3. EXPERIMENTAL METHODS

3.1 CHEMICALS AND REAGENTS

5-Amino-1-Pentanol, Copper(II) acetylacetonate, Nickel(II) acetylacetonate, Magnesium(II) acetylacetonate, Zinc(II) acetylacetonate, Iron(III) acetylacetonate, Cobalt(III) acetylacetonate, Manganese(III) acetylacetonate, Vanadyl acetylacetonate, Tetrabutylammonium perchlorate, Sodium perchlorate monohydrate, 10% palladium on carbon (50% wet) and Benzylchloroformate 50 % solution in Toluene were purchased from Aldrich.

Concentrated hydrochloric acid, Sulphuric acid, Hydroxylamine sulfate, Methylene chloride, Triethylamine, Methanesulfonyl chloride, Succinic anhydride, N,N’-Dicyclohexylcarbodiimide (DCC), N,N-Dimethyl acetamide (DMAc), Sodium hydroxide, Methanesulfonic acid, Ammonium hydroxide solution, Sodium carbonate and Sodium sulphate were purchased from S.D. Fine chemicals, Mumbai (INDIA).

Toluene, Hexanes, Methanol, Ethyl acetate, Isopropyl ether and Acetone were purchased from E.Merck (INDIA).

The supercoiled pBR322 DNA was purchased from Bangalore Genei (India). Superoxide dismutase (SOD), ethidium bromide (EB) and L-histidine, DAPI were obtained from Sigma. Tris(hydroxymethyl)aminomethane–HCl (Tris–HCl) buffer solution was prepared by using deionized and sonicated triple distilled water.
3.1.1 Purification of solvents

Reagents and solvents were purified by following standard procedures.\textsuperscript{131}

(i) Methanol

The commercial grade methanol and ethanol were refluxed for six hours with lime and distilled. The middle fraction was collected and used.

(ii) Dimethylformamide

Dimethylformamide has been purified by drying overnight over KOH pellets and then distilled from BaO under vacuum at 15-20 mm pressure.

3.2 INSTRUMENTAL METHODS

1) All the melting points were determined by using a Toshniwal melting point apparatus by open capillary tube method and were uncorrected.

2) The C, H, N contents of the ligands and complexes were carried out using a Carlo Erba Elemental analyzer Model 1106.

3) The IR spectrums were recorded on Perkin Elmer FTIR spectrometer with samples prepared as KBr pellets.

4) UV–Visible spectra were recorded using Perkin Elmer Lambda 35 spectrophotometer operating in the range 200–1000 nm with quartz cells and $\varepsilon$ values are given in M$^{-1}$ cm$^{-1}$.

5) The emission spectra were recorded on Perkin-Elmer LS-45 Fluorescence spectrometer.

6) NMR spectra were recorded using Varian Unity plus 400 MHz Spectrometer.

7) Mass spectra were recorded using LC/MS API 2000 AB SCIEX with positive ionisation mode using ESI probe.
8) EI mass spectra were recorded on a JEOL DX-303 EI mass spectrometer. Electrospray ionization mass spectral measurements were done using Thermo Finnigan LCQ-6000 Advantage Max-ESI mass spectrometer. The FAB-MS spectra were recorded on a Jeol SX 102/DA-6000 mass spectrometer using a p-nitrobenzyl alcohol (NBA) matrix.

9) Electrochemical measurements were performed at room temperature. The cyclic voltammograms of $10^{-3}$ M solution of complexes were obtained on a CHI600A electrochemical analyzer. The measurements were carried out under oxygen free condition using a three electrode cell in which glassy carbon electrode was the working electrode, saturated Ag/AgCl electrode was the reference and platinum wire was used as the auxiliary electrode. Tetra(n-butyl)ammonium perchlorate was used as supporting electrolyte and the concentration of TBAP was $10^{-1}$ M.

10) DNA binding experiments.

3.3. Experimental Procedures for DNA binding and cleavage

3.3.1. ABSORPTION SPECTRAL STUDIES

DNA binding experiments were performed in Tris-HCl/NaCl buffer (50 mM Tris-HCl/1 mM NaCl buffer, pH 7.5) using solution of the complexes copper(II), nickel(II), zinc(II), magnesium(II), iron(III), cobalt(III), manganese(III) and vanadium(IV) of ligand L$^1$ and L$^2$ in water. The concentration of CT DNA was determined from the absorption intensity at 260 nm with a $\varepsilon$ Value$^{132}$ of 6600 M$^{-1}$ cm$^{-1}$. Absorption titration experiments were made using different concentration of CT DNA, while keeping the
complex concentration as constant. Due correction was made for the absorbance of the CT DNA itself. Samples were equilibrated before recording each spectrum. The binding constants ($K_b$) have been determined from the spectroscopic titration data using the following equation: \(^{133}\)

$$\frac{[\text{DNA}]}{\varepsilon_a - \varepsilon_f} = \frac{[\text{DNA}]}{\varepsilon_b - \varepsilon_f} + \frac{1}{K_b (\varepsilon_b - \varepsilon_f)}$$

The ‘apparent’ extinction coefficient ($\varepsilon_a$) was obtained by calculating $A_{obs}/[\text{Cu}]$. The terms $\varepsilon_f$ and $\varepsilon_b$ correspond to the extinction coefficients of free (unbound) and the fully bound complexes. A plot of $[\text{DNA}]/(\varepsilon_a - \varepsilon_f)$ Vs $[\text{DNA}]$ will give a slope $1/(\varepsilon_b - \varepsilon_f)$ and an intercept $1/K_b (\varepsilon_b - \varepsilon_f)$. $K_b$ is the ratio of the slope and the intercept.

### 3.3.2. FLOURESCENCE SPECTRAL STUDIES

The fluorescence spectral method is used to study the binding of these complexes to CT-DNA. Ethidiumbromide (EB) emits intense fluorescence at about 600 nm in the presence of CT-DNA because of its strong interaction with CT-DNA base pairs. The addition of complex, which also binds to DNA would quench fluorescence intensity. The binding of complexes copper(II), nickel(II), zinc(II), magnesium(II), iron(III), cobalt(III), manganese(III) and vanadium(IV) of ligand $L^1$ and $L^2$ to CT-DNA was studied with an EB bound CT-DNA solution in Tris – HCl buffer (pH 7.2). Fluorescence intensities at 610 nm (excited at 510 nm) were measured at different complex concentrations. The reduction of emission intensity gives a measure binding propensity of complex to CT-DNA. Stern – Volmer quenching constant $K$ of the complexes CT-DNA were determined from the equation $I_o/I = 1 + Kr$. $I_o$ and $I$ are fluorescence intensities of EB-DNA in absence
and presence complex, respectively. \( K \) is a linear Stern – Volmer quenching constant. \( r \) is the ratio of the total concentration to that of DNA, \([M] / [DNA]\). In the linear fit plot of \( I_0 / I \) vs. \([\text{complex}]/[\text{DNA}]\), \( K \) is given by the ratio of the slope to intercept. The apparent binding constant (\( K_{\text{app}} \)) was calculated using the equation \( K_{\text{EB}}[\text{EB}]/K_{\text{app}}[\text{complex}] \) where \( K_{\text{EB}} = 1 \times 10^7 \), \([\text{EB}] = 4 \mu\text{M}, \) and \([\text{complex}]\) was the concentrations of the complexes were taken for observing a 50% reduction of the emission intensity of EB.\(^{134}\)

### 3.3.3. Viscosity measurements

To further clarify the binding mode of the present nickel complexes to CT-DNA, viscosity measurements were carried out on CT-DNA (0.5 mM) by varying the concentration of the complex (0.01 mM, 0.02 mM, 0.03 mM, 0.04 mM and 0.05 mM). Data were presented as \((\eta/\eta_0)\) versus binding ratio of concentration of complex to that of concentration of CT-DNA, where \(\eta\) is the viscosity of DNA in the presence of complex and \(\eta_0\) is the viscosity of DNA alone.

### 3.3.4. DNA CLEAVAGE EXPERIMENTS

The cleavage of plasmid DNA was monitored by agarose gel electrophoresis. The cleavage of supercoiled pBR322 DNA by copper(II), nickel(II) and zinc(II) complexes were studied in a medium of 50 mM Tris-HCl/NaCl Buffer (pH = 7.2) in the presence of \(\text{H}_2\text{O}_2\). All the samples were incubated for 30 minutes at 37ºC followed by its addition to the loading buffer containing 25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol (3 mL). All the samples were finally loaded on 0.8% agarose gel containing EB (1 mg mL\(^{-1}\)). Electrophoresis was carried out at 50 V for 1 h in TBE buffer (45 mM Tris, 45 mM \(\text{H}_3\text{BO}_3\), 1 mM EDTA, pH 8.3). Resulting bands were visualized by UV light and
photographed. Quantification was performed by fluorescence imaging by use of a GelDoc 1000 (BioRad) and data analysis with Multianalysis software (version 1.1) provided by the manufacturer using the volume quantification method. In all cases, background fluorescence was subtracted by reference to a lane containing no DNA. A correction factor was used for supercoiled DNA since the ability of ethidium bromide to intercalate into supercoiled DNA (form I) decreased relative to nicked (form II) and linear (form III). The fraction of each form of DNA was calculated by dividing the intensity of each band by the total intensities of all the bands in the lane.

3.4. Synthesis of ligand L¹ precursors

3.4.1 Preparation of N-Benzylxycarbonylaminopentan-1-ol (1)

To a 3 litre 4-necked RBF equipped with mechanical stirrer, thermo pocket and dropping funnel added 1100 ml of water and cooled to 15 ºC. 5-Amino-1-pentanol (100 g, 0.969 mol) and Sodium carbonate (123.2 g, 1.162 mol) were added and then stirred for 10 minutes at 15 ºC. To this a mixture, Benzylchloroformate (180 g, 1.055 mol) in 180 mL of toluene was added for 30 minutes at 5 ºC and flushed with 20 ml of toluene. The resulting mixture was stirred for 30 minutes at 0 - 5 ºC and the reaction mass temperature were raised to 32 ºC slowly. Further, it was stirred for 25 - 30 minutes at 30 - 32 ºC. The mixture was left for settling and the layers were separated. The aqueous layer was extracted with Toluene (2 x 150 ml) at 30 to 32 ºC and the toluene layers were combined. The toluene layer was washed with 200 ml water at 30 to 32 ºC, followed by 200 ml brine solution at 30 to 32 ºC. The toluene was distilled out completely under vacuum at 50 to 65 ºC. To the residue, 500 ml hexanes was added and stirred for about 6 hours at 20-25 ºC. The solid thus obtained was filtered, washed, with about 200 ml
hexanes and dried under vacuum at 25 to 30 ºC, to obtain the product 1 (218 g, 95%).
Melting point: 45 ºC. Elemental analysis data: calculated (%) for C_{13}H_{19}NO_{3} (237): C 65.80, H 8.07, N 5.90. Found (%): C 65.78; H 8.02, N 5.89. ESI MS: 237 [M]^+. IR Spectrum: 1535 cm\(^{-1}\) for N-H bending, 1685 cm\(^{-1}\) for ν C=O str, 2863-2943 cm\(^{-1}\) for C-H str, 3335 cm\(^{-1}\) for ν N-H str and 3400 cm\(^{-1}\) for ν O-H str. \(^1\)H NMR (CDCl\(_3\)): δ 7.3-7.4 (m, 5H, phenyl protons), δ 5.1 (s, 2H, methylene protons attached to phenyl ring), δ 4.8 (s, 1H, OH proton), δ 3.2 (t, 2H, J = 5.1 Hz, methylene protons), δ 2.9 (t, 2H, J = 6 Hz, methylene protons), δ 1.6 (m, 4H, methylene protons), δ 1.4 (m, 2H, methylene protons).

3.4.2 Preparation of 5-Benzylxycarbonylaminopentyl methanesulfonate (2)

To a 2 litre 4-necked RBF, equipped with mechanical stirrer, thermo pocket and dropping funnel, containing 300 mL of methylene chloride at 25 to 30 ºC, 1 (100 g, 0.42 mol) was added and stirred for about 10 to 15 minutes to get a clear solution. The reaction mass was cooled to -5 to -10 ºC. This was followed by the addition of Triethylamine (64 g, 0.63 mol) in one lot and the reaction mass temperature was maintained at -10 to -15 ºC. Meanwhile a mixture of 64 g of methane sulfonyl chloride (64 g, 0.558 mol) in 50 mL of methylene chloride at 25 to 30 ºC was prepared and added
dropwise for 15 to 20 minutes to the above reaction mass at -10 to -15 °C. The resulting reaction mixture was stirred for about 25 to 30 minutes while maintain the temperature at -10 to -15 °C. The temperature was the raised to 0 to 5 °C in 25 to 30 minutes and stirred for about 30 minutes. The reaction mass was quenched into 500 mL of precooled water at 3 to 5 °C. The temperature was then raised to 15 to 20 °C and stirred for 10 to 15 minutes. The mixture was allowed to stand until the layers were separated out. The organic layer was collected and aqueous layer was further extracted with about 150 mL of methylene. The organic layer separated was combined with the previous portion. The organic layer thus obtained was washed with 400 mL of brine solution. Methylene chloride was distilled off completely under vacuum at 30 to 40 °C to get the product 2 as a paste, (140 g) which was used as such for the Preparation of N-Benzylxycarboxamino-5-hydroxylaminopentane.

![5-Benzylxycarboxamino-5-hydroxylaminopentyl methanesulfonate](image)
3.4.3 Preparation of N-Benzylxycarbonylamino-5-hydroxylaminopentane (3)

In a RBF, 216 mL of water and Hydroxylamine sulphate (415.5 gm, 0.32 mol) were added at 25 to 30 °C and then cooled to 15 to 20 °C. Sodium hydroxide (200, 5 mol) was dissolved in 600 mL of water and then added drop wise in about 60 minutes at 15 to 20 °C. To the resultant reaction mass, added 700 mL of methanol at 15 to 20 °C. Meanwhile the N-Benzylxycarbonylamino-5-hydroxylaminopentane of 2 (140 g, 2.25 mol) obtained in the previous stage was dissolved in 500 mL of methanol and transferred into the above mass at 15 to 20 °C. The temperature of the reaction mass was further raised to 55 to 58 °C and stirred 120 to 150 minutes. The reaction mass was transferred into the mixture of water and methylene chloride (3400 mL and 1000 mL respectively) at 25 to 30 °C and stirred for 10 to 15 minutes at 25 to 30 °C. The mixture was allowed to stand and the organic layer was collected separately. The aqueous layer was further extracted using 500 mL of methylene chloride, the organic layer separated and combined to the previous portion. The methylene chloride was distilled off under vacuum at 30 to 40 °C until a solid product is obtained. This was followed by the addition of 130 mL of methanol and stirring for 5 to 10 minutes at 40 to 45 °C. The mass was then cooled to 20 to 25 °C and 500 mL of Isopropyl ether was added slowly in 45 to 60 minutes at 20 to 25 °C. The mixture was stirred for 4 hours at 20 to 25 °C. The solid product was then filtered under vacuum, washed with Isopropyl ether (2 x 75 mL) and dried under vacuum at about 50 °C for about 8 hours, to obtain 3 (75 g, 71%). Melting point: 107 °C. Elemental analysis data: calculated (%) for C_{13}H_{20}N_{2}O_{3} (252): C 61.88, H 7.99, N 11.10. Found (%): C 61.86, H 7.90, N 11.00. ESI MS: 253 [M+H]^+. IR Spectrum: 1690 cm\(^{-1}\) for ν C=O str, 2933 cm\(^{-1}\) for C-H str, 3257 cm\(^{-1}\) for ν N-H str and 3320 cm\(^{-1}\) for ν O-H str. \(^1\)H
NMR (CDCl$_3$): δ 7.3-7.4 (m, 5H, phenyl protons), δ 5.1 (s, 2H, methylene protons attached at phenyl ring), δ 4.85 (s, 1H, OH proton), δ 3.6 (t, 2H, J = 4.2 Hz, methylene protons), δ 3.2 (t, 2H, J = 4.8 Hz, methylene protons), δ 1.5-1.6 (m, 4H, methylene protons), δ 1.3-1.5 (m, 2H, methylene protons).

3.4.4 Preparation of N-Benzylxycarbonylamino-5-(N-succinylhydroxylamino)-pentane (4)

To a RBF containing 1600 mL of methylene chloride at 25 to 30 ºC, 3 (100 g, 0.39 mol) was added and flushed with 100 mL of methylene chloride. The mass was then cooled to -25 to -30 ºC. This was followed by the addition of succinic anhydride (14.3 g, 7 mol) in one lot and the mixture was stirred for 10 to 15 minutes at -25 to -30 ºC. The temperature was then raised to 0 to 5 ºC from -25 to -30 ºC in 60 to 70 minutes. Methylene chloride was distilled off completely under vacuum at 30 to 40 ºC. To this, about 800 mL of Isopropyl ether was added and the temperature was raised to 40 to 45 ºC. The mixture was stirred for 60 minutes at 40 to 45 ºC. The solid product was filtered off under vacuum, washed with 2 x 100 mL of isopropyl ether and dried under vacuum at 30-32 ºC for about 10 hours to obtain 4 (135 g, 97%). Melting point: 115 ºC. Elemental analysis data: calculated (%) for C$_{17}$H$_{24}$N$_2$O$_6$ (352): C 57.94, H 6.86, N 7.9. Found (%): C 57.91, H 6.83, N 7.5. ESI MS: 353 [M + H]$^+$. IR Spectrum: 1702 cm$^{-1}$ for v C=O str (carboxylic acid), 1686 cm$^{-1}$ for v C=O str (amide), 2879-2947 cm$^{-1}$ for C-H str, 3147 cm$^{-1}$.
$^1$ for ν N-H str and 3345 cm$^{-1}$ for ν O-H str. $^1$H NMR (CDCl$_3$): δ 7.3-7.4 (m, 5H, phenyl protons), δ 6.3 (s, 1H, NOH proton), δ 5.1 (s, 2H, s, methylene proton attached to phenyl ring), δ 3.75 (t, 2H, J = 4.5 Hz, methylene protons), δ 3.2 (t, 2H, J = 4.2 Hz, methylene protons), δ 2.8 (t, 2H, J = 4.7 Hz, methylene protons), δ 2.6 (t, 2H, J = 9 Hz, methylene protons), δ 1.7 (t, 2H, J = 4.5 Hz, methylene protons), δ 1.5 (t, 2H, J = 4.65 Hz, methylene protons) and δ 1.3 (m, 2H, methylene protons).

![Chemical Structure](image)

**3.4.5 Preparation of N-(5'-Benzyloxy carbonylamino pentyl) tetrahydro-3,6-dioxo-1,2-oxazine (5)**

1100 mL of methylene chloride was added in to a 4-necked RBF equipped with mechanical stirrer and thermo pocket at 25 to 30 ºC and then 4 (100 g, 0.28 mol) was added and flushed with 100 mL of methylene chloride. The reaction mass was cooled to 0 to 5 ºC. N,N'-Dicyclohexylcarbodiimide (64.2 g, 0.311 mol) was dissolved in 300 mL of methylene chloride and added into the above reaction mass. The mixture was stirred for 60 minutes at 0 to 5 ºC and later the temperature was raised to 25 to 30 ºC. Then it was stirred for about 3 hours at 25 to 30 ºC. The reaction mass was cooled to -10 to -20 ºC and stirred for about 60 minutes at the same temperature. The insoluble by product Dicyclohexyl urea was filtered off and washed with 200 mL of chilled methylene
chloride. The methylene chloride in the filtrate was distilled out under vacuum at 30 to 40 ºC till solid obtained. About 800 mL of Isopropyl ether was added under stirring and cooled to 0 to 5 ºC. The stirring was continued for 90 minutes. The product thus obtained was filtered and the wet cake was washed with 200 mL of chilled Isopropyl ether. The wet cake was dried under vacuum at 30 ºC for about 4 hours to obtain 5 (75 g, 95%). Melting point: 85°C. Elemental analysis data: calculated (%) for C_{17}H_{22}N_{2}O_{5} (334): C 61.07, H 6.63, N 8.38. Found (%): C 61.0, H 6.59, N 8.3. ESI MS: 334 [M]^+. IR Spectrum: 1770 cm\(^{-1}\) for \(\nu\) C=O str (carboxylic acid), 1657 cm\(^{-1}\) for \(\nu\) C=O str (amide), 2863-2935 cm\(^{-1}\) for C-H str, 3300 cm\(^{-1}\) for \(\nu\) N-H str. \(^1\)H NMR (CDCl\(_3\)): \(\delta\) 7.3-7.4 (m, 5H, phenyl protons), \(\delta\) 5.1 (s, 2H, methylene protons attached to phenyl ring), \(\delta\) 3.75 (t, 2H, J= 5.4 Hz, methylene protons), \(\delta\) 3.2 (t, 2H, J= 4.9 Hz, methylene protons), \(\delta\) 2.8 (t, 2H, J= 3.15 Hz, methylene protons), \(\delta\) 2.7 (t, 2H, J= 2.4 Hz, methylene protons), \(\delta\) 1.7 (t, 2H, J= 4.2 Hz, methylene protons), \(\delta\) 1.5 (t, 2H, J= 5.3 Hz, methylene protons) and \(\delta\) 1.3 (m, 2H, methylene protons).
3.4.6. Preparation of N-(5-Benzoyloxycarbonylaminopentyl)-N-(hydroxy)acetamide (6)

To a 200 mL of N, N-Dimethyl acetamide, 3 (100 g, 0.39 mol) was added at room temperature and then flushed with 100 mL of N, N-Dimethyl acetamide. The resultant mixture was cooled to 0 to -5 ºC. Anhydrous sodium carbonate (42 g, 0.39 mol) was then added in one lot at 0 to -5 ºC and stirred for 5-10 minutes. The mass was then further cooled to -10 to -15 ºC and Acetyl chloride (40.2 g, 0.51 mol) was added drop wise at -10 to -15 ºC in 40 minutes. The temperature of the mass was maintained at -10 to -15 ºC for 60 minutes. The temperature of the reaction mass was raised to 25 to 30 ºC. 100 mL of 18-20% aqueous ammonium hydroxide solution was added and stirred for 30 to 40 minutes. The reaction mass was transferred into the mixture of 1000 mL of water and 1000 mL of ethyl acetate at 25 to 30 ºC. The pH of reaction mass was adjusted to 1.5±0.2 using 16-18% aqueous hydrochloric acid solution. The mixture was stirred for 15 minutes at 25 to 30 ºC and allowed to stand until the two layers were separated. The organic layer was collected separately and the aqueous layer was further extracted using about 500 mL of ethyl acetate. The organic layer thus obtained was combined with the previously obtained organic layer. The combined ethyl acetate portion was washed with 800 mL of water and 200 mL of brine solution. The ethyl acetate layer thus obtained was subjected to distillation under vacuum at 35 to 45 ºC until a solid product appeared. To this, 700 mL of Isopropyl ether was added and stirred for 40 minutes at 20 to 22 ºC. The slurry was filtered, washed with 100 mL of Isopropyl ether and dried under vacuum at 30 ºC for about 6 hours, to obtain 6 (105 g, 90%). Melting point: 94 °C. Elemental analysis data: calculated (%) for C_{15}H_{22}N_{2}O_{4} (294): C 61.21, H 7.53, N 9.52. Found (%): C 61.12, H 7.51, N 9.49. ESI MS: 295 [M+H]^+. IR Spectrum: 1593 cm\(^{-1}\) for \(\nu\) C-N str, 1686 cm\(^{-1}\) for \(\nu\) C=O str (amide), 2863-2950 cm\(^{-1}\) for C-H str, 3125 cm\(^{-1}\) for \(\nu\) N-H str and 3347 cm\(^{-1}\)
for ν O-H str. $^1$H NMR (CDCl$_3$): δ 8.5 (1H, s, NH proton), δ 7.3-7.4 (m, 5H, phenyl protons), δ 5.0 (s, 2H, methylene protons attached to phenyl ring), δ 4.9 (s, 1H, NOH proton), δ 3.6 (t, 2H, J = 6 Hz, methylene protons), δ 3.2 (t, 2H, J = 4.5 Hz, methylene protons), δ 2.2 (s, 3H, methyl protons), δ 1.5-1.6 (m, 4H, methylene protons), δ 1.3 (m, 2H, methylene protons).

3.4.7 Preparation of N-(5-Aminopentyl)-N-(hydroxy)acetamide (7)

To a 1000 mL 4-necked RBF equipped with mechanical stirrer, thermo pocket 200 mL of Methanol, Sodium hydroxide (1.8 g, 0.045 mol) were added and stirred for about 15 minutes to get clear solution. Then 6 (12 g, 0.04 mol) was added and stirred for about 15 minutes at 20 to 30°C to get clear solution. The solution was transferred into a hydrogenator. This was followed by the addition of 0.6 g of 10% palladium on carbon (50% wet) and flushing with 10 mL of Methanol. The hydrogenator was flushed with nitrogen and was evacuated under vacuum. The reaction mass was stirred 20 to 30°C under hydrogen pressure of 40 to 50 psi for about 45 minutes. The palladium on carbon was filtered off from the reaction mixture using celite bed and the filtrate was collected.
Sulphuric acid solution was prepared by adding 1.8 g of sulfuric acid into 4 mL of water, cooled to 20-30°C and then added into the above filtrate in about 30 minutes at 20 to 30°C. The methanol was distilled out under vacuum at below 50°C to obtain a paste of 7 which was used as such for preparation of N-(5-Benzylloxycarbonylaminopentyl)-3-[[5-(N-hydroxyacetamido)pentyl]carbamoyl]propionohydroxamic acid. Elemental analysis data: calculated (%) for C_{7}H_{16}N_{2}O_{2} (160): C 52.48, H 10.07, N 17.48. Found (%): C 52.42, H 10.01, N 17.41. ESI MS: 161 [M+H]^+. \(^1\)H NMR (D_{2}O): \(\delta\) 3.4 (t, 2H, J = 4.26 Hz, methylene protons), \(\delta\) 2.8 (t, 2H, J = 4.65 Hz, methylene protons), \(\delta\)1.8 (s, 3H, methyl protons), \(\delta\) 1.4 (m, 4H, methylene protons), \(\delta\) 1.1 (m, 2H, methylene protons).

3.4.8 Preparation of N-(5-Benzylloxycarbonylaminopentyl)-3-[[5-(N-hydroxyacetamido) pentyl]carbamoyl]propionohydroxamic acid (8)

To the N-(5-Aminopentyl)-N-(hydroxy)acetamide obtained from the previous stage, added 10 ml of water and 60 ml of acetone at 20°C and stirred for about 60 minutes at 20°C. The precipitated sodium sulfate salt was filtered off and washed with mixture of 6 mL of water and 30 mL of acetone. The filtrate was collected and 55 mL of acetone and 5 (17.4 g, 0.05 mol) were added to the filtrate. The mixture was stirred for about 16 hours at 30°C. The solid product was filtered under vacuum and washed with 80
mL of acetone, 80 mL of water and again with 80 mL of acetone. The wet material was transferred into 150 mL of methanol, heated to 60°C and maintained for about 30 minutes at 60° to get clear solution. This solution was then cooled to 20 to 30°C to precipitate the product and stirred for about 5 hours at 20 to 30°C. The slurry mass was filtered and washed with 60 mL of methanol. The product was dried under vacuum at about 35°C for about 8 hours to obtain 8 (14.5 g, 72%). Melting point: 148°C. Elemental analysis data: calculated (%) for C_{24}H_{38}N_{4}O_{7} (494): C 58.28, H 7.74, N 11.33. Found (%): C 58.21, H 7.64, N 11.29. ESI MS: 494 [M]^+. IR Spectrum: 1533 cm^{-1} for ν C-N str, 1685 cm^{-1} for ν C=O str (carboxylic acid), 1652 cm^{-1} for ν C=O str (amide), 2863-2950 cm^{-1} for C-H str, 3126 cm^{-1} for ν N-H str and 3325 cm^{-1} for ν O-H str. ^1H NMR (DMSO): δ 8.1 (s, 1H, NH proton), δ 7.3-7.4 (m, 5H, phenyl protons), δ 5.0 (s, 2H, methylene protons attached to phenyl ring), δ 3.4 (t, 4H, J = 3.6 Hz, methylene protons), δ 3.0 (t, 4H, J = 6.3 Hz, methylene protons), δ 2.64 (t, 2H, J = 8.5 Hz, methylene protons), δ 2.33 (t, 2H, J = 5.1 Hz, methylene protons), δ 1.94 (s, 3H, methyl protons), δ 1.35-1.5 (m, 8H, methylene protons), δ 1.16-1.2 (m, 4H, methylene protons).
3.5. Synthesis of ligand $L^1$

3.5.1 Preparation of N-(5-Aminopentyl)-3-[[5-(N-hydroxyacetamido)pentyl]carbamoyl]propionohydroxamic acid $L^1$ (9)

In a 1000 mL 4-necked RBF equipped with mechanical stirrer, thermo pocket 220 mL of Methanol and Sodium hydroxide (2.34 g, 0.06 mol) were added and stirred for about 15 minutes to get clear solution. Then 8 (13 g, 0.03 mol) was added and stirred for about 15 minutes at 20 to 30°C to get clear solution. The solution was transferred into a hydrogenator. This was followed by the addition of 0.65 g of 10% palladium on carbon (50% wet) and flushing with 10 mL of Methanol. The hydrogenator was flushed with nitrogen and was evacuated under vacuum. The reaction mass was stirred 20 to 30°C under hydrogen pressure of 40 to 50 psi for about 45 minutes. The palladium on carbon was filtered off from the reaction mixture using celite bed and the filtrate was collected. Sulphuric acid solution was prepared by adding 2.34 g of sulfuric acid into 5 mL water. The solution was cooled to 20-30°C and added into the above filtrate in about 15 to 30 minutes at 20-30°C (observed pH: 7.5). The methanol was distilled out under vacuum at below 50°C. To the residue, added 10 mL of water and 60 mL of acetone and then stirred for about 60 minutes at 20-30°C. The precipitated sodium sulfate salt was filtered off and the filtrate was concentrated under vacuum. 100 mL of acetone was added to the concentrated mass and stirred for about 30 minutes at 20-30°C. The solid material was filtered and washed with 20 mL of acetone. The wet material was dried under vacuum at 20-30°C for about 5 hours to obtain $L^1$ (9) (8 g, 85% yield). Melting point: 140°C. Elemental analysis data: calculated (%) for $C_{16}H_{32}N_{4}O_{5}$ (360): C 53.31, H 8.95, N 15.54. Found (%): C 53.29, H 8.9, N 15.44. ESI MS: 361 [M+H]$^+$. IR Spectrum: 1530 cm$^{-1}$ for $\nu$
C-N str, 1622 cm\(^{-1}\) for ν C=O str (amide), 2859-2932 cm\(^{-1}\) for C-H str, 3092 cm\(^{-1}\) for ν N-H str and 3310 cm\(^{-1}\) for ν O-H str. \(^1\)H NMR (D\(_2\)O): δ 8.1 (s, 1H, NH proton), δ 3.3 (t, 4H, J = 4.6 Hz, methylene protons), δ 2.87 (t, 2H, J = 2.8 Hz, methylene protons), δ 2.6 (t, 2H, J = 5.7 Hz, methylene protons), δ 2.10 (t, 2H, J = 5.1 Hz, methylene protons), δ 1.8 (s, 3H, methyl protons), δ 1.34-1.54 (m, 6H, methylene protons), δ 1.1-1.25 (m, 2H, methylene protons), δ 0.9-1.08 (m, 4H, methylene protons). \(^{13}\)C NMR (D\(_2\)O): δ 173.7, 172.9, 170 (carbonyl carbons), δ 50.5, 48.2, 47.1, 29.786, 26.152, 24.336, 23.723, 23.556 (methylene carbons), δ 19.0 (methyl carbon).

3.6 synthesis of ligand L\(^2\) precursor

3.6.1 Preparation of N-[5-[3-[(5-benzyloxycarbonylamino)pentyl]hydroxylcarbamoyl]-propionamido]pentyl]-3-[[5-(N-hydroxyacetamido)pentyl]carbamoyl]-propiono-hydroxamic acid (10)

In a 250 mL RBF added 25 mL of water, 55 mL of acetone, \(\text{L}^1\) (10 g, 0.0277 mol) and 5 (11.3 g, 0.033 mol) at 25 to 30°C. The resultant reaction mass was stirred for about 16 hours at 25 to 30°C. The solid product was filtered under vacuum and washed with 80 mL of acetone. The solid product was further slurry washed with 190 mL water.
The product was filtered and washed with 40 mL of water and 80 mL of acetone. The wet material was dried under vacuum at 35°C for about 8 hours to obtain 9 (14.8 g, 81%).


^1H NMR (DMSO-d6): δ 7.78 (s, 2H, NH proton), δ 7.36 (m, 5H, phenyl protons), δ 4.99 (s, 2H, methylene protons attached to phenyl ring, δ 3.4 (t, 6H, J = 3.45 Hz, methylene protons), δ 3.02 (t, 4H, J = 4.35 Hz, methylene protons), δ 2.5 (m, 4H, methylene protons), δ 2.28 (t, 4H, J = 5.4 Hz, methylene protons), δ 1.93 (s, 3H, methyl protons), δ 1.49 (m, 6H, methylene protons), δ 1.34 (m, 6H, methylene protons), δ 1.2 (m, 6H, methylene protons).
3.7 Synthesis of ligand L²

3.7.1 Preparation of Deferoxamine B mesylate (L²)

To a 1000 mL 4-necked RBF equipped with a mechanical stirrer, thermo pocket added 110 mL of Methanol, Sodium hydroxide (2.5 g, 0.06 mol) and stirred for about 15 minutes to get clear solution. Then 9 (13 g, 0.02 mol) was added and stirred for about 15 minutes at 20 to 30°C to get clear solution. The solution was transferred into a hydrogenator. This was followed by the addition of 0.65 g of 10% palladium on carbon (50% wet) and flushing with 25 mL of methanol. The hydrogenator was flushed with nitrogen and evacuated under vacuum. The reaction mass was stirred at 20 to 30°C under hydrogen pressure of 40 to 50 psi for about 45 minutes. The palladium on carbon was filtered off from the reaction mixture using celite bed and the filtrate was collected. The celite bed was washed with 10 mL of methanol. The filtrate thus obtained was cooled to 0 to 8°C and then added Methanesulfonic acid (4.3 g, 0.04 mol) diluted with 10 mL of water dropwise over a period of 60 minutes until the reaction mass pH reached about 9.0 0 to 8°C. The slurry material was stirred for 5 hours at was at 0 to 8°C and filtered. The wet cake was washed with mixture of 50 mL of methanol and 50 mL of acetone. The wet solid was added into a RBF containing 60 mL of Methanol and 25 mL of water at 20 to 30°C. Methane sulfonic acid (1.7 g, 0.02 mol) was added dropwise into the above reaction mixture at 10 to 15°C and stirred for about 15 minutes to get clear solution (observed pH: 1.9). The resultant clear solution was filtered and the filtrate was collected. To the filtrate, 280 mL of acetone was added over a period of 60 minutes at 20 to 30°C and the products precipitated. The slurry mass was stirred for 4 hours at 20
to 30°C and the filtered. The wet material was washed with mixture of 5 mL of methanol and 25 mL of acetone. The wet material was dried under vacuum at 35°C for about 12 hours, to obtain Deferoxamine mesylate (L²) (8.6 g, 92%). Melting point: 140°C. Elemental analysis data: calculated (%) for C_{26}H_{52}N_{6}O_{11}S (656): C 47.55, H 7.98, N 12.8, Found (%): C 47.53, H 8.03, N 12.63. ESI MS: 561 [M + 1]^+.

IR Spectrum: 1538 cm\(^{-1}\) for \(\nu\) C\(-\)N str, 1624 cm\(^{-1}\) for \(\nu\) C=O str (amide), 2932 cm\(^{-1}\) for C-H str, 3130 cm\(^{-1}\) for \(\nu\) N-H str and 3330 cm\(^{-1}\) for \(\nu\) O-H str.

\(^1\)H NMR (D\(_2\)O): \(\delta\) 8.0 (s, 2H, NH proton), \(\delta\) 3.41 (t, 6H, J = 8.7 Hz, methylene protons), \(\delta\) 2.97 (t, 4H, J = 4.8 Hz, methylene protons), \(\delta\) 2.8 (t, 2H, J = 5.5 Hz, methylene protons), \(\delta\) 2.6 (m, 6H, methylene protons), \(\delta\) 2.3 (t, 4H, J = 5.25 Hz, methylene protons), \(\delta\) 1.94 (s, 3H, methyl protons), \(\delta\) 1.41 - 1.54 (m, 8H, methylene protons), \(\delta\) 1.30 - 1.37 (m, 4H, methylene protons), \(\delta\) 1.16 - 1.2 (m, 6H, methylene protons).

Deferoxamine B mesylate (L²)
3.8 Synthesis of Metal complexes with L₁

3.8.1 Synthesis of copper(II), nickel(II), zinc(II) and magnesium(II) complexes [M(II)L₁]

Metal complexes were prepared by a general procedure in which L₁ (1 mol) in water was reacted with metal(II) acetylacetonate (1.1 mol) in ethyl acetate under stirring for 20 h at 25°C. Layers were separated and the aqueous layer was washed with ethyl acetate. The resulting complex [M(II)L₁] (M(II) = Cu, Ni and Zn) was isolated from aqueous layer as solid by lyophilization. Thus obtained complexes were characterized by Elemental analysis, ESI-MS, FTIR, UV-Visible spectroscopic techniques.

3.8.1.1 Synthesis of CuL₁ complex (9a)

L₁ (5 g, 0.014 mol) was dissolved in 50 mL of water and then reacted with copper(II) acetylacetonate (4 g, 0.015 mol) in 50 mL of ethyl acetate for about 20 hours at 25°C. Then, Layers were separated and the aqueous layer was washed with 50 mL of ethyl acetate. The aqueous layer was filtered and then lyophilized to obtain about 4.7 g (80 %) of [CuL₁] complex. Elemental analysis data: calculated (%) for C₁₆H₃₀CuN₄O₅ (421): C 45.54, H 7.17, N 13.28, Found (%): C 45.51, H 7.12, N 13.21. IR Spectrum: 1640 cm⁻¹ for ν C=O str (amide), 2937 cm⁻¹ C-H str, 3100 cm⁻¹ for ν N-H str and 3433 cm⁻¹ for ν O-H str. UV-Visible: [λ/nm (ε/M⁻¹ cm⁻¹)] in H₂O: 211 (1,03,000), 614 (230). ESI MS: 422 [CuL₁+1]⁺.
3.8.1.2 Synthesis of NiL¹ complex (9b)

L¹ (5 g, 0.014 mol) was dissolved in 50 mL of water and then reacted with nickel(II) acetylacetonate (3.9 g, 0.015 mol) in 50 mL of ethyl acetate for about 20 hours at 25°C. Then, Layers were separated and the aqueous layer was washed with 50 mL of ethyl acetate. The aqueous layer was filtered and then lyophilized to obtain about 4.3 g (75 %) of [NiL¹] complex. Elemental analysis data: calculated (%) for C₁₆H₃₀N₄NiO₅ (416): C 46.07, H 7.25, N 13.43, Found (%): C 46.02, H 7.16, N 13.29. IR Spectrum: 1650 cm⁻¹ for ν C=O str (amide), 2932 cm⁻¹ C-H str, 3290 cm⁻¹ for ν N-H str. UV-Visible: [λ/nm (ε/M⁻¹ cm⁻¹)] in H₂O: 225 (99,000), 308 (93,000), 655 (110), 749 (80) and 1073 (100). ESI MS: 417 [NiL¹+1]⁺.

3.8.1.3 Synthesis of ZnL¹ complex (9c)

L¹ (5 g, 0.014 mol) was dissolved in 50 mL of water and then reacted with zinc(II) acetylacetonate (4.0 g, 0.015 mol) in 50 mL of ethyl acetate for about 20 hours at 25°C. Then, Layers were separated and the aqueous layer was washed with 50 mL of ethyl acetate and then lyophilized to obtain about 5.0 g (85 %) of [ZnL¹] complex. Elemental analysis data: calculated (%) for C₁₆H₃₀N₄O₅Zn (422): C 45.34, H 7.13, N 13.22, Found (%): C 45.31, H 7.08, N 13.19. IR Spectrum: 1651 cm⁻¹ for ν C=O str (amide), 2935 cm⁻¹ C-H str, 3435 cm⁻¹ for ν O-H str. UV-Visible: [λ/nm (ε/M⁻¹ cm⁻¹)] in H₂O: 220 (2,91,000), 312 (1,20,000). ESI MS: 423 [ZnL¹+1]⁺.
3.8.1.4 Synthesis of MgL¹ complex (9d)

L¹ (5 g, 0.014 mol) was dissolved in 50 mL of water and then reacted with magnesium(II) acetylacetonate (3.4 g, 0.015 mol) in 50 mL of ethyl acetate for about 20 hours at 25°C. Then, Layers were separated and the aqueous layer was washed with 50 mL of ethyl acetate. The aqueous layer was filtered and then lyophilized to obtain about 4.2 g (80 %) of [MgL¹] complex.

Elemental analysis data: calculated (%) for C₁₆H₃₀N₄O₅Mg (382): C 50.21, H 7.90, N 14.64, Found (%): C 50.15, H 7.84, N 14.58. IR Spectrum: 1625 cm⁻¹ for ν C=O str (amide), 2932 cm⁻¹ C-H str, 3406 cm⁻¹ for ν O-H str. UV-Visible: [λ/nm (ε/M⁻¹ cm⁻¹)] in H₂O: 210 (1,58,000), 310 (1,45,000). ESI MS: 383 [MgL¹ + 1]⁺.

3.8.2 Synthesis of Fe(III), Co(III) and Mn(III) complexes [M(III)L¹].ClO₄

Metal complexes were prepared by a general procedure in which L¹ (1 mol) was dissolved in a solution of sodium perchlorate monohydrate (1 mol) in water and then it was reacted with metal(III) acetylacetonate (1.1 mol) in ethyl acetate under stirring for 20 h at 25 °C. Layers were separated and the aqueous layer was washed with ethyl acetate. The resulting complex [M(III)L¹].ClO₄ (M(III) = Fe, Co and Mn) was isolated as solid from the aqueous layer by lyophilization. Thus obtained iron(III), cobalt(III) and manganese(III) complexes were characterized by Elemental analysis, ESI-MS, FTIR, UV-Visible spectroscopic techniques.
3.8.2.1 Synthesis of FeL¹.ClO₄ complex (9e)

L¹ (5 g, 0.014 mol), sodium perchlorate monohydrate (1.95 g, 0.014 mol) were dissolved in 50 mL of water and then reacted with iron(III) acetylacetonate (4.9 g, 0.015 mol) in 50 mL of ethyl acetate for about 20 hours at 25°C. Then, Layers were separated and the aqueous layer was washed with 50 mL of ethyl acetate. The aqueous layer was filtered and then lyophilized to obtain about 5.5 g (77 %) of [FeL¹]ClO₄ complex. Elemental analysis data: calculated (%) for C₁₆H₃₀ClFeN₄O₉ (513): C 37.41, H 5.89, N 10.91, Found (%): C 37.34, H 5.83, N 10.85. IR Spectrum: 1690 cm⁻¹ for ν C=O str (amide), 3120 cm⁻¹ for ν N-H str. UV-Visible: [λ/nm (ε/M⁻¹ cm⁻¹)] in H₂O: 220 (1,36,000) and 430 (100). ESI MS: 414 [FeL⁺1]⁺

3.8.2.2 Synthesis of CoL¹.ClO₄ complex (9f)

L¹ (5 g, 0.014 mol), sodium perchlorate monohydrate (1.95 g, 0.014 mol) were dissolved in 50 mL of water and then reacted with cobalt(III) acetylacetonate (5.44 g, 0.015 mol) in 50 mL of ethyl acetate for about 20 hours at 25°C. Then, Layers were separated and the aqueous layer was washed with 50 mL of ethyl acetate. The aqueous layer was filtered and then lyophilized to obtain about 5.3 g (74 %) of [CoL¹]ClO₄ complex. Elemental analysis data: calculated (%) for C₁₆H₃₀ClCoN₄O₉ (516): C 37.18, H 5.85, N 10.84, Found (%): C 37.10, H 5.79, N 10.78. IR Spectrum: 1710 cm⁻¹ for ν C=O str (amide), 3200 cm⁻¹ for ν N-H str. UV-Visible: [λ/nm (ε/M⁻¹ cm⁻¹)] in H₂O: 210 (88,000), and 614 (230). ESI MS: 418 [CoL⁺1]⁺
3.8.2.3 Synthesis of MnL₁.ClO₄ complex (9g)

L₁ (5 g, 0.014 mol), sodium perchlorate monohydrate (1.95 g, 0.014 mol) were dissolved in 50 mL of water and then reacted with manganese(III) acetylacetonate (5.4 g, 0.015 mol) in 50 mL of ethyl acetate for about 20 hours at 25°C. Then, Layers were separated and the aqueous layer was washed with 50 mL of ethyl acetate and then lyophilized to obtain about 5.6 g (79 %) of [MnL₁]ClO₄ complex. Elemental analysis data: calculated (%) for C₁₆H₃₀ClMnN₄O₉ (512): C 37.47, H 5.90, N 10.93, Found (%): C 37.32, H 5.81, N 10.84. IR Spectrum: 1680 cm⁻¹ for ν C=O str (amide), 3180 cm⁻¹ for ν N-H str. UV-Visible: [λ/nm (ε/M⁻¹ cm⁻¹)] in H₂O: 240 (80,000) and 710 (90). ESI MS: 413 [MnL⁺₁].

3.8.3 Synthesis of VO(IV) complex [VOL₁] (9h)

L₁ (5 g, 0.014 mol) was dissolved in 50 mL of water and then reacted with vanadyl acetylacetonate (4.0 g, 0.015 mol) in 50 mL of ethyl acetate for about 20 hours at 25°C. Then, Layers were separated and the aqueous layer was washed with 50 mL of ethyl acetate. The aqueous layer was filtered and then lyophilized to obtain about 4.8 g (81%) of [VOL₁] complex. Thus obtained VO(IV) complex was characterized by Elemental analysis, ESI-MS, FTIR, UV-Visible spectroscopic techniques. Elemental analysis data: calculated (%) for C₁₆H₃₀VN₄O₆ (425): C 45.18, H 7.11 N 13.17, Found (%): C 45.13, H 7.06, N 13.11. IR Spectrum: 1675 cm⁻¹ for ν C=O str (amide), 3150 cm⁻¹ for ν N-H str. UV-Visible: [λ/nm (ε/M⁻¹ cm⁻¹)] in H₂O: 210 (1,14,000) and 625 (80). ESI MS: 425 [VOL⁺₁].
3.9 Synthesis of Metal complexes with L^2

3.9.1 Synthesis of copper(II), nickel(II), zinc(II) and magnesium(II) complexes [M(II)L^2]

Metal complexes were prepared by a general procedure in which L^2 (1 mol) in water was reacted with metal(II) acetylacetonate (1.1 mol) in ethyl acetate under stirring for 20 h at 25 °C. Layers were separated and the aqueous layer was washed with ethyl acetate. The resulting complex [M(II)L^2] (M(II) = Cu, Ni, Zn and Mg) was isolated from aqueous layer as solid by lyophilization. Thus obtained copper(II), nickel(II), zinc(II), cobalt(III) and magnesium(II) complexes were characterized by Elemental analysis, ESI-MS, FT-IR, UV-Visible spectroscopic techniques.

3.9.1.1 Synthesis of CuL^2 complex (11a)

L^2 (5 g, 0.0076 mol) was dissolved in 50 mL of water and then reacted with copper(II) acetylacetonate (2.19 g, 0.0084 mol) in 50 mL of ethyl acetate for about 20 hours at 25°C. Then, Layers were separated and the aqueous layer was washed with 50 mL of ethyl acetate. The aqueous layer was filtered and then lyophilized to obtain about 3.9 g (72 %) of [CuL^2] complex. Elemental analysis data: Calculated for C_{26}H_{50}CuN_{6}O_{11}S (717) (%): C 43.47, H 7.02, N 11.70, Found: C 43.45, H 7.06, N 11.67. IR Spectrum: 1650 cm\(^{-1}\) for ν C=O str (amide), 2936 cm\(^{-1}\) C-H str, 3090 cm\(^{-1}\) for ν N-H str and 3440 cm\(^{-1}\) for ν O-H str. UV-Visible [λ/\(\varepsilon\)/nm (ε/M\(^{-1}\) cm\(^{-1}\))] in H\(_2\)O: 214 (96,000), 634 (130). ESI MS: 622 [CuL^2 + 1]^+. 
3.9.1.2 Synthesis of NiL$_2$ complex (11b)

L$_2$ (5 g, 0.0076 mol) was dissolved in 50 mL of water and then reacted with nickel(II) acetylacetonate (2.15 g, 0.0084 mol) in 50 mL of ethyl acetate for about 20 hours at 25°C. Then, layers were separated and the aqueous layer was washed with 50 mL of ethyl acetate. The aqueous layer was filtered and then lyophilized to obtain about 4.5 g (83 %) of [NiL$_2$] complex. Elemental analysis data: Calculated for C$_{26}$H$_{50}$NiN$_6$O$_{11}$S (712) (%): C 43.77, H 7.06, N 11.78. Found: C 43.74, H 7.08, N 11.79. IR Spectrum: 1650 cm$^{-1}$ for ν C=O str (amide), 2937 cm$^{-1}$ C-H str, 3100 cm$^{-1}$ for ν N-H str. UV-Visible: [λ/nm (ε/M$^{-1}$ cm$^{-1}$)] in H$_2$O: 221 (93,000), 667 (40), 765 (20), 1068 (60). ESI MS: 617 [NiL$_2$ + 1]$^+$. 

3.9.1.3 Synthesis of ZnL$_2$ complex (11c)

L$_2$ (5 g, 0.0076 mol) was dissolved in 50 mL of water and then reacted with zinc(II) acetylacetonate (2.0 g, 0.0084 mol) in 50 mL of ethyl acetate for about 20 hours at 25°C. Then, layers were separated and the aqueous layer was washed with 50 mL of ethyl acetate and then lyophilized to obtain about 4.4 g (81 %) of [ZnL$_2$] complex. Elemental analysis data: Calculated for C$_{26}$H$_{50}$ZnN$_6$O$_{11}$S (718) (%): C 43.36, H 7.00, N 11.67. Found: C 43.31, H 7.05, N 11.63. IR Spectrum: 1650 cm$^{-1}$ for ν C=O str (amide), 2935 cm$^{-1}$ C-H str, 3450 cm$^{-1}$ for ν O-H str. UV-Visible [λ/nm (ε/M$^{-1}$ cm$^{-1}$)] in H$_2$O: 223 (1,59,000). ESI MS: 623 [ZnL$_2$ + 1]$^+$. $^1$H NMR (D$_2$O): δ 8.0 (s, 2H,
NH proton), δ 3.51 (t, 6H, J = 6 Hz, methylene protons), δ 3.0 (t, 4H, J = 4.5 Hz, methylene protons), δ 2.8 (m, 2H, methylene protons), δ 2.6 (m, 6H, methylene protons), δ 2.3 (t, 4H, J = 4.5 Hz, methylene protons), δ 1.94 (s, 3H, methyl protons), δ 1.5 (m, 8H, methylene protons), δ 1.4 (m, 4H, methylene protons), δ 1.3 (m, 2H, methylene protons) and δ 1.2 (m, 4H, methylene protons).

3.9.1.4 Synthesis of MgL² complex (11d)

L² (5 g, 0.0076 mol) was dissolved in 50 mL of water and then reacted with magnesium(II) acetylacetonate (2.17 g, 0.0084 mol) in 50 mL of ethyl acetate for about 20 hours at 25°C. Then, layers were separated and the aqueous layer was washed with 50 mL of ethyl acetate. The aqueous layer was filtered and then lyophilized to obtain about 3.9 g (76 %) of [MgL²] complex. Elemental analysis data: Calculated for C₂₆H₅₀MgN₆O₁₁S (678) (%): C 45.99, H 7.42, N 12.38. Found: C 45.85, H 7.36, N 12.33. IR Spectrum: 1630 cm⁻¹ for ν C=O str (amide), 2935 cm⁻¹ C-H str, 3250 cm⁻¹ for ν N-H str. UV-Visible: [λ/nm (ε/M⁻¹ cm⁻¹)] in H₂O: 205 (30,000) and 300 (40,000). ESI MS: 583 [MgL² + 1]⁺.
3.9.2 Synthesis of Fe(III), Co(III) and Mn(III) complexes [M(III)L²]

Metal complexes were prepared by a general procedure in which L² (1 mol) was dissolved in water and then reacted with metal(III) acetylacetonate (1.1 mol) in ethyl acetate under stirring for 20 h at 25°C. Layers were separated and the aqueous layer was washed with ethyl acetate. The resulting complex [M(III)L²] (M(III) = Fe, Co and Mn) was isolated as solid from the aqueous layer by lyophilization. Thus obtained iron(III), cobalt(III) and manganese(III) complexes were characterized by Elemental analysis, ESI-MS, FTIR, UV-Visible spectroscopic techniques.

3.9.2.1 Synthesis of FeL² complex (11e)

L² (5 g, 0.0076 mol) was dissolved in 50 mL of water and then reacted with iron(III) acetylacetonate (2.96 g, 0.0084 mol) in 50 mL of ethyl acetate for about 20 hours at 25°C. Then, Layers were separated and the aqueous layer was washed with 50 mL of ethyl acetate. The aqueous layer was filtered and then lyophilized to obtain about 4.7 g (87 %) of [FeL²] complex. Elemental analysis data: Calculated for C₂₆H₄₉FeN₆O₁₁S (709) (%): C 44.01, H 6.96, N 11.84. Found: C 43.96, H 7.00, N 11.87. IR Spectrum: 1650 cm⁻¹ for ν C=O str (amide), 2935 cm⁻¹ C-H str, 3435 cm⁻¹ for ν O-H str. UV-Visible [λ/nm (ε/M⁻¹ cm⁻¹)] in H₂O: 210 (89,000), 431 (80). ESI MS: 614 [FeL²⁺]⁺.
3.9.2.2 Synthesis of CoL\textsuperscript{2} complex (11f)

L\textsuperscript{2} (5 g, 0.0076 mol) was dissolved in 50 mL of water and then reacted with cobalt(III) acetylacetonate (3.0 g, 0.0084 mol) in 50 mL of ethyl acetate for about 20 hours at 25°C. Then, layers were separated and the aqueous layer was washed with 50 mL of ethyl acetate. The aqueous layer was filtered and then lyophilized to obtain about 3.85 g (71 %) of [CoL\textsuperscript{2}] complex. [CoL\textsuperscript{2}] Elemental analysis: Calculated for C\textsubscript{26}H\textsubscript{49}CoN\textsubscript{6}O\textsubscript{11}S (712) (%): C 43.82, H 6.93, N 11.79. Found: C 43.78, H 7.0, N 11.76. IR Spectrum: 1650 cm\textsuperscript{-1} for ν C=O str (amide), 2935 cm\textsuperscript{-1} C-H str, 3420 cm\textsuperscript{-1} for ν O-H str. UV-Visible [λ/\text{nm} (ε/M\textsuperscript{-1} cm\textsuperscript{-1})] in H\textsubscript{2}O: 220 (1,85,000), 614 (23). ESI MS: 617 [CoL\textsuperscript{2+1}\textsuperscript{+}].

3.9.2.3 Synthesis of MnL\textsuperscript{2} complex (11g)

L\textsuperscript{2} (5 g, 0.0076 mol) was dissolved in 50 mL of water and then reacted with manganese(III) acetylacetonate (2.95 g, 0.0084 mol) in 50 mL of ethyl acetate for about 20 hours at 25°C. Then, layers were separated and the aqueous layer was washed with 50 mL of ethyl acetate. The aqueous layer was filtered and then lyophilized to obtain about 4.15 g (77 %) of [MnL\textsuperscript{2}] complex. Elemental analysis data: Calculated for C\textsubscript{26}H\textsubscript{49}MnN\textsubscript{6}O\textsubscript{11}S (708) : (%) C 44.06, H 6.97, N 11.86. Found: C 43.95, H 6.89, N 11.81. IR Spectrum: 1650 cm\textsuperscript{-1} for ν C=O str (amide), 2936 cm\textsuperscript{-1} C-H str, 3450 cm\textsuperscript{-1} for ν
O-H str. UV-Visible \[\lambda/\text{nm} (\varepsilon/\text{M}^{-1}\text{cm}^{-1})\] in H$_2$O: 210 (1,51,000), 310 (20,000), 580 (45).

ESI MS: 613 \[\text{[MnL}^2+]\]^{	ext{+}}

3.9.3 Synthesis of VO(IV) complexes [VOL$^2$] (11h)

L$^2$ (5 g, 0.0076 mol) was dissolved in 50 mL of water and then reacted with vanadyl acetylacetonate (2.2 g, 0.0084 mol) in 50 mL of ethyl acetate for about 20 hours at 25°C. Then, layers were separated and the aqueous layer was washed with 50 mL of ethyl acetate. The aqueous layer was filtered and then lyophilized to obtain about 3.7 g (78%) of [VOL$^2$] complex. Thus obtained VO(IV) complex was characterized by Elemental analysis, ESI-MS, FTIR, UV-Visible spectroscopic techniques.

Elemental analysis Calculated for C$_{25}$H$_{46}$VN$_6$O$_9$ (625) (%): C 48.00, H 7.41, N 13.43. Found: C 47.92, H 7.33, N 13.39. IR Spectrum: 1650 cm$^{-1}$ for ν C=O str (amide), 2937 cm$^{-1}$ C-H str, 3422 cm$^{-1}$ for ν O-H str. UV-Visible \[\lambda/\text{nm} (\varepsilon/\text{M}^{-1}\text{cm}^{-1})\] in H$_2$O: 210 (89,000), 570 (80). ESI MS: 625 [VOL$^2$]$^+$.