Synthesis of O,O-ethylene bridged bis-metallated macrocycles;
Selective enzyme models for catecholase activity.
CHAPTER IV

Experimental

Ethanolamine, formaldehyde, 1,2-dibromoethane (Merck), CuCl₂·2H₂O, NiCl₂·6H₂O (BDH) and triethylenetetramine (Fluka) were used. Microanalysis of the complexes were carried out on a Carlo Erba analyzer model 1108. Molar conductance was measured at room temperature on a Digisun electronic conductivity bridge. Infrared spectra (400-4000 cm⁻¹) were recorded on a Shimadzu 8201 PC spectrophotometer in Nujol mull. The electronic spectra were recorded on a USB 2000 Ocean Optics spectrometer. ¹H and ¹³C n.m.r. spectra were recorded on a Bruker DRX-300 spectrometer. The e.p.r. spectra was run on a Varian E112. High purity, H₂O/MeOH (95:5) solvent was employed for the cyclic voltammetric studies. Kinetic experiments of the dinuclear copper(II) complex were performed at room temperature by using USB 2000 Ocean Optics spectrometer at varying concentrations of pyrocatechol.

Synthesis of [C₁₀H₁₅N₅OCu₂(ClO₄)]₂

(2.00 g, 0.011 mol) of CuCl₂·2H₂O was dissolved in 5 cm³ of methanol. To this solution was added triethylenetetramine (1.63 cm³, 0.011 mol), ethanolamine (1.65 cm³, 0.011 mol) and formaldehyde (0.94 cm³, 0.022 mol) in a 1:1:1:2 molar ratio. The resulting green colored mixture was refluxed for ca. 24 h. The solution was cooled at room temperature and filtered. Excess of perchloric acid was added to the filtrate and the
mixture was kept in refrigerator. The green crystals which separated out, were washed thoroughly with methanol and dried in vacuo.

*Synthesis of [C_{10}H_{25}N_3ONi]_2(ClO_4)_2*

To a solution of NiCl_2·6H_2O (2.00 g, 0.008 mol) in MeOH (5 cm^3) was added triethylenetetramine (1.25 cm^3, 0.008 mol), ethanolamine (1.20 cm^3, 0.008 mol) and formaldehyde (0.688 cm^3, 0.016 mol) in a 1:1:1:2 molar ratio and the reaction mixture was refluxed for 24 h. The mixture was cooled at room temperature and filtered. To the filtrate, excess of perchloric acid was added and kept in refrigerator. The yellow crystals appeared, which were filtered, washed thoroughly with methanol and dried in vacuo.

*Synthesis of [C_{22}H_{32}N_{10}O_2Cu]_2(ClO_4)_4*

The mononuclear complex [C_{10}H_{25}N_3OCu](ClO_4)_2 (1.00 g, 0.004 mol) dissolved in DMF (18 cm^3) by heating on water bath. To this solution was added 1,2-dibromoethane (0.171 cm^3, 0.002 mol) in 2:1 molar ratio and the resulting mixture was refluxed for 5 h until a dark brown solution resulted. The solution was reduced to half the volume and left overnight in refrigerator. A dark brown colored complex appeared, which was filtered, washed with hexane and dried in vacuo.

*Synthesis of [C_{22}H_{32}N_{10}O_2Ni_2](ClO_4)_4*

To a solution of [C_{10}H_{25}N_3ONi](ClO_4)_2 (1.00 g, 0.004 mol) in DMF was added 1,2-dibromoethane (0.171 cm^3, 0.002 mol) in 2:1 molar ratio and boiled under reflux for 5 h. The resulting solution was concentrated to half its volume and kept in refrigerator. A
$\text{MCl}_2 + \text{Triethylene tetramine} + \text{Ethanolamine} + \text{HCHO}$

$\xrightarrow{-2\text{H}_2\text{O}}$

$\xrightarrow{-2\text{HBr}} \text{C}_2\text{H}_4\text{Br}_2$

Scheme 3, $M=\text{Cu(II)}, \text{Ni(II)}$
light brown complex is isolated, which was filtered, washed with hexane and dried in vacuo. The synthesis procedure is outlined in (Scheme 3).

**Results and discussion**

Dinuclear complexes have been synthesized by the reaction of mononuclear complexes with 1,2-dibromoethane in 2:1 molar ratio. The elemental analysis and other physical data are presented in (Table 5). The conductance values ($10^{-3}$ M) in DMF indicate that the mononuclear and dinuclear complexes are 2:1 and 4:1 electrolytes, respectively.

**I.r spectra**

The i.r spectra of the mononuclear and dinuclear complexes are displayed in (Table 6). The mononuclear complexes $[\text{C}_{10}\text{H}_{23}\text{N}_{5}\text{OCu}]_2\text{ClO}_4$ and $[\text{C}_{10}\text{H}_{23}\text{N}_{5}\text{Oni}]_2\text{ClO}_4$ exhibit characteristic $\nu$(N-H) of the coordinated secondary amines in the range of 3200 to 3210 cm$^{-1}$ [185] and show O-H stretching vibration of the pendant arm at 3400 cm$^{-1}$ [186]. The $\nu$(Cl$_2$) was observed in the range of 2926-2931 cm$^{-1}$ [187]. The appearance of broad absorption at 1113 cm$^{-1}$ indicates the crystallization of ionic perchlorates [188]. The dinuclear complexes show i.r bands similar to the mononuclear complexes except that there is disappearance of $\nu$(O-H) at 3400 cm$^{-1}$. This clearly demonstrates the formation of ethylene bridge through alcoholic O-H which is further authenticated by the presence of $\nu$(C-O) at 1242 cm$^{-1}$ in the dinuclear complexes [189]. The formation of dinuclear complexes is further supported by $^1$H n.m.r and $^{13}$C n.m.r data. The $\nu$(M-N),
\(v(N=2)\) and \(v(ClO_4^-)\) appears at 495-498 cm\(^{-1}\), 3222-3225 cm\(^{-1}\) and 1113-1120 cm\(^{-1}\) respectively [190-192].

**E.p.r spectra**

The solid state e.p.r spectrum of the mononuclear copper(II) complex at liquid nitrogen temperature LNT (77 K) (Figure-24) exhibits a large isotropic signal centered at \(g = 2.057\), corresponding to the square-planar Cu(II) ion [193]. In contrast, the e.p.r. spectrum of dinuclear Cu(II) complex was found to be anisotropic with two \(g\) values (Figure-25). The parameters \(g_{ll} = 2.094\), \(g_{l} = 2.025\) and \(g_{av} = 2.048\) were computed from the spectrum using TCNE free radicle as ‘g’ marker. The existence of \(g_{ll} > g_{l}\) suggests that \(d^9\) is the ground state for the \(d^9[\text{Cu}^{2+}]\) configuration i.e \((e_g)^4 (a_{1g})^2 (b_{2g})^2 (b_{1g})^1\) suggesting square-planar geometry around Cu(II) centers [194-195]. In addition, there is exchange coupling interaction between two copper centers explained by Hathaway expression \(G = (g_{ll}-2)/(g_{l}-2)\). According to Hathaway, if the value of \(G\) is greater than four, the exchange interaction is negligible, whereas when the value of \(G\) is less than four, a considerable interaction is indicated in the solid complex. For the dinuclear copper(II) complex, the value of \(G\) was found to be 3.76 indicating the exchange interaction between two copper centers.
Figure-24. The e.p.r spectrum of complex $[C_{19}H_{25}N_3OCu].(ClO_4)_2$ at LNT (77 K).

Figure-25. The e.p.r spectrum of complex $[C_{22}H_{32}N_{10}O_2Cu_2].(ClO_4)_4$ at LNT (77 K).
Electronic spectra

The electronic spectra of mononuclear complex \([\text{C}_{10}\text{H}_{25}\text{N}_{5}\text{OCu}]_{2}\text{(ClO}_4\text{)}_{2}\) displays a broad d-d band at 15,151 cm\(^{-1}\) due to the transition \(^2\text{B}_{1g} \rightarrow ^2\text{A}_{1g}\), which commensurates with the square-planar geometry around Cu(II) metal ion [196,197]. The electronic spectrum of dinuclear complex \([\text{C}_{22}\text{H}_{52}\text{N}_{10}\text{O}_{2}\text{Cu}_2]_{2}\text{(ClO}_4\text{)}_{4}\) in DMF is dominated by an intense band at 14,705 cm\(^{-1}\) suggesting square-planar geometry around Cu(II) ion. A single band centered at 22.271 cm\(^{-1}\) in the electronic spectrum of the complex \([\text{C}_{10}\text{H}_{25}\text{NiCl}]_{2}\text{(ClO}_4\text{)}_{2}\) indicate square-planar geometry around Ni(II) ion [198]. The spectrum of dinuclear nickel(II) complex exhibits a band at 21,739 cm\(^{-1}\) which is assigned to \(^1\text{A}_{1g} \rightarrow ^1\text{B}_{2g}\) transition, characteristic of square-planar geometry around the nickel(II) ion.

N.m.r spectra

\(^1\text{H}\) and \(^{13}\text{C}\) n.m.r spectra of the mononuclear \([\text{C}_{10}\text{H}_{25}\text{N}_{5}\text{ONi}]_{2}\text{(ClO}_4\text{)}_{2}\) and dinuclear \([\text{C}_{22}\text{H}_{52}\text{N}_{10}\text{O}_{2}\text{Ni}_2]_{2}\text{(ClO}_4\text{)}_{4}\) complex were recorded in DMSO-\(d_6\), and compared (Table 7 and 8). \(^1\text{H}\) n.m.r spectra of mononuclear Ni(II) complex exhibits signals in the range \(\delta 2.98-3.01, \delta 2.08-2.10 (\text{dd,}4\text{H}), \delta 2.40-2.89 (\text{m,}12\text{H})\) due to NH-CH\(_2\)-CH\(_2\)-N and HN-CH\(_2\)-CH\(_2\)-NH respectively [199]. Two more signals appear at 3.93 and 7.67 ppm due to CH\(_2\)OH and NH protons [200]. The n.m.r spectra of dinuclear complex \([\text{C}_{22}\text{H}_{52}\text{N}_{10}\text{O}_{2}\text{Ni}_2]_{2}\text{(ClO}_4\text{)}_{4}\) reveals no change in the above signals, except disappearance of the signal of OH at 3.93 ppm and new signal appear in the range of \(\delta 3.46-3.87 (\text{m,}4\text{H})\) due to O-CH\(_2\)-CH\(_2\)-O confirming the dinuclear complex formation [201].
$^{13}$C n.m.r spectra of mononuclear Ni(II) complex exhibit C-C, C-N, C-OH and $-\text{ClH}_2$ signals at 37.90-40.34, 42.76, 150 and 35.3-36.61 respectively [202]. The $^{13}$C carbon signals appear in the same range in dinuclear complex along with new signal at 68-70 ppm due to O-CH$_2$-CH$_2$-O which further authenticate the formation of $-\text{ClH}_2$-$\text{ClH}_2$-bridged dinuclear Ni(II) complex [203].

**Electrochemical studies**

The electrochemical behavior of the dinuclear complex [C$_{22}$H$_{52}$N$_{10}$O$_2$Cu$_2$](ClO$_4$)$_4$ has been studied by cyclic voltammetry. The cyclic voltammogram of the free dinuclear Cu(II) complex in H$_2$O/DMF (95:5) at a scan rate of 0.1Vs$^{-1}$ reveal one electron quasireversible wave attributed to redox couple Cu$^{II}$/Cu$^I$ with formal electrode potential $E_{1/2} = -0.565$V ($E_{pc} = -0.620$V, $E_{pa} = -0.510$V), the ratio of anodic to cathodic peak currents $i_{pa}/i_{pc} = 0.814$ and $\Delta E_p = 0.110$ V which is larger than Nernstian value observed for one electron transfer (Figure-26). After passing molecular oxygen to the solution of the complex, the cyclic voltammetric behavior differs considerably from the free complex as the formal electrode potential $E_{1/2} = -0.593$V ($E_{pc} = -0.655$V and $E_{pa} = -0.531$ V), $i_{pa}/i_{pc} = 2.93$ and $\Delta E_p = 0.124$ V (Figure-27). The shift in formal electrode potential $E_{1/2}$ as well as cathodic and anodic peak potentials indicate the binding of oxygen to the dinuclear Cu(II) complex. The redox behavior for the catechol cleaving dioxygenase
**Figure-26.** Cyclic voltammogram of dinuclear copper(II) complex \([C_{22}H_{32}N_{10}O_2Cu_2](ClO_4)_4 (1 \times 10^{-3} \text{ M})\) in DMF at a scan rate of 0.1 \(V\text{s}^{-1}\).

**Figure-27.** Cyclic voltammogram of dinuclear copper(II) complex in DMF after passing \(O_2\) gas.
activity of the present complex towards pyrocatechol was examined in presence of molecular oxygen at a scan rate of 0.1 Vs\(^{-1}\) (Figure-28). On addition of pyrocatechol to the complex solution in presence of O\(_2\), the cyclic voltammetric results of the catalytic reaction reflects the quasi-reversible wave for the one electron transfer Cu\(^{II}\)/Cu\(^{I}\) couple with \(E_{pc} = -0.655\) V, \(E_{pa} = -0.501\) V, \(E_{1/2} = -0.57\) V, with emergence of a new cathodic peak at 0.015 V. The cyclic voltammogram for the catecholase activity of the dicopper complex shows a shift of 0.001, 0.006 and 0.017 V in the cathodic and anodic peaks after sometime (Figure-29).

**Figure-28.** Cyclic voltammogram of dinuclear copper(II) complex taken initially in the presence of pyrocatechol.
Figure-29. Cyclic voltammogram of dinuclear copper(II) complex after the complete conversion of pyrocatechol into o-benzoquinone by the dinuclear copper(II) complex.

Kinetic studies for catecholase mimetic activities

The catalytic oxidation of pyrocatechol by the model complex \([C_{22}H_{52}N_{10}O_2Cu_2](ClO_4)_4\) was carried out spectrophotometrically. Nishida and co-workers [204] have found that dinuclear copper(II) complexes exhibit a high catalytic activity in comparison to mononuclear complexes and proposed that a determining factor is the presence of two metal centers located in close proximity to facilitate the binding of the two oxygen atoms of the catechol prior to the electron transfer [205]. Catalytic studies were performed in DMF solution owing to the good solubility of the dinuclear copper complex as well as substrate in DMF. To a (100 cm\(^3\)) solution of \([C_{22}H_{52}N_{10}O_2Cu_2].(ClO_4)_4\) (concentration
1x10^{-3} \text{ mol dm}^{-3}) \text{ was added (100 cm}^3\text{) catechol solution of varying concentration (3 x10^{-3}, 3.5 x10^{-3}, 4 x10^{-3}, 4.5 x10^{-3}, 5 x10^{-3}, 5.5 x10^{-3}, 6 x10^{-3} \text{ mol dm}^{-3}), (100 cm}^3\text{) of tris-HCl buffer pH 8.0 and (3.7 cm}^3\text{) of DMF saturated with molecular O}_2. \text{ The course of the reaction was followed by Uv-vis spectroscopy (Figure-30). The absorption spectra of the original solution in absence of pyrocatechol recorded two absorption maxima at 35,971 and 14,705 cm}^{-1} \text{ which were attributed to CT bands and d-d transition of the metal ion respectively. After the addition of pyrocatechol the uv-vis spectra was scanned at regular time intervals for 25 min. and was also recorded after the duration of 24 hr. There was increase in the absorption in 26,315-25,000 cm}^{-1} \text{ region, with the course of reaction a sharp absorption emerged at 25,000 cm}^{-1} \text{ which is characteristic of oxidation of catechol to substituted o-quinones [206]. Oxygen directly participates in the catalytic cycle by reoxidising copper(I) species back to the active copper(II) species.}

![Figure-30: Oxidation of pyrocatechol by the dinuclear copper(II) complex.](image)
Figure-31. A comparison of time-dependent formation of quinone by the complex 
\([\text{C}_{22}\text{H}_{52}\text{N}_{10}\text{O}_{2}\text{Cu}_2}\). \((\text{ClO}_4)_4\), at different concentrations of pyrocatechol 
representative curves (a) 3 \times 10^{-4} (b) 4 \times 10^{-3} (c) 5 \times 10^{-3} (d) 6 \times 10^{-3} \text{ mol dm}^{-3}

The kinetics of the oxidation of catechol was determined by the method of initial rates 
versus the concentration of dinuclear complex by monitoring the growth of the 25,000 
cm\(^{-1}\) band of the product o-benzoquinone. In order to determine the kinetic parameters, 
the Michaelis-Menten approach which originally developed for enzyme kinetics, was 
applied [207-209]. The parameters \(V_{\text{max}}\), \(K_M\) were determined from Lineweaver-Burk 
plots (Figure-32) and a value of \(V_{\text{max}}\) (2.8 \times 10^{-3} \text{ MS}^{-1}) and \(K_M\) (1.4 \times 10^{-3} \text{ mM}) for the 
dinuclear copper complex was obtained, which indicate the second order kinetics with
respect to different concentrations of pyrocatechol. The rate law which holds good for the kinetic reaction is,

\[ V = \frac{d[P]}{dt} = K_{+2}[ES] = K_{+2}[E_0][S]/[S] + K_M \]

where \( V \) is the rate of reaction at which the product is formed, or simplifying

\[ V = V_{max}[S]/[S] + K_M \]

Where \( S \) is the concentration of substrate, \( V_{max} \) is the maximum rate of reaction, which occurs when the enzyme is completely saturated with substrate and \( K_M \) is the Michaelis constant.

**Figure-32.** Kinetic parameters \( V_{max} \) and \( K_M \) have been determined by the plot of \( V_0 (MS^{-1}) \) versus concentrations of pyrocatechol.
Table 5. Physical and analytical data of the complexes.

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Colour</th>
<th>M.p(°C)</th>
<th>Yield(%)</th>
<th>Found(calcd.)( %)</th>
<th>Ω⁻¹cm²mol⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C</td>
<td>H</td>
</tr>
<tr>
<td>[C₁₀H₂₅N₃OCu].(ClO₄)₂</td>
<td>green</td>
<td>220</td>
<td>70</td>
<td>24.41</td>
<td>5.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(24.39)</td>
<td>(5.08)</td>
</tr>
<tr>
<td>[C₁₀H₂₅N₃ONi].(ClO₄)₂</td>
<td>yellow</td>
<td>250</td>
<td>71</td>
<td>24.61</td>
<td>5.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(24.59)</td>
<td>(5.12)</td>
</tr>
<tr>
<td>[C₁₂H₃₂N₁₀O₂Cu₂].(ClO₄)₄</td>
<td>dark brown</td>
<td>195</td>
<td>65</td>
<td>26.15</td>
<td>5.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(26.13)</td>
<td>(5.14)</td>
</tr>
<tr>
<td>[C₁₄H₃₂N₁₀O₂Ni₂].(ClO₄)₄</td>
<td>light brown</td>
<td>70</td>
<td>63</td>
<td>26.36</td>
<td>5.15</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>(26.34)</td>
<td>(5.18)</td>
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<table>
<thead>
<tr>
<th>Complexes</th>
<th>δ(CH₂)</th>
<th>δ(C-O)</th>
<th>δ(N-H)</th>
<th>δ(C-O)</th>
<th>δ(M-N)</th>
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<tbody>
<tr>
<td>[C₆H₆N₄Cu(CIO₄)₂]</td>
<td>2926</td>
<td>1289</td>
<td>3200</td>
<td>3400</td>
<td>495</td>
</tr>
<tr>
<td>[C₆H₆N₄Cu(CIO₄)]</td>
<td>2929</td>
<td>1289</td>
<td>3210</td>
<td>3410</td>
<td>495</td>
</tr>
<tr>
<td>[C₆H₆N₄O₄Cu²⁺Cu₃⁺(CIO₄)₄]</td>
<td>2930</td>
<td>1242</td>
<td>3222</td>
<td>498</td>
<td>1118</td>
</tr>
<tr>
<td>[C₆H₆N₄O₄Cu⁴⁺(CIO₄)₄]</td>
<td>2931</td>
<td>1245</td>
<td>3225</td>
<td>498</td>
<td>1120</td>
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Table 6. Infrared spectra of the complexes (cm⁻¹).
Table 7. $^1$H n.m.r spectra of the complexes (ppm).

<table>
<thead>
<tr>
<th>Complexes</th>
<th>NH-CH$_2$-N</th>
<th>CH$_2$-CH$_2$-N</th>
<th>HN-CH$_2$-CH$_2$-NH</th>
<th>CH$_2$OH</th>
<th>O-CH$_2$-CH$_2$-O</th>
</tr>
</thead>
<tbody>
<tr>
<td>[C$<em>{10}$H$</em>{25}$N$_5$ONi].(ClO$_4$)$_2$</td>
<td>2.98-3.01</td>
<td>2.08-2.10</td>
<td>2.40-2.89</td>
<td>3.93</td>
<td>-</td>
</tr>
<tr>
<td>[C$<em>{22}$H$</em>{32}$N$_{10}$O$_2$Ni$_2$].(ClO$_4$)$_4$</td>
<td>2.97-3.12</td>
<td>2.09-2.20</td>
<td>2.32-2.84</td>
<td>-</td>
<td>3.46-3.87</td>
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Table 8. $^{13}$C n.m.r spectra of the complexes (ppm).

<table>
<thead>
<tr>
<th>Complexes</th>
<th>C-C</th>
<th>C-N</th>
<th>C-OH</th>
<th>-CH$_2$</th>
<th>O-CH$_2$-CH$_2$-O</th>
</tr>
</thead>
<tbody>
<tr>
<td>[C$<em>{10}$H$</em>{25}$N$_5$ONi]$_2$(ClO$_4$)$_2$</td>
<td>37.90-40.34</td>
<td>42.76</td>
<td>150</td>
<td>35.3-36.61</td>
<td></td>
</tr>
<tr>
<td><a href="ClO$_4$">C$<em>{22}$H$</em>{32}$N$_{10}$O$_2$Ni$_2$</a>$_4$</td>
<td>39.5</td>
<td>42.7-42.9</td>
<td>148</td>
<td>34.4</td>
<td>68-70</td>
</tr>
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