

# Synthesis of new Metal Complex - Biomolecule Hybrids as Redox Sensors for Specific Nucleobases

G. Gasser<sup>\*</sup>, A.M. Bond, B. Graham, Z. Kosowski, and L. Spiccia<sup>‡</sup>

School of Chemistry, Monash University, Victoria 3800, Australia

<sup>\*</sup>gilles.gasser@sci.monash.edu.au

<sup>‡</sup>leone.spiccia@sci.monash.edu.au

## ABSTRACT

This article describes the synthesis, characterization and Zn(II) complexation properties of a macrocyclic tetraamine ligand derivative of 1,4,7,10-tetraazacyclododecane (cyclen) (**1**) bearing a ferrocenyl pendant arm. Functionalization of cyclen with the ferrocenyl group has been accomplished according to a published method. NMR spectroscopy has been used to establish that the ligand forms a Zn<sup>2+</sup> complex with a 1:1 stoichiometry. We have investigated the change in the redox properties of the metal-free ligand **1**, the Zn<sup>2+</sup> complex and the Zn<sup>2+</sup> complex following addition of the DNA base thymine (T) in acetonitrile as solvent. The electrochemical data indicate a significant change in the redox potential of the ferrocenyl group on addition of zinc ions to the macrocycle-ferrocenyl adduct. Addition of thymine to the complex caused only a minor change in electrochemical response, which indicated that this Zn(II)-ferrocene hybrid is unlikely to be useful as a thymine sensor under the conditions used in our experiments.

**Keywords:** Ferrocene-macrocycle derivative, zinc complex, electrochemistry, biosensor, nucleobase.

## 1 INTRODUCTION

There are a number of redox-active supramolecular receptors described in the literature, but those containing ferrocene as a redox-active reporter group are the most frequently cited. These ferrocene derivatives allow electrochemical detection of cations, anions and, more recently, neutral molecules like barbiturate or urea derivatives<sup>[1]</sup>. Chong *et al.* also showed that modified ferrocenyl conjugates can be used as signaling probes for the detection of nucleic acids by hydrogen bonding<sup>[2]</sup>.

Kimura *et al.* showed, recently, the ability of the tetraamine macrocycle cyclen to complex the Zn<sup>2+</sup> cation<sup>[3]</sup>. Furthermore, they discovered that Zn<sup>2+</sup>-cyclen could selectively bind to the thymidine (dT) nucleoside and its homologues via the deprotonated imide nitrogen to form stable 1:1 complexes in aqueous solution at physiological pH<sup>[4,5]</sup>. In comparison with the receptor of Chong *et al.*, in the present study we report initial efforts to develop a receptor for nucleobases that not only uses hydrogen bonding in binding the guest, but also allows for

coordination (dative) bonding to a metal centre, as this is expected to increase the association constant of the receptor-guest adduct.

With this background, our research group has therefore directed its attention to the preparation of new metal complexes-biomolecule hybrids, with the aim of sensing specific RNA/DNA sequences via electrochemical methods. In this article we present our initial research in this area which led us to synthesize a derivative of ferrocene (**1**) (figure 1) and to study the complexation of this assembly with Zn<sup>2+</sup> core and subsequently the binding of thymine to the Zn<sup>2+</sup> complex. Future application of such adducts in the sensing of specific DNA or RNA sequences featuring thymine and uracil bases, respectively can be envisaged.

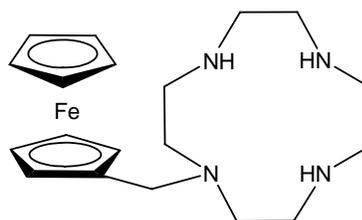


Figure 1: Receptor **1**

## 2 RESULTS AND DISCUSSION

### 2.1 Receptor Choice and its Synthesis

The organometallic receptor, 1-(ferrocenemethyl)-1,4,7,10-tetraazacyclododecane **1** has been chosen for our studies as it has a number of characteristics: readily prepared, cheap and with sufficient water solubility for sensor investigations.

The ferrocene pendant has been synthesized following the procedure published by Sisti *et al.* that uses a 3-step reaction sequence starting from commercially-available cyclen<sup>[6]</sup>. The ferrocene pendant should not interfere with the thymine binding to the Zn-cyclen, as shown in figure 2. The CH<sub>2</sub> linker between the macrocycle and the ferrocene moiety provides sufficient flexibility to allow the desired nucleobase adduct to form.

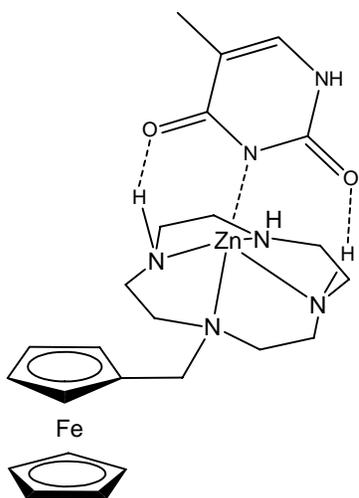


Figure 2: Receptor **1** complexed with  $Zn^{2+}$  and thymine

## 2.2 Stoichiometry of Zn(II) Complex

The stoichiometry of the complex between **1** and  $Zn^{2+}$  was established by measuring the variation in electrochemical response and change in the NMR spectrum as a function of  $Zn^{2+}$  concentration. These measurements indicated the formation of a 1:1 complex. A gradual shift of the singlet peak from 3.53 ppm of the  $CH_2$  bridging the ferrocene to the macrocycle was not observed upon the addition of aliquots of  $Zn^{2+}$ . Instead, a new signal was found at 3.81 ppm indicating of the formation of the complex. The 1:1 stoichiometry has been proved as the peak at 3.53 disappeared completely after the exact addition of one equivalent of  $Zn^{2+}$  to the ligand solution. The table 1 summarizes the percentage ratio between the peaks at about 3.53 ppm (corresponding to the ligand) and 3.81 ppm (corresponding to the complex) upon the addition of  $Zn^{2+}$ . Splitting of the peak at 3.53 ppm was observed when  $Zn^{2+}$  was added because the acidity of the metal cation generates free acid and leads to a change in the degree of protonation of the cyclen-ferrocene ligand and these protonated forms interchange slowly on the NMR time scale.

Table 1: Ratio (in %) of the NMR integration for the signals at 3.53 ppm (corresponding to the ligand) and 3.81 ppm (corresponding to the complex) as a function of added  $Zn^{2+}$ .

Eq. of $Zn^{2+}$	Ratio at 3.53 ppm	Ratio at 3.81 ppm
0	100	0
0.10	88	12
0.25	74	26
0.50	54	46
0.75	31	69
1.00	0	100
1.25	0	100
2.00	0	100
5.00	0	100

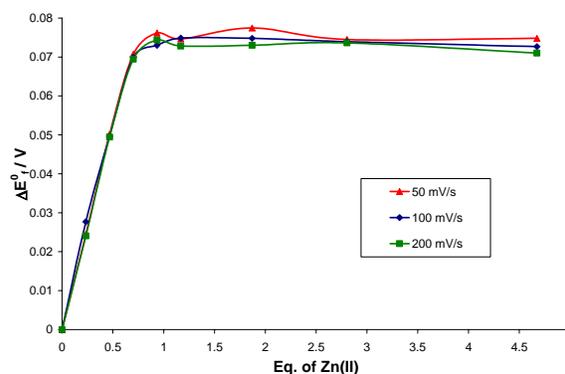


Figure 3: Electrochemical plot of the value of  $\Delta E_f^0$  for the  $Fc^+/Fc$  redox couple of the ligand (1 mM in  $CH_3CN$  with a GC working electrode) upon the addition of aliquots of  $Zn^{2+}$

## 2.3 Electrochemistry

In electrochemical studies, the difference in the reversible formal potential,  $\Delta E_f^0$ , (values calculated as the difference from the average of the oxidation and reduction peak potentials between the Zn-complex and the ligand), was measured in acetonitrile upon the addition of aliquots of  $Zn^{2+}$  and at different scan rates (50, 100 and 200 mV/s). The end-point was at a metal:**1** ratio of less than 1:1 (figure 3). However, the value of  $\Delta E_f^0$  was independent of zinc concentration after the addition of more than one equivalent. Similar curves were observed either with a Pt or a glassy carbon (GC) working electrode. More detailed analysis of data presented in figure 3 is required to fully interpret the voltammetric titration results.

Cyclic voltammetry was performed on ligand **1** in  $CH_3CN$  and the shift of the reversible formal potential upon the addition of, first, 1.0 equivalent of  $Zn^{2+}$  followed by addition of 1.0 equivalent of thymine and triethylamine was measured. The triethylamine was added in order to deprotonate the DNA base and enhance the binding with the Zn-complex. The formal potential of the  $Fe^{2+}/Fe^{3+}$  redox couple of the ferrocenyl macrocycle **1** was measured to be 112 mV against the Ag/AgCl reference electrode. As shown in figure 4, a shift of 67 mV to more positive potentials is observed upon the addition of 1.0 equivalent of  $Zn^{2+}$ . Thus, the  $Fe^{2+}/Fe^{3+}$  redox couple of the ferrocene has been made more difficult to oxidize to the ferrocenium derivative because of the positive charge of the cation complexed to the cyclen. Upon the subsequent addition of thymine to Zn-cyclen, a slight decrease in potential is observed (-10 mV). The trend to less positive potentials was expected as the DNA base withdraws electron density from the Zn-cyclen, making the  $Fe^{2+}/Fe^{3+}$  redox couple of ferrocene easier to oxidize. However, the shift in the formal potential observed under these conditions is quite small particularly given that the experimental uncertainty in each potential value is  $\pm 5$  mV.

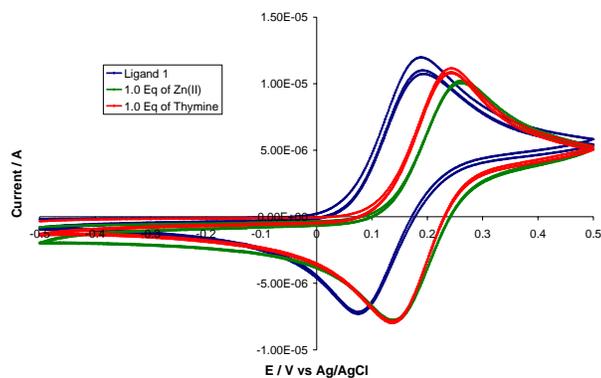


Figure 4: Cyclic voltammograms in  $\text{CH}_3\text{CN}$  of **1**, **1** + 1.0 equivalent of  $\text{Zn}^{2+}$ , and **1** + 1.0 equivalent of  $\text{Zn}^{2+}$  + 10.0 equivalents of thymine and triethylamine (4.38 mM in  $\text{CH}_3\text{CN}$  with a Pt working electrode at 200 mV/s).

### 3 CONCLUSION

This work allowed the observation that the complexation of a ferrocenyl macrocycle by  $\text{Zn}^{2+}$  measured by electrochemistry and NMR spectroscopy in acetonitrile, results in the formation of a Zn(II) complex with the expected 1:1 stoichiometry. Under the experimental conditions presented in this paper, addition of  $\text{Zn}^{2+}$  to the macrocycle-ferrocenyl adduct resulted in a significant positive shift in of potential, which indicated that the ferrocene becomes more difficult to oxidize in the presence of the positively charged  $\text{Zn}^{2+}$  ions. The shift upon the addition of the DNA base thymine is too small to be considered useful for the development of biosensors for the detection of specific RNA/DNA sequences. Consequently, new experimental conditions or modification of the ligand structure will be investigated to increase the electrochemical response upon the addition of the thymine. In this perspective, further electrochemical studies in water will be undertaken. The electrochemical detection of thymidine instead of thymine will be also investigated in order to assess whether the negative charge of the phosphate backbone changes the electrochemical response. Crystallization of the different complexes (Zn-cyclen and thymine-Zn-cyclen) is being attempted with a view to establish the structures of these complexes by X-ray crystallography.

## 4 EXPERIMENTAL

### 4.1 General Comments

All chemicals were of reagent grade quality or better, obtained from commercial suppliers and used without further purification. Solvents were used as received or dried over 4 Å molecular sieves. **1** was prepared by slight modification of literature procedures<sup>[6]</sup>. Zinc solutions for use in NMR and electrochemical experiments were

prepared from either  $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  or  $\text{Zn}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$  and standardized by routine EDTA titration<sup>[7]</sup>.

**Caution:** Although no problems were encountered in this work, metal perchlorate complexes are potentially explosive. They should be prepared in small quantities and handled with care.

### 4.2 Physical Measurements

$^1\text{H}$  NMR spectra of **1** were recorded in  $\text{CD}_3\text{CN}$  on an Avance DRX400 Bruker spectrometer at  $30^\circ\text{C}$ . The residual  $\text{CHD}_2\text{CN}$  has been used as an internal reference. CHN analyses were performed by the Campbell Microanalytical Services, University of Otago, Dunedin, NZ.

NMR titrations were carried out by adding aliquots of a solution of a standardized  $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  to a solution of the ferrocene ligand **1** ( $C=10$  mM), in the same solvent ( $\text{CD}_3\text{CN}$ ).

### 4.3 Electrochemistry

Voltammetric experiments were performed with a VoltaLab PGZ 301 Radiometer Instrument using a standard 4-necked electrochemical cell that allowed reproducible positioning of the working, reference and auxiliary electrodes and nitrogen inlet tube. 3 mm diameter glassy carbon (GC) (IJ Cambria) and 3.3 mm diameter Pt were used as the working electrodes. All electrodes were polished with a 0.3 mm alumina slurry on Microcloth polishing cloth and dried with tissue paper (Kimwipe) prior to use. The Ag/AgCl (aqueous 3 M KCl) reference electrode was separated from the test solution by a salt bridge containing acetonitrile (0.1 M  $\text{Bu}_4\text{NPF}_6$ ). Check of the reference electrode stability was made by frequent measurement of the Ag/AgCl reference electrode potential against that for the  $\text{Fc}^{0/+}$  ( $\text{Fc}$  = ferrocene) scale (153 mV). The auxiliary electrode was made from platinum mesh. Voltammetric experiments were commenced after degassing the acetonitrile (0.1 M  $\text{Bu}_4\text{NPF}_6$ ) electrolyte solutions with solvent saturated nitrogen for at least 10 minutes.

Aliquots of a standardized solution of to a solution of the ferrocene ligand **1**, in the same solvent ( $\text{CH}_3\text{CN}$ ) were added in order to get the electrochemical plot of the value of  $\Delta E_f^0$  for the  $\text{Fc}^+/\text{Fc}$  redox couple of the ligand.

## 5 ACKNOWLEDGEMENT

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