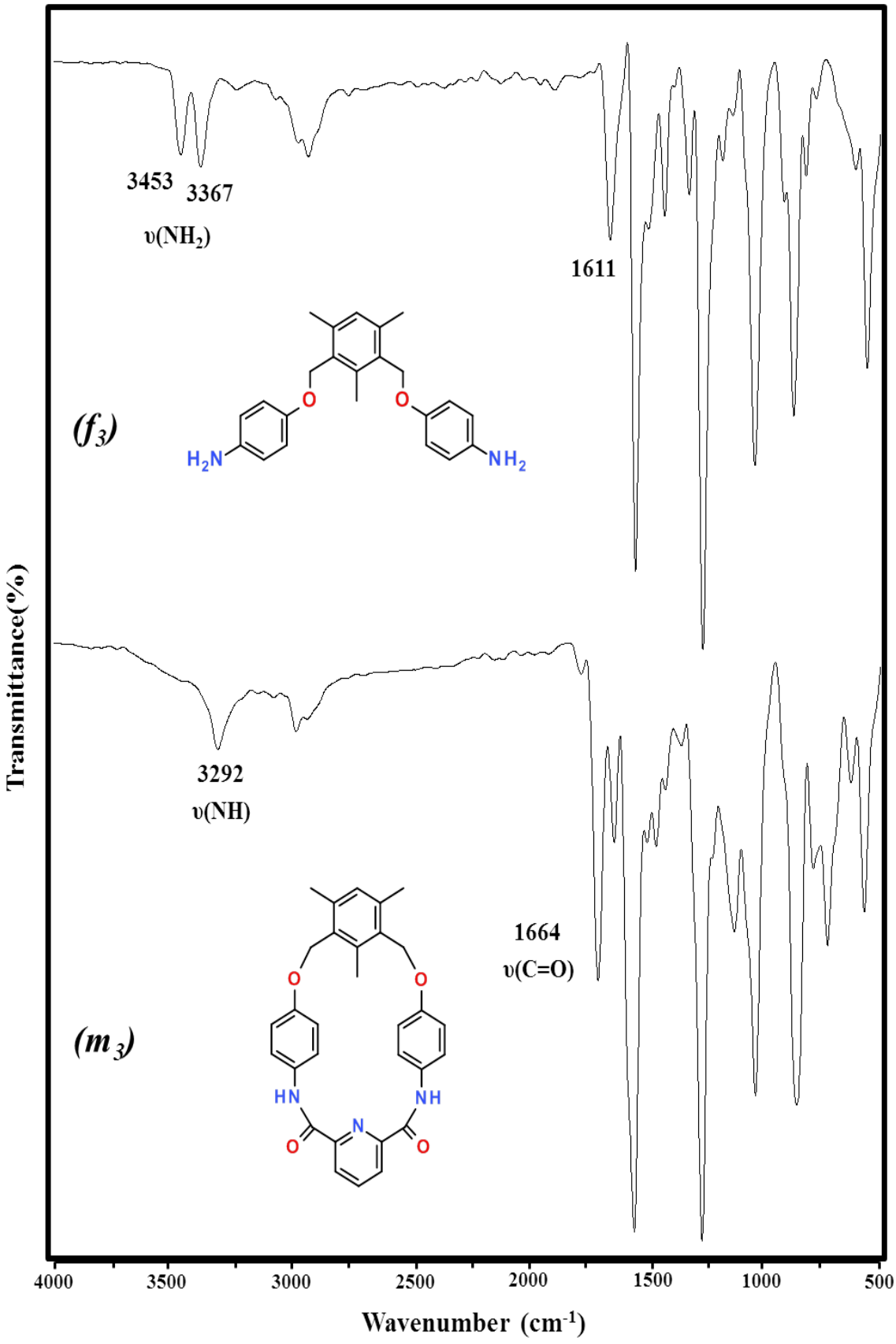

Figure 3.10 Comparative FT-IR spectra of diamine (f_3) and macrocyclic (m_3).

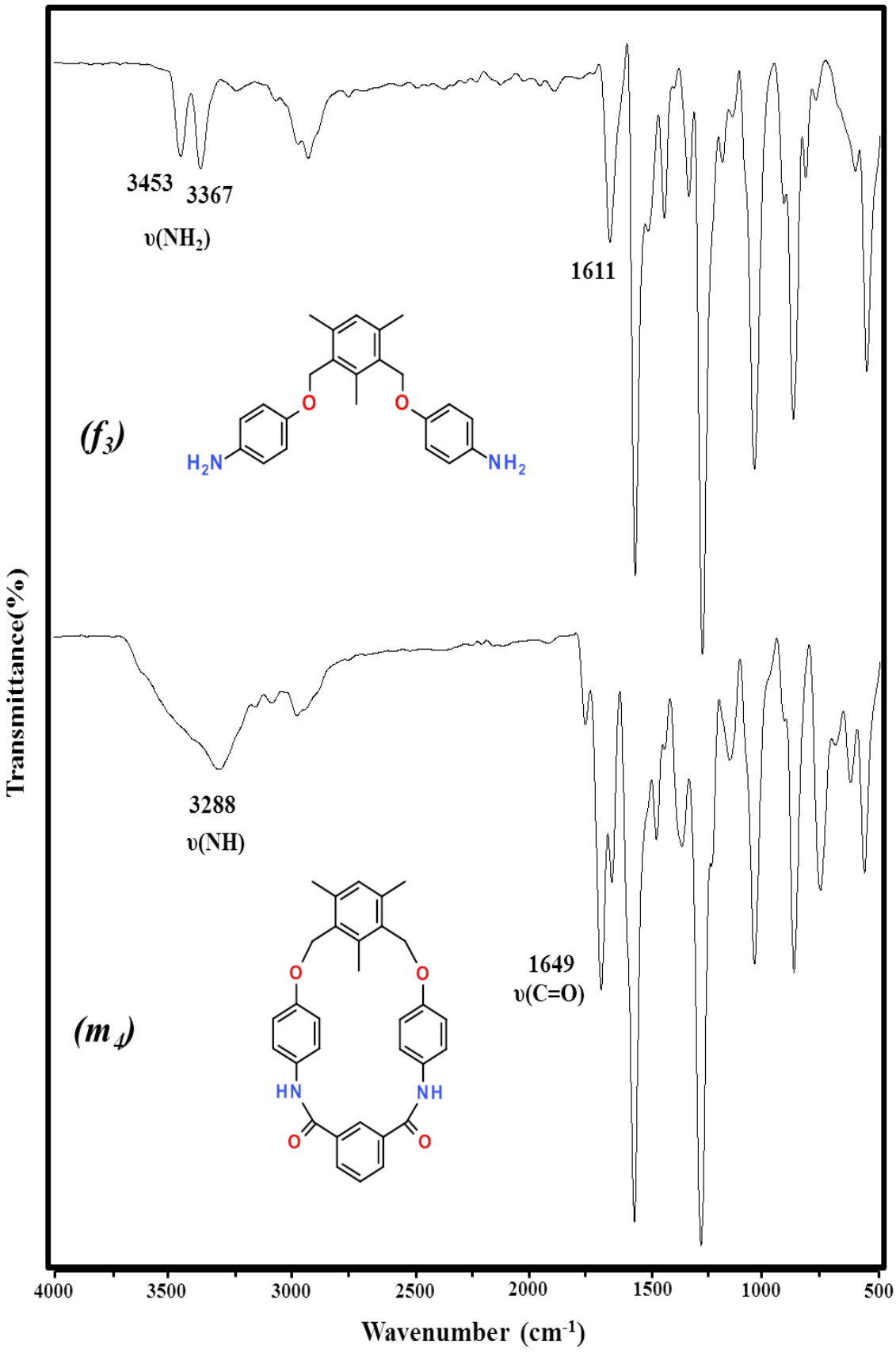

Figure 3.11 Comparative FT-IR spectra of diamine (f_3) and macrocyclic (m_4).

3.2 NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

3.2.1 NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPIC STUDY OF DI-AMIDES ($e_1 – e_3$)

^{1}H NMR of di-amides ($e_1 – e_3$) shows a singlet for (CH_2) protons at 5-5.20 ppm, and (NH) protons at 8.5-10 ppm. Also ^{1}H NMR of di-amides shows a monomethyl (CH_3) and two dimethyl ($2CH_3$) protons within the region of 1.5-2.50 ppm with integrations of (3H) for monomethyl and (6H) for each dimethyl. The integration for aromatic protons is significantly consistent with all the structure of the di-amides.

^{13}C NMR of all di-amides shows expected number of carbon atoms as in the chemical structure. Di-amides ($e_1 – e_3$) shows 13-15 carbons, while it was depending on their positional isomerism. Di-amides ($e_1 – e_3$) show monomethyls (CH_3) at ~ 15 and two dimethyl ($2CH_3$) at ~ 20 ppm. (CH_2) carbons were observed around the region 65-67 ppm . carbonyle group (C=O) at ~ 168 ppm was observed and (C-O) between 150-160 ppm.

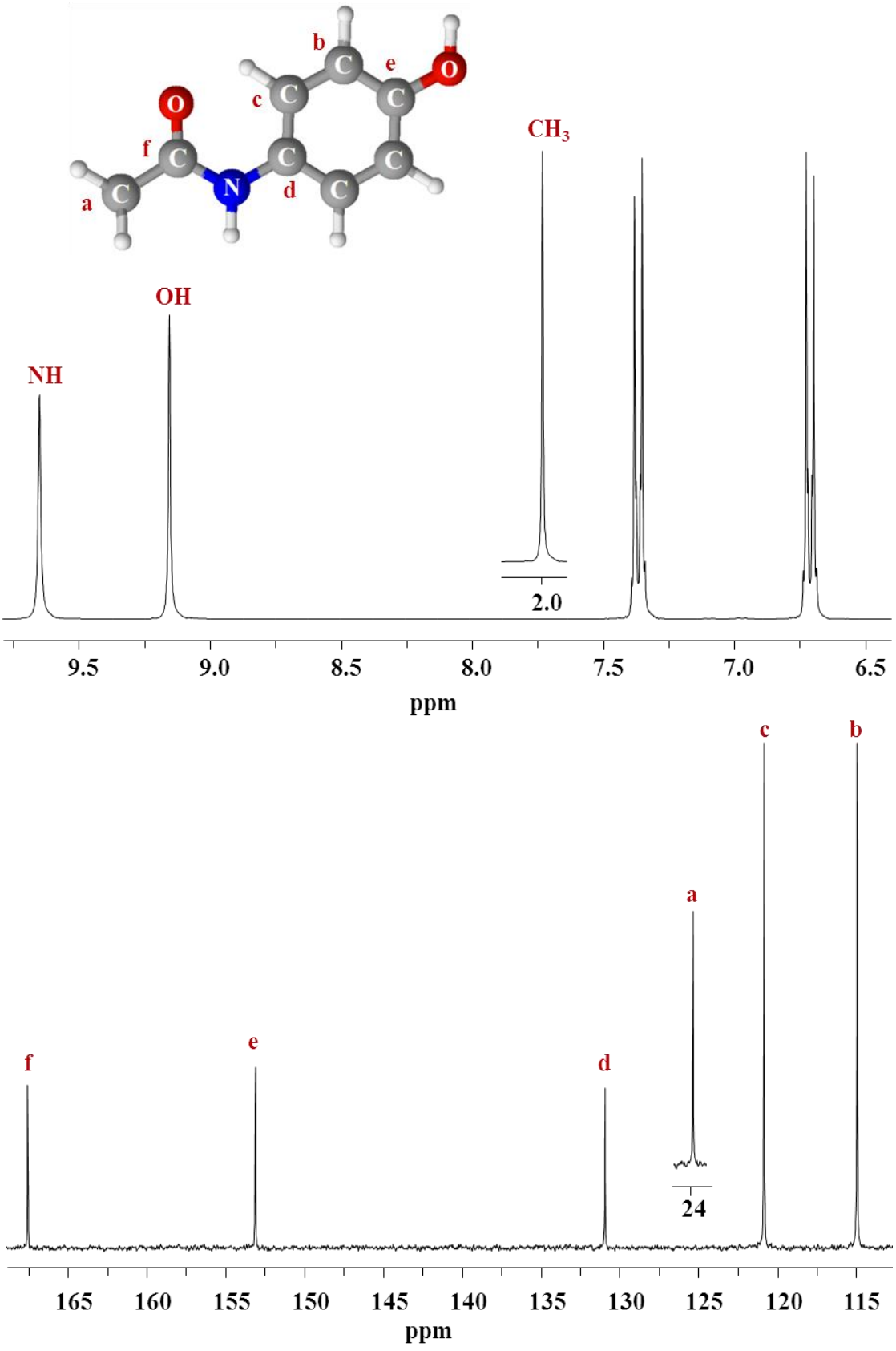

Figure 3.12 ^{1}H NMR and ^{13}C NMR spectra of 4-acetamidophenol (c_3).

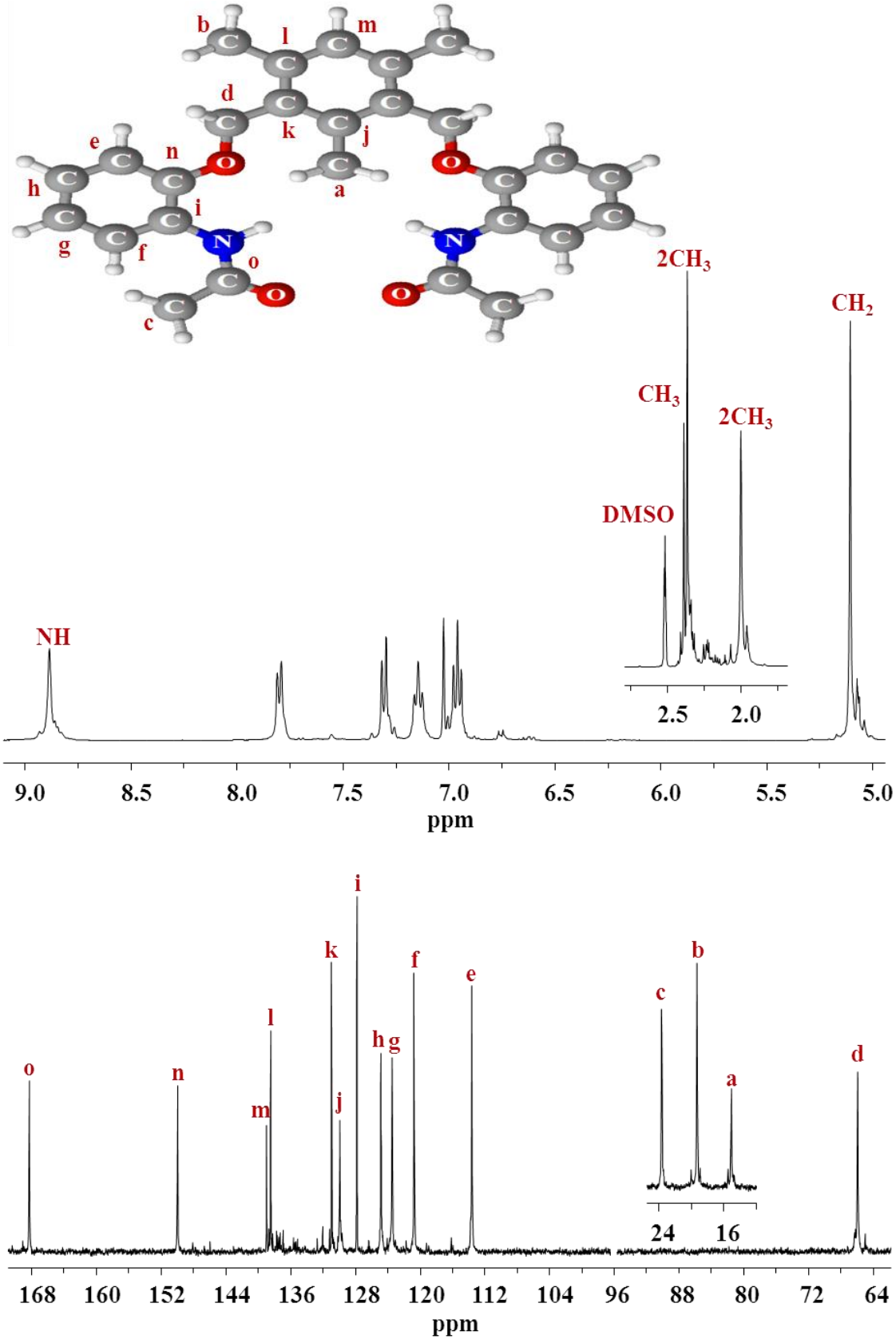

Figure 3.13 ^{1}H NMR and ^{13}C NMR spectra of diamide (e_1).

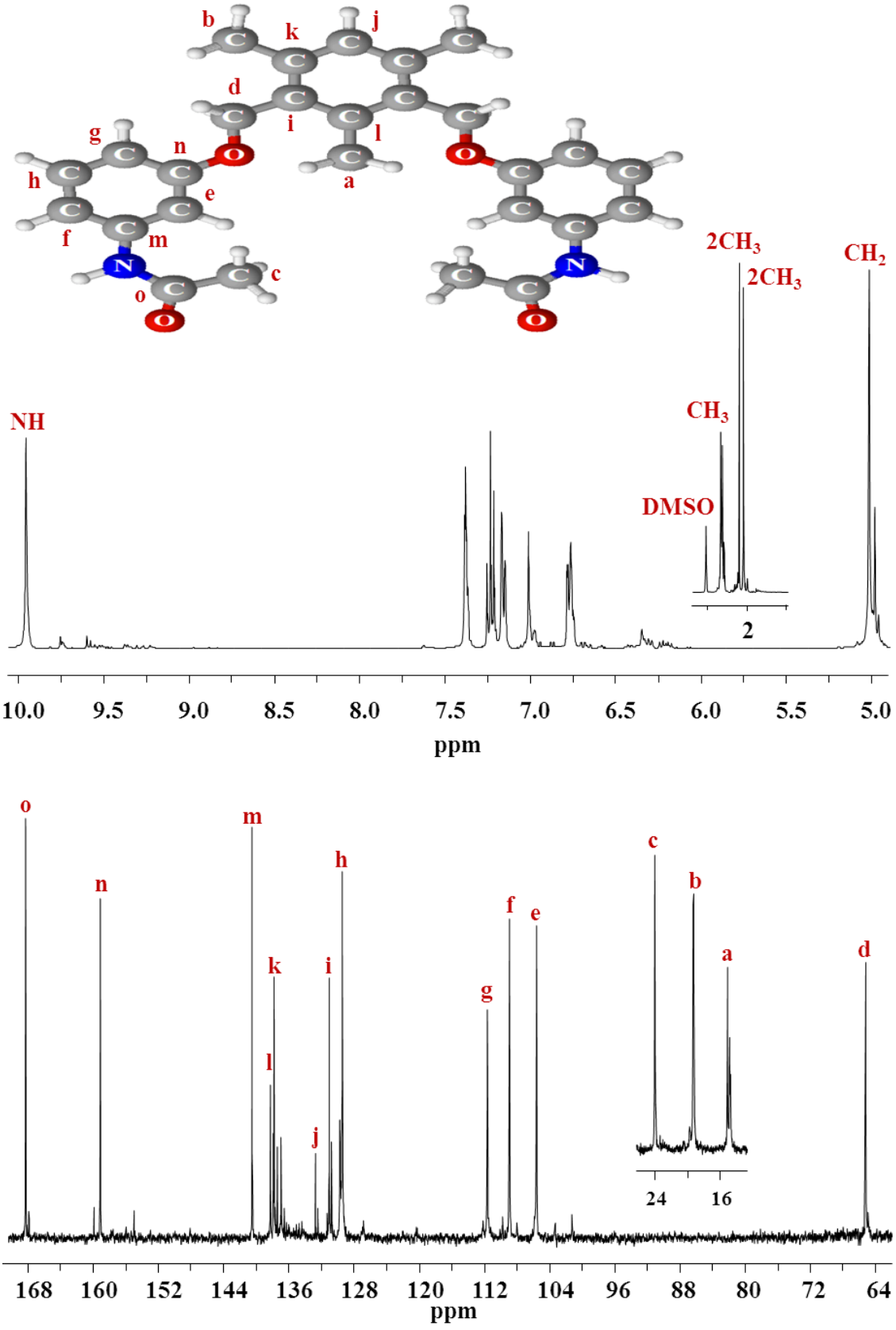

Figure 3.14 ^{1}H NMR and ^{13}C NMR spectra of diamide (e_2).

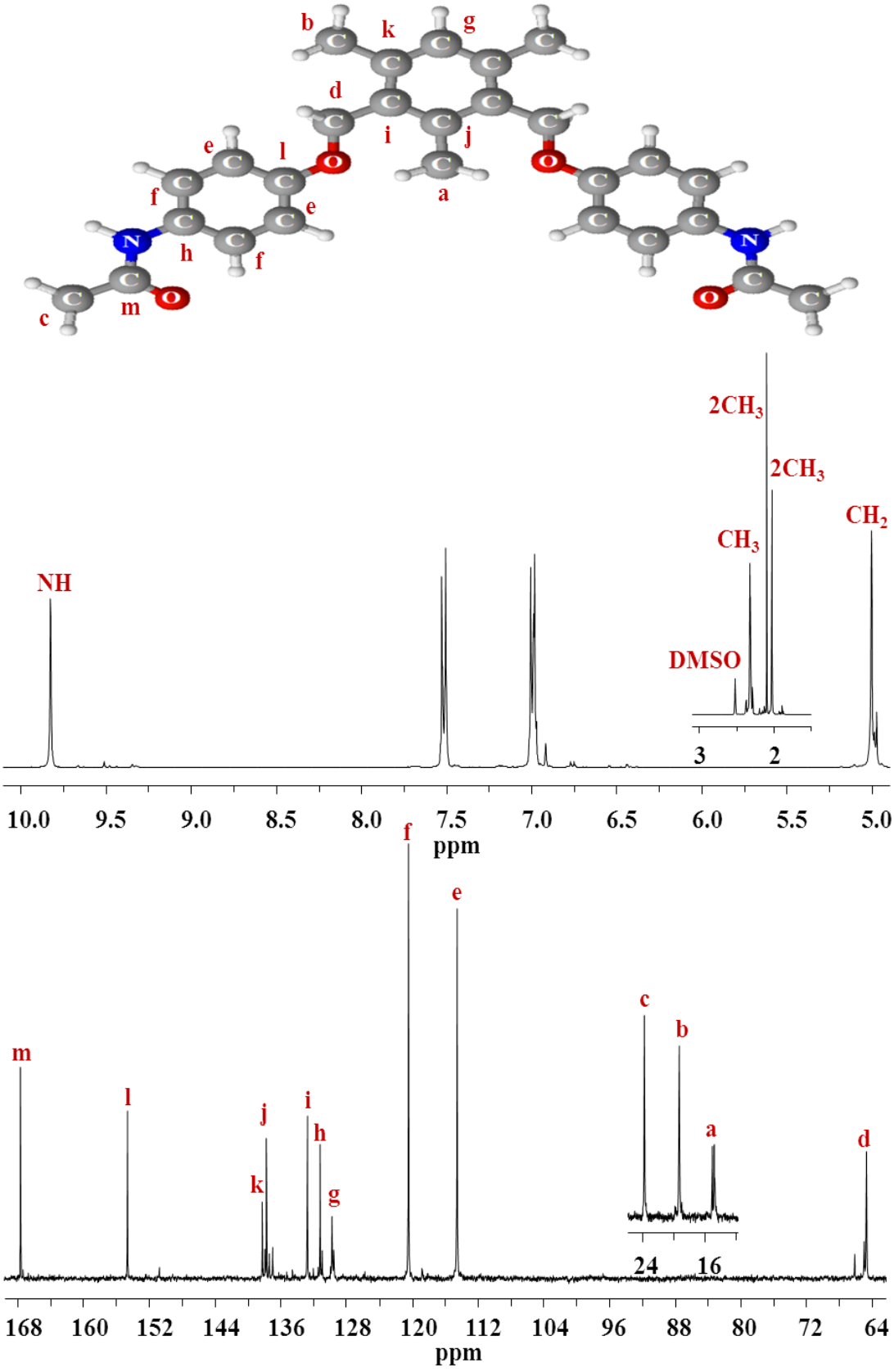

Figure 3.15 ^{1}H NMR and ^{13}C NMR spectra of diamide (e_3).

3.2.2 NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPIC STUDY OF DI-AMINES ($f_1 – f_3$)

^{1}H NMR of di-amines ($f_1 – f_3$) shows a singlet for (CH_2) protons at 5-5.20 ppm, and (NH_2) protons at 4-5 ppm. Also ^{1}H NMR of di-amines shows a monomethyl (CH_3) and dimethyl ($2CH_3$) protons within the region of 2.3-2.5 ppm with integrations of (3H) for monomethyl and (6H) for dimethyl respectively. The integration for aromatic protons is significantly consistent with all the structure of the di-amines.

^{13}C NMR of all di-amines shows expected number of carbon atoms as in the chemical structure. Di-amines ($f_1 – f_3$) shows 11-13 carbons, while it was depending on their positional isomerism. Di-amides ($f_1 – f_3$) show monomethyls (CH_3) and dimethyl ($2CH_3$) at ~ 25 ppm. (CH_2) carbons were observed around the region 65-67 ppm and and the carbonyl peak at ~ 168 ppm was not observed. (C-O) carbon between 144-160 ppm.

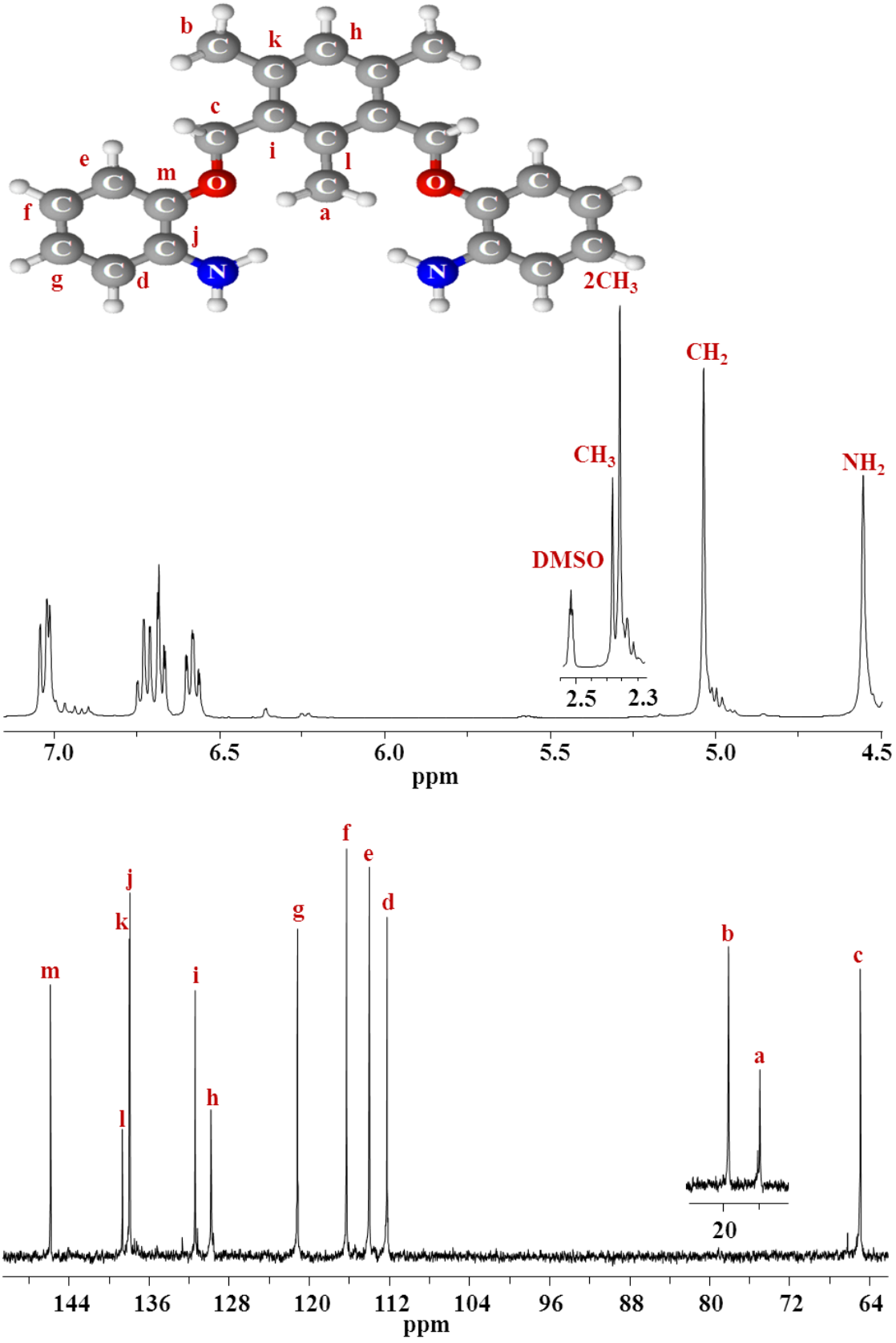

Figure 3.16 ^{1}H NMR and ^{13}C NMR spectra of diamine (f_1).

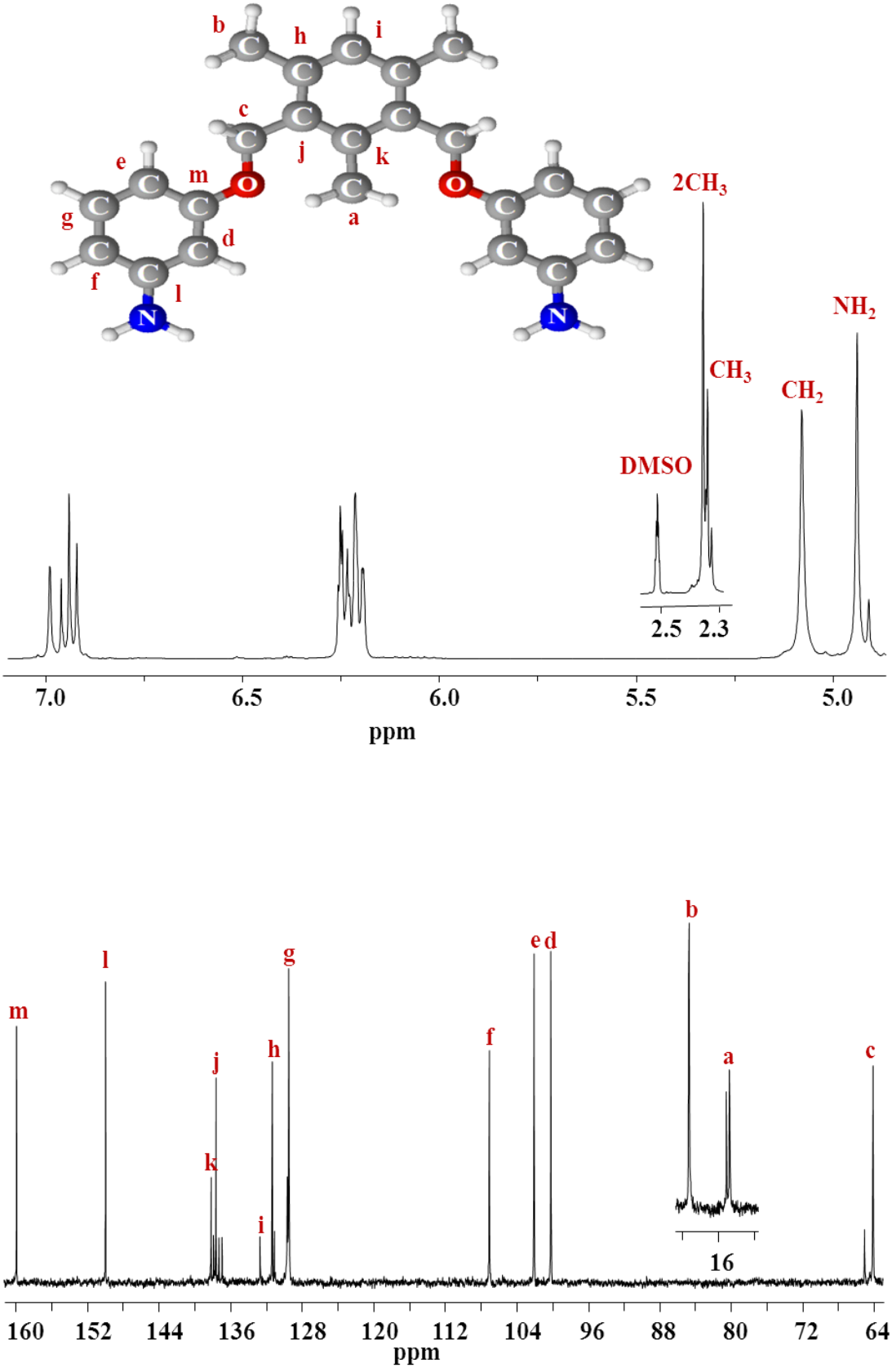

Figure 3.17 ^{1}H NMR and ^{13}C NMR spectra of diamine (f_2).

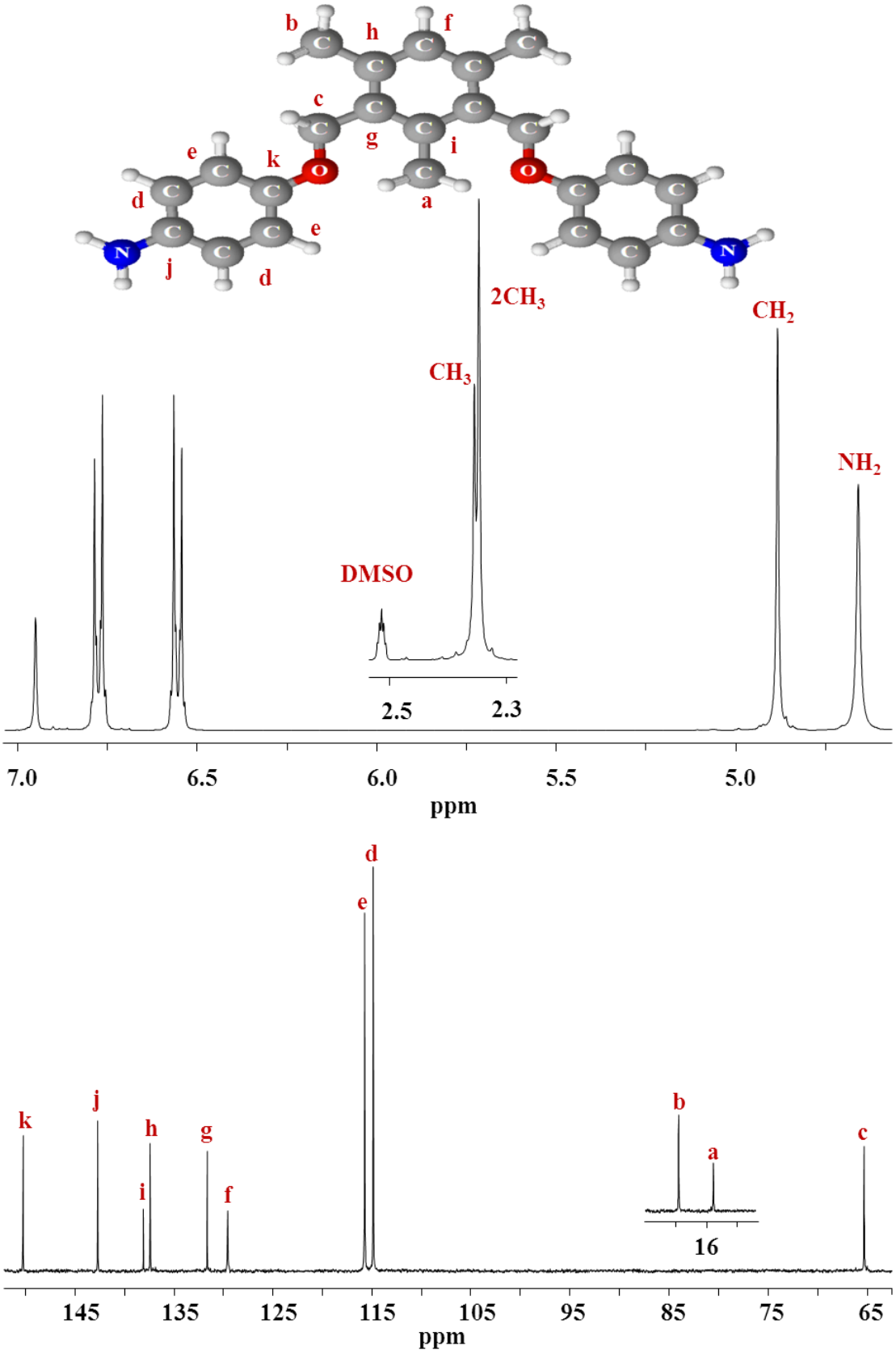

Figure 3.18 ^{1}H NMR and ^{13}C NMR spectra of diamine (f_3).

3.2.3 NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPIC STUDY OF MACROCYCLICS (m_1- m_4)

The ^1H NMR spectra of the macrocyclic compounds do not give any signal corresponding to aniline $–NH_2$ at ~ 4.20 ppm, instead sharp bands appear in the regions 10-11 ppm corresponding to amide (2NH) protons. As expected no significant changes are observed for the methylene proton chemical shifts compared with the starting materials. They appear around 5 ppm as a sharp singlet (4H, $2CH_2$) for each of the cyclic compounds.

Macrocyclic formation does not seem to affect considerably the CH_2 methylene carbon chemical shift values. They appear around 64 ppm (2C, $2CH_2$). The peaks corresponding to acid chlorides COCl ν(C=O) (≈ 170 ppm) groups are not observed in the ^{13}C NMR spectra of the cyclic products, instead the signals which appeared for each product in the regions 160-165 ppm are attributed to a (CONH) group.

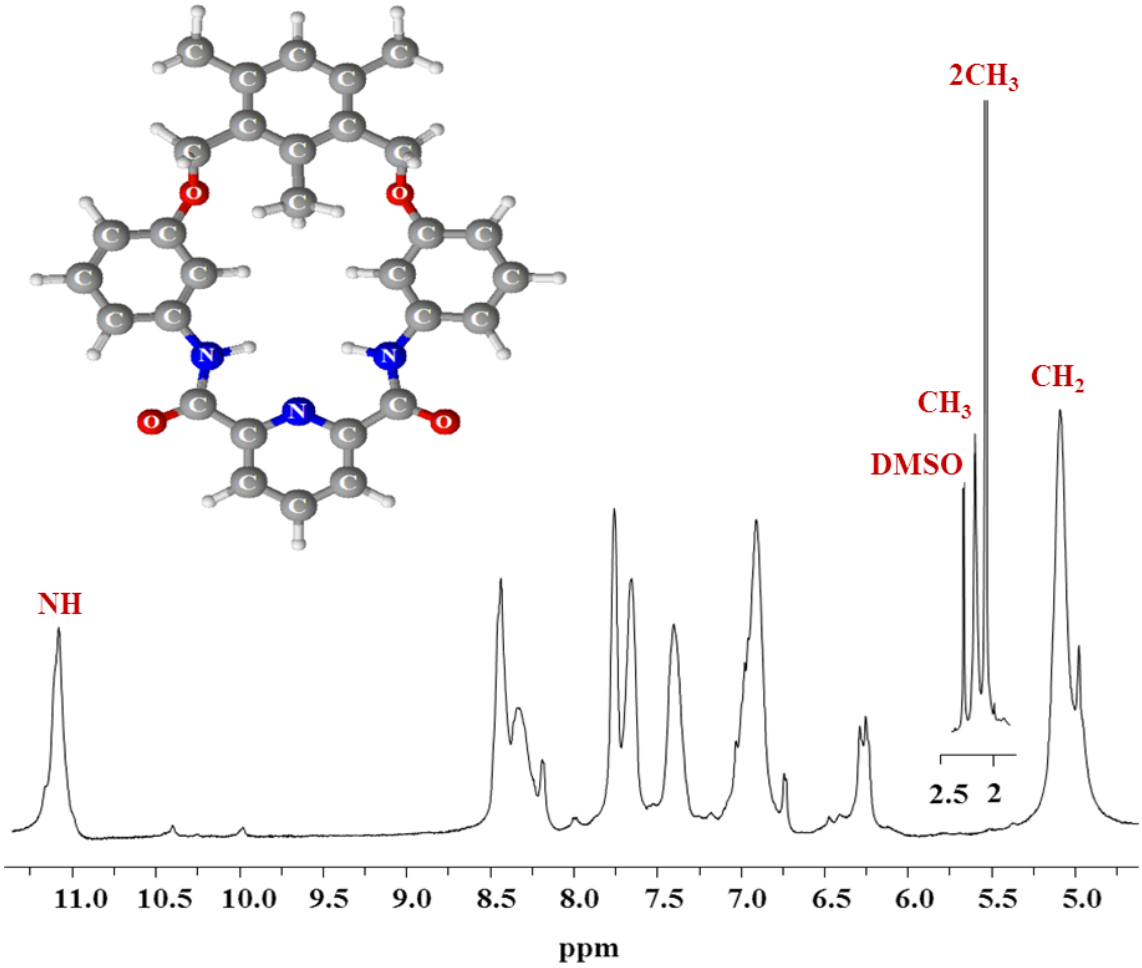

Figure 3.19 ^{1}H NMR spectra of macrocyclic (m_1).

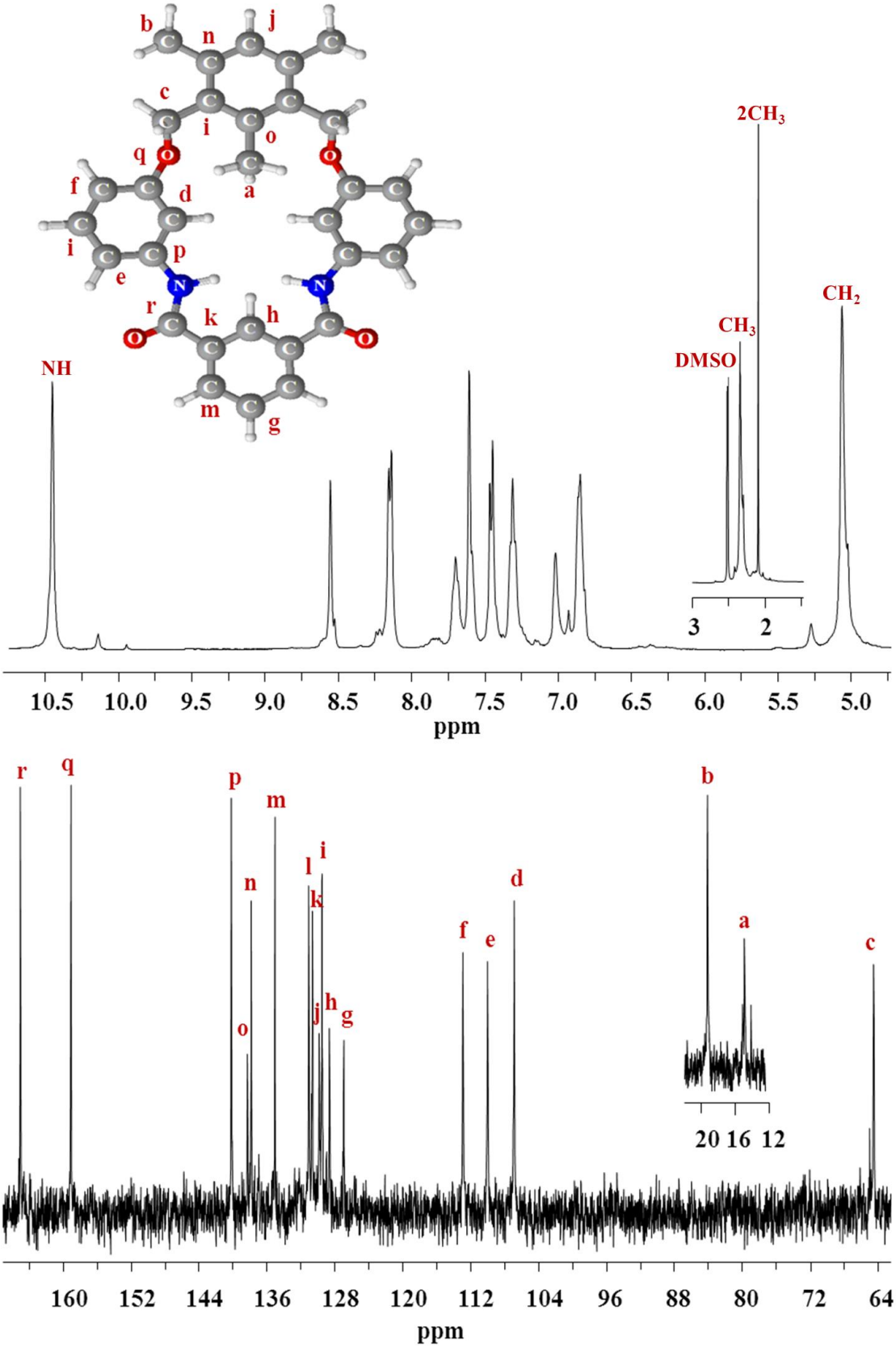

Figure 3.20 ^{1}H NMR and ^{13}C NMR spectra of macrocyclic (m_2).

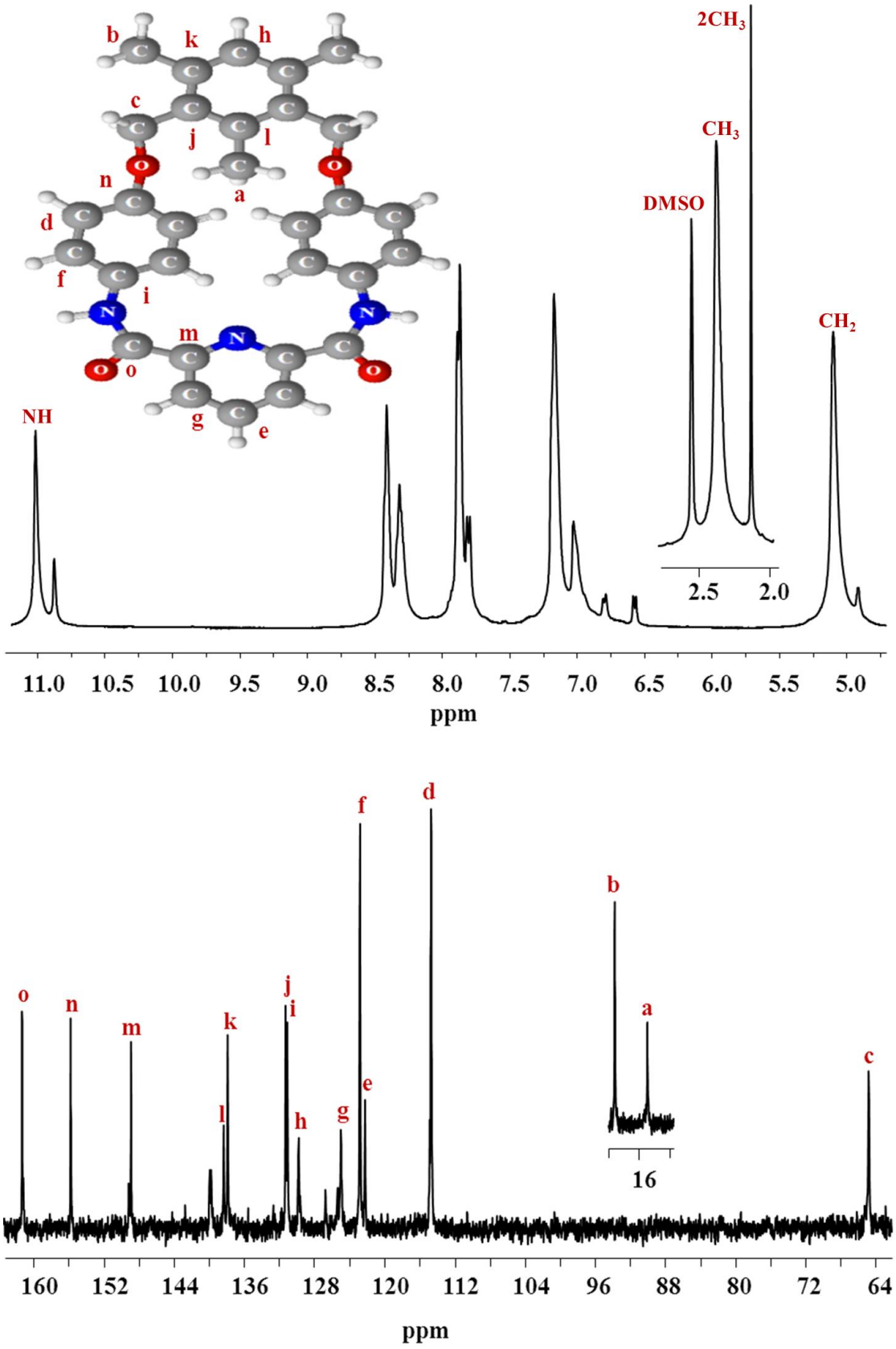

Figure 3.21 ^{1}H NMR and ^{13}C NMR spectra of macrocyclic (m_3).

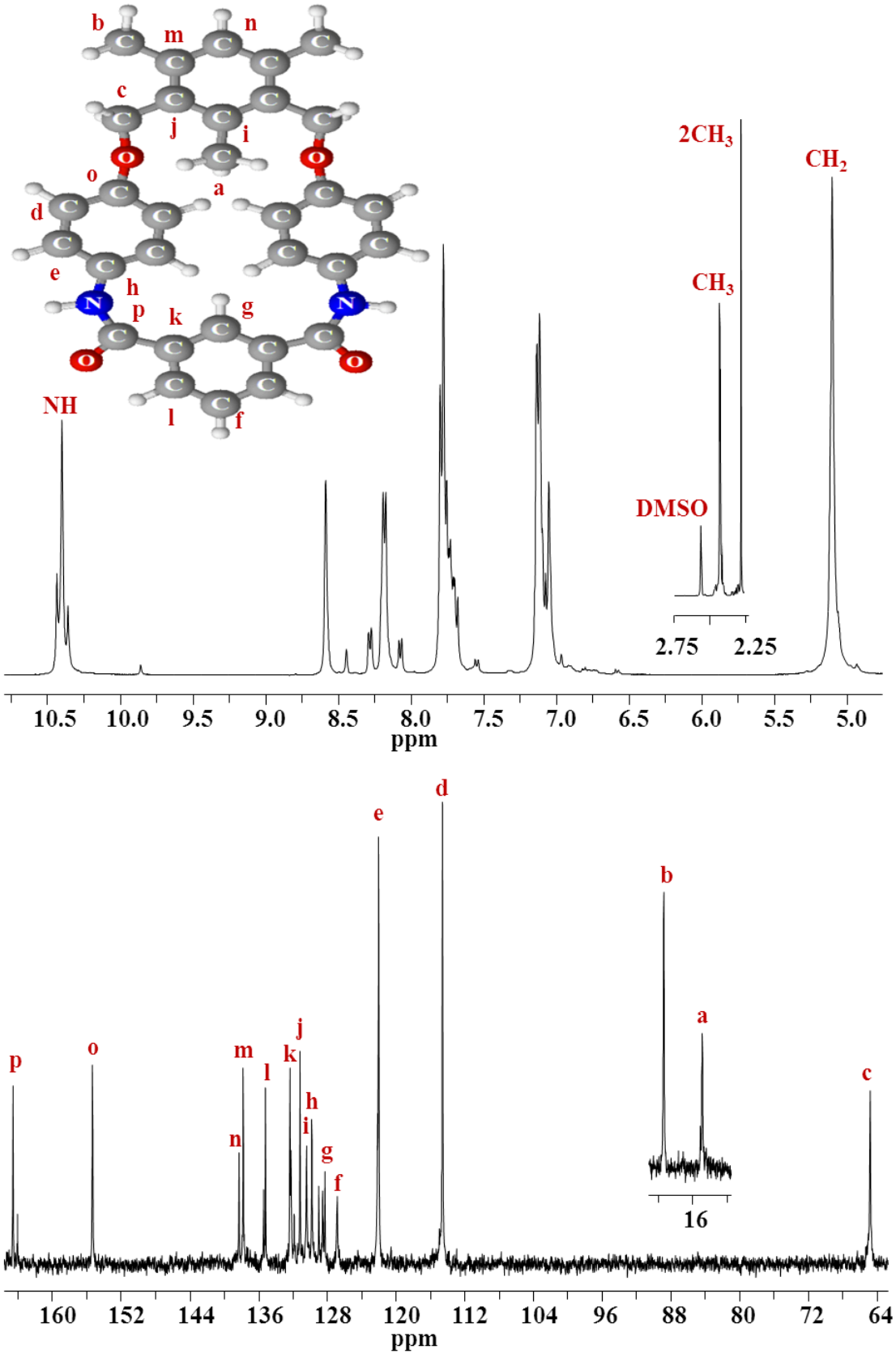

Figure 3.22 ^{1}H NMR and ^{13}C NMR spectra of macrocyclic (m_4).

3.3 Microbial activity

The results concerning *in vitro* antimicrobial activities of the macrocycles together with inhibition zone (mm) and (MIC) values of compared antibiotic and antifungal reagents are listed in Tables 1 and 2. The overall macrocycles can interact through van der Waals and hydrophobic interactions, while the individual hetero atoms present in the structure particularly nitrogen atoms, could interact by strong hydrogen bonding. As far as hydrogen bonding is concerned, there is an important electronegative aspect regarding to nitrogen atoms. The nitrogen atom is the most common atom involved as hydrogen bond acceptor in biological systems. Moderate biological activity shown by the compounds may be related to their hydrogen bonding capability due to the presence of several hetero atoms capable of hydrogen bonding with biomolecules. Comparing the inhibition zone values, in most cases the (m_1 and m_3) compound seems to show slightly higher biological activity than the (m_2-m_4) macrocycles (Table 2). So the inhibition activity seems to be governed to a certain degree by the numbers of nitrogen presence in these compounds, because the (m_1 and m_3) macrocycles contains higher numbers of nitrogen atoms compared with the other two compounds. This pattern repeats itself for both inhibition zone and MIC values (Tables 1, 2).

Of all the test compounds tested, (m_3) showed higher activity against both the Gram-positive pathogen *Bacillus cereus* (12.5 μg mL^{-1}) and Gram-negative bacteria *Proteus vulgaris* (12.5 μg mL^{-1}) compared with Gentamycin (6.25 μg mL^{-1}, Tables 2). Showing moderate antibacterial activity on both Gram-positive and Gram-negative bacteria, these compounds could be considered to possess a broad-spectrum potency.

The inhibition zone and (MIC) values indicate that almost all the compounds exhibited a moderate antifungal activity against most of the yeast cultures. Once more the compound (m_1) shows slightly higher activity (zone 22 mm and 12.5 μg mL^{-1}) against *Hanseniaspora guilliermondii* in comparison with Nystatin (zone 21 mm and MIC 3.125 μg mL^{-1}).

Table 3.1 In vitro antimicrobial activity (mm zone) of (m_1-m_4) and standard reagents.

Microorganisms/compounds	m_1	m_2	m_3	m_4	PE	AM	CT	VA	OF	TE	NY	KE	CL
Staphylococcus aureus$^+$	24	12	23	16	13	16	12	13	24	26	-	-	-
Bacillus cereus$^+$	26	16	28	17	14	12	14	18	30	25	-	-	-
Micrococcus luteus$^+$	24	14	26	15	36	32	32	34	28	22	-	-	-
Mycobacterium smegmatis$^+$	22	14	24	15	15	21	11	20	32	24	-	-	-
Listeria monocytogenes$^+$	28	12	22	16	10	12	16	26	30	28	-	-	-
Escherichia coli$^-$	20	14	22	14	18	12	10	22	30	28	-	-	-
Proteus vulgaris$^-$	28	12	24	15	10	16	18	20	28	26	-	-	-
Klebsiella pneumoniae$^-$	20	14	18	13	18	14	13	22	28	30	-	-	-
Pseudomonas aeruginose$^-$	26	12	28	14	8	10	54	10	44	34	-	-	-
Kluyveromyces fragilis	20	12	18	14	-	-	-	-	-	-	18	16	18
Rhodotorula rubra	20	12	18	15	-	-	-	-	-	-	18	22	16
Candida albicans	18	12	20	14	-	-	-	-	-	-	20	21	15
Hanseniaspora guilliermo.	22	15	20	14	-	-	-	-	-	-	21	24	22
Debaryomyces hansenii	18	14	20	12	-	-	-	-	-	-	16	14	18

For every one three tests were performed and the average was taken as last data.

Table 3.2 in vitro antimicrobial activity (MIC, µg mL^{-1}) of (m_1-m_4) and standard reagents.

Microorganisms/compounds	m_1	m_2	m_3	m_4	GEN	NY
Staphylococcus aureus$^+$	12.5	25	12.5	12.5	25	-
Bacillus cereus$^+$	6.25	12.5	12.5	12.5	6.25	-
Micrococcus luteus$^+$	6.25	25	12.5	25	25	-
Mycobacterium smegmatis$^+$	12.5	12.5	12.5	12.5	12.5	-
Listeria monocytogenes$^+$	6.25	25	12.5	12.5	12.5	-
Escherichia coli$^-$	12.5	25	6.25	12.5	6,25	-
Proteus vulgaris$^-$	12.5	25	12.5	25	6.25	-
Klebsiella pneumoniae$^-$	6.25	12.5	12.5	25	6.25	-
Pseudomonas aeruginose$^-$	6.25	50	12.5	25	6,25	-
Kluyveromyces fragilis	12.5	12.5	6.25	12.5	-	6,25
Rhodotorula rubra	25	25	12.5	12.5	-	6,25
Candida albicans	3.125	12.5	6.25	12.5	-	3,125
Hanseniaspora guilliermo.	12.5	12.5	6.26	6.25	-	3,125
Debaryomyces hansenii	12.5	12.5	6.25	25	-	12,5

For every one three tests were performed and the average was taken as last data.

CHAPTER 4

CONCLUSION

Conclusively, the thesis proposed and accomplished the synthesis and structural characterization of three new di-amides, three new di-amines and four new macrocyclics from acetamidophenols, 2,4-Bis(chloromethyl)mesitylene, pyridine-2,6-dicarbonyl dichloride and isophthaloyl chloride. Their melting temperatures, FT-IR Spectra, ^{1}H NMR and ^{13}C NMR were also recorded. The FT-IR shows all the expected from their structures functional groups in each set of di-amides, di-amines and macrocyclics. A comparative FT-IR spectroscopy studies reveal a clear success of the synthetic pathways by comparing the synthetic steps involved in the synthesis of the compounds.

^{1}H NMR of the compounds shows all the prominent peaks as in the chemical structure. The integration of the peaks gives the exact number of hydrogen atoms from which the peak is obtained, this also indicate the purity of these compounds. ^{13}C NMR show equal number of carbon atoms as expected in the chemical structure which confirmed the formation of the compounds and success of the synthesis. The purity of each compound was also checked and assured by T. L .C. test after subjecting the compounds through several purification procedures. In the T. L. C. test single sport was observed for all the compounds accept where isomerism occurs. Reduction of di-amides to di-amines depends of the time, temperature and the amount of sodium hydroxide. Reduction of di-amides to di-amines by sodium hydroxide need 3-4 days if added about 10 times of sodium hydroxide, but if added less than 10 times it need more than 4 days to converted di-amides to di-amines. Moreover, this study focused mainly on the synthetic aspect and structural characterization of the compounds. It is recommended that further research particularly on the applications of these compounds in the field of

medicine, biology and pharmacy should be investigated. Recent research revealed wide application of such compounds in medical diagnosis, treatment, prevention and control of many diseases and biological disorders. The inexhaustible diversity of molecular architecture in macromolecula systems natural or synthetic allows the design of novel substrates whose chemical facets have dominant influences upon specific biological and physical properties.

It is also recommended that complexes of the macrocyclics should be made and analyzed for ion transfer and related medical and biological applications, this is because the biological studies revealed that many similar sort of hetero macromolecules had exhibited a significant antibacterial properties and were actively used in the treatment of cancer, atherosclerosis, angiogenesis, neurodegenerative diseases, and inflammatory disease.

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