An investigation of transition metal complex chemistry:
enzyme mimicry and Zn(II) detection

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ABSTRACT

This thesis presents research in the field of transition metal complex chemistry.

The major project, reported in Part I, is focussed on the mimicking of enzymes in order to produce simple catalyst molecules that enhance the rate of ester hydrolysis. This work involved a systematic characterisation of a suite of potential enzyme mimics which were based on the simple template molecules tris(2-aminoethyl)amine (tren) and 1,4,7-triazacyclononane (tacn). The effects of substituting tren and tacn with methyl groups and β-cyclodextrin and then complexing these with Zn$^{2+}$, Cu$^{2+}$, Cd$^{2+}$ and Ni$^{2+}$ were examined. The aim was to produce stable complexes with lowered pK$_a$s for their associated aqua ligands (‘pK$_{a\text{H}_2\text{O}}$’), such that the catalytically active hydroxo ligands (responsible for the ester hydrolysis) were readily available over a broad pH range.

The thirty two resulting transition metal complex systems were characterised by potentiometric titration under identical experimental conditions (aqueous NaClO$_4$ solution, $I = 0.10$ mol dm$^{-3}$, 298.2 K). For selected systems which were characterised by large stability constants and relatively low aqua ligand pK$_{a\text{H}_2\text{O}}$s, their ability to enhance the rate of hydrolysis of 4-nitrophenyl acetate was measured using UV-Visible spectroscopy. A modified Michaelis-Menten enzyme kinetics analysis was developed specifically for this study. Information about the possible mechanism of catalysis was also obtained by a qualitative investigation of the UV-Vis spectra of several systems over the course of the reaction.

The chapters comprising Part I therefore describe the synthesis of the ligands (including two new compounds; 6$^A$-[(E)-2-((E)-2-phenyl)ethenyl]-6$^A$-deoxy-$\beta$-cyclodextrin (βCDMe$_3$tren) and 6$^A$-1,4,7-trimethyl-1,4,7-triazacyclononan-1-yl)-6$^A$-deoxy-$\beta$-cyclodextrin (βCDMe$_2$tacn)), potentiometric titrations to establish speciation of the systems and the subsequent monitoring of the hydrolysis of 4-nitrophenyl acetate using UV-Vis spectroscopy.

The secondary project, reported in Part II, is focussed on the detection of physiological Zn$^{2+}$ using a newly characterised ligand; 2-((E)-2-phenyl)ethenyl-8-((N-4-methylbenzenesulfonyl)aminoquinol-6-yloxyacetic acid. A styryl functional group was added to the commercially available Zn$^{2+}$-selective fluorophore “Zinquin” which detects physiological Zn$^{2+}$ by complexing it and producing a fluorescent signal exclusively on Zn$^{2+}$ binding. The
intention of the styryl addition was to enhance the bulk of the Zinquin analogue and thereby hope to improve the selectivity of this fluorophore for free, intracellular Zn$^{2+}$ over the Zn$^{2+}$ found in the structures and catalytic centres of proteins and enzymes. This styryl Zinquin derivative was characterised using potentiometric titrimetry, UV-Vis spectroscopy and fluorimetry. This includes an analysis of the photoisomerism introduced by the styryl functionality. The absorbance and fluorescence characteristics of the free and Zn$^{2+}$-complexed ligand were measured and stability constants for the Zn$^{2+}$ complexes were determined. The results were compared to Zinqu in under the same experimental conditions. Like Zinqu, the new styryl analogue was found to be Zn$^{2+}$ selective.
DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. The author acknowledges that copyright of those works resides with the copyright holder(s) of those works. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide.

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B.Sc. (Hons), University of Adelaide

27/10/2015
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Mum and Dad, mcsquared, I would be absolutely nowhere without you. Thank you for your never ending love and support.

“\textit{It was, he thought, the difference between being dragged into the arena to face a battle to the death and walking into the arena with your head held high. Some people, perhaps, would say that there was little to choose between the two ways, but Dumbledore knew - and so do I, thought Harry, with a rush of fierce pride, and so did my parents - that there was all the difference in the world.}”

Harry Potter and the Half-Blood Prince
ABBREVIATIONS

1. General

\( \delta \quad \) chemical shift (parts per million)

\( \nu \quad \) frequency (s\(^{-1}\))

\( \Phi \quad \) quantum yield

\( \lambda \quad \) wavelength (nm)

\( A_{\text{infinity}} \quad \) UV-Vis absorbance at an infinite reaction time

\( c \quad \) speed of light, \( 3 \times 10^8 \) m s\(^{-1}\)

CHEF \quad chelation enhanced fluorescence

\( \text{dm} \quad \) decimeter

\( E \quad \) enzyme (Pat I)

\( E \quad \) observed electrode potential (millivolts) (Part II)

\( E_0 \quad \) standard electrode potential (millivolts)

EPR \quad electron paramagnetic resonance

E•S \quad enzyme-substrate Michaelis complex

\( \text{et al.} \quad \text{et alia} \)

\( F \quad \) Faraday constant, \( 9.6487 \times 10^4 \) C mol\(^{-1}\)

\( h \quad \) Planck’s constant, \( 6.63 \times 10^{-34} \) J s

\( [H^+] \quad \) proton concentration (mol dm\(^{-3}\))

\( I \quad \) constant ionic strength (mol dm\(^{-3}\))

\( J \quad \) coupling constant (context; NMR) or joules

\( K \quad \) stability constant

\( K_1 \quad \) stability constant for the 1:1 (M\(^{2+}\):ligand) transition metal complex
\( K_2 \)  
stability constant for the 2:1 (M\(^{2+}\):ligand) transition metal complex

\( K_a \)  
acid dissociation constant

\( K_{aH2O#1} \)  
acid dissociation constant for the first aqua ligand deprotonation

\( K_{aH2O#2} \)  
acid dissociation constant for the second aqua ligand deprotonation

\( K_{aH2O#3} \)  
acid dissociation constant for the third aqua ligand deprotonation

\( k_{cat} \)  
rate constant (s\(^{-1}\)) for the catalysed rate of hydrolysis of 4-nitrophenyl acetate

\( k_{obs} \)  
rate constant for the overall observed rate of hydrolysis of 4-nitrophenyl acetate

\( k_{un} \)  
rate constant for the uncatalysed rate of hydrolysis of 4-nitrophenyl acetate

\( K_D \)  
dissociation constant for the reformation of the free enzyme and substrate from the Michaelis complex (E•S)

\( K_M \)  
Michaelis constant

\( L \)  
free, deprotonated ligand

\( LH^+ \)  
free, monoprotonated ligand

\( LH_2^{2+} \)  
free, diprotonated ligand

\( LH_3^{3+} \)  
free, triprotonated ligand

\( LH_4^{4+} \)  
free, tetraprotonated ligand

\( MHz \)  
Megahertz

\([MLH]^{3+}\)  
1:1 M\(^{2+}\):monoprotonated ligand complex (Part I)

\([ML]^{2+}\)  
1:1 M\(^{2+}\):deprotonated ligand (Part I)

1:1 stZQA:M\(^{2+}\) (Part 2)

\([MLOH]^+\)  
1:1:1 M\(^{2+}\):ligand:hydroxo

\([ML(OH)_2]\)  
1:1:2 M\(^{2+}\):ligand:2×hydroxo
[M(L)$_2$]$^{2+}$ 1:2 M$^{2+}$:2×ligand

Note: the five complexes above may or may not contain water ligands as is noted for each specific example in the text.

mV  millivolts

nm  nanometer

NMR  nuclear magnetic resonance

pH  -log[H$^+$]

$pK_a$  -log[$K_a$]

$pK_{aH_2O#1}$  -log[$K_{aH_2O#1}$]

$pK_{aH_2O#2}$  -log[$K_{aH_2O#2}$]

$pK_{aH_2O#3}$  -log[$K_{aH_2O#3}$]

ppm  parts per million

R  Ideal gas constant, 8.314 J K$^{-1}$ mol$^{-1}$

S  substrate (in this study; ester 4-nitrophenyl acetate – Part I)

$S_0$  ground electronic state (Part II)

$S_1$  first excited electronic state (Part II)

T  Temperature (°C or Kelvin)

t$_{infinity}$  An infinite time into a reaction

UV-Vis  UV-Visible

$v_0$  ground vibrational state

$v_1$  first excited vibrational state

Zn$^{2+}_{Ad}$  adventitious Zn$^{2+}$
### 2. Chemicals

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<th>Name</th>
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<td>ACES</td>
<td>2-(carbamoylmethylamino)ethanesulfonic acid (buffer)</td>
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<tr>
<td>βCD</td>
<td>β-cyclodextrin</td>
</tr>
<tr>
<td>βCDMe₂tacn</td>
<td>6^A-(1,4,7-trimethyl-1,4,7-triazacyclononan-1-yl)-6^A-deoxy-β-cyclodextrin</td>
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<tr>
<td>βCDMe₅tren</td>
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<tr>
<td>βCDtren</td>
<td>6^A-{2-[bis(2-aminoethyl)amino]ethylamino}^-6^A-deoxy-β-cyclodextrin</td>
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<tr>
<td>Bicine</td>
<td>2-(bis(2-hydroxyethyl)amino)acetic acid (buffer)</td>
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<td>DEPP</td>
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<td>HEPES</td>
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<td>Tren</td>
<td>tris(2-aminoethyl)amine</td>
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<td>Acronym</td>
<td>Full Name</td>
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<tr>
<td>---------</td>
<td>-----------</td>
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<tr>
<td>TRIS</td>
<td>2-amino-2-hydroxymethyl-propane-1,3-diol (buffer)</td>
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<td>stZQA</td>
<td>2-((E)-2-Phenyl)ethenyl-8-(N-4-methylbenzenesulfonyl)aminoquinol-6-yloxyacetic Acid (styryl Zinquin A)</td>
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<td>ZQA</td>
<td>2-methyl-8-p-toluenesulfonamido-6-quinolyloxyacetic acid (Zinquin A)</td>
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