A New Class of Macrocyclic Complexes of Bivalent Manganese: Template Synthesis, Spectroscopic, Electrochemical and Biological Studies

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Abstract

New series of Mn(II) macrocyclic complexes have been synthesized by the template condensation of the ligand (MacL) with appropriate diamine in the presence of MnCl₂·4H₂O in 1:1:1 molar ratio in methanol. The macrocyclic ligand, bis(benzil)-4-fluoro-1,2-phenylenediamine(MacL) has been synthesized by the condensation of benzil and 4-fluoro-1,2-phenylenediamine (2:1 molar ratio) in ethanol. The ligand and complexes have been characterized with the help of elemental analysis, molecular weight determinations, melting point determinations, magnetic measurements, electronic, infrared, ESR spectral studies and X-ray diffraction for structure elucidation. On the basis of the spectral studies the binding sites are proposed as the nitrogen atom of the macrocycles and an octahedral geometry has been assigned for the metal complexes.

Keywords: Mn(II) macrocyclic complexes, Spectral studies, Electrochemical studies and Biological activities

Introduction

The field of the macrocyclic chemistry of transition metals is developing very fast because of its variety of applications and importance in the area of coordination chemistry. During the past decade macrocyclic chemistry has attracted much attention and has become a growing area of research in inorganic and bioinorganic chemistry in view of its biological significance. Many synthetic routes to macrocyclic ligands involve the use of metal ion template to orient the reacting groups of linear substrates in the desired conformation for the ring to close. A number of nitrogen donor macrocyclic derivatives have long been used in analytical, industrial and biomedical applications. Macrocyclic metal complexes are of great importance due to their resemblance to many natural systems such as porphyrins and cobalaminies [1-4].

A variety of transition metals can be chelated, depending on the size and flexibility of the polydentate binding site. While open polydentate ligands have been much studied, macrocycles are the most stable form of polydentate ligands and are commonly found in biological redox systems where some structural flexibility is required. Macrocyclic ligands are considerably attractive in the quest for new chemistry, because they offer a wide variety of donor atoms, ionic charges, coordination numbers, and geometry of the resultant complexes. Studies on complexes of Schiff base macrocyclic ligands with different ring sizes and donor atoms with a variety of metals have been published.
Schiff bases have therefore provided a foundation stone for the building of contemporary macrocyclic chemistry. A wide range of Schiff base macrocycles evolved from the early studies [5-8]. Metal ion complexes with macrocyclic ligands are significant, also for the development of new methodologies in separation science. These ligands are also of theoretical interest as they are capable of furnishing an environment with controlled geometry and ligand field strength [9,10].

During our efforts to synthesize and study of macrocyclic ligands, we have prepared and characterized complexes of the tetraazamacrocyclic ligand (MacL) with manganese(II) using different diamines.

**Experimental**

**Methods and Materials**

The chemicals used were of AR grade. These chemicals and solvents were dried and purified by standard methods. Molecular weights were determined by the Rast Camphor Method. Manganese was estimated complexometrically with EDTA using Eriochrome Black T as an indicator. Sulfur and nitrogen were determined by Messenger’s and Kjeldahl's methods respectively. Chlorine was estimated by Volhard’s method. Infrared spectra were recorded on a Model Nicolet Megna FTIR-550 spectrophotometer using KBr pellets. The electronic spectra were recorded on a Hitachi U-2000 spectrophotometer (range 200–600 nm) using methanol as the solvent at I.I.T. Madras, Chennai. ESR spectra of the complexes were monitored on Varian E-4X band spectrometer at SAIF, IIT, Madras, Chennai.

**Preparation of Macrocyclic Ligand**

Bis(benzil)-4-fluoro-1,2-phenylenediamine was prepared by the condensation of benzyl (2.0 g, 9.51 mmol) and 4-fluoro-1,2-phenylenediamine (0.595 g, 4.75 mmol) (2:1 molar ratio) in ethanol. The reaction mixture was refluxed for 6-7 h. After reducing the solvent, the solution was cooled and the coloured crystalline compound thus obtained was recrystallized from ethanol and dried in vacuo. The resulting products were analysed before use. The synthetic route of the ligand is shown below (Figure 1):

![Synthetic route of the ligand](https://via.placeholder.com/150)

Figure 1 Synthetic route of the ligand

**Preparation of the Macrocyclic Complexes**

The macrocyclic complexes were prepared by template condensation method (Figure 2). A weighed amount of Bis(benzil)-4-fluoro-1,2-phenylenediamine (MacL) (3.09 g, 6.06 mmol) was taken into a dry 100mL round bottom
flask. The solution of ligand was treated with various diamines: 4-fluoro-1,2-phenylenediamine (0.759 g, 6.06 mmol), 4-chloro-1,2-phenylenediamine (0.865 g, 6.06 mmol), 1,2-diaminopropane (0.450 g, 6.06 mmol), 1,3-diaminopropane (0.450 g, 6.06 mmol), 1,4-diaminobutane (0.535 g, 6.06 mmol), 2,4-diaminotoluene (0.741 g, 6.06 mmol), 3,4-diaminotoluene (0.741 g, 6.06 mmol), and MnCl₂·4H₂O (1.2 g, 6.06 mmol) in 1:1:1 molar ratio. The reaction mixture was heated under reflux for 7-10 hours. The reaction solution was concentrated to half of its volume. After cooling, the solution was transferred to an evaporating dish and kept overnight at room temperature in a desiccator. The formed crystalline solid was collected, washed with methanol, dried under reduced pressure and recrystallized from methanol. The physical properties and analytical data are given in Table 1.

![Synthetic route of the Mn(II) macrocyclic complexes](image)

Figure 2: Synthetic route of the Mn(II) macrocyclic complexes

Where, n = 1-7

R₁ = 4-fluoro-1,2-phenylenediamine
R₂ = 4-chloro-1,2-phenylenediamine
R₃ = 1,2-diaminopropane
R₄ = 1,3-diaminopropane
R₅ = 1,4-diaminobutane
R₆ = 2,4-diaminotoluene
R₇ = 3,4-diaminotoluene.

Table 1: The physico-chemical properties and analytical data of the ligands and their complexes

<table>
<thead>
<tr>
<th>Compound</th>
<th>M.P. and Colour</th>
<th>M.Wt. (Calcd.)</th>
<th>Yield (%)</th>
<th>Elemental analysis found (Calcd.)%</th>
<th>Magnetic moment (B.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₂₃H₂₂N₂O₂F (Mac-L)</td>
<td>76-78 Light brown</td>
<td>508.20 (510.56)</td>
<td>89</td>
<td>C: 78.97 (79.03), H: 4.49 (4.54), N: 5.75 (5.48)</td>
<td>-</td>
</tr>
<tr>
<td>Mn(C₄₀H₂₆N₄F₂Cl₂)</td>
<td>140-143 Brown</td>
<td>718.12 (726.47)</td>
<td>85</td>
<td>C: 66.08 (66.13), H: 3.51 (3.60), N: 7.52 (7.71)</td>
<td>79.53 (9.76), 7.49 (7.56)</td>
</tr>
<tr>
<td>Mn(C₄₀H₂₆N₄Cl₃)</td>
<td>148-150 Dark brown</td>
<td>736.74 (742.93)</td>
<td>75</td>
<td>C: 64.59 (64.66), H: 3.48 (3.52), N: 7.19 (7.54)</td>
<td>14.16 (14.31), 7.20 (7.39)</td>
</tr>
<tr>
<td>Mn(C₃₇H₂₉N₄F₂Cl₂)</td>
<td>115-117 Yellowish Brown</td>
<td>659.99 (675.47)</td>
<td>69</td>
<td>C: 65.72 (65.79), H: 4.43 (4.47), N: 8.01 (8.29)</td>
<td>10.22 (10.49), 8.00 (8.13)</td>
</tr>
<tr>
<td>Mn(C₃₇H₂₉N₄FCl₂)</td>
<td>125-128 Light Brown</td>
<td>662.14 (674.46)</td>
<td>78</td>
<td>C: 65.80 (65.89), H: 4.28 (4.33), N: 8.02 (8.30)</td>
<td>10.39 (10.51), 8.06 (8.14)</td>
</tr>
</tbody>
</table>
Results and Discussion

ESR Spectral Analysis and Magnetic Moment

The ESR spectrum of a polycrystalline sample gives one broad isotropic signal centered at 2.0155, i.e. approximately the free electron g-value ($g_0 = 2.0023$). The broadening of the spectrum is due to spin relaxation [11]. The μ values for all the complexes are in the range 5.73-5.90 B.M. These values of the Mn(II) tetraazamacroyclic complexes [12], at room temperature indicate the presence of high spin $d^5$ configuration of Mn(II). The μ eff values are close to the expected values for five unpaired electrons as these complexes will not dimerise due to the presence of the bulkier phenyl groups.

Infrared Spectral Studies

It was noted that a pair of bands corresponding to $\nu (\text{NH}_2)$ at 3273–3309 cm$^{-1}$ are present in the spectrum of diamine but are absent in the infrared spectra of all the complexes. Furthermore, no strong absorption band was observed near 1700 cm$^{-1}$, indicating the absence of >C=O of benzil and confirming the condensation of carbonyl groups of benzil and amino groups of diamine [13,14]. These results provide strong evidence for the formation of the macrocyclic frame [15]. A strong absorption band in the region ca. 1600–1615 cm$^{-1}$ may be attributed to the C=N group. The lower values of (C=N) may be explained on the basis of drift of a lone pair density of azomethine nitrogen towards the metal atom [16]. In the Mn(II) complexes of macrocycles derived from benzil, medium intensity bands in the region 1550–1570 cm$^{-1}$ are attributed to $\nu (\text{C}=\text{C})$ of phenyl groups and bands at 740–800 cm$^{-1}$ to C–H out of plane bending of phenyl groups. The phenyl ring absorption appears in the 1462-1494 and 1354-1385 cm$^{-1}$ region are assigned to $\nu_{\text{asym}} \text{C}_9 \text{H}_7$ and $\nu_{\text{sym}} \text{C}_9 \text{H}_7$ respectively. Moreover, the bands due to Mn-N and Mn-Cl obtained at 420-460 cm$^{-1}$ and 250-300 cm$^{-1}$ respectively.

Electronic Spectral Analysis

The electronic spectra exhibited four weak intensity absorption bands at 18,330–19,684, 22,280–23,466, 28,022–28,334 and 38,450–38,618 cm$^{-1}$ which may be assigned as $^6A_{1g} \rightarrow ^4T_{1g} (4^G)$; $^6A_{1g} \rightarrow ^4E_g$; $^4A_{1g} (4^F)$ (10B + 5C), $^6A_{1g} \rightarrow ^4E_g$ (4$^P$) (17B + 5C) and $^6A_{1g} \rightarrow ^4T_{1g} (4^P)$ transitions, respectively. The B and C values were calculated using the transitions, $^6A_{1g} \rightarrow ^4E_g$, $^4A_{1g} (4^F)$ and $^6A_{1g} \rightarrow ^4E_g$; (4$^P$). This is due to the fact that the energies of these two transitions are independent of the crystal field splitting energy and depend only on the parameters B and C. The B and C values are 946.8 and 4356.4 cm$^{-1}$ respectively. The value for the parameter Dq can be evaluated with the help of the curve transition energy versus Dq, given by Orgel, using an energy level due to the transition $^6A_{1g} \rightarrow ^4T_{1g}$. The value of the Dq parameter could not be obtained using transitions $^6A_{1g} \rightarrow ^4E_g$, $^4A_{1g} (4^F)$; $^6A_{1g} \rightarrow ^4E_g$ (4$^P$) because, having almost zero slope, these transitions are independent of the Dq value. The above studies are in agreement of the octahedral geometry of the Mn(II) complexes [17]. The value of molar extinction coefficient (ε) for each transition shown by the complexes is also calculated by the Beer Lambert’s law i.e. $\varepsilon = \frac{A}{cl}$ where, l = 1cm and c = 10$^{-3}$ M. The value of molar extinction coefficients (ε) for $^6A_{1g} \rightarrow ^4T_{1g} (4^G)$; $^6A_{1g} \rightarrow ^4E_g$; $^4A_{1g} (4^F)$ (10B + 5C), $^6A_{1g} \rightarrow ^4E_g$ (4$^P$) (17B + 5C) and $^6A_{1g} \rightarrow ^4T_{1g} (4^P)$ transitions are 5400, 9800, 13200 and 14500 M$^{-1}$ cm$^{-1}$, respectively.
Mass Spectra

The FAB mass spectrum of the [Mn(C\textsubscript{37}H\textsubscript{29}N\textsubscript{4}FCl\textsubscript{2})] complex was studied as a representative case and some important peaks have been described. Peaks of appreciable intensity were observed at \textit{m/z} values 674.52, 603.77, 548.83 and 510.05. The molecular ion peak for the complex [Mn(C\textsubscript{37}H\textsubscript{29}N\textsubscript{4}FCl\textsubscript{2})] observed at \textit{m/z} 674.52 is in good agreement with its molecular weight, which suggests the monomeric nature of the complex and confirms the proposed formula (Figure 3). The proposed molecular formula of the complex was confirmed by comparing its molecular formula weight with \textit{m/z} value. This data is in good agreement with the respective molecular formula. The peak at \textit{m/z} 603.77 indicates that the molecular ion gave a fragment ion [Mn(C\textsubscript{37}H\textsubscript{29}N\textsubscript{4}F)] by losing two chloride ions. This fragment ion undergoes demetallation to form the species [C\textsubscript{37}H\textsubscript{29}N\textsubscript{4}F] and [C\textsubscript{34}H\textsubscript{23}N\textsubscript{2}O\textsubscript{2}F] which gave the fragment ion peaks at \textit{m/z} 548.83 and 510.05, respectively, as follows:

\[
\begin{align*}
\text{[Mn(C}_{37}\text{H}_{29}\text{N}_{4}\text{FCl}_{2})] - 2\text{Cl} & \rightarrow \text{[Mn(C}_{37}\text{H}_{29}\text{N}_{4}\text{F}]}, & \text{m/z}=674.52 \\
\text{[Mn(C}_{37}\text{H}_{29}\text{N}_{4}\text{F}] - \text{Cl}} & \rightarrow \text{[C}_{37}\text{H}_{29}\text{N}_{4}\text{F} + \text{[C}_{34}\text{H}_{23}\text{N}_{2}O_{2}\text{F}]}, & \text{m/z}=603.77,548.83,510.05
\end{align*}
\]

![Figure 3](image.png)

**Figure 3** Mass spectrum of the [Mn(C\textsubscript{37}H\textsubscript{29}N\textsubscript{4}FCl\textsubscript{2})] complex

Electrochemical Studies

The electrochemical behavior of the compound Mn(C\textsubscript{41}H\textsubscript{29}N\textsubscript{4}FCl\textsubscript{2}) was studied by cyclic voltammetric techniques on HMDE. Compound gave two well defined reduction peaks at -0.68 and -0.77mV in the nonaqueous solution which are attributed to the reduction of Mn(II) at HMDE. First peak can be explained with the help of one electron transfer during reduction of Mn(II) to Mn(I) and second peak appears corresponding to another one electron transfer step during which Mn(I) undergoes a further reduction to form Mn(0). No peak could be observed in anodic direction of
the reverse scans suggesting the irreversible nature of the electrode process. The peak potential shifted towards more negative values with increase in scan rate confirming the irreversible nature of the reduction process.

The effect of scan rate ($\nu^{1/2}$) on stripping peak current ($i_p$) was examined under the above experimental conditions (Figure 4 and 5). As the sweep rate is increased from 100 to 500 mV/s at a fixed concentration ($3 \times 10^{-3}$ M) of Mn(C$_{41}$H$_{29}$N$_4$FCl$_2$);

(i) The peak potential shifted cathodically and
(ii) The peak current increased steadily.

A straight line is obtained when $i_p$ is plotted against $\nu^{1/2}$, which may be expressed by the equation:

For first reduction peak: $y(i_p) = 0.0803 \nu^{1/2} (\text{mV/s}) + 0.0009 (\mu\text{A})$, $r^2 = 0.9991$

For second reduction peak: $y(i_p) = 0.176 \nu^{1/2} (\text{mV/s}) + 0.0423 (\mu\text{A})$, $r^2 = 0.998$

All these facts pointed towards the diffusion-controlled nature of the electrode process [18], and support the proposed structure of the complex.

**Figure 4, 5** Plot of peak current vs scan rate for first and second reduction peak of Mn(C$_{41}$H$_{29}$N$_4$FCl$_2$)

**X-ray Diffraction Study**

**Figure 6** X-ray powder diffraction pattern for [Mn(C$_{40}$H$_{26}$N$_4$F$_2$Cl$_2$)]
The possible lattice dynamics of the finely powdered product, [Mn(C_{40}H_{26}N_{4}F_{2}Cl_{2})] has been deduced on the basis of X-ray powder diffraction pattern (Figure 6). The observed interplanar spacing values ('d' in Å) have been measured from the diffractogram of the compound and the Miller indices h, k and l have been assigned to each d value and 2-Theta angles are reported in Table 2. The X-ray diffraction analysis of the compound [Mn(C_{40}H_{26}N_{4}F_{2}Cl_{2})] confirms the orthorhombic crystal system having unit cell dimensions as a=9.50, b=17.30, c=21.00, maximum deviation of 2-Theta= 0.058° and alpha= 90, beta= 90 and gama=90.

<table>
<thead>
<tr>
<th>h</th>
<th>k</th>
<th>l</th>
<th>2θ (°) (Exp.)</th>
<th>2θ (°) (Calc.)</th>
<th>2θ (°) (Diff.)</th>
<th>d(A) (Exp.)</th>
<th>d(A) (Calc.)</th>
<th>Intensity (Exp.)</th>
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<tr>
<td>1</td>
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<td>2</td>
<td>24.140</td>
<td>24.127</td>
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<td>1.56172</td>
<td>1.56237</td>
<td>1.93</td>
</tr>
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</table>

On the basis of analytical data and spectral evidences, the following hexa-coordinated environment has been established around the metal atom for the Mn(II) complexes (Figure 7).

![Figure 7 Proposed structure of [Mn(C_{40}H_{26}N_{4}F_{2}Cl_{2})] complex](image-url)
Biological Activities

Antibacterial Screening:

*In vitro* antibacterial screening is generally performed by disc diffusion method [19], for primary selection of the compounds as therapeutic agents. The antibacterial activity of the ligand and its Mn(II) complexes were evaluated against two bacteria including Gram-positive (*B. subtilis*) and Gram-negative (*P. aeruginosa*). The nutrient agar medium having the composition peptone 5g, beef extract 5g, NaCl 5g, agar-agar 20g and distilled water 1000 mL was pipetted into petridish. When it solidified, 5mL of warm seeded agar was applied. The seeded agar was prepared by cooling the molten agar to 40°C and then added the 10 mL of bacterial suspension. The compounds were dissolved in methanol in 500 and 1000 ppm concentrations. Paper discs of Whatman No.1 filter paper measuring diameter of 5mm were soaked in these solutions of varied concentrations. The discs were dried and placed on the medium previously seeded with organisms in petriplates at suitable distance. The petriplates were stored in an incubator at 28±2°C for 24 h. The diameters of the zone of inhibition produced by the compounds were compared with the standard antibiotic (Tetracycline). The zone of inhibition thus formed around each disc containing the test compounds was measured accurately in mm (Figure 8).

![Figure 8](image)

**Figure 8** Antibacterial activity of the ligand and its Mn(II) complexes

Antifungal Screening:

Bioefficacy of the compounds synthesized were checked *in vitro*. The antifungal activity of the ligand and its Mn(II) complexes have been evaluated against two pathogenic fungi, *F. oxysporum* and *M. phaseolina* by the agar plate technique [20]. The potato dextrose agar (PDA) medium was prepared in the laboratory to maintain the fungal growth. For PDA preparation, 20g potato was extracted with distilled water (100mL) at 100 ºC for 1 h and it was filtered off by cotton filter. The potato juice was then mixed with 2g dextrose and 1.5g agar and finally the pH of the prepared PDA media was adjusted at 7. Solutions of the test compounds in methanol at 100 and 200 ppm concentrations were prepared and then were mixed with the medium. The medium then was poured into petri plates and the spores of fungi were placed on the medium with the help of inoculum’s needle. These petriplates were wrapped in the polythene bags containing a few drops of alcohol and were placed in an incubator at 25±2°C. The activity was determined after 96 hrs of incubation at room temperature (25 ºC). The controls were also run and three replicates were used in each case. The linear growth of the fungus was obtained by measuring the diameter of the fungal colony after four days and the percentage inhibition was calculated as:

\[ \text{% inhibition} = 100 \times \frac{(C-T)}{C}, \]
where \( C = \) diameter of the fungus colony in the control plate after 96 h and \( T = \) diameter of the fungal colony in the test plates after the same period. The antifungal screening data of compounds were compared with the standard (Fluconazole) (Figure 9).

\[ \text{Figure 9 Antifungal activity of the ligand and its Mn(II) complexes} \]

Antimicrobial Assay

All the metal complexes were found to be more active against all the organisms used than the ligand (Figure 8 and 9). The enhanced antimicrobial activity of the metal chelates over their corresponding chelating agents may be explained on the basis of Overtone’s concept [21], and the Tweedy’s chelation theory [22,23]. According to Overtone’s concept of cell permeability, the lipid membrane that surrounds the cell favours the passage of only lipid-soluble materials due to which lipophilicity is an important factor which controls the antimicrobial activity. On chelation, the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, it increases the delocalisation of \( \pi \)-electrons over the whole chelate ring and enhances the lipophilicity of the complexes. This increased lipophilicity enhances the penetration of the complexes into lipid membranes and thus blocking the various metabolic activities of microorganisms.

The higher activity of the metal complexes can be attributed to the involvement of a metal ion in the normal cell processes [24]. Generally, this can be achieved through the following properties of the metal complexes:

(a) The complex should possess sufficient lipid solubility to permit transport of metal ions across the membranes.
(b) The metal complexes should be highly thermodynamically stable to reach the site without being dissociated.

Conclusion

We describe the synthesis, characterization and biological activity of Mn(II) macrocyclic complexes. On the basis of magnetic, analytical and spectral data an octahedral geometry has been proposed for the Mn(II) macrocyclic complexes. The electrochemical properties of metal complexes revealed the irreversible two electron transfer redox process. The antimicrobial activity results indicated that the complexes showed promising antibacterial and antifungal activities. The enhanced activity of the macrocyclic complexes than the parent ligands has been explained on the basis of chelation theory.

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References


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