Chapter II
Experimental

Chemicals and Reagents

The following chemicals and reagents were used for the synthesis of the compounds.

4-nitrophenol (Sigma-Aldrich)
4-chlorophenol (Sigma-Aldrich)
4-Bromophenol (Sigma-Aldrich)
N,N,N’-trimethylethylenediamine (Sigma-Aldrich)
N,N,N’-triethylethylenediamine (Sigma-Aldrich)
Copper(II) acetate (Sigma-Aldrich)
Copper(II) bromide (Sigma-Aldrich)
Copper(II) chloride (Sigma-Aldrich)
Copper(II) carbonate (Sigma-Aldrich)
Paraformaldehyde (Sigma-Aldrich)

were obtained commercially and used as such. Commercially available ethanol and methanol were refluxed with quick lime for 8 h, decanted the solution and finally distilled from magnesium. The middle fraction was collected onto 3 Å molecular sieves and used. Acetonitrile (HPLC grade) was dried by stirring over potassium carbonate for 24 h and stored over 3 Å sieves and used. The AR solvents diethyl ether, dichloromethane and chloroform were purchased from S. D. Fine chemicals and distilled from P₂O₅ and stored in 3 Å sieves.
2.1 Synthesis of tridentate Mannich based ligands

The ligands were obtained by following Mannich reaction, using one equivalents of amines (N,N,N’-trimethylethylenediamine, N,N,N’-triethylethylenediamine) with one equivalent of 4-substituted phenol and formaldehyde in alcoholic medium.

2.1.1 Synthesis of 4-Nitro-2-[N-(2-{dimethylamino}ethyl-N’-ethyl)aminomethyl phenol [HL₁]

4-Nitrophenol (0.038 mol) in ethanol (150 mL) was mixed with N,N,N’-trimethylethylenediamine (0.038 mol) under stirring and cooled in ice. Formaldehyde (35%, 0.113 mol) was added dropwise with constant stirring. The mixture was stirred at room temperature (28°C) for 24 h and then refluxed for 8 h. The solvent was removed under vacuo and the resulting oily product was neutralized with saturated sodium carbonate and extracted with chloroform (4 × 50 mL), the organic layers were combined and dried over anhydrous magnesium sulphate followed by filtration afforded a yellow solid product. This was purified by column chromatography on silical gel (CHCl₃/MeOH). M. F. C₁₂H₁₈N₃O₃; Yield 2.21 g (87%); mp 112°C; ¹H NMR (400 MHz, CDCl₃) δ 8.10-6.80 (m, 3H, ArH), 3.56 (s, 2H, benzylic CH₂), 2.67-2.56 (m, 4H, NCH₂CH₂N), 2.31 (s, 6H, N(CH₃)₂), 2.26 (s, 3H, NCH₃); ¹³C NMR (100 MHz, CDCl₃) δ 165.4, 125.8, 123.6, 117.3, 77.48, 57.1, 55.8, 53.3, 44.7, 42.4; IR (KBr, cm⁻¹): 3400-3600, 2900-2850, 1477, 1267.

The remaining ligands [HL₂], [HL₃] and [HL₄] were synthesized in similar way to [HL₁] with the appropriate phenols and secondary amines.
2.1.2 Synthesis of 4-Nitro-2-[N-(2-{diethylamino}ethyl-N’-ethyl)aminomethyl]phenol [HL₂]

A yellow solid was obtained. M. F. C₁₄H₂₄N₃O₃; Yield 6.48 g (88%); mp 80° C; ¹H NMR (400 MHz, CDCl₃) δ 8.10-6.80 (m, 3H, ArH), 3.68 (s, 2H, benzylic CH₂), 2.67-2.48 (m, 4H, NCH₂CH₂N), 2.33 (t, 9H, NCH₂CH₃), 2.66 (q, 6H, NCH₂CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 166.3, 138.9, 125.7, 123.5, 117.2, 54.8, 49.3, 47.3, 46.2, 10.9, 10.6; IR (KBr, cm⁻¹): 3400-3500, 2920-2800, 1492, 1377.

2.1.3 4-Chloro-2-[N-(2-{dimethylamino}ethyl-N’-methyl)aminomethyl]phenol [HL₃]

A yellow semi-solid was obtained. M. F. C₁₂H₁₈N₂OCl; Yield 9.59 g (87%); ¹H NMR (400 MHz, CDCl₃) δ 7.10 (s, 2H, ArH), 3.64 (s, 4H, benzylic CH₂), 2.60-2.57 (t, 8H, NCH₂CH₂N), 2.52-2.50 (q, 12H, NCH₂CH₃), 1.06-1.04 (t, 6H, NCH₂CH₃), 1.02-0.98 (t, 12H, NCH₂CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 154.5, 127.3, 125.7, 122.7, 53.8, 50.9, 50.4, 47.3, 11.3; IR (KBr, cm⁻¹): 3400-3500, 2900-2800, 1357, 1463, 887.

2.1.4 4-Bromo-2-[N-(2-{dimethylamino}ethyl-N’-methyl)aminomethyl]phenol [HL₄]

A yellow solid was obtained. M. F. C₁₂H₁₈N₂OBr; Yield 2.21 g (87%); mp 112° C; ¹H NMR (400 MHz, CDCl₃) δ 8.10-6.80 (m, 3H, ArH), 3.59 (s, 2H, benzylic CH₂), 2.67-2.56 (m, 4H, NCH₂CH₂N), 2.33 (s, 6H, N(CH₃)₂), 2.28 (s, 3H, NCH₃); ¹³C NMR (100 MHz, CDCl₃) δ 165.2, 152.6, 125.6, 123.5, 117.1, 102.6, 57.2, 55.8, 53.3, 44.7, 42.2; IR (KBr, cm⁻¹): 3400-3600, 2900-2850, 1452, 1317.
2.1.5 Synthesis of 2-[(4-methyl-pyridin-2-ylimino)-methyl]-phenol (Hsalamp)[HL₅]

The Schiff’s base ligand Hsalamp is synthesized by direct mixing of 1:1 molar ratio of 4-methyl-2-aminopyridine (2.12 g) with salicylaldehyde (4 ml) under reflux for about 6 hours in double distilled dry EtOH. Slow evaporation resulted in the formation yellow diamond shaped crystals. These crystals are dissolved in dry MeOH and recrystallized. The yield is around 80% and the melting point of the ligand is 383(1) K. IR (KBr disc): 1594 cm⁻¹ (C=N stretch); 1477 cm⁻¹ (phenolic OH stretch); 1267 cm⁻¹ (phenolic C=O); 2944 cm⁻¹ (intramolecular phenolic OH stretch).

2.2 Preparation of copper(II) perchlorate hexahydrate

To 15 g of copper(II) carbonate suspended in 10 mL of water, 100 mL of concentrated perchloric acid was added dropwise and stirred thoroughly until the effervescence ceases. The solution was left undisturbed for about 15 minutes, when the settled precipitate was filtered. The filtrate was concentrated by heating at 80° C and cooled to 0° C. Crystalline blue colour solid was separated, which was washed with sufficient quantity of ice-cold water to eliminate traces of perchloric acid, adhering to the crystals. Finally the product was washed with minimum quantity of cold diethyl ether and kept for drying in a desiccator. (Caution: All the perchlorate salts are highly explosive, hence care should be taken while handling this salt).
2.3 Synthesis of mononuclear copper(II) complexes

2.3.1 Copper(II) acetato complexes \([CuHL_1(CH_3COO)H_2O] \), (IA) An aqueous methanol (1:4, H_2O: CH_3OH) solution of copper acetate (25 mL, 0.002 mol) was mixed with a methanolic solution containing ligand 4-Nitro-2-[N-(2-{dimethylamino}ethyl-N’-ethyl)aminomethyl phenol [HL_1] (50 mL, 0.001 mol). The final dark green solution was allowed to evaporate slowly at room temperature. The resulting green compound was recrystallized from methanol. Dark green Plate shaped crystals suitable for X-ray diffraction.

2.3.2 Copper(II) Imidazole complexes \([Cu(HL_1)(Im)H_2O]ClO_4 \), (IIA) The methanolic solution of copper(II) perchlorate (10 mL, 0.002 mol) was added dropwise to a methanolic solution of ligand 4-Nitro-2-[N-(2-{dimethylamino}ethyl-N’-ethyl)aminomethyl phenol [HL_1] (75 mL, 0.002 mol), followed by the methanolic imidazole (20 mL, 0.002 mol) with constant stirring. The resulting green solution was stirred for 3 h and kept in room temperature for slow evaporation. The green powder sample was recrystallized from methanol. Light Green crystals were obtained suitable for X-ray diffraction.

2.3.3 Copper(II) pyrazole complexes \([Cu(HL_1)(Pyz)H_2O]ClO_4 \), (IIIA) The methanolic solution of copper(II) perchlorate (10 mL, 0.002 mol) was added dropwise to a methanolic solution of ligand 4-Nitro-2-[N-(2-{dimethylamino}ethyl-N’-ethyl)aminomethyl phenol [HL_1] (75 mL, 0.002 mol), followed by the methanolic pyrazole (20 mL, 0.002 mol) with constant stirring. The resulting green solution was stirred for 3 h and kept in room temperature for slow evaporation. The dark green powder sample was...
recrystallized from methanol. Dark Green crystals were obtained suitable for X-ray diffraction.

2.3.4 **Aqua-copper(II) complexes** $[\text{Cu(HL}_1\text{)(H}_2\text{O})_2\text{]}\text{ClO}_4$ (IVA) The methanolic solution of copper(II) perchlorate (10 mL, 0.002 mol) was added dropwise to a methanolic solution of ligand 4-Nitro-2-[N-(2-{dimethylamino}ethyl-N’-ethyl)aminomethyl phenol $[\text{HL}_1\text{]}$ (75 mL, 0.002 mol) with constant stirring. The resulting green solution was stirred for 3 h and kept in room temperature for slow evaporation. The green powder sample was recrystallized from methanol. Green plate shaped crystals were obtained suitable for X-ray diffraction.

2.3.5 **Copper(II) chloride complexes** $[\text{Cu(HL}_5\text{)(L}_1\text{)}\text{(Cl)}_2\text{]}$, (VA) To the methanolic solution of ligand 2-[(4-methyl-pyridine-2-ylimino)-methyl]phenol (0.01 moles), methanolic solution of copper chloride (0.01 moles) was added, on further addition of the methanolic solution of 4-methylpyridin-2-amine (0.01 moles) solution becomes dark green. The whole solution is then kept under reflux for 2 hours around 330 k. slow evaporation of this solution resulted in the form of the development of small crystals of shiny green colour suitable for X-ray diffraction.

2.3.6 **Copper(II) azide complexes** $[\text{Cu(HL}_5\text{)}_2\text{(N}_3\text{)}_2\text{]}$ (VIA) The methanolic solution of copper(II) perchlorate (10 mL, 0.002 mol) was added dropwise to a methanolic solution of HSALAMP (75 mL, 0.002 mol), followed by the aqueous methanolic (1:3, $\text{H}_2\text{O}:\text{CH}_3\text{OH}$) sodium azide (20 mL, 0.002 mol) with constant stirring. The resulting green solution was stirred for 3 h and kept in room temperature for slow evaporation. The
green powder sample was recrystallized from methanol. Green square shaped crystals were obtained suitable for X-ray diffraction.

2.4 Synthesis of binuclear copper(II) complexes

2.4.1 Di-μ\textsubscript{1,3}-azido-bis(4-Nitro-2-[N-(2-{dimethylamino}ethyl-N'-methyl)amino methyl] phenol)-dicopper(II) complexes, (1a) To an ethanol solution (10 ml) of 4-Nitro-2-[N-(2-{dimethylamino}ethyl-N'-methyl) amino methyl]phenol[HL\textsubscript{1}] (0.1 mmol,) and NaN\textsubscript{3} (0.1 mmol,) was added an aqueous solution (1 mL) of Cu(ClO\textsubscript{4})\textsubscript{2}.6H\textsubscript{2}O (0.1 mmol,) with continuous stirring at room temperature. The final clear dark green solution was allowed to stand at room temperature for several days. Green Plate shaped crystals suitable for X-ray diffraction were collected and dried in air. The copper compounds prepared with ligands HL\textsubscript{1} –HL\textsubscript{4} are:

\[ [\text{Cu}_2(\text{HL}_1)_2(\mu_{1,3}-\text{N}_3)_2], \text{(1a)} \]

\[ [\text{Cu}_2(\text{HL}_2)_2(\mu_{1,3}-\text{N}_3)_2], \text{(1b)} \]

\[ [\text{Cu}_2(\text{HL}_3)_2(\mu_{1,3}-\text{N}_3)_2], \text{(1c)} \]

\[ [\text{Cu}_2(\text{HL}_4)_2(\mu_{1,3}-\text{N}_3)_2], \text{(1d)} \]

2.4.2 Aqua-bis(μ-phenoxyo)-4-Bromo-2-[N-(2-{dimethylamino}ethyl-N'-methyl) amino methyl]dicopper(II) perchlorate complexes, [Cu\textsubscript{2}(HL\textsubscript{4})\textsubscript{2}(H\textsubscript{2}O)(ClO\textsubscript{4})]\textsubscript{2} \text{ClO}_4 \text{(2d)} To an ethanol solution (10 ml) of 4-Bromo-2-[N-(2-{dimethylamino}ethyl-N'-methyl) amino methyl]phenol[HL\textsubscript{4}] (0.1 mmol, 29 mg) was added an aqueous solution (1 mL) of Cu(ClO\textsubscript{4})\textsubscript{2}.6H\textsubscript{2}O (0.1 mmol, 37.0 mg) with continuous stirring at room temperature. The final clear dark green solution was allowed to stand at room
temperature for several days. Green Plate shaped crystals suitable for X-ray diffraction were collected and dried in air. Similarly the copper compounds prepared with ligands HL₁ – HL₄ are:

\[
\text{[Cu}_2\text{(HL}_1\text{)}_2\text{(H}_2\text{O})(\text{ClO}_4\text{)]ClO}_4 \text{ (2a)}
\]

\[
\text{[Cu}_2\text{(HL}_2\text{)}_2\text{(H}_2\text{O})(\text{ClO}_4\text{)]ClO}_4 \text{ (2b)}
\]

\[
\text{[Cu}_2\text{(HL}_3\text{)}_2\text{(H}_2\text{O})(\text{ClO}_4\text{)]ClO}_4 \text{ (2c)}
\]

\[
\text{[Cu}_2\text{(HL}_4\text{)}_2\text{(H}_2\text{O})(\text{ClO}_4\text{)]ClO}_4 \text{ (2d)}
\]

2.4.3 Di-Azido-bis(μ-phenoxo)-(4-Bromo-2-[N-(2-{dimethylamino}ethyl-N′-methyl)amino methyl] dicopper(II) complex, [Cu₂(HL₄)₂(N₃)₂] (3d) To an ethanol solution (10 ml) of 4-Bromo-2-[N-(2-{dimethylamino}ethyl-N′-methyl) amino methyl] phenol[HL₄] (0.1 mmol, 29 mg) and NaN₃ (0.1 mmol, 6.5 mg) was added with continuous stirring at room temperature. The final clear dark green solution was allowed to stand at room temperature for several days. Green Plate shaped crystals suitable for X-ray diffraction were collected and dried in air. Similarly the copper compounds prepared with ligands HL₁ – HL₄ are:

\[
\text{[Cu}_2\text{(HL}_1\text{)}_2\text{(N}_3\text{)}_2\text{], (3a)}
\]

\[
\text{[Cu}_2\text{(HL}_2\text{)}_2\text{(N}_3\text{)}_2\text{], (3b)}
\]

\[
\text{[Cu}_2\text{(HL}_3\text{)}_2\text{(N}_3\text{)}_2\text{], (3c)}
\]

\[
\text{[Cu}_2\text{(HL}_4\text{)}_2\text{(N}_3\text{)}_2\text{], (3d)}
\]

2.4.4 Di-Chloro-bis(μ-phenoxo)-4-Bromo-2-[N-(2-{dimethylamino}ethyl-N′-methyl)amino methyl] dicopper(II), [Cu₂(HL₄)₂(Cl)₂] (4d) To an ethanol solution (10
ml) of 4-Bromo-2-[N-(2-{dimethylamino}ethyl-N'-methyl) aminomethyl]phenol (0.1 mmol) was added an aqueous solution (5 mL) of CuCl$_2$(0.1 mmol) with continuous stirring at room temperature. The final clear green solution was allowed to stand at room temperature for several days. Light green shaped crystals suitable for X-ray diffraction were collected and dried in air. Similarly the copper compounds prepared with ligands HL$_1$ –HL$_4$ are:

$$[\text{Cu}_2(\text{HL}_1)_2(\text{Cl})_2]$$ (4a)

$$[\text{Cu}_2(\text{HL}_2)_2(\text{Cl})_2]$$ (4b)

$$[\text{Cu}_2(\text{HL}_3)_2(\text{Cl})_2]$$ (4c)

$$[\text{Cu}_2(\text{HL}_4)_2(\text{Cl})_2]$$ (4d)

### 2.4.5 Di-Bromo-bis($\mu$-phenoxo)-(4-Chloro-2-[N-(2-{dimethylamino}ethyl-N' -methyl) aminomethyl]dicopper(II), $[\text{Cu}_2(\text{HL}_3)_2(\text{Br})_2]$ (5c)

To an ethanol solution (10 ml) of 4-Chloro-2-[N-(2-{dimethylamino}ethyl-N’-methyl) aminomethyl]phenol (0.1 mmol) was added an aqueous solution (5 mL) of CuBr$_2$(0.1 mmol) with continuous stirring at room temperature. The final clear green solution was allowed to stand at room temperature for several days. Light green shaped crystals suitable for X-ray diffraction were collected and dried in air. Similarly the copper compounds prepared with ligands HL$_1$ –HL$_4$ are:

$$[\text{Cu}_2(\text{HL}_1)_2(\text{Br})_2]$$ (5a)

$$[\text{Cu}_2(\text{HL}_2)_2(\text{Br})_2]$$ (5b)

$$[\text{Cu}_2(\text{HL}_3)_2(\text{Br})_2]$$ (5c)

$$[\text{Cu}_2(\text{HL}_4)_2(\text{Br})_2]$$ (5d)
2.5 Instrumental methods

2.5.1 Infrared spectra

Infrared spectra of the ligands and complexes were recorded on a Perkin-Elmer IR 598 spectrometer, FT-IR IFS66U spectrometer, ABB Bomem MB-104 FTIR spectrometer and also Hitachi Infrared spectrophotometer using KBr pellet disk.

2.5.2 Electronic spectra

Electronic absorption spectra were obtained in acetonitrile solution with a Hitachi-320 UV-visible double beam spectrometer using 1 cm quartz cells and Perkin Elmer-UV/VIS spectrometer Lambda 20 with solution concentration of $10^{-3}$ mol dm$^{-3}$. Catalysis experiments were carried out on Ocean Optics, SD 1000 fibre optic spectrometer using methanol as a solvent. A methanol solution of 3,5-DTBC (0.1 mmol dm$^{-3}$) and copper(II) complexes (0.001 mmol dm$^{-3}$) in a 25 mL standard flask were kept under ice cold condition. Absorbance was monitored at intervals of 10 min immediately after exposing the solution to air.

2.5.3 $^1$H NMR and $^{13}$C NMR Spectra

$^1$H NMR and $^{13}$C NMR spectra were recorded on a JEOL FX-400 FT-NMR and AMX 400 FT-NMR spectrometer in CDCl$_3$ solution, using TMS as the internal standard chemical shifts are reported in $\delta$ units downfield from TMS. Abbreviation used $s$: singlet, $d$: doublet, $t$: triplet, $m$: multiplet.
2.5.4 ESR Spectra

ESR spectra were recorded with a JEOL TES 100 ESR spectrometer with 100 kHz field modulation. The spectra were calibrated with diphenylpicrylhydrazine (DPPH), \( g = 2.0036 \). Low temperature measurements were made using a liquid nitrogen Dewar and JEOL DVT3 variable temperature accessory. The error in temperature is around \( \pm 2^{\circ}\ ).

2.5.5 Variable temperature magnetic measurements

Variable temperature magnetic susceptibility data of the powdered samples were measured in the range 80-300 K using a Princeton Applied Research Model 155 VSM instruments in 5000 G magnetic field. Diamagnetic susceptibility corrections for ligand susceptibility were made using Pascal’s constants. The instrument was calibrated using metallic Ni. The variable temperature magnetic susceptibility data were fitted to the Bleaney-Bowers equation** to evaluate the best fit parameters \(-2J, P \) and \( g \). where \( \chi_M \) is

\[
\chi_M = \{Ng^2\beta^2/kT\}[3 + \exp (-2J/kT)]^{-1}(1-p) + (Ng_i^2\beta^2/4kT)P + N_\alpha
\]

the paramagnetic susceptibility per molecule after the correction for diamagnetism, \( p \) is the percentage of the monomeric impurity, \( g_i \) is the average \( g \) factor of the paramagnetic impurity, \(-2J\) is the singlet-triplet energy separation and \( N_\alpha \) is the TIP assumed to be \( 60 \times 10^{-6} \text{ cm}^3 \text{ mol}^{-1} \), \( \beta \) is Bohr magneton \((eh/4\pi\text{Imc})\), \( k \) is Boltzmann’s constant and \( T \) is temperature. Effective magnetic moments were calculated using the formula \( \mu_{\text{eff}} = 2.828(\chi_M T)^{1/2} \). The discrepancy factor between the theoretical and experimental susceptibility values was evaluated using the relationship \( R(\chi) = \left[ \sum(\chi_{\text{obser}} - \chi_{\text{Calc}})^2 / \sum(\chi_{\text{obser}})^2 \right]^{1/2} \).
2.5.6 Antibacterial activity by well diffusion method

The antibacterial activity was carried out by well diffusion method as described by Tagg and McGiven[107]. Bacillus subtilis which is a gram positive bacterium and Pseudomonas fluorescens which is a gram negative bacterium were used in this study. Overnight grown bacterial culture was mixed with molten nutrient agar at 55° C and poured into the petriplates under sterile conditions. After solidification, wells were made in the plate using sterile cork borer. In each plate, 3 wells of 8mm diameter were made. These wells were filled with the test compounds at a concentration if 900μg/30μl methanol. The antibacterial activity of the monomeric Cu(II) complex was compared with that of standard antibiotic streptomycin at a concentration 900μg/30μl methanol/well. Methanol was used as solvent control. The plates were incubated at 37° C for 24hrs. The inhibition was measured in millimeter using antibiotic zone scale (HiMedia, Mumbai)

2.5.7 Superoxide dismutase

Superoxide dismutase activity of complexes and the ligands were determined by using their ability to inhibit the reduction of nitro blue tetrazolium (NBT) [108-109]. The reaction system contained 0.2 mM xanthine and 0.6 mM NBT in 0.1 M phosphate buffer pH 7.8. The tested compounds were dissolved in DMF and the final concentration of DMF in the reaction mixture was 1% in 0.1 M phosphate buffer pH 7.8. The measured concentrations of the compounds were 50 μg/mL, 100 μg/mL, 250 μg/mL and 500 μg/mL. 0.07 U/mL, xanthine oxidase was added to the reaction to start. Each experiment was performed in triplicate.