Synthesis, Characterization and Cellular Uptake of Magnesium Complexes of Pyrones and Amino Acids

Derek Robert Case
Syracuse University

Follow this and additional works at: https://surface.syr.edu/etd

Part of the Chemistry Commons

Recommended Citation
https://surface.syr.edu/etd/1519

This Dissertation is brought to you for free and open access by the SURFACE at Syracuse University at SURFACE at Syracuse University. It has been accepted for inclusion in Dissertations - ALL by an authorized administrator of SURFACE at Syracuse University. For more information, please contact surface@syr.edu.
Abstract
This thesis focuses on the treatment of micronutrient deficiency, specifically hypomagnesemia, through the synthesis and solution- and solid-state characterization of “pharma grade”, biorelevant magnesium complexes (nutraceuticals) utilizing short-chain peptides (di- and tri-), pyrones, and amino acids. There is further emphasis placed on determining the in vitro uptake of all produced complexes. This work aims to mitigate the shortcomings of current magnesium supplements and better illustrate an understanding of the factors impacting magnesium uptake.

Specifically, 1) There is a paucity of information regarding the coordination of biorelevant ligands to magnesium, most of which was investigated between the 1950s and 1980s, and that provided inconclusive structural determination of the resultant complexes and their impurities.

2) Current magnesium supplements suffer from a lack of proper characterization given an absence of strict regulations as are present in pharmaceuticals

3) The most ubiquitously used magnesium supplements often place emphasis on a high percentage magnesium composition, but exhibit poor water solubility and subsequently poor bioavailability thus resulting in less substantial magnesium uptake

Q1. (Chapter 2) What is the expected ligand coordination of magnesium complexes and how does Mg\(^{2+}\) coordination manifest when probed utilizing 1D and 2D \(^1\text{H}/\text{C}\) NMR?

**Goals:** Synthesize and characterize a series of Mg\(^{2+}\) complexes utilizing pyrones and amino acids and employ 1D and 2D \(^1\text{H}/\text{C}\) NMR to determine the mode of coordination assumed by the Mg\(^{2+}\) coordinate ligands. Furthermore, better outline how Mg\(^{2+}\) coordination is observed and manifested on NMR spectra (e.g., signal intensity reduction, resolution changes, shifting, etc.)
Q2. (Chapter 3) Given the affinity for water of Mg\(^{2+}\), can solid-state methods be employed to determine the degree of complex hydration and elucidate the general composition of the Mg\(^{2+}\)-complex core composition and provide additional insight as to the coordination mode of Mg\(^{2+}\) coordinate ligands?

*Goals:* To utilize thermal analyses (TGA/DSC) and elemental analysis to determine the degree of hydration exhibited by the synthesized Mg\(^{2+}\) complexes and to determine the core structure of said synthesized complexes.

Q3. (Chapter 4) How does Mg\(^{2+}\) complex solubility impact the overall cellular uptake of Mg\(^{2+}\) from synthesized complexes?

*Goals:* To determine the solubility of synthesized Mg\(^{2+}\) complexes and determine if there is a relationship between the solubility of the complexes and the observed *in vitro* uptake of Mg\(^{2+}\) from the respective complexes.

Q4. (Chapter 6) Can other complexes using similar biorelevant metals (e.g., Zn\(^{2+}\)) be characterized in a similar way and does ligand coordination differ from that observed for Mg\(^{2+}\)?

*Goals:* To determine if analogous complexes utilizing different biorelevant divalent cations (e.g., Zn\(^{2+}\)) can be synthesized and characterized in a similar fashion. Additionally, to determine if different metals exhibit different observables when characterized utilizing NMR, given difference in electron configuration, electropositivity/electronegativity.
Synthesis, Characterization and Cellular Uptake of Magnesium Complexes of Pyrones and Amino Acids

By
Derek R. Case
B.S. Chemistry, Utica College 2017

DISSERTATION
Submitted in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in Chemistry

Syracuse University
May 2022
Table of Contents

**Chapter 1. Introduction**

1.1. Introduction to Magnesium

1.2. Biological Significance of Mg$^{2+}$
1.2.1. Distribution of Mg$^{2+}$ in the Body
1.2.2. Absorption of Mg$^{2+}$ in the Gastrointestinal (GI) Tract
1.2.3. Introduction to the Mg$^{2+}$ Uptake Pathways
1.2.3.1. Mechanism of the PPP
1.2.3.2. Mechanism of the TP
1.2.4. Renal Filtering of Mg$^{2+}$

1.3. Determining Mg$^{2+}$ Status in Humans
1.3.1. Urinary Analysis and “Loading Test” to Evaluate Mg$^{2+}$ Status
1.3.2. Evaluating Mg$^{2+}$ Status Isotopically

1.4. Hypomagnesaemia
1.4.1. Hypomagnesaemia in Chronic Disease
1.4.1.1. Mg$^{2+}$ in Heart Health
1.4.1.2. Mg$^{2+}$ in Osteoporosis
1.4.1.3. Mg$^{2+}$ in T2DM

1.5. Mg$^{2+}$ Supplementation to Treat Hypomagnesaemia
1.5.1. Introduction to Mg$^{2+}$ Supplements and Understanding Oxides and Salts
1.5.2. Introduction to Mg$^{2+}$-Chelates
1.5.2.1. A Brief Discussion of Characterized Biorelevant Mg$^{2+}$ Complexes 15 – 17

1.5.3. Nutraceuticals 17

1.6. Summary 17 – 19

1.7. References 19 – 26

Chapter 2. The Effect of Magnesium Coordination on the $^1$H/$^{13}$C Nuclear Magnetic Resonance of Magnesium-Coordinate Ligands

2.1. Introduction 27 – 29

2.2. NMR Characterization of Maltol and 1 29

2.2.1. 1H NMR Characterization of Maltol and 1 29 – 31

2.2.2. Confirmation of 1 Proton and Carbon Signal Determination Utilizing Heteronuclear Single Quantum Coherence (HSQC) and Heteronuclear Multiple Bond Correlation (HMBC) 31 – 36

2.2.3. Characterization of 1 Via $^{13}$C NMR 36 – 39

2.3. NMR Characterization of Ethylmaltol and 2 39

2.3.1. 1H NMR Characterization of Ethylmaltol and 2 39 – 41

2.3.2. Confirmation of 2 Proton and Carbon Signal Determination Utilizing HSQC and HMBC 42 – 46

2.3.3. Characterization of 2 Via $^{13}$C NMR 46 – 47

2.4. NMR Characterization of HG$_2$ and 3 48

2.4.1. 1H NMR Characterization of HG$_2$ and 3 48 – 50

2.4.2. Confirmation of 3 Proton and Carbon Signal Determination Utilizing HSQC and HMBC 50 – 54

2.4.3. Characterization of 3 Via $^{13}$C NMR 54 – 56
2.5. NMR Characterization of HG\textsubscript{3} and \textbf{4} \\
2.5.1. 1H NMR Characterization of HG\textsubscript{3} and \textbf{4} \\
2.5.2. Confirmation of \textbf{4} Proton and Carbon Signal Determination Utilizing HSQC and HMBC \\
2.5.3. Characterization of \textbf{4} Via \textsuperscript{13}C NMR \\
2.6. Conclusion \\
2.7. References \\

\textbf{Chapter 3. Determining Complex Composition and Varying Degrees of Mg\textsuperscript{2+}-Complex Hydration Utilizing Solid-State Analyses and Potentiometry} \\
3.1. Introduction \\
3.2. FT-IR Supports Ligand Coordination Modes and the Presence of Water \\
3.2.1. FT-IR Analysis of \textbf{1} \\
3.2.2. FT-IR Analysis of \textbf{2} \\
3.2.3. FT-IR Analysis of \textbf{3} \\
3.2.4. FT-IR Analysis of \textbf{4} \\
3.3. Determining the Composition of Complexes \textbf{1} – \textbf{4} \\
3.3.1 Determining the Composition of \textbf{1} \\
3.3.1.1. Thermal and Elemental Analyses of \textbf{1} \\
3.3.1.2. Mass Spectrometry Supports the Mg\textsuperscript{2+}:Maltol Stoichiometry of \textbf{1} \\
3.3.1.3. Discussing the Core Structure of \textbf{1} \\
3.3.2. Determining the Composition of \textbf{2} \\
3.3.2.2. Thermal and Elemental Analyses of \textbf{2}
3.3.2.3. Mass Spectrometry Supports the Mg$^{2+}$:Ethylmaltol Stoichiometry of 2 81 - 82
3.3.2.4. Discussing the Core Structure of 2 82
3.3.3. Determining the Composition of 3 83
3.3.3.1. Thermal and Elemental Analyses of 3 83 - 84
3.3.3.2. Mass Spectrometry Supports the Mg$^{2+}$:HG$_2$ Stoichiometry of 3 84
3.3.3.3. Discussing the Core Structure of 3 85
3.3.4. Determining the Composition of 4 85
3.3.4.1. Thermal and Elemental Analyses of 4 85 - 87
3.3.4.2. Mass Spectrometry Supports the Mg$^{2+}$:HG$_3$ Stoichiometry of 4 87
3.3.4.3. Discussing the Core Structure of 4 88
3.4. Conclusions 88 - 89
3.5. References 89 - 90

Chapter 4. Evaluating the Solubility and In Vitro Cellular Uptake of 1 – 4 in Colorectal Carcinoma (CaCo-2) Cells

4.1. Introduction 91 - 92
4.2. Aqueous Solubility and In Vitro Uptake of 1 – 4 92
4.2.1. Determining the Water Solubility of 1 – 4 92
4.2.2. Determining the In Vitro Cell Uptake of 1 – 4 in CaCo-2 Cells 93
4.2.2.1. Determining the Uptake of 1 and 2 93
4.2.3.1. Determining the Uptake of 3 and 4 93 - 95
4.4. Conclusions 95
4.5. References 96 - 97
Chapter 5. Experimental

5.1. Materials and Methods

5.1.1. Chemicals and Equipment

5.1.2. Cell Lines and Culture Conditions

5.1.2.1. Colorectal Carcinoma (CaCo-2) Cells

5.1.2.2. Mg$^{2+}$ Uptake Assay

5.2. Syntheses

5.2.1. Synthesis of Magnesium Maltol (Mg(C$_6$H$_5$O$_3$)$_2$•$X$H$_2$O) (1)

5.2.2. Synthesis of Magnesium Ethylmaltol (Mg(C$_7$H$_7$O$_3$)$_2$•$x$H$_2$O) (2)

5.2.3. Synthesis of Magnesium Glycylglycine (Mg(C$_4$H$_7$N$_2$O$_3$)(H$_2$O)$_2$(OH)) (3) 102 – 103

5.2.4. Synthesis of Magnesium Glycylglycylglycine (Mg(C$_6$H$_{10}$N$_3$O$_4$)(H$_2$O)$_2$(OH)) (4) 103 – 104

5.3. Crystal Structure Determination of 5 and 6 (see thumb drive for CIF Files)

5.3.1. Data Collection and Refinement

5.3.1.1. Data Collection of 5 104 – 105

5.3.1.2. Structural Refinement of 5 105 – 106

5.3.2. Data Collection and Refinement of 6 106

5.3.2.1. Data Collection of 6 106

5.3.2.2. Structural Refinement of 6 106 – 107

5.4. References 107
Chapter 6. Current and Future Work with Other Biorelevant Divalent Metals (Zn$^{2+}$)

6.1. Introduction 108 – 109

6.2. Current Work 109

6.2.1. Solid- and Solution-State Characterization of Zinc Complexes 109

6.2.1.1. Zinc Diglycine (5) Preliminary Data 109

6.2.1.1.1. Synthesis of 5 109 – 110

6.2.1.1.2. Compositional Determination of 5 Via Thermal and Elemental Analyses 110 – 111

6.2.1.1.3. Structural Determination of 5 Via Fourier Transform Infrared (FT-IR) 112

6.2.1.1.4. Determining the Coordination Mode of HG$_2$ Via $^1$H and $^{13}$C NMR 113 – 115

6.2.1.1.5. Isolation of X-ray Quality Crystals of 5 115

6.2.1.1.6. Determining the Crystal Structure of 5 Via Single Crystal Analysis 115 – 119

6.2.1.2. Zinc Maltol (6) Preliminary Data 119

6.2.1.2.1. Isolation of X-ray Quality Crystals of 6 119

6.2.1.2.2. Single Crystal X-ray Structure of 6 119 – 121

6.3. Future Work 121

6.3.1. Synthesis and Characterization of a Library of Mg$^{2+}$-Amino Acid (MgAA) Complexes 121 – 122

6.3.2. Completing Mg$^{2+}$-Complex Uptake In Vitro and In Vivo 122 – 123

6.3.3. Assaying Cellular Uptake of Zn$^{2+}$- Complexes in CaCo-2 Cells 123

6.3.3.1. Assaying the In Vitro Uptake of Zn$^{2+}$ from 5 and 6 123

6.3.4. Crystallization of Synthesized Mg$^{2+}$ and Zn$^{2+}$-Complexes 123 – 124
6.4. References 124 – 126

Appendices 127

Appendix A: Publications 127

Appendix B: Crystallographic Information Files (CIFs) (see USB) 127

Curriculum Vitae 128 – 131
# List of Figures

<table>
<thead>
<tr>
<th>Chapter 1</th>
<th></th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fig 1.</strong></td>
<td>Structural Depiction of the Extracellular magnesium-ATP complex found in serum</td>
<td>2</td>
</tr>
<tr>
<td><strong>Fig 2.</strong></td>
<td>Distribution of Mg(^{2+}) in the body and the distribution of intracellular Mg(^{2+})</td>
<td>3</td>
</tr>
<tr>
<td><strong>Fig 3.</strong></td>
<td>Structures of magnesium carbonate, magnesium citrate, and magnesium citrate formed by serum Mg(^{2+}). “X” indicates the ability to form complexes of variable molar ratio.</td>
<td>4</td>
</tr>
<tr>
<td><strong>Fig 4.</strong></td>
<td>Passage of Mg(^{2+}) through TJ facilitated by claudins utilizing the PPP.</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Created with BioRender.</td>
<td></td>
</tr>
<tr>
<td><strong>Fig 5.</strong></td>
<td>Uptake of Mg(^{2+}) via the TP as facilitated by TRPM6 and TRPM7.</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Created with BioRender.</td>
<td></td>
</tr>
<tr>
<td><strong>Fig 6.</strong></td>
<td>Reabsorption of Mg(^{2+}) in different parts of the nephron before renal passing (made in BioRender)</td>
<td>9</td>
</tr>
<tr>
<td><strong>Fig 7.</strong></td>
<td>Proposed ligands utilized to generate Mg(^{2+})-chelate nutraceuticals.</td>
<td>18</td>
</tr>
</tbody>
</table>
Chapter 2

Fig 1. Structures of maltol, ethylmaltol, glycyglycine, and glycyglycylglycine 28

Fig 2. $^1$H NMR of maltol 39

Fig 3. $^1$H NMR of 1 30

Fig 4. $^1$H NMR overlay of maltol and 1 31

Fig 5. $^1$H/$^{13}$C 2D HSQC of maltol 32

Fig 6. $^1$H/$^{13}$C 2D HMBC of maltol 33

Fig 7. $^1$H/$^{13}$C 2D HSQC of 1 35

Fig 8. $^1$H/$^{13}$C 2D HMBC of 1 36

Fig 9. $^{13}$C NMR of maltol 37

Fig 10. $^{13}$C NMR overlay of maltol and 1 38

Fig 11. Predicted resonance structure of maltol upon forming 1 39

Fig 12. $^1$H NMR of ethylmaltol 40

Fig 13. $^1$H NMR of 2 41

Fig 14. $^1$H NMR overlay of ethylmaltol and 2 41

Fig 15. $^1$H/$^{13}$C 2D HSQC of ethylmaltol 42

Fig 16. $^1$H/$^{13}$C 2D HMBC of ethylmaltol 43

Fig 17. $^1$H/$^{13}$C 2D HSQC of 2 44

Fig 18. $^1$H/$^{13}$C 2D HMBC of 2 45

Fig 19. $^{13}$C NMR of ethylmaltol 46

Fig 20. $^{13}$C NMR overlay of ethylmaltol and 2 47

Fig 21. $^1$H NMR of HG2 48
Fig 22. $^1$H NMR of 3

Fig 23. $^1$H NMR overlay of HG$_2$ and 3

Fig 24. $^1$H/$^{13}$C 2D HSQC of HG$_2$

Fig 25. $^1$H/$^{13}$C 2D HMBC of HG$_2$

Fig 26. $^1$H/$^{13}$C 2D HSQC of 3

Fig 27. $^1$H/$^{13}$C 2D HMBC of 3

Fig 28. $^{13}$C NMR of HG$_2$

Fig 29. $^{13}$C NMR overlay of HG$_2$ and 3 (Full)

Fig 30. $^{13}$C NMR overlay of HG$_2$ and 3 (sp$^2$ and sp$^3$ regions zoomed)

Fig 31. $^1$H NMR of HG$_3$

Fig 32. $^1$H NMR of 4

Fig 33. $^1$H NMR overlay of HG$_3$ and 4

Fig 34. $^1$H NMR of HG$_3$ and 4 in DMSO-$d_6$

Fig 35. $^1$H/$^{13}$C 2D HSQC of HG$_3$

Fig 36. $^1$H/$^{13}$C 2D HMBC of HG$_3$

Fig 37. $^1$H/$^{13}$C 2D HSQC of 4

Fig 38. $^{13}$C NMR of HG$_3$

Fig 39. $^{13}$C NMR of 4

Fig 40. $^{13}$C NMR overlay of HG$_3$ and 4
Chapter 3

Fig 1. Possible ligand coordination methods (inner sphere, outer sphere, and mixed sphere) to Mg$^{2+}$

Fig 2. FT-IR overlay of maltol and 1

Fig 3. FT-IR overlay of ethylmaltol and 2

Fig 4. FT-IR overlay of glycyglycine (HG$_2$) and 3

Fig 5. FT-IR overlay of glycyglycylglycine and 4

Fig 6. Thermal analyses of maltol and 1 (including TGA of maltol, TGA of 1, and DSC of 1).

Fig 7. ESI-MS of 1

Fig 8. Predicted core structure of 1

Fig 9. Thermal analyses of ethylmaltol and 2 (including TGA of ethylmaltol, TGA of 2, and DSC of 2).

Fig 10. ESI-MS of 2

Fig 11. Predicted core structure of 2

Fig 12. Thermal analyses of HG$_2$ and 3 (including TGA of HG$_2$, TGA of 3, and DSC of 3).

Fig 13. ESI-MS of 3

Fig 14. Predicted core structure of 3

Fig 15. Thermal analyses of HG$_2$ and 4 (including TGA of HG$_3$, TGA of 4, and DSC of 4).

Fig 16. ESI-MS of 4

Fig 17. Predicted core structure of 4
Chapter 4

Fig 1. Cellular uptake of 1 and 2 relative to magnesium chloride (MgCl$_2$•6H$_2$O) in CaCo-2 cells plotted as linear regression of optical density at 450 nm against [Mg$^{2+}$] (including SEM, upper/lower CI, slope, and R$^2$ value). Mg$^{2+}$ concentrations were

Fig 2. Cellular uptake of 3 and 4 relative to magnesium chloride (MgCl$_2$•6H$_2$O) and magnesium bisglycinate (MgBG) in CaCo-2 cells plotted as linear regression of optical density at 450 nm against [Mg$^{2+}$] (including SEM, upper/lower CI, slope, and R$^2$ value). Mg$^{2+}$ concentrations were

Chapter 6

Fig 1. Thermal analyses of HG$_2$ and 5 (including TGA of HG$_2$, TGA of 5, and DSC of 5).

Fig 2. FT-IR spectra of HG$_2$ and 5

Fig 3. $^1$H NMR overlay of HG$_2$ and 5

Fig 4. $^{13}$C NMR overlay of HG$_2$ and 5

Fig 5. Reported possible metal coordination modes of the HG$_2$ ligand

Fig 6. ORTEP plot of the asymmetric unit of 5. Thermal ellipsoids are shown at the 50% probability level. Hydrogen atoms are omitted for clarity.
Fig 7. A portion of the packing diagram of 5 showing the hydrogen bonding scheme. Hydrogen bonds are shown as dotted lines. Lesser contributing disordered atoms have been omitted for clarity.

Fig 8. ORTEP plot of the asymmetric unit of 6. Thermal ellipsoids are shown at the 50% probability level.

Fig 9. Figure of the supported coordination mode of maltol to biorelevant metals.

Fig 10. A view of the crystal packing in 6 with hydrogen bonds shown as dotted lines.
<table>
<thead>
<tr>
<th>List of Abbreviations</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mg(C₆H₅O₃)₂(H₂O)₂ • xH₂O</td>
</tr>
<tr>
<td>2</td>
<td>Mg(C₇H₇O₃)₂(H₂O)₂ • xH₂O</td>
</tr>
<tr>
<td>3</td>
<td>Mg(C₄H₇N₂O₃)(H₂O)(OH) • xH₂O</td>
</tr>
<tr>
<td>4</td>
<td>Mg(C₆H₁₀N₂O₄)(H₂O)₂(OH) • xH₂O</td>
</tr>
<tr>
<td>5</td>
<td>Zn(C₄H₇N₂O₃)₄(H₂O)₂</td>
</tr>
<tr>
<td>6</td>
<td>Zn(C₆H₆O₃)₂Cl₂</td>
</tr>
<tr>
<td>ATCC</td>
<td>American Type Culture Collection</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
</tr>
<tr>
<td>CA</td>
<td>Citric Acid</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>Calcium</td>
</tr>
<tr>
<td>CaCo-2</td>
<td>Colorectal Carcinoma Cells</td>
</tr>
<tr>
<td>DCT</td>
<td>Distal Convoluted Tubule</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s Modified Eagle Medium</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential Scanning Calorimetry</td>
</tr>
<tr>
<td>EA</td>
<td>Elemental Analysis</td>
</tr>
<tr>
<td>ESI-MS</td>
<td>Electrospray Ionization Mass Spectrometry</td>
</tr>
<tr>
<td>EtMa</td>
<td>Ethylmaltol</td>
</tr>
<tr>
<td>FT-IR</td>
<td>Fourier Transform Infrared</td>
</tr>
<tr>
<td>G₂¹⁻</td>
<td>Glycylglycine monoanion</td>
</tr>
<tr>
<td>G₃¹⁻</td>
<td>Glycylglyclyglycine monoanion</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal tract</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>GSH</td>
<td>Glutathione</td>
</tr>
<tr>
<td>HbA1C</td>
<td>Hemoglobin A1C</td>
</tr>
<tr>
<td>HBSS</td>
<td>Hank’s Balanced Salt Solution</td>
</tr>
<tr>
<td>HG2</td>
<td>Glycylglycine</td>
</tr>
<tr>
<td>HG3</td>
<td>Glycylglycylglycine</td>
</tr>
<tr>
<td>HMBC</td>
<td>Heteronuclear Multiple Bond Correlation</td>
</tr>
<tr>
<td>HSQC</td>
<td>Heteronuclear Single Quantum Coherence</td>
</tr>
<tr>
<td>ICP</td>
<td>Inductively Coupled Plasma</td>
</tr>
<tr>
<td>K+</td>
<td>Potassium</td>
</tr>
<tr>
<td>KBr</td>
<td>Potassium Bromide</td>
</tr>
<tr>
<td>Malt</td>
<td>Maltol</td>
</tr>
<tr>
<td>Mg^{2+}</td>
<td>Magnesium</td>
</tr>
<tr>
<td>MgBG</td>
<td>Magnesium bisglycinate</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>Magnesium Chloride</td>
</tr>
<tr>
<td>MgCl₂•6H₂O</td>
<td>Magnesium Chloride Hexahydrate</td>
</tr>
<tr>
<td>MgO</td>
<td>Magnesium Oxide</td>
</tr>
<tr>
<td>MgOH</td>
<td>Magnesium Hydroxide</td>
</tr>
<tr>
<td>Na⁺</td>
<td>Sodium</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>PPP</td>
<td>Passive Paracellular Pathway</td>
</tr>
<tr>
<td>PT</td>
<td>Proximal Tubule</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic Acid</td>
</tr>
<tr>
<td>T2DM</td>
<td>Type 2 Diabetes Mellitus</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>TAL</td>
<td>Thick Ascending Loop</td>
</tr>
<tr>
<td>TGA</td>
<td>Thermogravimetric Analysis</td>
</tr>
<tr>
<td>TJ</td>
<td>Tight Junctions</td>
</tr>
<tr>
<td>TP</td>
<td>Active Transcellular Pathway</td>
</tr>
<tr>
<td>TRPM 6</td>
<td>Transient Receptor Potential Melastatin 6</td>
</tr>
<tr>
<td>TRPM 7</td>
<td>Transient Receptor Potential Melastatin 7</td>
</tr>
<tr>
<td>Zn$^{2+}$</td>
<td>Zinc</td>
</tr>
</tbody>
</table>
### Crystal Structures of Compounds Synthesized by the Author

<table>
<thead>
<tr>
<th>Code use in Thesis</th>
<th>Chemical Formula</th>
<th>Space Group</th>
<th>CCDC Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>[Zn$_2$(HG$_2$)$_2$(H$_2$O)$_2$]·5.32H$_2$O</td>
<td>$P-1$</td>
<td>2101440</td>
</tr>
<tr>
<td>6</td>
<td>Zn(Malt)$_2$Cl$_2$</td>
<td>$P2_1/n$</td>
<td>pending</td>
</tr>
</tbody>
</table>

*Crystal Data was refined by Dr. William W. Brennessel at the University of Rochester, Rochester, NY, USA.*
Chapter 1. Introduction

1.1 Introduction to Magnesium (Mg$^{2+}$)

Magnesium (Mg$^{2+}$) is a grayish metal,\(^1\) First recognized as an element as early as 1755, and isolated in 1808 by Sir Humphry Davy.\(^2,3\) Mg$^{2+}$ has atomic number 12 and an atomic weight of 24.3 g/mol.\(^1\) Other physical and chemical properties include a melting point of 649°C, a density of 1.738 g/cm\(^3\), and an electronegativity of 1.56.\(^4\) Mg$^{2+}$ is found predominantly in the earth’s crust as complex minerals, with the most biologically available source located in the hydrosphere.\(^4,5\) Mg$^{2+}$ exhibits an extensive hydration sphere, with the hydrated radius being approximately 400 times larger than the dehydrated radius.\(^4\) When complexed and in solution, Mg$^{2+}$ acts as an electropositive di-cation (Mg$^{2+}$), making it ideal for the coordination of Lewis bases.\(^6\)

1.2 Biological Significance of Magnesium

Evidence supporting the biorelevance of Mg$^{2+}$ is abundant and clear\(^4,7,8\) Mg$^{2+}$ is the fourth most common element in the human body and the second most common intracellular cation, after potassium.\(^4,8\) Mg$^{2+}$ is implicated for some 80% of metabolic function and acts as a cofactor for more than 300 enzymes and 800 proteins.\(^4,8,9\) Most notably, Mg$^{2+}$ is required for oxidative phosphorylation, glycolysis, and energy production, where it acts as a cofactor for the production of adenosine triphosphate (ATP) – Figure 1.\(^4,8,10\)
**Figure 1.** Structural depiction of the extracellular magnesium-ATP complex found in blood.\(^4\)

\(\text{Mg}^{2+}\) is also required for the synthesis of deoxyribonucleic acid (DNA),\(^{11,12}\) ribonucleic acid (RNA),\(^{13,14}\) and the antioxidant glutathione (GSH).\(^{15}\) \(\text{Mg}^{2+}\) also facilitates the active transport of calcium (Ca\(^{2+}\)) and potassium (K\(^{+}\)) ions across cell membranes, demonstrating its importance in functions such as muscle contraction and nerve impulse conduction.\(^{16–18}\) Given the impact that \(\text{Mg}^{2+}\) has on calcium transport, it is not surprising that it is also required to maintain calcium homeostasis between bone and blood.\(^{19}\)

### 1.2.1 Distribution of Mg\(^{2+}\) in the Body

The average adult body contains ~25 g (1000 mmol) of \(\text{Mg}^{2+}\).\(^{20,21}\) However, the distribution of \(\text{Mg}^{2+}\) in the human body is vastly disproportionate, with approximately 99% of \(\text{Mg}^{2+}\) existing intracellularly (90% stored in bone and muscle tissue and < 1% distributed in blood).\(^{22–24}\) Of all intracellular magnesium, only 2 – 3% is considered free and the concentration of this magnesium is 0.5 – 0.6 mmol/L.\(^{21}\) Approximately 50 – 60% of all intracellular \(\text{Mg}^{2+}\) is stored exclusively in bone,\(^4\) with \(\text{Mg}^{2+}\) concentrations in muscle and soft tissues accounting for 27% and 19%, respectively.\(^{22}\) Given high concentrations of \(\text{Mg}^{2+}\) in bone, there is a delicate homeostasis maintained between serum \(\text{Mg}^{2+}\) and available \(\text{Mg}^{2+}\) residing in outer layers of bone.\(^{25}\)
Only ~1% of total body Mg$^{2+}$ is found in serum, at concentrations of 0.7 – 1.1 mmol/L (Figure 2). Of the Mg$^{2+}$ in serum, approximately 55% exists in the free or ionized state and accounts for the greatest level of biological activity, most notably for Ca$^{2+}$ transport, and as a Ca$^{2+}$ antagonist (e.g. decreased free serum [Mg$^{2+}$] causes increased [Ca$^{2+}$], which results in increased contractility of smooth muscles (i.e. cardiac)). Approximately 30% of serum magnesium is found bound to proteins, with albumin representing the majority. The remaining 15% of serum magnesium is found complexed to biological anions such as ATP (i.e. phosphate (PO$_4^{3-}$); see Figure 1), carbonate (CO$_3^{2-}$), or citrate (C$_6$H$_5$O$_7^{3-}$) (Figure 3).
Figure 3. Structures of magnesium carbonate, magnesium citrate, and magnesium citrate formed by serum Mg$^{2+}$. “X” indicates the ability to form complexes of variable stoichiometric ratio.

1.2.2 Absorption of Mg$^{2+}$ in the Gastrointestinal (GI) Tract

There is a delicate maintenance of Mg$^{2+}$ homeostasis, with contributions from bone (e.g. exchange of bone and serum Mg$^{2+}$),$^4$ the kidneys (e.g. renal excretion),$^{29,30}$ and the intestines (e.g. absorption).$^4$ Mg$^{2+}$ uptake in the body occurs in the lower GI, with independent sections (e.g. duodenum, jejunum, ileum, etc.) exhibiting unequal levels of absorption,$^4,31–39$ with the majority of uptake occurring in the ileum (~56%).
Table 1. Mg\(^{2+}\) uptake from sections of the GI.

<table>
<thead>
<tr>
<th>GI Tract Segment</th>
<th>pH</th>
<th>Contribution of Magnesium Uptake (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duodenum</td>
<td>5.9–6.8</td>
<td>11</td>
<td>4, 31 - 39</td>
</tr>
<tr>
<td>Colon</td>
<td>5.7–7.2</td>
<td>11</td>
<td>4, 31 - 39</td>
</tr>
<tr>
<td>Jejunum</td>
<td>5.9–6.8</td>
<td>22</td>
<td>4, 31 - 39</td>
</tr>
<tr>
<td>Ileum</td>
<td>7.3–7.6</td>
<td>56</td>
<td>4, 31 - 39</td>
</tr>
</tbody>
</table>

The pH of the GI varies per section with an overall range of 5.7 – 7.6 (ΔpH = 1.9).\(^{32}\) Absorption of Mg\(^{2+}\) in the GI has subsequently been correlated to pH, as has been illustrated by the work of Thongon et al.\(^{31,40}\) The increased uptake of Mg\(^{2+}\) in the ileum is largely attributed to a more acidic luminal pH.\(^{33}\)

1.2.3 Introduction to the Mg\(^{2+}\) Uptake Pathways

There are two pathways that contribute to the uptake of Mg\(^{2+}\) once absorbed in the lower GI.: the saturable, active Transcellular Pathway (TP) and the non-saturable Passive Paracellular Pathway (PPP).\(^{4,7,8}\) The TP is responsible for the uptake of small magnesium loads (~125 – 250 mg) while the PPP is responsible for the uptake of larger magnesium loads (≥ ~250 mg).\(^{4}\)
1.2.3.1 Mechanism of the PPP

The PPP is responsible for the uptake of 80 – 90% of total Mg$^{2+}$ in the body (accounting for Mg$^{2+}$ loads of ≥ 250mgs), although the exact mechanism is still unknown. Absorption via the PPP is gradient driven electrochemically and also by solvent drag of Mg$^{2+}$ between intestinal enterocytes. This effect is a result of high luminal concentrations of Mg$^{2+}$ (1 – 5 mmol/L) and the lumen-positive transepithelial voltage (~ 5 mV). The PPP depends upon the passage of ions such as Mg$^{2+}$ through tight junctions (TJ), which is complicated by the extensive hydration sphere exhibited by Mg$^{2+}$. For passage through TJ, a series of proteins called claudins are employed by the PPP (Figure 4).

Figure 4. Passage of Mg$^{2+}$ through TJ facilitated by claudins utilizing the PPP. Created with BioRender.
In general, claudins are responsible for stripping Mg\(^{2+}\) of its extensive hydration sphere to allow for passage through TJ. Currently, there are 26 identifiable claudins and subsets of these claudins serve differing barrier functions.\(^{43,44}\) Claudins -1, -3, -4, -5, -8, -11, -14, and -19 have been identified in an extensive review by Amasheh et al. as barrier builders, claudins -2 and -10 as permeability mediators, and claudins -7, -12, and -15 as ambiguous.\(^{43}\) An extensive review by de Baaij et al.\(^{8}\) further highlights claudin function, indicating that claudins -16 and -19 are most responsible for Mg\(^{2+}\) uptake, as was further illustrated by Konrad et al.\(^{45}\) upon Claudin -16 and -19 mutation resulting in renal wasting of Mg\(^{2+}\) as well as renal failure.\(^{45}\) The true function of claudin-16 is currently debated, but it is believed that it acts to mediate the epithelial voltage gradient by controlling the Na\(^+\) back-leak.\(^{8,46–48}\) While less extensively explored, claudin-19 has been further implicated in Mg\(^{2+}\) uptake.\(^{49}\)

1.2.3.2 Mechanism of the TP

The TP is responsible for smaller Mg\(^{2+}\) loads (~125 – 250 mg).\(^{4}\) Uptake in the TP is controlled by the presence of transient receptor potential channel melastatins – predominantly members 6 (TRPM6) and 7 (TRPM7) (Figure 5).\(^{8}\) TRPM7 is ubiquitously expressed, however, TRPM6 has been shown to be expressed predominantly in the lower intestine of murine tissue, although not confirmed in humans.\(^{8,50}\) Expression of TRPM6 and TRPM7 is limited to enterocytes – namely within the luminal membrane.\(^{8}\) The exact mechanism of transport has yet to be elucidated, but could be gradient based as is the case with the PPP.\(^{8}\)
1.2.4 Renal Filtering of Mg$^{2+}$

As part of homeostatic maintenance, excess Mg$^{2+}$ must be excreted renally.$^{4,7,8}$ The glomeruli is responsible for the filtering of approximately 2400 mg/day of Mg$^{2+}$. Of the 2400mg of Mg$^{2+}$ filtered, only about 100 mg/day are excreted in urine – the other 90 – 95% of Mg$^{2+}$ is reabsorbed along the nephron.$^{7}$ As is the case with Mg$^{2+}$ uptake along the GI, Mg$^{2+}$ reabsorption is also unequal along the nephron. The nephron can be broken into three main sections: the proximal tubule (PT), the thick ascending limb (TAL), and the distal convoluted tubule (DCT).
Figure 6. Reabsorption of Mg$^{2+}$ in different parts of the nephron before renal passing (made in BioRender).

As shown (Figure 6), Henle’s Loop is responsible for the majority of Mg$^{2+}$ reabsorption accounting for up to 70% - with predominant focus on the TAL. The TAL consists of a cortical segment, where magnesium uptake occurs, and a medullary segment, which is not responsible for any magnesium reabsorption. Uptake in the TAL is predominantly paracellular.

In newborns, the PT constitutes ~70% of overall magnesium reabsorption. In adults, the PT accounts for the second highest level of magnesium reabsorption – constituting approximately 10 – 25% of the overall total. Absorption in the PT is gradient based and paracellular in nature at high Mg$^{2+}$ concentrations.
The DCT accounts for the smallest amount of Mg\(^{2+}\) reabsorption at only ~ 10% of Mg\(^{2+}\). Unlike the PT and the TAL, Mg\(^{2+}\) reabsorption in the DCT is predominantly transcellular and requires the presence of the TRPM6 pathway for Mg\(^{2+}\) to enter the cell.

Considering the DCT to be the end of the Mg\(^{2+}\) reabsorption pathway, the kidney is then responsible for excreting Mg\(^{2+}\). Renal excretion of magnesium varies in cases of increased Mg\(^{2+}\) intake or in renal failure when the kidney will excrete Mg\(^{2+}\) fractionally to maintain homeostasis as required until onset hypomagnesaemia.

1.3 Determining Mg\(^{2+}\) Status in Humans

It is estimated that some 50 – 60% of the U.S. population is afflicted by chronically low Mg\(^{2+}\) levels (hypomagnesaemia), however, these estimates may be even lower than the real number of individuals impacted. Determining Mg\(^{2+}\) status in humans is complicated by the previously mentioned unequal distribution of Mg\(^{2+}\) in the body. Analyzing serum Mg\(^{2+}\) remains the most used method for determining overall Mg\(^{2+}\) status. However, given the disparity in Mg\(^{2+}\) distribution, serum analysis is not sufficient to determine true body Mg\(^{2+}\) values. There are other methods of body Mg\(^{2+}\) determination including isotopic analysis as well urine analysis and retention testing, but these, too, are not without shortcomings.

1.3.1. Urinary Analysis and “Loading Test” to Evaluate Mg\(^{2+}\) Status

Urinary analysis provides information mostly beneficial in determining the degree of renal wasting of Mg\(^{2+}\) or inadequate Mg\(^{2+}\), but does not indicate actual Mg\(^{2+}\) quantity. Mg\(^{2+}\) loading, or an Mg\(^{2+}\) retention test, provides a means of determining whether or not an individual is in a
hypomagnesaemic state by evaluating serum Mg\(^{2+}\) after an oral administration of Mg\(^{2+}\), but may also be evaluated by examining bone Mg\(^{2+}\) content.\(^4\) Increases in serum Mg\(^{2+}\) after acute oral dosing are attributed to adequate absorption of Mg\(^{2+}\) by the GI.\(^4,22,57\) Furthermore, evaluation of Mg\(^{2+}\) status is correlated to bone levels of Mg\(^{2+}\), and can quantify Mg\(^{2+}\).\(^4,57\) As pointed out by Fawcett et al., however, this method of analysis is not fully standardized.\(^27\)

**1.3.2. Evaluating Mg\(^{2+}\) Status Isotopically**

Mg\(^{2+}\) status may be evaluated isotopically.\(^4\) There are three non-radioactive Mg\(^{2+}\) isotopes (\(^{24}\)Mg, \(^{25}\)Mg, and \(^{26}\)Mg) which occur in relative abundance of 78.7%, 10.1%, and 11.2%, respectively.\(^58\) Although isotopic Mg\(^{2+}\) allows for a direct quantification method of Mg\(^{2+}\), analysis is complicated by short half-life times of the respective isotopes.\(^4,22\) The fourth Mg\(^{2+}\) isotope, \(^{28}\)Mg, is radioactive and decays by emission of high-energy beta or gamma particles.\(^4\) Given the shortcomings of Mg\(^{2+}\) isotopes, they are typically restricted to research use only and not clinical.\(^4\)

**1.4. Hypomagnesaemia**

Mg\(^{2+}\) deficiency, or hypomagnesaemia (defined as < 0.75 mmol/L in serum),\(^55\) is not a novel concept. In fact, hypomagnesaemia has been documented as far back as the early 20\(^{th}\) century.\(^59-62\) Hypomagnesaemia is a micronutrient deficiency that drastically impacts the developed world. In fact, it is estimated to impact some 50 – 60% of the American population alone,\(^63-65\) but as outlined in section 1.3, given complications in evaluating Mg\(^{2+}\) status, this number is probably substantially higher. There are many factors that contribute to hypomagnesaemia, such as increased renal wasting due to disease states (e.g. alcoholism, Type 2 Diabetes Mellitus (T2DM), etc.) or poor absorption in the GI.\(^23\) However, the prevalence of hypomagnesaemia in developed countries is attributed predominantly to poor diet.\(^23\) Inadequate
intake is further attributed to changes in diet that favor fast foods and processed foods as opposed to more organic, micro-nutrient rich foods. While rather widespread, hypomagnesaemia often goes undiagnosed, with chronic latent hypomagnesaemia further correlated to other chronic disease states.

1.4.1 Hypomagnesaemia in Chronic Disease

Hypomagnesaemia has been identified as an impacting factor in several chronic disease states. Specifically, hypomagnesaemia has been implicated in cardiac disorders (e.g. arrythmia), bone disorders (e.g. osteoporosis), hypertension, headaches/seizures, and T2DM.

1.4.1.1 Mg²⁺ in Heart Health

Given the impact that Mg²⁺ has on nerve impulse conduction, it is no surprise that presence of the cation definitively impacts heart health. It has been shown that a deficiency of Mg²⁺ increases the presence of sodium and calcium in the heart; increased concentrations of these ions contribute to vasoconstriction, arterial spasm, and subsequently may cause cardiac arrest. Furthermore, Mg²⁺ supplementation has been shown to decrease blood pressure and may be used intravenously to reduce the number of experienced arrythmias.

1.4.1.2. Mg²⁺ in Osteoporosis

Mg²⁺ has been shown to impact osteoporosis as discussed briefly in an extensive review on the clinical impact of hypomagnesaemia by de Baaij et al. In general, studies indicate that osteoporosis may be attributed to hypomagnesaemia, as it may be treated via oral Mg²⁺ supplementation and bone loss may be observed upon Mg²⁺ reduction. Most studies are conducted on postmenopausal women, but others have been conducted on the elderly and have
indicated, that although marginally, Mg\(^{2+}\) supplementation increases Mg\(^{2+}\) loading, thus indicating substantial Mg\(^{2+}\) uptake.\(^8^1\)

**1.4.1.3. Mg\(^{2+}\) in T2DM**

In a study conducted by Rodriguez-Moran *et al.*, Mg\(^{2+}\) supplementation was shown to positively impact patients with T2DM.\(^8^2\) As reported, when treated with Mg\(^{2+}\) in the form of MgCl\(_2\), treated subjects exhibited a decrease in overall fasting glucose as well as Hemoglobin A\(_1c\) (HbA\(_1c\)) and other diabetic metrics.\(^8^2\) Thus indicating that Mg\(^{2+}\) supplementation has a positive impact on T2DM.

**1.5. Mg\(^{2+}\) Supplementation to Treat Hypomagnesaemia**

**1.5.1. Introduction to Mg\(^{2+}\) Supplements and Understanding Oxides and Salts**

Provided the biological impact of Mg\(^{2+}\) on general health, methods of palliating hypomagnesaemia have been utilized, with both acute and chronic Mg\(^{2+}\) supplementation at the fore.\(^4,8^2–8^4\) Mg\(^{2+}\) supplementation has been studied in pregnant women,\(^8^5\) athletes,\(^8^6\) and patients with osteoporosis (given the delicate homeostasis maintained between Mg\(^{2+}\) and Ca\(^{2+}\)).\(^5^9,8^0,8^7\) To date, the most commonly used Mg\(^{2+}\) supplements are the oxides/hydroxides of magnesium (MgO/MgOH) and magnesium salts (e.g. magnesium chloride (MgCl\(_2\) \(\cdot\) xH\(_2\)O) and magnesium citrate). However, several other supplements are utilized (*Table 2*).
Table 2. Commonly used Mg\(^{2+}\) supplements and form, acid/base chemistry, solubility, and %Mg composition.

<table>
<thead>
<tr>
<th>Form (Oxide/Salt/Chelate)</th>
<th>Acid/Base Chemistry</th>
<th>Solubility (g/100 mL H(_2)O)</th>
<th>MW g/mol (%Mg)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium Oxide</td>
<td>Alkaline</td>
<td>0.010</td>
<td>40.30 (60.3)</td>
<td>88</td>
</tr>
<tr>
<td>Magnesium Citrate</td>
<td>Acidic (pKa(_1) = 3.13)</td>
<td>20</td>
<td>214.41 (11.3)</td>
<td>1</td>
</tr>
<tr>
<td>Magnesium Chloride</td>
<td>Neutral</td>
<td>54.0</td>
<td>95.21 (25.5)</td>
<td>89</td>
</tr>
<tr>
<td>Magnesium Sulfate</td>
<td>Acidic (pKa(_1) = 3.0; pKa(_2) = 1.99)</td>
<td>35.7</td>
<td>120.37 (20.1)</td>
<td>88</td>
</tr>
<tr>
<td>Magnesium Orotate</td>
<td>Acidic (pKa(_1) = 2.83)</td>
<td>Slightly Soluble</td>
<td>334.48 (7.2)</td>
<td>90</td>
</tr>
<tr>
<td>Magnesium Taurate</td>
<td>Acidic (pKa = 1.50)</td>
<td>Slightly Soluble</td>
<td>272.57 (8.9)</td>
<td>91</td>
</tr>
<tr>
<td>Magnesium Aspartate</td>
<td>Acidic (pKa(_1) = 1.88; pKa(_3) = 3.65)</td>
<td>4.0</td>
<td>288.49 (8.5)</td>
<td>92</td>
</tr>
<tr>
<td>Magnesium Threonate</td>
<td>Acidic (pKa(_1) = 3.4)</td>
<td>Soluble</td>
<td>294.50 (8.3)</td>
<td>93</td>
</tr>
<tr>
<td>Magnesium Malate</td>
<td>Acidic (pKa(_1) = 3.46; pKa(_2) = 5.10)</td>
<td>Slightly Soluble</td>
<td>156.37 (15.5)</td>
<td>94,95</td>
</tr>
<tr>
<td>Magnesium Hydroxide</td>
<td>Alkaline</td>
<td>0.00069</td>
<td>58.32 (41.7)</td>
<td>88</td>
</tr>
<tr>
<td>Magnesium Carbonate</td>
<td>Weakly Alkaline</td>
<td>0.18</td>
<td>84.31 (28.8)</td>
<td>1</td>
</tr>
</tbody>
</table>

MgO is most frequently used given its high %Mg composition, whereas MgCl\(_2\) and magnesium citrate are utilized given biocompatibility and substantial aqueous solubility. There are, however, shortcomings to the oxide and salt forms of Mg\(^{2+}\) supplements: MgO exhibits low aqueous solubility relative to other Mg\(^{2+}\) supplements at only 0.010 g/100 mL,\(^{88}\) and the laxative effects of Mg\(^{2+}\) salts are well defined.\(^4\) Other Mg\(^{2+}\) supplements, such as magnesium malate\(^{95}\) and
magnesium threonate\textsuperscript{93} represent a third supplemental form: Mg\textsuperscript{2+}-chelates, which provide a means of successfully navigating the shortcomings of current oxide and salt Mg\textsuperscript{2+} supplements.

\textbf{1.5.2. Introduction to Mg\textsuperscript{2+}-Chelates}

Mg\textsuperscript{2+}-chelates are typically synthesized utilizing biorelevant chelate ligands such as ligands implicated in biological processes (formic acid/formate)\textsuperscript{96} or amino acids,\textsuperscript{97} to aid in overall complex aqueous solubility as well as stability given coordination of a higher degree (mono, bi, tridentate, \textit{etc}.). Increased solubility is desirable given a correlation between complex solubility and bioavailability.\textsuperscript{98–100} While promising, Mg\textsuperscript{2+}-chelates are often poorly characterized which impacts metrics of supplemental use such as appropriate dosing and modes of action. In fact, very few Mg\textsuperscript{2+}-chelates are fully characterized.

\textbf{1.5.2.1. A Brief Discussion of Characterized Biorelevant Mg\textsuperscript{2+} Complexes}

Few Mg\textsuperscript{2+}-chelate complexes have been fully characterized, outside of select few outlined in the works of Schmidbauer \textit{et al.}\textsuperscript{97,101–105} and others outlined in an extensive review by Case \textit{et al.} (Table 3).\textsuperscript{106} Early studies of Mg\textsuperscript{2+}-chelate complexes, such as those conducted by Martell \textit{et al.} on short-chain glycine peptides, were limited to titrimetric analysis, which was used to determine complex speciation,\textsuperscript{107–109} but provided tentative assignments and could not confirm the assumed coordination mode of ligand. While crystallization would provide definitive information as to complex composition and assumed coordination mode, crystallization of Mg\textsuperscript{2+}-chelates is often complicated by the extensive hydration sphere observed for Mg\textsuperscript{2+},\textsuperscript{4} and the inherent disorder imparted by such waters. While Mg\textsuperscript{2+}-chelates are a promising means of Mg\textsuperscript{2+} supplementation, other means of characterization must be employed to definitively confirm species coordination.
mode and composition, as to generate fully-characterized, pharmaceutical-grade Mg\textsuperscript{2+}
supplements – or nutraceuticals.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>pKa\textsubscript{1}</th>
<th>pKa\textsubscript{2}</th>
<th>pKa\textsubscript{3}</th>
<th>Lewis Bases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orotic Acid</td>
<td>2.83</td>
<td>---</td>
<td>---</td>
<td>1</td>
</tr>
<tr>
<td>Mandelic Acid</td>
<td>3.41</td>
<td>---</td>
<td>---</td>
<td>2</td>
</tr>
<tr>
<td>Anthranilic Acid</td>
<td>2.14</td>
<td>4.85</td>
<td>---</td>
<td>2</td>
</tr>
<tr>
<td>Formic Acid</td>
<td>3.75</td>
<td>---</td>
<td>---</td>
<td>1</td>
</tr>
<tr>
<td>Glycine</td>
<td>2.34</td>
<td>9.60</td>
<td>---</td>
<td>2</td>
</tr>
<tr>
<td>Malic Acid</td>
<td>3.40</td>
<td>5.11\textsuperscript{*}</td>
<td>---</td>
<td>3</td>
</tr>
<tr>
<td>Maleic Acid</td>
<td>1.83</td>
<td>---</td>
<td>---</td>
<td>2</td>
</tr>
<tr>
<td>Aspartic Acid</td>
<td>1.88</td>
<td>9.60</td>
<td>---</td>
<td>3</td>
</tr>
<tr>
<td>Glutamic Acid</td>
<td>2.19</td>
<td>9.67</td>
<td>---</td>
<td>3</td>
</tr>
</tbody>
</table>
Table 3. Characterized and reported Mg$^{2+}$ supplements outlined in the review by Case et al.$^{106}$

1.5.3. Nutraceuticals

The term nutraceutical was first coined by Dr. Stephen DeFelice in 1989 as a portmanteau of the words “nutrition” and “pharmaceutical”. For a time, there was no regulatory operative definition of a nutraceutical, and thus there was a discrepancy between what constituted a nutraceutical or a functional food. As of 2003, Dr. Ekta K. Kalra, proposed a means of redefining nutraceuticals as based upon the Dietary Supplement and Health Education Act (DSHEA) of 1994, which outlined criteria to constitute a dietary supplement. As such, Dr. Kalra proposed the operative definition of a nutraceutical such that it must not only supplement diet, but aid in the treatment of disease. Facing scrutiny from the FDA as to what constitutes justifiable claims and safety of nutraceuticals, further adaptation of the operative definition of nutraceutical is required, and as such the term should be amended to include full complex characterization.

1.6. Summary

The aim of this research was to synthesize Mg$^{2+}$ complexes utilizing biorelevant ligands, with the intent of outlining methods that may be used to confirm ligand coordination to Mg$^{2+}$ and hydrated state, thus allowing for full characterization in both the solution- and solid-state, as to
generate novel nutraceuticals for the treatment of hypomagnesaemia. Utilizing the biorelevant ligands maltol, ethylmaltol, glycyglycine (HG$_2$) and glycyglyclyglycine (HG$_3$) shown in Figure 7.

![Maltol](image1.png) ![Ethylmaltol](image2.png)

**Maltol:** 3-hydroxy-2-methyl-4H-pyran-4-one  **Ethylmaltol:** 2-ethyl-3-hydroxy-4H-pyran-4-one

![Glycyglycine](image3.png) ![Glycyglyclyglycine](image4.png)

**Glycyglycine (HG$_2$):** (2-Aminoacetamido)acetic acid  **Glycyglyclyglycine (HG$_3$):** 2-[(2-[(2-aminoacetyl)amino]acetyl]amino]acetic acid

**Figure 7.** Proposed ligands utilized to generate Mg$^{2+}$-chelate nutraceuticals.

Maltol (IUPAC: 3-hydroxy-2-methyl-4H-pyran-4-one), HG$_2$ (IUPAC – (2-Aminoacetamido)acetic acid), and HG$_3$ (2-[(2-[(2-aminoacetyl]amino]acetyl]amino]acetic acid) were selected given their natural occurrence and isolation,$^{112,113}$ and ethylmaltol (IUPAC: 2-ethyl-3-hydroxy-4H-pyran-4-one) was selected given its generally regarded as safe (GRAS) status.$^{114}$ All ligands were further promising given access to multidentate coordination (bi, tri, and tetradentate) coordination modes. Additionally, all three ligands exhibit substantial aqueous solubility.$^{115–117}$

Chapter 2 focuses predominantly on the characterization of complexes 1 – 4 in the solution state via 1D and 2D $^1$H/$^{13}$C NMR and how Mg$^{2+}$ coordination manifests as an observable. Chapter
3 focuses on determining the hydrated state of complexes 1 – 4 and determining overall complex composition. Chapter 4 evaluates the solubility of 1 – 4 and the resulting observed cellular uptake as it relates to %Mg$^{2+}$ composition. Given the positive results exhibited by 1 – 4, Chapter 6 focuses on the application of determined synthetic methods and utilized ligands to other biorelevant metals such as zinc (Zn$^{2+}$).

1.7. References


(74) Piuri, G.; Zocchi, M.; Porta, M. Della; Ficara, V.; Manoni, M.; Zuccotti, G. V.; Pinotti, L.;


(91) McCarty, M. F. Magnesium Taurate and Other Mineral Taurates. 5,582,839, 1996.


Chapter 2. The Effect of Magnesium Coordination on the $^1$H/$^13$C Nuclear Magnetic Resonance of Magnesium-Coordinate Ligands

All work in this chapter was completed by the author. Work in this chapter was published in:


2.1. Introduction

As more fully described in chapter 1, Mg$^{2+}$ readily coordinates with hard Lewis bases as exemplified by the monodentate magnesium chelates of formic acid,$^1$ orotic acid,$^2$ maleic acid,$^3$ the bidentate magnesium chelates of mandelic acid$^4$ and malic acid$^5$ and the tridentate magnesium chelate of citric acid.$^6,7$ It is generally accepted that ligand chelation to the divalent Mg$^{2+}$ cation is often characterized by an observable upfield shift in the $^1$H nuclear magnetic resonance (NMR) spectrum, or a change in signal resolution, of the proton signals adjacent to the Lewis bases of the ligand, due to the electropositive character of the metal.

Additionally, Mg$^{2+}$ coordination is manifested in $^{13}$C NMR either by shifting, most often downfield, or via a change in carbon signal intensity/resolution.$^8,9$ These shift/intensity changes are exhibited by the carbons adjacent to the coordinate ligands, moieties that are implicated in Mg$^{2+}$ coordination. Occurrences of this type are notably exemplified by magnesium complexes of ofloxacin and levofloxacin.$^8$
While 1-Dimensional $^1$H/$^{13}$C NMR is satisfactory to determine Mg$^{2+}$-complex formation, it is not enough to fully assign all proton and carbon identities, and as such is inconclusive in fully confirming the ligand coordination mode. Therefore, 2-Dimensional $^1$H/$^{13}$C NMR in the form of heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond correlation (HMBC) were employed herein to investigate coordination modes. To this end, a combination of both 2D NMR techniques confirmed the identities of all proton and carbon signals thus ascertaining the ligand coordination mode. Given the impact that concentration may have on the

**Figure 1.** Structures of the magnesium coordinate ligands maltol, ethylmaltol, glycylglycine (HG$_2$) and glycylglycylglycine (HG$_3$), including IUPAC naming conventions, used in this study.
shifting of proton and carbon signals, it is important to note here that all samples were compared and analysed at equimolar concentration.

Coordination of maltol (Figure 1a) and ethylmaltol (Figure 1b) to Mg$^{2+}$ is predicted to occur in a bidentate fashion utilizing the ketone moiety and the deprotonated terminal alcohol, whereas the coordination of HG$_2$ (Figure 1c) and HG$_3$ (Figure 1d) is expected to be tridentate and tetrade ntate, respectively. Thus, we set out to assay chelate ligands across a spectrum of possible coordinative modes with Magnesium, with each ligand (a)-(d) discussed below.

2.2. NMR Characterization of Maltol and 1

2.2.1. $^1$H NMR Characterization of Maltol and 1

Figure 2. The full $^1$H NMR of maltol with insets indicating the enhanced aromatic region (left) and the enhanced aliphatic region (right) to display shifting and integration. NMR was conducted in D$_2$O.
The $^1$H NMR of uncoordinated commercial maltol exhibits (Figure 2) three proton signals: two aromatic signals exhibiting doublet splitting at 8.00/7.99 ppm and 6.51/6.50 ppm, and one aliphatic singlet at 2.38 ppm, attributed to $H_2$, $H_1$, and $H_3$, respectively (Figure 2). The observed integration for these signals was 1:1:3, as predicted.

Upon coordination of maltol to Mg$^{2+}$, three proton signals attributed to the maltol ligand were observed with the same splitting pattern: two aromatic doublets at 7.97/7.96 ppm and 6.50/6.49 ppm, and an aliphatic singlet at 2.35 ppm (Figure 3). A small but observable upfield shift of all proton signals was observed however for 1. Upfield shifting was 0.03 ppm for $H_2$, 0.01-0.02 ppm for $H_1$, and 0.03 ppm for $H_3$, respectively (Figure 4). Additionally, the integration of maltol and 1 is conserved. Upfield shifting of the observed proton signals is consistent with the formation of 1 and the conservation of the integration is consistent with no change in the maltol ligand – suggesting no other reactions independent of the formation of 1.

**Figure 3.** $^1$H NMR of 1. NMR was conducted in D$_2$O.
NMR analysis of 1 also exhibited a quartet with peaks between 2.51 – 2.72 ppm which is attributed to magnesium citrate. Presence of magnesium citrate was confirmed via ¹H NMR and is a result of utilizing citric acid to solubilize the magnesium oxide starting material. The integration of the peaks attributed to magnesium citrate indicate a percent composition of 7.2% magnesium citrate (Figure 3 and Figure 4).

Figure 4. ¹H NMR overlay of maltol (top) and 1 (bottom) at equimolar concentration indicating the observed proton upfield signal shifting. NMR was conducted in D₂O.

2.2.2. Confirmation of 1 Proton and Carbon Signal Determination Utilizing Heteronuclear Single Quantum Coherence (HSQC) and Heteronuclear Multiple Bond Correlation (HMBC)

Both 2D ¹H/¹³C HSQC and HMBC NMR were utilized to confirm the proton and carbon assignments for maltol and 2. As shown in Figure 5, the HSQC of maltol exhibited only one
coherence point between the only saturated carbon and its only aliphatic proton. This is consistent with the predicted $^1$H/$^{13}$C spectrum of maltol.

![Diagram of maltol]

**Figure 5.** Full $^1$H/$^{13}$C HSQC NMR of maltol showing a zoomed (inset middle) image of only one observed coherence point. NMR was conducted in D$_2$O.

Along with the denoted integration of three observed for this proton on the $^1$H NMR spectrum of maltol, this confirms the signal at 2.39 ppm as the H$_3$ proton and confirms the identity as the terminal methyl group of maltol. The carbon assignment at 13.95 ppm is also confirmed as C$_6$ given that this is the only carbon within a single bond length of this proton signal.
The HMBC of maltol, as shown in Figure 6, was more elaborate, indicating the presence of seven distinct signals – the number of observed signals was consistent with the predicted number of observed signals and allowed for the complete assignment of all carbon signals.

As illustrated, the H₂ proton exhibits three correlative points: 113.40 ppm, 154.50 ppm, and 175.20 ppm. The signal at 113.40 ppm is assigned to the C₂ carbon signal given that this is the only carbon that H₂ would interact with across multiple bonds and there is no interaction of this
carbon with the H\textsubscript{1} proton, which is the result of this carbon being only one bond length away from the H\textsubscript{1} proton. The signal at 154.50 ppm is attributed to the C\textsubscript{4} carbon assignment given the distance of this interaction is three bonds. While C\textsubscript{3} and C\textsubscript{4} are not substantially different, there is no correlation between the H\textsubscript{2} proton and C\textsubscript{3} for the same reason that there is no interaction between H\textsubscript{1} and the assigned C\textsubscript{2}. Lastly, the signal at 175.20 ppm is attributed to the C\textsubscript{1} assignment. This assignment is not only consistent with the generally predicted region for ketone carbons but is also supported by the secondary observed interaction of the C\textsubscript{1} carbon with the H\textsubscript{1} proton assignment, which is only two bonds away. The interaction of both the H\textsubscript{1} and H\textsubscript{2} protons with this carbon confirms the C\textsubscript{1} assignment.

The H\textsubscript{3} proton signal exhibited correlation to two carbon signals: 154.50 ppm and 142.00 ppm. Analysis of these points confirms the previously established C\textsubscript{4} assignment at 154.50 ppm and supports the assignment of C\textsubscript{5} at 142.00 ppm. These assignments arise from differences in signal intensity – given that signal at 142.00 ppm has a weaker signal intensity, it is predicted to be farther way from the H\textsubscript{3} proton, and thus is assigned as C\textsubscript{5}. Process of elimination subsequently confirms the signal at 156.00 ppm is attributed to the C\textsubscript{3} carbon assignment.

There were observed changes to the respective HSQC (Figure 7) and HMBC (Figure 8) spectra of maltol upon coordination to Mg\textsuperscript{2+}. Most notably, both spectra exhibited the presence of peaks attributed to magnesium citrate, which is the result of citric acid utilization during complex synthesis. Furthermore, while the HSQC observed signal remained the same with only one observed signal, attributed to the interaction between the H\textsubscript{3} proton and the C\textsubscript{6} carbon, the ratio of observed interactions on the HMBC spectra was substantially different. The ratio of observed signals for the H\textsubscript{1}, H\textsubscript{2}, and H\textsubscript{3} protons (previously observed as 3:3:2) had changed to an observed 2:3:1 ratio. With the proton assignments remaining the same, this change in the observed ratio of
interaction points was attributed to changes in correlative carbon environments and was subsequently probed utilizing $^{13}$C NMR.

**Figure 7.** Full $^1$H/$^{13}$C HSQC NMR of 1 with observed signals zoomed (inset bottom left). NMR was conducted in D$_2$O.
2.2.3. Characterization of 1 Via $^{13}$C NMR

Missing proton/carbon interactions as observed on the HMBC spectrum of 1 prompted $^{13}$C NMR analysis of 1 relative to maltol. Upon comparison, the respective spectra were noticeably different.
The $^{13}$C NMR spectrum of maltol (Figure 9) exhibited six carbon signals with only one signal in the region attributed to saturated carbons (-CH$_3$) and the other five signals within the region attributed to C=C carbons and C=O carbons. These observations are consistent with the predicted $^{13}$C NMR spectrum of maltol as based upon the structure. The carbon assignments for these signals were made based upon the $^1$H/$^{13}$C HSQC and HMBC analyses of maltol as previously mentioned (see section 4.2.4.1).

As predicted, the $^{13}$C NMR spectrum of 1 was substantially different comparatively. As was the case with the $^1$H NMR of 1, so too did the $^{13}$C NMR exhibit signals attributed to magnesium citrate (see Figure 10).
Most striking, however, is the reduction in signal intensity of carbons C₁ and C₂, as well as the complete disappearance of the C₅ carbon – the ketone carbon, the carbon nearest the ketone, and the carbon attributed to the terminal alcohol. These observations are consistent with the maltol ligand coordinating to Mg²⁺ about the ketone and the deprotonated terminal alcohol. The decrease in signal intensity of these carbons may be easily explained by the deprotonation of the terminal alcohol and the coordination of the substantially electropositive Mg²⁺ causing a change in the dipole moment of these moieties.

Figure 10. $^{13}$C NMR overlay of maltol and 1. * Indicates peaks attributed to magnesium citrate. NMR was conducted in D₂O.
Additionally, resonance is expected upon Mg$^{2+}$ coordination (Figure 11) – subsequently, a change in the D1 scan time to a wider window allowing for a longer analysis of relaxation might give rise to the presence of these peaks. These observations support the coordination of maltol to Mg$^{2+}$ to be bidentate in nature about the ketone and the terminal alcohol.

2.3. NMR Characterization of Ethylmaltol and 2

2.3.1. $^1$H NMR Characterization of Ethylmaltol and 2

As predicted, the $^1$H NMR of ethylmaltol (Figure 12) exhibited four proton signals with splitting like that of maltol, which is expected given only the addition of a -CH$_2$ moiety to the terminal methyl (-CH$_3$) group. Signals are as follows: two aromatic doublets at 8.02/8.01 ppm and 6.50/6.49 ppm, an aliphatic quartet with signals at 2.77/2.76/2.73/2.72 ppm, and an aliphatic triplet with signals at 1.22/1.20/1.18 ppm. These signals are attributed to H$_1$, H$_2$, H$_3$, and H$_4$, respectively. The observed integration of these signals is 1:1:2:3.

Like that of 1, upon coordination to Mg$^{2+}$, the proton signals attributed to the ethylmaltol ligand exhibit a consistent splitting pattern: two aromatic doublets at 7.95/7.94 ppm and 6.45/6.43 ppm, an aliphatic quartet with signals at 2.77/2.75/2.73/2.71 ppm, and an aliphatic triplet with

![Figure 11. Predicted resonance of maltol upon coordination to Mg$^{2+}$.](image-url)
signals at 1.20/1.18/1.16 ppm. Additionally, these signals are unilaterally upfield shifted and the integration is conserved (Figure 13).

**Figure 12.** Full 1H NMR of the ethylmaltol ligand with insets indicating the enhanced aromatic region (left) and the enhanced aliphatic region (right) to display chemical shifting and integration. NMR was conducted in D$_2$O.

Upfield shifting was 0.01ppm, 0.01ppm, 0.01ppm, and 0.01ppm for H$_1$ – H$_4$, respectively. As was noted of 1, conserved integration and upfield shifting are consistent with ethylmaltol coordination to Mg$^{2+}$ devoid of the formation of new signals that would indicate secondary side reactions. The $^1$H NMR signals are attributed to H$_1$, H$_2$, H$_3$, and H$_4$, respectively. $^1$H NMR analysis of 2 also exhibited a quartet with peaks between 2.51 – 2.72 ppm, which is attributed to magnesium citrate (Figure 14). Presence of magnesium citrate was confirmed via $^1$H NMR and is the result of
utilizing citric acid to solubilize the magnesium oxide starting material. The integration of the peaks attributed to magnesium citrate indicate a percent composition of 6.1%.

Figure 13. Full $^1$H NMR of 2 with insets indicating the enhanced aromatic region (left) and the enhanced aliphatic region (right) to displaying chemical shifting and integration. NMR was conducted in D$_2$O.

Figure 14. $^1$H NMR Overlay of ethylmaltol (top) and 2 (bottom) indicating the observed shifting of ethylmaltol proton signals upon Mg$^{2+}$ coordination. NMR was conducted in D$_2$O.
2.3.2. Confirmation of 2 Proton and Carbon Signal Determination Utilizing HSQC and HMBC

As for 2, 2D $^1$H/$^{13}$C HSQC and HMBC was again employed to confirm all proton and carbon assignments for the ethylmaltol chelate analog. Considering the addition of only a methyl spacer to the side chain, resulting in two saturated carbons and an additional aliphatic proton, two signals were now expected on the HSQC spectrum.

![Diagram of HSQC NMR spectrum](image)

**Figure 15.** Full $^1$H/$^{13}$C HSQC NMR of ethylmaltol with signals zoomed for clarity (inset bottom left). NMR was conducted in D$_2$O.
As predicted, two signals are observed (Figure 15). The assignments of the carbon signals are relatively easy given that each proton has only one carbon within a single bond distance. As such the carbon signal at 9.99 ppm is assigned as C<sub>7</sub>, and the carbon signal at 21.52 ppm is assigned as C<sub>6</sub>.

The assignment of all carbon signals via HMBC was more elaborate, however, as a total of ten signals were observed in a ratio of 4:3:2:1 for H<sub>1</sub> – H<sub>4</sub>, respectively (Figure 16).

**Figure 16.** Full <sup>1</sup>H/<sup>13</sup>C HMBC NMR of ethylmaltol with the aromatic (inset bottom right) and aliphatic (inset top right) regions highlighted for clarity. NMR was conducted in D<sub>2</sub>O.
Employing the same logic as before, all carbons were assigned with C\textsubscript{1} at 178.60 ppm, and C\textsubscript{3}, C\textsubscript{4}, C\textsubscript{5}, and C\textsubscript{2} at 158.10 ppm, 154.90 ppm, 141.40 ppm, and 111.20 ppm, respectively. Given the determined coordination mode of maltol, and the structural similarity to ethylmaltol, particular interest was given to the C\textsubscript{1}, C\textsubscript{2}, and C\textsubscript{5} carbon assignments.

The HSQC and HMBC of Ethylmaltol were compared to 2 in the same fashion as the maltol and Mg\textsuperscript{2+}-maltol pair. Similarly, there was minimal difference between the HSQC of ethylmaltol and Mg\textsuperscript{2+}-ethylmaltol (Figure 17). Like the HSQC of ethylmaltol, the HSQC of Mg\textsuperscript{2+}-ethylmaltol

Figure 17. Full \textsuperscript{1}H/\textsuperscript{13}C HSQC NMR of 2 with signals zoomed (inset bottom left) for clarity. NMR was conducted in D\textsubscript{2}O.
also exhibited two signals, suggesting no change to these protons or carbons, as expected given no presence of Lewis bases for coordinative participation.

Figure 18. Full $^1$H/$^{13}$C HMBC NMR of 2 showing both the aromatic (inset bottom right) and aliphatic (inset top right) regions for clarity. NMR was conducted in D$_2$O.
There was, however, a substantial difference in the respective HMBC spectra of ethylmaltol and 2 (Figure 18); most notably, as was the case with 1, the disappearance of the interaction between H_1 and C_5, and a substantial decrease in the C_1 and C_2 carbon signal intensity. Given no change in proton signal assignment, this could only be the result of changes in carbon environment, and as such, $^{13}$C NMR was utilized to confirm the coordination mode of ethylmaltol.

### 2.3.3. Characterization of 2 Via $^{13}$C NMR

The $^{13}$C NMR spectra of ethylmaltol was predicted to exhibit seven carbon signals. As observed, the $^{13}$C NMR spectra of ethylmaltol exhibited seven carbons: two sp$^3$-hybridized saturated carbons and five carbons within the region attributed to C=O carbons and C=C carbons (Figure 19 and Figure 20).

**Figure 19.** Full $^{13}$C NMR of ethylmaltol. NMR was conducted in D$_2$O.
The carbon environments of interest were the C₁, C₂, and C₅ environments noted at 175.46 ppm, 113.28 ppm, and 141.19 ppm, respectively. These carbons were of interest given the predicted bidentate coordination mode about the terminal alcohol and ketone, as was observed for 1.

Like that of the ¹³C NMR of 1, the ¹³C NMR of 2 exhibited a change in the expected carbon signals (Figure 20). Most notable, is a substantial decrease in the signal intensity of C₁ and C₂, as well as a complete disappearance of C₅ signal. These observations are consistent with coordination about the ketone and the terminal acid, as was similarly deduced as the coordination mode of maltol to Mg²⁺.

Figure 20. ¹³C NMR overlay of ethylmaltol (top) and 2 (bottom). *Indicates peaks attributed to magnesium citrate. NMR was conducted in D₂O.
2.4. NMR Characterization of HG$_2$ and 3

2.4.1. $^1$H NMR Characterization of HG$_2$ and 3

The $^1$H NMR spectrum of HG$_2$ (Figure 21) exhibited two proton signals with a combined integration of four in a ratio of 2:2; the observed signals were a sharp singlet at 3.84ppm ($H_1$) and a doublet centered around 3.80ppm ($H_2$).

![Figure 21. $^1$H NMR of HG$_2$ exhibiting two proton signals in the aliphatic region. Aliphatic region is enlarged for clarity (inset right). NMR was conducted in D$_2$O.](image)

Additionally, the $^1$H NMR of 3 exhibited a combined integration of four. These observations were consistent with the hypothesized observations. Like the $^1$H NMR spectrum of HG$_2$, spectral analysis 3 exhibited two proton signals with a combined integration of four (Figure 22). Unlike HG$_2$, the observed proton signals of 2 were both singlets, and each singlet exhibited a substantial upfield shift: $H_1 = 3.38$ppm ($\Delta$ppm = 0.46) and $H_2 = 3.76$ ($\Delta$ppm = 0.04) (Figure 22.
and Figure 23). Furthermore, spectral analysis of 2 revealed a quartet between 2.50 – 2.70 ppm attributed to magnesium citrate via $^1$H NMR, which is a result of citric acid utilization during synthesis.

**Figure 22.** Full $^1$H NMR of 3 exhibiting two singlets in the aliphatic region. Aliphatic region is enlarged for clarity (inset left). NMR was conducted in D$_2$O. * indicates signals attributed to magnesium citrate.
2.4.2. Confirmation of 3 Proton and Carbon Signal Determination Utilizing HSQC and HMBC

The HSQC spectrum of HG₂ (Figure 24) indicated two correlation points: one point attributed to H₁ and one point attributed to H₂. The point attributed to H₁ corresponded to the carbon signal at 40.7ppm, confirming this to be C₁, and the point attributed to H₂ corresponded to the carbon signal at 43.4ppm, confirming this carbon to be C₃.
The HMBC of HG₂ (Figure 25) showed three correspondence points of ratio 1:2. The proton signal attributed to H₂ exhibited two correspondence points. Given that H₁ is within range of only one carbon, this indicates that the observed correspondence point for H₁ at 167.1 ppm is attributed to C₂. Additionally, H₂ shows two correspondence points. Given that the C₂ carbon signal corresponds to two protons, this confirms the assignment of the C₂ proton given its proximity to both protons, and the remaining carbon signal at 176.5 ppm is confirmed as C₄ given that it is out of range of the H₁ proton (Figure 25).

**Figure 24.** Full 2D $^{1}$H/$^{13}$C HSQC of HG₂ with signals zoomed for clarity. NMR was conducted in D₂O.
HSQC analysis of 3 (Figure 26) indicated two correspondence points, like that of the HG₂ ligand (Figure 23). Unlike the HSQC of HG₂, both correspondence points are observed after 40 ppm (43.2 ppm and 43.7 ppm, respectively). The signal at 43.2 ppm is attributed to the C₃ carbon and the signal at 43.7 ppm is attributed to the C₁ carbon. The substantial downfield shifting of the C₁ carbon supports coordination of the HG₂ ligand via the amine – this is consistent with the proton shift of H₁ observed in the proton NMR spectrum of 3 relative to HG₂.
In contrast to the HMBC of the free HG$_2$ ligand, the ratio of correspondence points observed for 3 is switched (2:1) (Figure 27). Given the observations made for the HMBC of HG$_2$, this indicates the more substantial upfield shift attributed to the H$_1$ proton during $^1$H NMR analysis is consistent with terminal amine participation in coordination. Additionally, the HMBC confirms the H$_2$ assignment and implicates the terminal carboxylic acid moiety, which was previously supported by infrared analysis.

Figure 26. Full $^1$H/$^{13}$C HSQC NMR of 3. Signals are zoomed for clarity (inset bottom left). NMR was conducted in D$_2$O.
2.4.3 Characterization of 3 Via $^{13}$C NMR

$^{13}$C NMR was conducted with the aim of confirming the conclusions derived from infrared and $^1$H NMR analyses. The $^{13}$C NMR of 3 was conducted relative to HG$_2$. Spectral analysis HG$_2$ (Figure 28) revealed four (4) carbon signals: two signals between the range of 40 – 50ppm attributed to the sp$^3$-hybridized R-CH$_2$-R carbon moieties (C$_1$ – 40.7ppm, C$_3$ – 43.4ppm) and two signals between 160 – 180ppm attributed to the sp$^2$-hybridized R-CO-R moieties (C$_2$ – 167.1ppm, C$_4$ – 176.4ppm).

Figure 27. Full $^1$H/$^{13}$C HMBC NMR of 3. Signals are zoomed for clarity (see inset). NMR was conducted in D$_2$O.
$^{13}$C NMR analysis of 3 exhibited four (4) carbon signals in the same regions as HG$_2$ (Figure 29). In contrast to the $^{13}$C spectrum of HG$_2$, the signal separation was substantially diminished, a result of the downfield shift of the C$_1$ ($\Delta$ppm = 3) and C$_2$ ($\Delta$ppm = 8) signals to 43.2ppm and 175ppm, respectively. These observations are consistent with those reported by Drevenšek et al.$^8$ and Chang et al.$^{10}$ during their studies of magnesium testosterone and magnesium ofloxacin/levofloxacin, respectively, and are consistent with magnesium coordination in regions adjacent to these moieties. Additionally, in agreement with $^1$H NMR analysis, $^{13}$C NMR analysis of 3 exhibited four signals attributed to magnesium citrate (Figures 29 and Figure 30) and no new ligand-based carbon signals were observed, further supporting the formation of 3 as a 1:1 complex.

Figure 28. Full $^{13}$C NMR of HG$_2$. NMR was conducted in D$_2$O.
Figure 29. Full $^{13}$C NMR Overlay of HG$_2$ and 3. NMR was conducted in D$_2$O. * Indicates peaks attributed to magnesium citrate.

Figure 30. Full $^{13}$C NMR Overlay of HG$_2$ (top) and 3 (bottom) with ketone carbon (left) and saturated carbon (right) regions zoomed for clarity. NMR was conducted in D$_2$O. * Indicates peaks attributed to magnesium citrate.
2.5. NMR Characterization of HG₃ and 4

2.5.1. ¹H NMR Characterization of HG₃ and 4

The HG₃ ligand exhibited three aliphatic singlets at 4.04, 3.91 and 3.79 ppm. These signals were attributed to H₂, H₁, and H₃, respectively. The integration of the observed proton signals was in a ratio of 2:2:2. These observations were consistent with those predicted (Figure 31).

![Figure 31](image)

Figure 31. Full ¹H NMR of glycyclglycyglycine (HG₃). NMR was conducted in 6:1 H₂O:D₂O.

The ¹H NMR of 4 exhibited three signals attributed to HG₃ (Figure 32). The splitting of these signals was the same as HG₃, with three aliphatic singlets observed. However, unlike HG₃ upon coordination to Mg²⁺, there was an observable upfield shift of all observed signals attributed to the HG₃ ligand. 4 exhibited three proton signals at 3.97, 3.75, 3.41, respectively, indicating a
confirmed upfield shift of 0.5 ppm for H₁, 0.1 ppm for H₂, and 0.04 ppm for H₃. Furthermore, there was no observed change to the integration of the signals attributed to HG₃ consistent with no further reactions after the formation of 4.

Figure 32. Full ¹H NMR of 4 with inset indicating the enhanced aliphatic region (left) to display chemical shifting and integration. NMR was conducted in D₂O.
Figure 33. $^1$H NMR overlay of HG$_3$ and 4 indicating the upfield shift of HG$_3$ proton signals as well as the conserved integration. NMR was conducted in D$_2$O.

Given the more substantial upfield shift of the H$_1$ proton suggests coordination to the terminal amine (Figure 33). However, provided the established hard acid-base chemistry of magnesium, it is more likely for Mg$^{2+}$ to coordinate to the terminal acid. As such, the participation of the terminal acid moiety in Mg$^{2+}$ coordination was probed.

The $^1$H NMR of both HG$_3$ and 4 was conducted in deuterated dimethyl sulfoxide (DMSO-$d_6$) to evaluate the participation of the terminal carboxylic acid in Mg$^{2+}$ coordination. The $^1$H NMR of HG$_3$ in DMSO exhibited three aliphatic proton signals: two singlets and one double with signals at 4.00, 3.85, and 3.75/3.74 ppm attributed to H$_2$, H$_1$, and H$_3$, respectively. Given the tendency of
polar aprotic solvents such as DMSO to participate in hydrogen bonding, there is an observed splitting of the H₃ signal nearest the terminal carboxylic acid, which is consistent with DMSO hydrogen bonding to the protonated terminal acid thus resulting in an unequal distribution of electron density about the acid that manifests itself in the observed doublet splitting of the H₃ proton.

When analyzing the ¹H NMR of 4 in DMSO-d₆ (Figure 34), no splitting of the H₃ proton was observed, indicating the deprotonation of the terminal acid. Additionally, this indicates that the now deprotonated G₃⁻ ligand has coordinated to Mg²⁺ about the terminal acid moiety, whose electropositive character stabilizes the electron distribution resulting in no observed proton signal splitting.

Figure 34. ¹H NMR of HG₃ (left) and 4 (right) in DMSO-d₆ at equimolar concentration.
$^1$H NMR analysis of 4 is consistent with the deprotonated $\text{G}_3^-$ coordinating via both the terminal amine and the deprotonated terminal acid. It is, however, unlikely for the deprotonated $\text{G}_3^-$ to form a 10-membered ring structure and as such it is likely that either the backbone ketone moieties or the backbone amides are also participating in coordination to $\text{Mg}^{2+}$ and provide insight into the formation of an entropically-favored tetradentate coordination mode. This is supported by the work of Martell conducted in the 1950s and 60s, who theorized a tetradentate coordination mode based upon titrimetric analyses.\textsuperscript{11,12} Further $^{13}$C NMR studies were conducted to evaluate the participation of the backbone ketone moieties.

### 2.5.2. Confirmation of 4 Proton and Carbon Signal Determination Utilizing HSQC and HMBC

The HSQC spectrum of $\text{HG}_3$ (Figure 35) exhibited only three correspondence points, with each proton exhibiting only one correspondence to each carbon. Given the assignments of each proton from the 1D $^1$H NMR, the subsequent assignment of each $sp^3$-carbon was simplified. The carbon exhibiting correspondence to the $H_1$ proton was assigned as $C_1$, the carbon corresponding to the $H_2$ proton signal was assigned as $C_3$, and the carbon corresponding to the $H_3$ proton was assigned as $C_5$ with the carbons exhibiting chemical shifts of 40.48, 42.35, and 43.18 ppm.
The 2D $^1\text{H}/^{13}\text{C}$ HMBC NMR of HG$_3$ (Figure 36) exhibited five correspondence points in a ratio of 2:1:2 for H$_2$, H$_1$, and H$_3$, respectively. H$_1$ was predicted to exhibit correspondence with only one ketone carbon given the presence of only one such carbon within a 2 – 3 bond distance. The carbon correspondence point for H$_1$ is observed at 167.77 ppm and is assigned as C$_2$. This assignment is further supported by a secondary correspondence of this carbon to H$_2$, which is the only other proton within distance of this carbon. The second correspondence point observed for

**Figure 35.** Full 2D $^1\text{H}/^{13}\text{C}$ HSQC NMR of HG$_3$ with zoomed sp$^3$-hybridized carbons highlighted in inset (bottom left).
H₂ is at 170.79 ppm and is attributed to the C₄ assignment. This assignment is corroborated by a secondary correspondence exhibited by this carbon to the H₃ proton – the only other proton within distance of this carbon. The final correspondence point for H₃ is observed at 176.36 ppm and is attributed to C₆. Given the predicted correspondence of the C₆ carbon to only the H₃ within plausible distance, these observations are consistent.

**Figure 36.** Full 2D \(^1\)H/\(^{13}\)C HMBC NMR of HG₃ with the zoomed sp\(^2\)-hybridized region observed as the inset (bottom left).
Upon Mg$^{2+}$ coordination, there were observed changes to the correspondence points attributed to the HG$_3$ ligand of both the HSQC and HMBC spectra of 4.

![Figure 37. Full 2D $^1$H/$^{13}$C HSQC NMR of 4 with zoomed sp$^3$-hybridized carbons highlighted in inset (bottom left).](image)

While only three correspondence points were observed like that of the HSQC of HG$_3$, the correspondence points are no longer observed as a triangle, but more so as a straight line (Figure 37). Given the predicted coordination mode, the proton signals observed at 3.76, 3.53, and 3.21 ppm were assigned as H$_2$, H$_3$, and H$_1$, respectively. The carbon signals observed at 42.36, 43.14, and 43.51 ppm, were assigned C$_3$, C$_5$, and C$_1$ as based upon their respective corresponding proton signals. As such, C$_1$ observes the most substantial shift in signal ($\Delta = 2.9$ ppm). This observation supports the coordination of the terminal amine moiety.
2.5.3. Characterization of 4 Via $^{13}$C NMR

Mg$^{2+}$ coordination to oxygen-containing moieties (e.g. ketones and carboxylic acids) has been shown to result in a substantial impact to the observed carbon signals of the coordinate ligand (i.e. signal intensity reduction or changes in signal resolution), as was the case for the magnesium complexes of the steroids ofloxacin and levofloxacin.\(^8\) As such, $^{13}$C NMR was utilized to determine the participation of the backbone ketones of HG\(_3\) in Mg$^{2+}$ coordination.

The $^{13}$C NMR of HG\(_3\) (Figure 38) exhibited six carbon signals: three sp\(^2\)-hybridized carbon signals at 176.48, 170.84, and 167.79 ppm, as well as three sp\(^3\)-hybridized carbon signals at 43.18, 42.35, and 40.48 ppm. These carbon signals are attributed to C\(_2\), C\(_4\), C\(_6\), C\(_5\), C\(_3\), and C\(_1\), respectively, and are consistent with the predicted six carbon signals expected. The sp\(^3\)-hybridized carbons are attributed to saturated backbone carbons. The sp\(^2\)-hybridized carbons are attributed to the terminal carboxylic acid and the backbone ketones, which are predicted to exhibit substantial change upon coordination to Mg\(^{2+}\).

Comparatively, the $^{13}$C NMR of 4 (Figure 39) also exhibited six observed carbon signals and showed a conserved three sp\(^2\)-hybridized signals and three sp\(^3\)-hybridized signals. Notably, the $^{13}$C NMR spectrum of 4 exhibited a substantial shift of the C\(_6\) carbon assigned as the terminal carboxylic acid which is consistent with the observations made for the $^1$H NMR and provides further support for the participation of the terminal carboxylic acid in Mg$^{2+}$ coordination. Additionally, there is a substantial shift of the C\(_1\) carbon assigned as the carbon nearest the terminal amine, which is also consistent with the observations made for the $^1$H NMR and provides further support for the coordinative participation of this moiety (Figure 40).
**Figure 38.** Full $^{13}$C NMR of HG$_3$ indicating three sp$^2$-hybridized carbons (inset left) and three sp$^3$-hybridized carbons (inset right). NMR was conducted in D$_2$O.

**Figure 39.** Full $^{13}$C NMR of 4 exhibiting three sp$^2$-hybridized carbon signals (inset left) and three sp$^3$-hybridized carbon signals (inset right). NMR conducted in D$_2$O.
Unexpectedly, there is little to no change of the C$_2$ and C$_4$ carbons, assigned to the backbone ketones, which is consistent with no participation of these moieties in metal binding. Considering the unlikelihood of the formation of a ten-membered ring structure, these observations provide further support for a tetralentate coordination mode of the G$_3$-ligand utilizing the backbone amine moieties.

Lastly, the conservation of six carbon signals provides further support for the absence of secondary reactions resulting in the formation of undesired products. This is again consistent with the observations made for the $^1$H NMR of 4.

**Figure 40.** Full $^{13}$C NMR overlay of HG$_3$ and 4 exhibiting three sp$^2$-hybridized carbon signals (inset left) and three sp$^3$-hybridized carbon signals (inset right). NMR conducted in D$_2$O.
2.6. Conclusion

A combination of 1D and 2D $^1$H/$^{13}$C NMR provides ample insight into assumed coordinative mode of a ligand when coordinating Mg$^{2+}$. In general, ligand coordination to Mg$^{2+}$ will result in an observable upfield shift of the protons nearest coordination sights with a diminishing impact further away from the sight of coordination. Noticeably, amines are much more susceptible to this observed shifting, relative to carboxylic acids, as was the case with 3 and 4. This may be attributed to the less electron dense amine’s inability to accommodate the largely electropositive character of Mg$^{2+}$ like the more electron dense acid is able to do. Moreover, a substantial shift of the adjacent amine protons is attributed to Mg$^{2+}$ coordination about this moiety. Furthermore, $^1$H NMR analysis in DMSO may be utilized to confirm the deprotonation of the terminal acid and provide insight to the availability of this moiety for coordination, as was observed for 4.

$^{13}$C NMR may be even more telling in terms of determining ligand coordination mode given the impact that Mg$^{2+}$ coordination can have on ketones. Consistent with previous findings, Mg$^{2+}$ coordination to ketone moieties will result in a substantial decrease in signal intensity, as was the case with 1 and 2.

While Mg$^{2+}$ coordinative manifestations are plentiful, determining all proton and carbon identities is essential and 2D HSQC/HMBC are ideal for this purpose. The combination all types of NMR analysis provides a reliable means of determining ligand coordination mode to Mg$^{2+}$. 
2.7. References


Chapter 3. Determining Complex Composition and Varying Degrees of Mg$^{2+}$-Complex Hydration Utilizing Solid-State Analyses and Potentiometry

All work in this chapter was completed by the author unless otherwise indicated.

3.1 Introduction

While 1D and 2D NMR analysis is sufficient to determine the coordination mode of the utilized ligands to Mg$^{2+}$ in solution, it provides no means of elucidating the chemical structure of the isolated Mg$^{2+}$-complexes in the solid state. Solid-state analysis of the Mg$^{2+}$-complexes was employed not only to provide support for the coordination modes of the utilized ligands as predicted from NMR analysis in the solution state, but also to determine the overall composition of all isolated Mg$^{2+}$ complexes. Solid-state analysis of Mg$^{2+}$ complexes is, however, substantially complicated by the innate chemical properties of Mg$^{2+}$ itself.

Mg$^{2+}$ will bind hydration water tighter than other biorelevant cations such as calcium (Ca$^{2+}$), sodium (Na$^{+}$), and potassium (K$^{+}$). As such, the hydrated radius of Mg$^{2+}$ is approximately 400 times greater than the dehydrated Mg$^{2+}$ radius, whereas the hydrated radius of Ca$^{2+}$ is approximately 25 times greater than that of its fully dehydrated radius. A similar hydrated/dehydrated radius ratio is observed for Na$^{+}$, while the hydrated radius of K$^{+}$ is only ~4 times greater than its dehydrated radius.
The ability of Mg$^{2+}$ to readily hydrate so extensively provides insight into the modes of Mg$^{2+}$ uptake and the requirement of claudins to facilitate transport through tight junctions (TJ), as well as the different types of Mg$^{2+}$ coordination modes in solution (Figure 1). The tendency of Mg$^{2+}$ to bind water so tightly further complicates Mg$^{2+}$ dehydration and gives rise to Mg$^{2+}$(L)$_x$•xH$_2$O species, such as the magnesium citrate 14-hydrate complex extensively described in the solid-state by Mansour et al. Given the complications in formulation brought about by Mg$^{2+}$ hydration, we assayed for the presence of water across complexes 1–4 via Fourier Transform Infrared (FT-IR) and thermal analyses (thermogravimetric analysis (TGA)/differential scanning calorimetry (DSC)) to qualify and quantify complex hydration. Compositional determinations supported by these findings were further corroborated utilizing elemental analysis (EA). In the case of 1:1 Mg$^{2+}$:monoanion complexes (e.g., 3 and 4), compositional findings were supplemented with potentiometric analyses to confirm required complex charge balance via hydroxide anion (OH$^-$), which cannot be singularly distinguished from waters of hydration via the other methods noted above.

**Figure 3.** Possible Mg$^{2+}$-ligand coordination modes observed in solution.
3.2. FT-IR Supports Ligand Coordination Modes and the Presence of Water

3.2.1. FT-IR Analysis of 1

The FT-IR spectrum of 1 was analyzed relative to the FT-IR spectrum of maltol. The FT-IR spectrum of maltol was analyzed between 1500 – 1700 cm\(^{-1}\) and 3000 – 3500 cm\(^{-1}\) with peaks of interest at 1621, 1655, and 3260 cm\(^{-1}\) – these peaks were attributed to the ketone and the terminal alcohol, respectively. The FT-IR spectrum exhibited the almost complete disappearance of the band attributed to the terminal alcohol and was observed at 3264 cm\(^{-1}\) and the ketone peaks were observed at 1617 and 1655 cm\(^{-1}\), respectively (Figure 2).

![FT-IR spectrum of maltol and 1](image)

**Figure 4.** Full FT-IR spectrum of maltol (blue)/1 (red) with the ketone fingerprint region enlarged (inset) and signal assignments provided (table at right).

<table>
<thead>
<tr>
<th>Complex</th>
<th>IR Frequency (cm(^{-1}))</th>
<th>Assignment</th>
<th>Change (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maltol</td>
<td>3260</td>
<td>v(OH), C-OH</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>1655</td>
<td>v(C=O)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>1021</td>
<td>v(C=O)</td>
<td>–</td>
</tr>
<tr>
<td>1</td>
<td>3435</td>
<td>v(OH), H(_2)O</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>3264</td>
<td>v(OH), C-OH</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1655</td>
<td>v(C=O)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>1617</td>
<td>v(C=O)</td>
<td>4</td>
</tr>
</tbody>
</table>

The observed change in the frequency of the signals attributed to the ketone moiety of 1 are higher in energy relative to maltol,\(^4\) which is consistent with magnesium coordination about the
ketone, and a shift to slightly higher energy is consistent with magnesium coordination as reported by Nara et al.\textsuperscript{5} However, this is different than the signal shifts observed for other divalent metal-maltol complexes such as bismaltolato zinc (II),\textsuperscript{6} which exhibited shifts to lower energy. This may be the result of zinc being less electropositive in character than magnesium, thus resulting in less ionic character upon coordination, but may also be attributed to differences in ionic radii of the two metals.

Strikingly, an intense, broad signal was observed at 3435 cm\textsuperscript{-1} (Figure 2) and qualitatively confirms the presence of coordinated water, which is predicted from synthetic conditions and agrees with the tendency of Mg\textsuperscript{2+} to take on water. Given the 2:1 stoichiometry of maltol to Mg\textsuperscript{2+}, charge balance is inherently provided by the ligand, mitigating the requirement of hydroxide anion to zero.

### 3.2.2. FT-IR Analysis of 2

FT-IR analysis of the 2 complex was conducted relative to ethylmaltol (Figure 3) in a method comparable to the analysis of the maltol/1 pair. The FT-IR spectrum of 2 (Figure 3) was much more difficult to analyze given a lack of resolution and signal intensity in the fingerprint region for the ketone, and thus was inconclusive in providing further support to the predicted coordination mode of the ligand. However, there was an intense broad signal strikingly observed at 3447 cm\textsuperscript{-1}, which was again attributed to the presence of coordinated water. Given similarities in reaction stoichiometry and ethylmaltol acting as a monoanion, it is again predicted that there is no requirement for hydroxide to provide charge balance, and as such provides further support that the attribution of this signal to coordinated water is correct.
3.2.3. FT-IR Analysis of 3

The FT-IR of 3 relative to HG\textsubscript{2} exhibited a substantial change in the frequency region that corresponds specifically to the −OH stretching mode attributed to the carboxylic acid of the HG\textsubscript{2} ligand at 3287 cm\textsuperscript{-1}. HG\textsubscript{2} exhibited a sharp stretching band in this region that is not observed for 3, providing support for the deprotonation of the acid (pKa = 3.14). Additionally, HG\textsubscript{2} exhibits a broad signal at 2055 cm\textsuperscript{-1}, which is attributed to the terminal amine (−NH\textsubscript{3}\textsuperscript{+}), a signal not observed for 3. This indicates that both the terminal amine and the terminal acid are in coordination. The FT-IR spectra of HG\textsubscript{2} and 3 are shown in Figure 4.

Figure 5. Full FT-IR spectrum of the ethylmaltol (blue)/2 (red) pair with the fingerprint region for ketones enlarged (inset) and signal assignments provided (table at right).
Given a predicted tetradeutate coordination mode of the HG\(_3\) ligand upon deprotonation of the terminal acid, particular interest was paid to the region between 3000 – 3500 cm\(^{-1}\) as well as the region between 900 – 1200 cm\(^{-1}\). Most notably, the FT-IR spectrum of HG\(_3\) (Figure 5) exhibited a sharp, intense signal at 3297 cm\(^{-1}\) and a broader, less intense signal at 2635 cm\(^{-1}\), which were attributed to the terminal carboxylic acid. After reacting with Mg\(^{2+}\), the signals at 3297 cm\(^{-1}\) and 2635 cm\(^{-1}\) were no longer observed and provided support for the predicted deprotonation of the terminal acid. Evidence of the coordination of the anionic G\(_3^-\) ligand to Mg\(^{2+}\) via the deprotonated acid is provided by the observable shift of the C-O signal for the unreacted HG\(_3\) ligand observed at 991 cm\(^{-1}\) and the same signal of 4 shifted (Δcm\(^{-1}\) = 37) to lower energy observed at 1028 cm\(^{-1}\) (Figure 5).

**Figure 6.** FT-IR spectra of HG\(_2\) (blue) and 3 (red) conducted in KBr.

### 3.2.4. FT-IR Analysis of 4

![FT-IR spectra](image)

<table>
<thead>
<tr>
<th>Complex</th>
<th>IR Frequency (cm(^{-1}))</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>HG(_2)</td>
<td>3297, 2055</td>
<td>v(OH), C-O</td>
</tr>
<tr>
<td></td>
<td></td>
<td>v((\tilde{\nu}_{\text{NH}_2}))</td>
</tr>
<tr>
<td>3</td>
<td>3360</td>
<td>v(OH), H(_2)O</td>
</tr>
<tr>
<td></td>
<td></td>
<td>v((\tilde{\nu}_{\text{NH}_2}))</td>
</tr>
</tbody>
</table>
Also, of interest was the intense, broad signal at \( \sim 3050 \text{ cm}^{-1} \) observed for 4 which was again attributed to coordinated water. Provided the monoanionic \( \text{G}_3^- \) ligand was reacted with \( \text{Mg}^{2+} \) in a 1:1 stoichiometry, it was predicted that the isolated solid achieved charge balance via a hydroxy anion, which was subsequently confirmed via potentiometric analysis.

### 3.3. Determining the Composition of Complexes 1 – 4

#### 3.3.1 Determining the Composition of 1

##### 3.3.1.1. Thermal and Elemental Analyses of 1

Maltol exhibited a continuous percent weight loss onset at \( \sim 70^\circ \text{C} \) to 200°C and stopped decreasing in percent weight at approximately 5% (Figure 7), thus suggesting decomposition of
maltol between 160 and 200°C, which is consistent with the known melting point of maltol at 160°C.\textsuperscript{7}

TGA analysis of 1 exhibited a similar decomposition trend differing only with the percent weight loss exhibited by 1 reaching a minimum at approximately 40%. The DSC spectrum of 2 exhibited two endotherms: a broad endotherm with an apex at approximately 120°C attributed to the loss of coordinated water from 1, and a secondary more intense, sharper endotherm attaining apogee at approximately 160°C. This endotherm is attributed to the thermal decomposition of the maltol ligand (Figure 6), which is consistent with the TGA of maltol. The endotherm at 120°C corresponds to a percent weight decrease of 22.70% observed on the TGA of 1, which is attributed to the loss of four water molecules given a predicted percent weight change of 20.80%. While the EA of 1 suggests only three waters with values of $\{[\text{Mg(C}_6\text{H}_5\text{O}_3)_2(\text{H}_2\text{O})_2].\text{H}_2\text{O}\}$: C = 43.17%, H

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure8}
\caption{Thermal analyses of 1 with maltol TGA (green), Mg\textsuperscript{2+}-maltol TGA (red) and 1 DSC (blue).}
\end{figure}
= 4.75%; Exp: C = 43.33%, H = 4.49%, this difference is attributed to different hydrated states given the propensity of magnesium to take on water.\textsuperscript{2,3} The composition of 1, including the presence of waters was supported by EA analyses, which indicated the presence of three waters. While thermal analyses indicated an additional water, this is easily attributed to differences in hydrated states.

3.3.1.2. Mass Spectrometry Supports the Mg\textsuperscript{2+}:Maltol Stoichiometry of 1

Figure 9. Mass spectrum of 1. ESI-MS conducted in 50/50 MeOH/H\textsubscript{2}O.

ESI-MS of 1 exhibits two signals at 127 m/z and 306 m/z, respectively (Figure 7). The signal observed at 127 m/z is attributed to maltol and the signal at 306 m/z is attributed to the methanol adduct of a 1:2 Mg\textsuperscript{2+}:maltol species. Observance of the signal at 306 m/z coincides with the predicted stoichiometry as a result of synthetic conditions as well as the conclusions drawn from thermal analyses of 1. This provides further confirmation of the hypothesized 1:2 Mg\textsuperscript{2+}:Maltol stoichiometry of 1.
3.3.1.3. Discussing the Core Structure of 1

The core structure of 1 is provided below as Figure 8. In general, Mg\(^{2+}\) assumes a six-coordinate octahedral geometry, which is consistent with the predicted core structure.

![Figure 8. Precited core structure of 1.](image)

Provided the NMR analyses discussed in chapter 2, the deprotonated maltol act as a bidentate ligand and coordinate via an O,O donor set about the deprotonated terminal alcohol and the ketone. Compositional determination via thermal and elemental analyses, as well as stoichiometric support from ESI-MS confirms a 1:2 Mg\(^{2+}\):maltol stoichiometry. Thermal and elemental analysis confirm a rounded-out core structure consisting of two waters with excess waters of hydration expected in varying degrees.

3.3.2. Determining the Composition of 2

3.3.2.2. Thermal and Elemental Analyses of 2

As observed with maltol, ethylmaltol exhibited only one continuous percent weight decrease from approximately 70°C - 200°C and stops decreasing in weight at approximately 5%
weight (Figure 9). This profile is attributed to the thermal decomposition of the ethylmaltol ligand, which is predicted to be roughly the same as maltol at ~160°C.

The TGA of 2 differed to that of ethylmaltol in that it exhibited two distinguishable percent weight decreases and stopped decreasing in percent weight at approximately 35%. Both percent weight changes correspond to two separate endotherms observed on the DSC of Mg$^{2+}$-ethylmaltol – one broad endotherm apexed at approximately 110°C and a secondary sharp, and substantially more intense, endotherm with an apex at approximately 320°C, respectively.

The first broad endotherm observed on the DSC of complex 2 shows a corresponding percent weight change of 15.33%, which corresponds to the loss three waters from the overall [Mg(EtMa)$_2$(H$_2$O)$_2$]$\cdot$H$_2$O] complex supported by the EA with a predicted weight percent change of 15.15%. The secondary, more intense, endotherm at approximately 320°C is attributed to the decomposition of the ethylmaltol ligand. The number of waters observed for 2 via thermal analyses

![Figure 11](image-url)
predict two waters directly coordinated to the magnesium core, and two additional waters of crystallization. The presence of three waters is consistent with theoretical EA values for \{[\text{Mg}(\text{C}_7\text{H}_7\text{O}_3)_2(\text{H}_2\text{O})_2]\cdot\text{H}_2\text{O}\}: C = 46.32\%, H = 5.47\%; \text{Exp: C = 46.95\%, H = 5.05\%}. However, magnesium readily absorbs water and differing drying conditions and/or sample preparations likely have contributed to the different hydration states noted.\textsuperscript{2} The three waters observed for 2 support two coordinated waters and one water of crystallization.

3.3.2.3. Mass Spectrometry Supports the Mg\textsuperscript{2+}:Ethylmaltol Stoichiometry of 2

**Figure 12.** Mass spectrum of 2. ESI-MS conducted in 50/50 MeOH/H\textsubscript{2}O.

Similar to the mass spectrum of 1, ESI-MS analysis of 2 exhibited two peaks at 141 m/z and 334 m/z, respectively (**Figure 10**). The signal observed at 141 m/z is attributed to ethylmaltol and the signal observed at 334 m/z is attributed to the methanol adduct of a 1:2 Mg\textsuperscript{2+}:ethylmaltol species. This is again consistent with the predicted 1:2 Mg\textsuperscript{2+}:ethylmaltol stoichiometry as based
upon synthetic conditions, as well as the conclusions drawn from thermal analyses of 2. This provides further confirmation of the hypothesized 1:2 Mg\textsuperscript{2+}:ethylmaltol stoichiometry of 1.

### 3.3.2.3. Discussing the Core Structure of 2

The core structure of 2 is provided below as Figure 11. In general, Mg\textsuperscript{2+} assumes a six-coordinate octahedral geometry,\textsuperscript{2} which is consistent with the predicted core structure.

![Predicted core structure of 2.](image)

**Figure 13.** Predicted core structure of 2.

Like 1, NMR analysis of 2 (see chapter 2) indicates an O,O bidentate coordination mode of ethylmaltol via the deprotonated terminal alcohol and the ketone. Thermal analyses, as well as ESI-MS, support a 1:2 Mg\textsuperscript{2+}:ethylmaltol stoichiometry. Given the monoanionic character of ethylmaltol and the predicted stoichiometry, 2 retains charge balance via ethylmaltol coordination. Additionally, thermal and elemental analyses, indicate that the remaining two Mg\textsuperscript{2+} coordination sites available are occupied by water with additional waters of hydration expected and observed.
3.3.3. Determining the Composition of 3

3.3.3.1. Thermal and Elemental Analyses of 3

TGA of HG\textsubscript{2} exhibited a single, gradual percent weight decrease onset at 220°C (inflection point observed at 270°C), which is consistent with the melting point of HG\textsubscript{2} at 220°C. TGA analysis of 3 indicated a gradual decline in percent weight onset from 30°C until just before 200°C. The weight change accounts for a loss of 21%, which is consistent with the loss of 2 waters (calculated to 19%) (Figure 12). This result is consistent with the tendency of magnesium to take on water in a rapid fashion.\textsuperscript{2,3} Thus suggesting a core magnesium species of Mg(G\textsubscript{2})(H\textsubscript{2}O)(OH), where rapid acquisition of subsequent water molecules is likely.

\textbf{Figure 14.} Overlay of HG\textsubscript{2} TGA (green) and TGA of 3 (red).

The presence of two waters for 3 via thermal is consistent with ICP analysis of 3, which was consistent with a species of composition Mg(G\textsubscript{2})(H\textsubscript{2}O)(OH) × 5 H\textsubscript{2}O, with theoretical nitrogen and magnesium values of Mg = 8.66% and N = 9.99% compared to experimental values of Mg
= 8.78% and N = 9.89%. Utilizing the Mg:N ratio, the experimental ratio of M:N = 0.89 and is consistent with the theoretical value of Mg:N = 0.87.

3.3.3.2. Mass Spectrometry Supports the Mg\(^{2+}\):HG\(_2\) Stoichiometry of 3

The mass spectrum of 3 exhibited several signals of note. Most notable are the signals observed at 155 m/z and 210 m/z, respectively (Figure 13). The signal at 155 is attributed to a 1:1 Mg\(^{2+}\):G\(_2\) species and the signal at 210 m/z is attributed to a trihydrate 1:1 Mg\(^{2+}\):G\(_2\)\((-1\)) species. Both species are consistent with the predicted 1:1 Mg\(^{2+}\):G\(_2\) species, and the presence of the trihydrate species is consistent with Mg\(^{2+}\) water affinity.\(^1\) These observations are consistent with the observations made for the thermal analysis of 3 and are consistent with the determined 1:1 Mg\(^{2+}\):G\(_2\)^{-}\ complex composition.

Figure 15. Mass spectrum of 3. ESI-MS conducted in 50/50 MeOH/H\(_2\)O.
3.3.3.3. Discussing the Core Structure of 3

The predicted structure of 3 is provided as Figure 14. Provided the NMR analyses conducted on 3, it is predicted that the deprotonated G\( _2 \)\(^- \) ligand acts in a tridentate fashion and coordinates via an N\( _2 \)O donor set utilizing the terminal amine, backbone amide, and deprotonated terminal acid. The predicted 1:1 Mg\(^{2+} \):G\( _2 \)\(^- \) stoichiometry of 3 is confirmed via thermal analyses and ESI-MS. Compositionally, thermal and elemental analyses confirm the presence of three waters, which is confirmed as two waters and a hydroxide anion, which was confirmed via potentiometric measurements – the hydroxide is required for complex charge balance given the presence of only one monoanionic G\( _2 \)\(^- \). Excess waters of hydration are expected upon sample analysis.

3.3.4. Determining the Composition of 4

3.3.4.1. Thermal and Elemental Analyses of 4

It was hypothesized that the two coordination sites of the magnesium remaining beyond the HG\(_3 \) ligand donor set (N\(_3\)O) would be occupied by water, while charge balance would be achieved via a hydroxy anion.
The TGA of 4 was strikingly different than that of HG₃, which exhibited only one continuous weight percent change beginning at approximately 240 °C (Figure 15). 4 exhibited three weight percent changes: 6.6% at 109 °C, 14.1% at 191.5 °C, and one continuous change at approximately 240 °C. The first weight percent change corresponds to loss of a hydroxy anion—further validated by conductivity which showed a monoanionic character of 4. The second weight percent change corresponds to the loss of two coordinating waters from a parent complex of [Mg(G₃)(H₂O)₂]OH, and the last corresponds to the degradation of HG₃. These three independent events were further supported by the presence of three separate observable endotherms on the DSC at ~109, 191 and 240 °C (Figure 15). Elemental analysis values for 4 confirmed the presence of two waters and a hydroxide theoretical values of C = 29.00%, H = 5.68%, N = 16.90% and experimental values are of C = 29.22%, H = 6.22%, N = 16.03%. Differentiation between an additional water or a hydroxide was probed utilizing potentiometric analyses which indicated that
4 exhibited a potential less than that observed for a KCl standard that acts as a 1:1 electrolyte. This supports that 4 achieves charge balance with a hydroxide and acts as a salt given this presence.

3.3.4.2. Mass Spectrometry Supports Mg$^{2+}$:HG$_3$ Stoichiometry of 4

![Mass spectrum](image)

**Figure 18.** Mass spectrum of 4. ESI-MS conducted in 50/50 MeOH/H$_2$O.

The mass spectrum of 4 exhibited three signals of interest at 190 m/z, 230 m/z, and 252 m/z, respectively (Figure 13). These signals are attributed to the HG$_3$ ligand, a 1:1 Mg$^{2+}$:G$_3$$^{-}$ monohydrate species, and the sodium adduct of the monohydrate species, respectively. All species coincide with the predicted 1:1 Mg$^{2+}$:G$_3$$^{-}$ species and are consistent with the conclusions drawn from thermal analyses of 4. These results further support an overall 1:1 Mg$^{2+}$:G$_3$$^{-}$ complex stoichiometry.
3.3.4.3. Discussing the Core Structure of 4

The predicted structure of 4 is provided as Figure 17. As shown, 4 consists of a 1:1 Mg$^{2+}$:G$_3^-$ stoichiometry which is supported by both thermal analyses and ESI-MS. Additionally, thermal analyses are consistent with the presented number of waters. Similar to 3, 4 exhibits the G$_3^-$ ligand acting in an entropically-favored tetradentate fashion utilizing an N$_3$O donor set. 4 exhibits an octahedral coordination sphere that is completed by two coordinating waters. Given only one monoanionic G$_3^-$ ligand, charge balance is achieved via a hydroxy anion, the presence of which was determined via potentiometry. It is possible, that the hydroxide anion is coordinated, but is unlikely given the affinity for water of Mg$^{2+}$. It is expected that excess waters of hydration will be observed.

![Figure 19. HG$_3$ (left) and the predicted core structure of 4 (right).](image)

3.4. Conclusion

Compositional analyses of 1 – 4 supplement solution-state analyses, which determine only ligand coordination, by confirming complex composition and Mg$^{2+}$:ligand stoichiometry. In the case of 1 and 2, both maltol and ethylmaltol act as bidentate ligands via an O,O donor set utilizing the deprotonated terminal alcohol and the ketone. Additionally, both complexes exhibited a six-coordinate octahedral coordination sphere completed by a minimum of two coordinated waters as
confirmed via thermal and elemental analyses. Provided the confirmed 1:2 Mg$^{2+}$/maltol/ethylmaltol stoichiometry, 1 and 2 achieve charge balance solely via maltol and ethylmaltol coordination.

3 and 4 were determined to exist in a 1:1 Mg$^{2+}$/G$_2^-$/G$_3^-$ stoichiometry. As expected, both 3 and 4 observed the G$_2^-$ ligand acting as a tridentate ligand and the G$_3^-$ acting in a tetradeinate fashion via an N$_2$O and N$_3$O donor set, respectively. Given the presence of only one monoanion, both complexes achieve charge balance via a hydroxide anion and act as 1:1 electrolytes in solution. As was expected for all complexes, access waters of hydration were observed and are consistent with the tendency of Mg$^{2+}$ to rapidly take on water.$^1$

3.5. References


Chapter 4. Evaluating the Solubility and *In Vitro* Cellular Uptake of 1 – 4 in Colorectal Carcinoma (CaCo-2) Cells

All work in this chapter was completed by the author. Work in this chapter was published in:


### 4.1 Introduction

As more comprehensively described in Chapter 1, the majority of Mg$^{2+}$ uptake occurs in the GI, with the majority of uptake occurring in the jejunum$^{1,2}$, and varying degrees of uptake occurring unequally throughout the length of the GI.$^{1,2}$

It is generally accepted in the nutraceutical field that the greater the %Mg composition of a supplement, the greater will be the uptake, irrespective of solubility. This is observed prominently via the administration of magnesium oxide as one of the most ubiquitously used Mg$^{2+}$ supplement, with some 60% Mg$^{2+}$ composition but low solubility in water (0.010 g/100 mL)$^{3,4}$

There has been, however, in reality a real dearth of attention given to Mg$^{2+}$ uptake as it specifically relates to complex solubility, with interest growing in recent years.$^{5-7}$ with most studies comparing different formulations of magnesium oxide to each other,$^{8}$ magnesium oxide to magnesium citrate,$^{9}$ and small studies of magnesium oxide to other organic magnesium formulations, such as those conducted by Uysal *et al.*$^{3}$ and Kappeler *et al.*$^{7}$

To this end, the aim of evaluating the *in vitro* cell uptake was to analyze uptake relative to complex solubility for 1 – 4 (fully described in chapters 2 and 3) with the intention of providing
more comprehensive support for increasing Mg\(^{2+}\) bioavailability relative to increasing complex solubility.

4.2. Aqueous Solubility and *In Vitro* Uptake of 1 – 4

4.2.1. Determining the Aqueous Solubility of 1 – 4

The water solubility of complexes 1 – 4 is shown in Table 1. In general, complexation of coordinate ligands to Mg\(^{2+}\) results in an increase in overall solubility. This may be due, in part, to the affinity that Mg\(^{2+}\) has for water.\(^1\) Additionally, in the case of diglycine and triglycine, this could be attributed to an increased salting-in effect as a result of increased solution ionic strength in the presence of cationic Mg\(^{2+}\).\(^12\) As noted for 1 and 2, the trend for solubility is conserved with 2 exhibiting greater solubility than 1, much like ethylmaltol exhibits greater solubility than maltol. Interestingly, 4 exhibits more substantial water solubility than 2 even though diglycine retains greater water solubility than triglycine. This may be attributed to a more extensive hydrogen bond network exhibited by triglycine, as highlighted by Srikrishnan *et al.*,\(^{15}\) relative to diglycine.

<table>
<thead>
<tr>
<th>Complex Name</th>
<th>Molecular Weight (g/mol)</th>
<th>Formula</th>
<th>%Mg in Compound</th>
<th>Solubility (g/100mL)</th>
<th>Ligand Solubility (g/100mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnesium Chloride</td>
<td>203.3</td>
<td>MgCl(_2)(\cdot)6H(_2)O</td>
<td>11.9</td>
<td>54</td>
<td>--</td>
</tr>
<tr>
<td>MgO</td>
<td>40.3</td>
<td>MgO</td>
<td>60.3</td>
<td>0.010</td>
<td>--</td>
</tr>
<tr>
<td>1</td>
<td>310.5</td>
<td>Mg(C(_6)H(_5)O(_2))(H(_2)O)(_2)(\cdot)xH(_2)O</td>
<td>7.8</td>
<td>15.6 ± 1.17</td>
<td>1.2(^{10})</td>
</tr>
<tr>
<td>2</td>
<td>338.6</td>
<td>Mg(C(_7)H(_7)O(_3))(H(_2)O)(_2)(\cdot)xH(_2)O</td>
<td>7.2</td>
<td>16.2 ± 0.75</td>
<td>5.84(^{11})</td>
</tr>
<tr>
<td>3</td>
<td>191.5</td>
<td>Mg(G(_2))(H(_2)O)(OH)(\cdot)xH(_2)O</td>
<td>17.3</td>
<td>39.40 ± 2.84</td>
<td>22.8(^{12})</td>
</tr>
<tr>
<td>4</td>
<td>265.5</td>
<td>[Mg(G(_3))(H(_2)O)(_2)]OH</td>
<td>9</td>
<td>169 ± 12.5</td>
<td>6.4 – 6.9(^{13,14})</td>
</tr>
</tbody>
</table>

Table 1. Aqueous solubilities of 1 - 4.
4.2.2. Determining the *In Vitro* Cell Uptake of 1 – 4 in CaCo-2 Cells

4.2.2.1. Determining the Uptake of 1 and 2

The uptake of 1 and 2 was evaluated relative to MgCl₂, and done so with the understanding that the complexes contained ~6 – 7% magnesium citrate, respectively, and the concentrations are based upon a total concentration of magnesium contributed from both species as based upon molecular weight. The uptake of Mg²⁺ from 1 and 2 was substantial and complex 2 exhibited slightly greater Mg²⁺ uptake relative to 1 although containing less overall Mg²⁺ (7.2% versus 7.8%, respectively) (Figure 1). It should be noted, however, that the uptake exhibited by 1 and 2 is relatively similar, as is expected given similarities in solubility and percent Mg²⁺ composition. Strikingly, however, both 1 and 2 exhibit greater overall uptake than MgCl₂.

![Graph](image.png)

**Figure 20.** Cellular uptake of MgCl₂, 1, and 2 in CaCo-2 cells. Slope/R² MgCl₂: 5x10⁻⁵/0.7373. Slope/R² 1: 0.0001/0.8198. Slope/R² 2:0.0001/0.8433. SEM: MgCl₂ = ±0.0006, 1 = ±0.001, 2 = ±0.001; Upper 95% C.I.: MgCl₂ = 0.004, 1 = 0.008, 2 = 0.011; Lower 95% C.I. = MgCl₂ = 0.001, 1 = 0.001, 2 = 0.001).

4.2.3.1. Determining the Uptake of 3 and 4

Cellular uptake of 3 and 4 was also evaluated relative to MgCl₂. Additionally, uptake of magnesium bisglycinate (MgBG – the 1:2 (Mg²⁺:glycine) Mg²⁺-complex of the glycine monomer)
was incorporated to provide insight into the impact that peptide length has on uptake as well. Uptake is plotted as a linear regression (Figure 2).

Figure 21. Cellular uptake of 3 (orange) plotted with Magesium Bisglycinate (red), 4 (green) and MgCl₂ (blue) in CaCo-2 cells. Slope/R² MgCl₂: 5x10⁻⁵/0.7373. Slope/R² 3: 5x10⁻⁴/0.7231. Slope/R² MgBG: 5x10⁻⁴/0.8477. Slope/R² 4: 7x10⁻⁴/0.8019. SEM: MgCl₂ = 0.0006, MgBG = 0.0006, 3 = 0.0007, 4 = 0.001; Upper 95% C.I.: MgCl₂ = 0.004, MgBG = 0.003, 3 = 0.004, 4 = 0.006; Lower 95% C.I.: MgCl₂ = 0.001, MgBG = 8.98x10⁻⁵, 3 = 1.11x10⁻⁵, 4 = 0.0005.

Analysis of cellular uptake data indicates that 3 exhibits uptake less than that of MgCl₂ and comparable to MgBG. Furthermore, observed uptake of 3 was only about half that of 4. The uptake of 3 relative to MgBG is interesting because similarities. While 3 exhibits slightly greater uptake than MgBG at higher concentration, it appears as though uptake is within error. That said, uptake tentatively increases with increased peptide length from MgBG, 3, and 4. This observation is relatively consistent with the uptake observed in humans for the free mono-, di- and tripeptide glycine ligands observed by Craft et al.¹⁶ Craft explains that the rate of cellular uptake of the free ligands is directly related to the available mole quantity of the ligand, and the corresponding rate of uptake as it pertains to the amount of ligand available.¹⁶ It is believed that in the case of the uptake of magnesium chelates of glycine-based complexes as reported, Craft’s discussion supports the observable uptake trend. The trend of increasing uptake with a corresponding increase in
peptide length is also supported by the findings of Hellier et al. who observed greater intestinal absorption of HG2 relative to glycine in human studies.\textsuperscript{17}

More interestingly, however, is that while uptake of the glycine oligopeptides seems to trend in favor of increasing peptide length, relative to MgCl\textsubscript{2}, 1, and 2, the uptake of 3 and 4 is substantially less, save for 4 exhibiting greater uptake than MgCl\textsubscript{2} at higher concentrations. Given only a minute presence of magnesium citrate, contributions from this species are negligible. While solubility may explain the relationship between 4 and MgCl\textsubscript{2}, it does not explain why 1 and 2, exhibiting substantially less water solubility exhibit substantially greater uptake.

\textbf{4.4. Conclusions}

The hypothesis that increased complex solubility corresponds to increased bioavailability of Mg\textsuperscript{2+} was inconclusive. Complexes 1 and 2 exhibit the greatest Mg\textsuperscript{2+} uptake while retaining the lowest complex solubility. Interestingly, however, when comparing only complexes 3 and 4, uptake appears to increase with increasing peptide length. These findings are consistent with the observed uptake for the free ligands. Additionally, the slope of uptake for 4 is substantially greater than that for MgCl\textsubscript{2}, and at higher concentrations, 4 exhibits greater overall uptake than MgCl\textsubscript{2}. While solubility may not be the main contributory factor, it must also be noted that percent Mg\textsuperscript{2+} composition is also not the main factor impacting Mg\textsuperscript{2+} uptake, as 1 and 2 also have the lowest Mg\textsuperscript{2+} composition. Greater uptake of 1 and 2 relative to 3 and 4 may be attributed to the predicted tri- and tetradeutate coordination of 3 and 4, and only a bidentate coordination of 1 and 2. Increased entropic stability of 3 and 4 may suggest greater uptake over a longer period relative to more substantial uptake of 1 and 2 initially. Future \textit{in vitro} studies are required to confirm these findings.
4.5. References


(4) Ropp, R. C. *Group 16 (O, S, Se, Te) Alkaline Earth Compounds*; 2013; Vol. 16.


(15) Srikrishnan, T.; Winiewicz, N.; Parthasarathy, R. New Patterns of Hydrogen Bonded


Chapter 5. Experimental

5.1. Materials and Methods

5.1.1. Chemicals and Equipment

Laboratory grade chemicals and assorted solvents were purchased from Sigma Aldrich (St. Louis, MO, USA), Fischer Scientific (Waltham, MA, USA), or Balchem Corp. (New Hampton, NY). Both deionized and distilled water were provided in-house. When required, all centrifugation of isolated synthetic compounds was conducted on a Sorvall RT instrument at a speed of 4000 rpm for 10 min. Fourier Transform Infrared (FT-IR) was conducted on a Nicolet Infrared Spectrophotometer utilizing a potassium bromide (KBr) pellet and typically scanned from 400 – 4000 cm\(^{-1}\). Thermogravimetric Analysis (TGA) was conducted in-house on a TA Instrument Q500 series (with the TGA of 3 conducted by the ThINC facility at Stoneybrook University (Stoneybrook, NY, USA). Differential Scanning Calorimetry (DSC) was conducted on a TA Instrument Q2000 series, respectively. Typical TGA analyses consisted of a 10°C/min ramp rate on samples of 5 – 20 mg placed on a platinum pan and analyzed from 30 - 800°C. Typical DSC analyses consisted of 5 – 10 mg samples crimped in T-zero aluminum pans scanned with a ramp rate of 10°C/min from 30°C - 300°C. TGA and DSC spectral analysis was performed utilizing the TA Instruments Universal Analysis 2000 software. Elemental Analyses (EA – C, H, N) and %Mg composition were determined via inductively coupled plasma – optical emission spectrometry (ICP-OES) analysis as necessary were conducted independently by Intertek Pharmaceutical Services (Whitehouse, NJ, USA). Electrospray Ionization Mass Spectrometry (ESI-MS) was conducted on a Shimadzu 8040 LC-MS/MS with samples typically analyzed from 100 – 600m/z in a solvent system of 50/50 H\(_2\)O/MeOH with 0.1% TFA. All Nuclear Magnetic Resonance (NMR)
spectroscopy, both 1D- and 2D $^1$H/$^{13}$C NMR (e.g. heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond correlation (HMBC)) was carried out on a Bruker Avance III HD 400 MHz instrument. Crystal data, when possible, were collected and refined by Dr. William W. Brennessel at the X-Ray crystallographic center at the University of Rochester on a Rigaku XtaLAB Synergy-S Dualflex diffractometer equipped with a HyPix-6000HE HPC area detector.

5.1.2. Cell Lines and Culture Conditions

5.1.2.1. Colorectal Carcinoma (CaCo-2) Cells

The CaCo-2 cell line was purchased from ATCC (Manassas, VA, USA). CaCo-2 cells were taken from liquid N$_2$ stocks and rapidly thawed using a water bath at 37°C. Cryopreservation media was removed with a micropipette after cells were pelleted via centrifugation for 2 min at 125g. Cells were resuspended in 1mL of Dulbecco’s Modified Eagle Medium (DMEM) that had been incubated at 37°C and cultured in DMEM (total volume of 5mLs) with a seeding density of $3.6 \times 10^4$ cells/cm$^2$ in a T-25cm$^2$ culture flask and left to grow in an incubator at 37°C and 5% CO$_2$. When cultures reached 90%+ confluency, cells were detached with manual scraping and gentle agitation, and pipetted. Two T-25cm$^2$ culturing flasks were combined and centrifuged into a pellet for 2min at 125g – the old media was pipetted off and cells were resuspended in 11mL fresh DMEM. Cells were plated in a 96-well plated at 100μL/well and left to grow to 90%+ confluency as to form a monolayer. Plated cells were used to determine magnesium uptake.
5.1.2.2. Mg\textsuperscript{2+} Uptake Assay

A colorimetric magnesium uptake assay kit for use with a 96-well plate was purchased from BioVision (Milpitas, CA, US). Sample solutions for use with the kit were prepared in-house utilizing magnesium/calcium free Hank’s Balanced Salt Solution (HBSS). The samples tested were magnesium chloride hexahydrate (MgCl\textsubscript{2}•6H\textsubscript{2}O) and 1 – 4. The kit provided standard for linearity confirmation begins as a 150nm/μl stock – as such MgCl\textsubscript{2}•6H\textsubscript{2}O, utilized as an internal standard, was prepared at this concentration – containing 17.93mM Mg\textsuperscript{2+}. Complexes 1 – 4 were prepared to contain the same amount of Mg\textsuperscript{2+} as to evaluate Mg\textsuperscript{2+} uptake in a relative fashion. DMEM was removed from the plated cells and cells were subsequently washed three times with HBSS in 100μl volume. MgCl\textsubscript{2}•6H\textsubscript{2}O and 1 – 4 were administered at 150μl/well as triplicate independent dilutions. Cells were treated for 1 – 2hr at 37°C and 5% CO\textsubscript{2}. After incubating, sample volume was removed from each well and the cells were again washed three times with HBSS. Cells were lysed utilizing 200μl/well of kit assay buffer, the post-lysis volume collected, and each sample centrifuged at 14000g for 10 minutes. The resulting supernatants were replated in the same order in 50μl volume. 50μl of kit-provided enzyme/buffer/developer mix was added to each well with a multichannel micropipette, and the plate incubated for 40 minutes at 37°C. Some wells were left blank for required background subtraction. The kit provided standard was diluted to 0, 3, 6, 9, 12, and 15nmol/μl in DI H\textsubscript{2}O and administered and developed in the same volumes as MgCl\textsubscript{2}•6H\textsubscript{2}O and 1 – 4 and was used only to determine kit linearity. Each well was analyzed for endpoint value over nine full plate scans with triplet scans/well/plate scan (a total of 27 scans per well) and the reported value of each well was the average value of these scans after background subtraction. All samples were analyzed in triplicate. Data was collected at 40 minutes. Raw data was reduced and plotted as absorbance against magnesium concentration of each well. All assays were repeated in
triplicate \( \text{Slope/R}^2 \) \( \text{MgCl}_2\cdot6\text{H}_2\text{O} \): \( 5\times10^{-5}/0.7373 \). \( \text{Slope/R}^2 \) 1: 0.0001/0.8198. \( \text{Slope/R}^2 \) 2: 0.0001/0.8433. \( \text{Slope/R}^2 \) 3: \( 5\times10^{-4}/0.7231 \). \( \text{Slope/R}^2 \) 4: \( 7\times10^{-4}/0.8019 \). SEM: \( \text{MgCl}_2\cdot6\text{H}_2\text{O} = \pm0.0006, 1 = \pm0.001, 2 = \pm0.001, 3 = 0.0006, 4 = 0.001 \); Upper 95% C.I.: \( \text{MgCl}_2\cdot6\text{H}_2\text{O} = 0.004, 1 = 0.008, 2 = 0.011, 3 = 0.004, 4 = 0.006 \); Lower 95% C.I. = \( \text{MgCl}_2\cdot6\text{H}_2\text{O} = 0.001, 1 = 0.001, 2 = 0.001, 3 = 1.11\times10^{-5}, 4 = 0.0005 \).

**5.2. Syntheses**

**5.2.1. Synthesis of Magnesium Maltol (\( \text{Mg(C}_6\text{H}_5\text{O}_3)_2\cdot\text{xH}_2\text{O} \) (1))**

A 1.00g sample of maltol (7.93 mmol; 2 eq.) was dissolved in 10mLs of DI \( \text{H}_2\text{O} \) in a 50mL round-bottom flask, with constant stirring at 90°C. A separate solution of 192.2 mgs of magnesium oxide (\( \text{MgO} – \text{mmol; 1.2 eq.} \)) was taken up in 10mLs of \( \text{H}_2\text{O} \), with an addition of 190.2 mgs of citric acid (CA - 0.25 eq), constantly stirred and heated to 90°C. The MgO/CA solution was subsequently added to the maltol solution in small increments over ~5 min. Upon addition, the mixture was a translucent white color that solubilized in about 30 seconds; each subsequent addition was administered when the previous addition had become wholly soluble. After all additions, the reaction was noted as colorless and clear. The reaction was conducted for one hour, whereupon the solution was noted as yellow and clear. The reaction was allowed to cool to room temperature and the pH was noted as 7.80. The solution was dried \textit{in vacuo} – producing a tan solid, which was used for subsequent analyses. The yield of 1 was stoichiometric relative to maltol with a purity of 92.8% based on \(^1\text{H NMR.} \) The solubility of 1 was determined to be 15.6 ± 1.17 g/100 mL \( \text{H}_2\text{O} \). \(^1\text{H NMR (D}_2\text{O, 400MHz): \( \delta 4.79 (s, 1\text{H}), 7.95-7.94 (d, 1\text{H}, \text{H}_2, J = 5.26\text{Hz}), 6.48-6.46 (d, 1\text{H}, \text{H}_1, J = 5.38\text{Hz}), 2.33 (s, 1\text{H}, \text{H}_3). \) EA: Theo for \{[\text{Mg(C}_6\text{H}_5\text{O}_3)_2(\text{H}_2\text{O}_2)].\text{H}_2\text{O}\}: \text{C} = 43.17\%, \text{H} = 4.75\%; \text{Exp: C} = 43.33\%, \text{H} = 4.49\% \).
5.2.2. Synthesis of Magnesium Ethylmaltol (Mg(C<sub>7</sub>H<sub>7</sub>O<sub>3</sub>)<sub>2</sub>xH<sub>2</sub>O) (2)

A 1.01g sample of ethylmaltol (EtMa – 7.14mmol; 2eq.) was dissolved in 10mLs of DI H<sub>2</sub>O in a 50mL round-bottom flask, with constant stirring at 90°C. A separate solution of 158.6mgs magnesium oxide (MgO – 3.93mmol; 1.1eq.) was taken up in 10mLs of DI H<sub>2</sub>O, with an addition of 172.3mgs of citric acid (CA - 0.25eq), constantly stirred and heated to 90°C. The MgO/CA solution was subsequently added to the ethylmaltol solution in small increments over 5 min. Upon addition, the mixture was a translucent white color that solubilized in about 30 seconds; each subsequent addition was administered when the previous addition had become wholly soluble. After all additions, the reaction was noted as colorless and clear. The reaction was conducted for one hour, whereupon the solution was noted as clear and amber/orange in color. The solution was allowed to cool to room temperature and the pH was noted as 7.80. The solution was dried in vacuo, at which time a tan solid was observed. The yield was found to be stoichiometric relative to ethylmaltol, and the purity was 93.9% based on <sup>1</sup>H NMR. The solubility of 2 was determined to be 16.2 ± 0.75 g/100mL H<sub>2</sub>O. 2: <sup>1</sup>H NMR (D<sub>2</sub>O, 400MHz): δ 4.79 (s, 1H), 7.99-7.98 (d, 1H, H1, J = 5.26Hz), 6.48-6.47 (d, 1H, H2, J = 5.50Hz), 2.76-2.71 (q, 2H, H3, J1 = 22.62Hz, J2 = 7.70Hz), 1.19-1.15 (t, 3H, H4, J = 15.16Hz). EA: Theo for {[Mg(C<sub>7</sub>H<sub>7</sub>O<sub>3</sub>)(H<sub>2</sub>O)]·H<sub>2</sub>O}: C = 46.32%, H = 5.47%; Exp: C = 46.95%, H = 5.05%.

5.2.3. Synthesis of Magnesium Glycylglycine (Mg(C<sub>4</sub>H<sub>7</sub>N<sub>2</sub>O<sub>3</sub>)(H<sub>2</sub>O)<sub>2</sub>(OH)) (3)

Glycylglycine (HG<sub>2</sub>) (1.02 g, 7.57 mmol) was dissolved in DI H<sub>2</sub>O (~20 mL) in a 50 mL round-bottom flask, with constant heating at 90°C and stirring. A separate solution of magnesium
oxide (MgO – 0.336 g, 8.33 mmol) was taken up in DI H2O (~20 mL), with an addition of citric acid (CA – 0.364 g, 2.08 mmol (0.25 eq.)), constantly stirred and heated to 90°C. The MgO/CA solution was added to the HG2 solution – upon addition, the combined solution turned an opaque white, and was observed as translucent white/clear after about 10 minutes and up until reaction completion. The reaction was left to run for 1hr at 90°C. The reaction was cooled to room temperature, centrifuged to pellet any remaining solid, and the supernatant filtered through a 40μm filter. The pH of the solution was noted as 10.2. The solution was concentrated in vacuo to approximately 3 mL and the solid precipitated with anhydrous ethanol. Centrifugation was employed to pellet the solid, and the ethanol was decanted off. The solid was triturated with diethyl ether ad libitum and then recentrifuged. The ether was decanted off. To ensure no trace solvent, the solid was reconstituted in DI H2O (~50 mL), flash frozen, and dried in vacuo on a lyophilizer. The dried material was collected and massed to 1.28 g. Yield was found to be 72%, relative to magnesium, and 82.6% pure with a 17.3% impurity attributed to magnesium citrate. The solubility of 3 was determined to be 39.40 ± 2.84 g/100 mL. 3: 1H NMR (D2O, 400MHz): δ 3.76 (s, 2H, H2), 3.38 (s, 2H, H1). ESMS m/z: [Mg(G2) + H+] calcd for Mg(C6H10N3O4)(H2O)155.4; found 155, [3 + H+] calcd for [MgC4H12N2O6 +H+] 209.5; found 210. By ICP (Mg(C6H10N3O4)(H2O)(OH) • 5H2O): Mg = 8.78% and N = 9.89%. These values are consistent with a species of composition Mg(G2)(H2O)(OH) × 5 H2O, with theoretical nitrogen and magnesium values for this species are Mg = 8.66% and N = 9.99%. Utilizing the Mg:N ratio, the experimental ratio of M:N = 0.89 and is consistent with the theoretical value of Mg:N = 0.87.

5.2.4. Synthesis of Magnesium Glycylglycylglycine (Mg(C6H10N3O4)(H2O)2(OH)) (4)

A 1.0025g sample of triglycine (HG3 – 5.29 mmol; 1 eq.) was dissolved in 10mLs of 18 MΩ H2O in a 50mL round-bottom flask, with constant stirring at 90°C. A separate solution of
215.5 mgs magnesium oxide (MgO – 5.29 mmol; 1eq.) was taken up in 10mLs of 18MΩ H2O, with an addition of 253.6 mgs of citric acid (CA - 0.25 eq), constantly stirred and heated to 90°C. The MgO/CA solution was subsequently added to the triglycine solution. Immediately upon addition, the combined solution turned a milky white color, whereupon this became a clear solution after ~ 20min with stirring at 90°C. The reaction was run for 2 hr, and then cooled to room temperature. The pH of the solution was noted as 10.2. The solution was concentrated to approximately 3 mL via rotary evaporation, and a white solid was precipitated with anhydrous ethanol. The solid was isolated by centrifugation at 4000rpm at room temperature for 10min, and the ethanol decanted off. The solid was trituted with diethyl ether, recentrifuged as before, the ether decanted off, and the solid dried in vacuo overnight. The dried white material was collected, and the yield obtained. The solubility of 4 was determined to be 169 ± 12.5 g/100 mL H2O and the purity was found to be 90+% via 1H NMR integration.

5.3. Crystal Structure Determination of 5 and 6 (see thumb drive for CIF files)

All work in this chapter was completed by the author unless otherwise stated. Crystal structure data collection and refinement was completed by Dr. William W. Brennessel at the University of Rochester, Rochester, New York, United States.

5.3.1. Data Collection and Refinement of 5

5.3.1.1. Data Collection of 5

A crystal (0.201 x 0.046 x 0.026 mm) of 5 was placed onto a thin glass optical fiber or a nylon loop and mounted on a Rigaku XtaLAB Synergy-S Dualflex diffractometer equipped with a HyPix-6000HE HPC area detector for data collection at 100.0(1) K. A preliminary set of cell constants and an orientation matrix were calculated from a small sampling of reflections. A short
pre-experiment was run, from which an optimal data collection strategy was determined. The full data collection was carried out using a PhotonJet (Cu) X-ray source with frame times of 0.24 and 0.96 seconds and a detector distance of 31.2 mm. Series of frames were collected in 0.50º steps in \( w \) at different \( 2q, k, \) and \( f \) settings. After the intensity data were corrected for absorption, the final cell constants were calculated from the \( xyz \) centroids of 11301 strong reflections from the actual data collection after integration.

### 5.3.1.2. Structural Refinement of 5

The structure was solved using SHELXT and refined using SHELXL.\(^{2,3}\) The space group \( P-1 \) was determined based on intensity statistics. Most or all non-hydrogen atoms were assigned from the solution. Full-matrix least squares/difference Fourier cycles were performed which located any remaining non-hydrogen atoms. All non-hydrogen atoms were refined with anisotropic displacement parameters. N-H and O-H hydrogen atoms on ligands that are involved in hydrogen bonding were found from the difference Fourier map and refined freely. Due to the disorder of the solvate water molecules over general and special positions, hydrogen atoms were placed in positions reasonable for hydrogen bonding and given rigid riding models; it should be noted that there may be other hydrogen bonding scenarios in addition to the one applied. All C-H hydrogen atoms were placed in ideal positions and refined as riding atoms with relative isotropic displacement parameters. The final full matrix least squares refinement converged to \( R1 = 0.0443 \) \( (F^2, I > 2s(I)) \) and \( wR2 = 0.1222 \) \( (F^2, \text{all data}) \).

Structure manipulation and figure generation were performed using Olex2.\(^4\) Unless noted otherwise all structural diagrams containing anisotropic displacement ellipsoids are drawn at the 50 % probability level. Images of the crystal packing were generated using CrystalMaker\(^\text{®}5\).
1 provides a summary of the crystal data and structure refinement for the structural studies of compound 1. CCDC 2101440 contains the supplementary crystallographic data for 5.

5.3.2. Data Collection and Refinement of 6

5.3.2.1. Data Collection of 6

A crystal (0.347 x 0.073 x 0.035 mm$^3$) was placed onto a thin glass optical fiber or a nylon loop and mounted on a Rigaku XtaLAB Synergy-S Dualflex diffractometer equipped with a HyPix-6000HE HPC area detector for data collection at 100.00(10) K. A preliminary set of cell constants and an orientation matrix were calculated from a small sampling of reflections. A short pre-experiment was run, from which an optimal data collection strategy was determined. The full data collection was carried out using a PhotonJet (Cu) X-ray source with frame times of 0.31 and 1.24 seconds and a detector distance of 34.0 mm. Series of frames were collected in 0.50º steps in $\omega$ at different $2\theta$, $k$, and $f$ settings. After the intensity data were corrected for absorption, the final cell constants were calculated from the xyz centroids of 12348 strong reflections from the actual data collection after integration.$^1$ See flash drive for additional crystal and refinement information.

5.3.2.2. Structural Refinement of 6

The structure was solved using SHELXT$^3$ and refined using SHELXL.$^2$ The space group $P2_1/n$ was determined based on systematic absences. Most or all non-hydrogen atoms were assigned from the solution. Full-matrix least squares / difference Fourier cycles were performed which located any remaining non-hydrogen atoms. All non-hydrogen atoms were refined with anisotropic displacement parameters. The O-H hydrogen atoms were found from the difference Fourier map and refined freely. All other hydrogen atoms were placed in ideal positions and refined as riding
atoms with relative isotropic displacement parameters. The final full matrix least squares refinement converged to $R1 = 0.0387 \ (F^2, \ I > 2s(I))$ and $wR2 = 0.1116 \ (F^2, \ all \ data)$. Structure manipulation and figure generation were performed using Olex2. Unless noted otherwise all structural diagrams containing anisotropic displacement ellipsoids are drawn at the 50% probability level. Images of the crystal packing were generated using CrystalMaker®. Table 1 provides a summary of the crystal data and structure refinement for the structural studies of compound 1.

5.4. References


Chapter 6. Current and Future Work with Other Biorelevant Divalent Metals (Zn\(^{2+}\))

All work in this chapter was completed by the author unless otherwise indicated. Work in this chapter resulted in the paper


And a novel crystal structure deposited to the CCDC:

CCDC 2101440; [Zn\(_2\)(HG\(_2\))\(_4\)(H\(_2\)O)\(_2\)]·5.32H\(_2\)O

A paper pursuant to the latter structure, with chemical formula Zn(C\(_6\)H\(_6\)O\(_3\))\(_2\)Cl\(_2\), is currently being drafted.

6.1. Introduction

While the extent of this thesis explores the complexes produced between Mg\(^{2+}\) and pyrones as well as short-chain (di- and tri-) peptides, there is still a plethora of work to be done. Analysis of scientific literature reveals that only a handful of characterized Mg\(^{2+}\) complexes have been produced, and strikingly, most of these complexes forgo the most fundamental biorelevant ligands that may be utilized to produce viable Mg\(^{2+}\) supplements: monomeric amino acids. To this end, the synthesis and characterization of a library of compounds of this type should be fervently evaluated.

Additionally, while the implications of Mg\(^{2+}\) deficiency were discussed at length, this does not mean that the negative impacts of Mg\(^{2+}\) on optimum biological function are singular or mutually exclusive. Much like Mg\(^{2+}\), other divalent metals such as zinc (Zn\(^{2+}\)) have far-reaching implications in biology.
One of the most abundant trace elements, second only to iron (Fe$^{2+/3+}$), Zn$^{2+}$ is found ubiquitously in biology and plays a critical role in many biological processes.\textsuperscript{1} Zn$^{2+}$ deficiency impacts cognitive function.\textsuperscript{2} More specifically, high concentrations of Zn$^{2+}$ in “zinc containing” neurons in the forebrain\textsuperscript{2} and implications in the release of neurotransmitters (\textit{e.g.} GABA, \textit{etc.})\textsuperscript{2,3} suggest a substantial role in neuronal excitation. Furthermore, Zn$^{2+}$ has been shown to impact the development of children,\textsuperscript{4,5} as well as immune system function.\textsuperscript{6}

The use of Zn$^{2+}$ supplements has been shown to effectively assuage the negative impacts of Zn$^{2+}$ deficiency.\textsuperscript{7} As such, having observed the promise of the synthesized Mg$^{2+}$ complexes, the synthesis of Zn$^{2+}$ analogues was probed with the aim of full solution- and solid-state characterization, which provides imperative insight for proper dosing as well as possible modes of action. Preliminary data for the syntheses of these compounds is provided and a means of determining cellular uptake in future studies is discussed.

6.2. Current Work

6.2.1. Solid- and Solution-State Characterization of Zinc Complexes

6.2.1.1. Zinc Diglycine (5) Preliminary Data

6.2.1.1.1. Synthesis of 5

A 1.0 g sample of HG$_2$ (7.57 mmol, 2eq.) was suspended in ca. 10 mL of DI H$_2$O in a round-bottom flask and heated with constant stirring to 90°C. In a separate vessel, a 0.308 g sample of zinc oxide (3.78 mmol, 1eq.) was suspended in 10 mL of DI H$_2$O and heated with constant stirring to 90°C. The solutions were combined via slow addition over 10 min until the zinc oxide
was no longer wholly soluble, and a white, semi-translucent solution remained. The solution was
reacted for up to 1 hr at 90°C. The solution was cooled to room temp. After cooling, the mixture
was centrifuged to pellet the solid at 2000 rpm for 5 min, and the remaining supernatant was
decanted and filtered through a 45 μm filter. The now clear and colorless solution was flash frozen
in liquid nitrogen and dried in vacuo on a lyophilizer. The isolated product was massed to 1.03 g
resulting in a yield of 79% based on zinc. Elemental Analysis Calcd. for Zn₂C₁₆H₃₂N₈O₁₄,
[Zn(HG₂⁻)₄(H₂O)₂]: C, 27.8; H, 4.67; N, 16.2. Found: C, 27.0; H, 4.95, N, 15.7. IR: 3264 cm⁻¹,
3090 cm⁻¹.

6.2.1.1.2. Compositional Determination of 5 Via Thermal and Elemental Analyses

Thermogravimetric analysis (TGA) of HG₂ exhibited a single, gradual percent weight
decrease onset at 220°C (inflection point observed at 270°C), which is consistent with the melting
point of HG₂ at 220°C. TGA analysis of 5 exhibited three observable percent weight changes that
corresponded to three distinct endothermic events on the differential scanning calorimetry (DSC)
spectrum at 140°C, 220°C, and 280°C, respectively (Figure 1).

The endotherm observed at 140°C coincides with a 5.5% weight decrease and is attributed
to loss of coordinated water (5.21% calculated). The secondary endotherms at 220°C and 280°C
are attributed to HG₂⁻ decomposition and exhibit a gradual weight loss of nearly 80.0%, which is
consistent with the projected weight loss calculated for the loss of four HG₂⁻ ligands (76%) Additionally, given the equivalent intensity of the endotherms at 220°C and 280°C it is believed
these are attributed to the different coordination modes of the HG₂⁻ ligand, thus resulting in
different decomposition temperatures. It is most likely the case that the endotherm at 220°C is
attributed to the HG$_2$$^\text{1-}$ ligand forming a five-member ring and the endotherm at 280$^\circ$C is the more thermodynamically stable bridging HG$_2$$^\text{1-}$ ligand.

*Figure 22.* Thermal analyses spectra of 5 including the TGA of HG$_2$ (green), TGA of 5 (red) and the DSC of 5.

Elemental analysis values are consistent with thermal analyses observations and provide experimental values of C = 27.0, H = 4.95, and N = 15.7. These values were consistent with the elemental values predicted for a complex composition of Zn$_2$C$_{16}$H$_{32}$N$_8$O$_{14}$, [Zn(HG$_2$$^\text{1-}$)$_4$(H$_2$O)$_2$]: C = 27.8, H = 4.67, and N = 16.2.
6.2.1.1.3. Structural Determination of 5 Via Fourier Transform Infrared (FT-IR)

Infrared analysis indicated a substantial shift to lower frequency of the band attributed to the carboxylic acid of HG₂ (~26 cm⁻¹) (Figure 2). This shift to lower frequency is assigned to zinc coordination, at the carboxylic acid, consistent with prior literature.⁸

![FT-IR spectra of HG₂ and 5.](image)

**Figure 23.** FT-IR spectra of HG₂ and 5.
6.2.1.1.4. Determining the Coordination Mode of HG₂ Via $^1$H and $^{13}$C NMR

The $^1$H NMR spectrum of 5 exhibited an upfield shift of the signal attributed to the proton of the carbon adjacent to the amine group in comparison to that of HG₂, indicative of coordination to the amine nitrogen (Figure 3). The shift was substantial in nature having shifted from 3.84 ppm to 3.69 ppm ($\Delta = 0.15$ ppm). While the observed shift is not as large in magnitude as the shift observed upon coordination to Mg$^{2+}$ (see Chapter 2), this is readily attributed to differences in electropositivity.

![NMR spectrum overlay](image)

**Figure 24.** $^1$H NMR overlay of HG₂ (top) and 5 (bottom). NMR conducted in D$_2$O.

There was no observed change to the integration of the peaks attributed to HG₂. Additionally, there were no additional peaks observed after the reaction of HG₂ and zinc, suggesting no production of products secondary to the observed 5.
Comparison of the $^{13}$C NMR spectra of 5 and HG$_2$ showed only a substantial downfield shift of the signal attributed to the carbonyl group of the peptide linkage (Figure 4). Furthermore, there is a substantial decrease in the signal intensity of this peak relative to that observed for HG$_2$ (Figure 4). These observations are consistent with those made for magnesium maltol (see Chapter 2).

![Figure 25. $^{13}$C NMR overlay of HG$_2$ and 5. NMR conducted in D$_2$O.](image)

The shift and decrease in intensity of the signal suggest zinc coordination to the oxygen of this carbonyl group. Similar observations have been made for the coordination of other divalent cations (e.g., Mg$^{2+}$) coordinating to carbonyls.$^9$,$^{10}$ As was the case with 3, 2D NMR confirmed the
assignments of all carbon and proton signals (see carbon and proton assignments for HG$_2$ in Chapter 2).

**6.2.1.5. Isolation of X-ray Quality Crystals of 5**

In a 15mL falcon tube, 1 was added to 10 mL H$_2$O unto saturation (determined by residual solid remaining at the bottom of the tube). The solution was centrifuged at 4000 rpm for 5 min, when the remaining solid was sufficiently pelleted. After centrifugation, the saturated supernatant was pipetted and transferred to a clean 20 mL glass vial, and two or three drops of water were added. The vial was sealed with parafilm, which was pierced with several small holes to allow for evaporation. After 7 to 8 days, small, needle-like crystals formed, which were collected for SC-XRD analysis.

**6.2.1.6. Determining the Crystal Structure of 5 Via Single Crystal Analysis**

The solid-state chemistry of metal-HG$_2$ complexes exhibits considerable versatility, as expected from the presence of four potential binding sites on the ligand: the amine nitrogen, the carbonyl oxygen of the amide linkage, the amide nitrogen, and the carboxylate oxygen. A number of metal-HG$_2$ complexes have been reported across the literature. As shown in Figure 5, the most common coordination mode is Type I, tridentate coordination through the amine nitrogen, the peptide nitrogen, and the carboxylate oxygen with the ligand in the deprotonated G$_2^{2-}$ form.$^{11-14}$ Protonated and bridging variants of this coordination mode have also been described, Types II and IIa.$^{15,16}$ Bidentate coordination through the amine nitrogen and the peptide carbonyl group has also been observed as in Type III$^{17-19}$ and the bridging variants Types IIIa and IIIb.$^{20,21}$ Monodentate zwitterionic coordination through the peptide carbonyl group or through a carboxylate oxygen are also known, Types IV and Iva.$^{22,23}$ Bridging through the carboxylate oxygen atoms as in the copper
acetate prototype with the ligand in the zwitterionic state has been reported in several instances, Type V.\textsuperscript{24,25}

\textbf{Figure 26.} Reported coordination modes of metal-HG\textsubscript{2} complexes.
As shown in **Figure 6**, the structure of \([\text{Zn}_2(\text{HG}_2\text{H}^-)_4(\text{H}_2\text{O})_2]\) consists of a centrosymmetric binuclear complex with the zinc(II) sites exhibiting \{N\_O\_4\} six coordination through bonding to the amine termini and peptide carbonyl oxygen donors of two HG\_2\text{H}^- ligands, the carboxylate oxygen of one of the HG\_2\text{H}^- ligands on the adjacent zinc site, and the oxygen of an aqua ligand. The nitrogen donors adopt a trans orientation, while the aqua ligand is trans to one amide carbonyl oxygen and carboxylate oxygen is trans to the second amide carbonyl oxygen donor.
Two different binding modes are adopted by the \( \text{HG}_2^{1+} \) ligands. More specifically, Type III (Figure 5), with the terminal amine and the amide carbonyl oxygen of one ligand at each zinc site binding in a bidentate fashion, and Type IIIa (Figure 5), again with the amine nitrogen and the peptide carbonyl oxygen binding in the same way but with the terminal carboxylate oxygen bridging to the adjacent zinc center of the binuclear unit. Thus, each zinc site binds exclusively to one bidentate \( \text{HG}_2^{1+} \) ligand, while coordinating in a bidentate fashion to a second \( \text{HG}_2^{1+} \) ligand that additionally bridges through the carboxylate oxygen to the second zinc site. The coordination sphere at the zinc sites is completed by aqua ligation, resulting in six-coordinate geometry. The coordination modes produce two five-membered chelate rings at each zinc site \{ZnNCCO\}, as well as an unusual cyclic fourteen-membered bridging unit \{ZnOCNCCO\}_2.

**Figure 28.** A view of the crystal packing of 5, showing the hydrogen bonding scheme. Color code: Zn, large gray spheres; oxygen, red spheres; nitrogen, blue spheres; carbon, black spheres; hydrogen, small gray spheres. Hydrogen bonds are shown as dotted lines. Lesser contributing disordered atoms have been omitted for clarity.
While there is some disorder of the water molecules of crystallization, these reside in the regions between stacked \([\text{Zn}_2(\text{HG}^1)_4(\text{H}_2\text{O})_2]\) molecules, as observed viewing normal to the bc crystallographic axes in Figure 7. Extensive hydrogen bonding is present between the crystallization water molecules and the amine nitrogen atoms, as well as between the aqua ligands and the carboxylate oxygen atoms of neighboring binuclear units and between the waters of crystallization. Crystal data was collected and refined by Dr. William W. Brennessel at the University of Rochester in Rochester, New York, United States.

6.2.1.2. Zinc Maltol (6) Preliminary Data

6.2.1.2.1. Isolation of X-ray Quality Crystals of 6

In a 15mL falcon tube, 6 was added to 10 mL H_2O unto saturation (determined by residual solid remaining at the bottom of the tube). The solution was centrifuged at 4000 rpm for 5 min, when the remaining solid was sufficiently pelleted. After centrifugation, the saturated supernatant was pipetted and transferred to a clean 20 mL glass vial, and two or three drops of water were added. The vial was sealed with parafilm, which was pierced with several holes to allow for evaporation. After allowing slow evaporation for 7 to 8 days, small, yellow, needle-like crystals formed, which were collected for SC-XRD analysis.

6.2.1.2.2. Single Crystal X-ray Structure of 6

As shown in Figure 8, the structure of Zn(Malt)_2Cl_2 exhibits one zinc metal center assuming a four-coordinate, tetrahedral geometry with two coordinated, neutral maltol ligands and charge balance achieved via two chloride anions; the maltol ligands are coordinated through the keto oxygen, and act as a monodentate ligand.
Coordination in this fashion is peculiar given a well-outlined bidentate coordination of maltol to metals of this type through the deprotonated terminal alcohol and the ketone (Figure 9). 26–28

![Tetrahedral Structure of 6](image1.png)

**Figure 29.** A view of the tetrahedral structure of 6, showing the atom-labeling scheme and 50% thermal ellipsoids.

![Coordination Mode](image2.png)

**Figure 30.** Supported coordination mode of maltol to biorelevant metals.

Reported crystal data provided to the CCDC indicates approximately 70 crystal structures of metal-maltol complexes, and a smaller subset comprised of biorelevant metals such as zinc, copper, iron, nickel, and cobalt. 29 Of these complexes, there are only three reported structures utilizing zinc, and all reported structures exhibited a bidentate coordination mode as shown in
Although coordination through the terminal alcohol and the ketone is more likely given the increased stability upon the formation of a bidentate chelate, it is likely coordination occurs in the described fashion given a lack of deprotonation of the terminal alcohol (pKa = 8.68) resulting from reaction pH at 3.1, which provides no charge balance from the ligand itself, requiring charge balance be provided by the presence of chloride anion.

The crystal packing for 6, shown in Figure 10, exhibits no presence of extensive hydrogen bonding or interstitial waters. Furthermore, there is little observed disorder. Additionally, the unit cell exhibits 6 packing in a stacked fashion as sheets.

6.3. Future Work

6.3.1. Synthesis and Characterization of a Library of Mg$^{2+}$- Amino Acid (MgAA) Complexes
Provided the success of the Mg\(^{2+}\) complexes reported in this thesis and considering only a pittance of fully characterized Mg\(^{2+}\) complexes throughout the literature\(^{31-33}\) there is a need to develop and characterize a library of complexes comprised of MgAA compounds given that amino acids are the most fundamental biorelevant ligands feasible.

In lieu of magnesium oxide (MgO) as a starting material, magnesium carbonate (MgCO\(_3\)) will be utilized given a substantial increase in water solubility relative to MgO as a means of forgoing the presence of magnesium citrate as observed from the synthesis of the Mg\(^{2+}\)-complexes reported within.

The synthesized MgAA complexes will be characterized in a similar fashion to the previously reported Mg\(^{2+}\)-complexes with both 1D and 2D \(^1\)H/\(^{13}\)C NMR as the primary means of determining preliminary ligand coordination to Mg\(^{2+}\) and solid-state analyses utilized to confirm complex composition. It is hypothesized that most amino acids will exhibit Mg\(^{2+}\) coordination predominantly to the deprotonated carboxylic acid in favor of the hard-hard acid-base chemistry of Mg\(^{2+}\), however, where possible, ligand coordination may occur in favor of entropically favored ring structures (e.g. aspartic acid, glutamic acid, phenylalanine, etc.), or in higher order (tri- and tetradentate) thus providing stability through increased entropy as well (e.g. arginine, threonine, etc.). Furthermore, the varying chemical characteristics of these ligands (acidic/basic, polar/nonpolar, aliphatic/aromatic, etc.) provide integral insight into what truly impacts observed Mg\(^{2+}\) uptake.

### 6.3.2. Completing Mg\(^{2+}\)-Complex Uptake *In Vitro and In Vivo*

Successful screening of the previously discussed Mg\(^{2+}\) complexes suggest the provided
Mg\(^{2+}\) uptake assay protocol as a viable means of determining Mg\(^{2+}\) uptake *in vitro*. As such, all complexes produced in the future will be screened for *in vitro* uptake in the same fashion. Having screened all Mg\(^{2+}\) complexes *in vitro*, the most promising compounds will be screened *in vivo* in rats.

**6.3.3. Assaying Cellular Uptake of Zn\(^{2+}\) Complexes in CaCo-2 Cells**

**6.3.3.1. Assaying the *In Vitro* Uptake of Zn\(^{2+}\) from 5 and 6**

As was the case concerning the *in vitro* evaluation of the uptake of Mg\(^{2+}\) from the Mg\(^{2+}\)-complexes, the cellular uptake of Zn\(^{2+}\) from the Zn\(^{2+}\)-complexes may be evaluated in the same fashion. Provided the crystal structures of 5 and 6, determining dosing is much more simplified.

Zn\(^{2+}\) uptake may be evaluated utilizing a colorimetric assay again provided by BioVision (Milpitas, CA, USA). CaCo-2 Cells will be treated in with the zinc complexes relative to zinc chloride (ZnCl\(_2\)) for a period of 1-2 hrs, cells will be lysed, and intracellular contents replated and developed to confirm presence of Zn\(^{2+}\).

**6.3.4 Crystallization of Synthesized Mg\(^{2+}\) and Zn\(^{2+}\)Complexes**

While the combination of both solution- and solid-state analyses has proved advantageous in determining complex composition and ligand coordination mode, attaining crystal structures of the produced compounds is ideal. Crystallization attempts in the form of slow evaporation, vapor-liquid diffusion, liquid-liquid diffusion, and sol-gel will be utilized. Additionally, different solvent systems will be employed to take advantage of differences in compound solubility.

While Mg\(^{2+}\)-complex crystallization is complicated by the substantial amount of disorder afforded to said complexes attributed to rapid hydration, the successful crystallization of Zn\(^{2+}\) complexes should be much more fruitful given promising results for Zn\(^{2+}\)-complex crystallization.
presented previously in this chapter and a decrease in overall disorder imparted by water.

6.4. References


(27) Thompson, K. H.; Barta, C. A.; Orvig, C. Metal Complexes of Maltol and Close Analogues}

125

(28) Barret, M. C.; Mahon, M. F.; Molloy, K. C.; Steed, J. W.; Wright, P. Synthesis and Structural Characterization of Tin (II) and Zinc (II) Derivatives of Cyclic r-Hydroxyketones, Including the Structures of Sn(Maltol)\(_2\), Sn(Tropolone)\(_2\), Zn(Tropolone)\(_2\), and Zn(Hinokitiol)\(_2\). *Synthesis (Stuttg)*. **2001**, *2* (ii), 4384–4388.


Appendices

Appendix A: Publications


Appendix B: Crystallographic Information Files (USB)

1. [Zn$_2$(HGG$^{1-}$)$_4$(H$_2$O)$_2$].5.32H$_2$O, 5

2. Zn(C$_6$H$_6$O$_3$)$_2$Cl$_2$, 6
BIOGRAPHICAL SKETCH
Provide the following information for the Senior/key personnel and other significant contributors. Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Derek R. Case

eRA COMMONS USER NAME (credential, e.g., agency login): DRCASE

POSITION TITLE: Doctoral Candidate

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE (if applicable)</th>
<th>Completion Date MM/YYYY</th>
<th>FIELD OF STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Utica College, New York</td>
<td>B.S.</td>
<td>05/2017</td>
<td>Chemistry (ACS Certified)</td>
</tr>
<tr>
<td>Syracuse University, New York</td>
<td>M. Phil.</td>
<td>05/2019</td>
<td>Inorganic Chemistry</td>
</tr>
<tr>
<td>Syracuse University, New York</td>
<td>Ph.D. (ABD)</td>
<td>08/2021</td>
<td>Inorganic Chemistry</td>
</tr>
</tbody>
</table>

A. **Personal Statement**

I am an inorganic chemist with interests in transition metal organic-inorganic hybrid small molecules for photocatalysis, and alkali metal amino acid chelates for the synthesis of novel nutraceuticals. I have a background in inorganic synthesis, photocatalysis, amino acid chelates, cell growth and culture propagation, X-Ray crystallography, solid-state characterization, NMR characterization, and assay development for the quantification of micronutrient uptake in a gastrointestinal model. In 2017, I graduated from Utica College, *cum laude*, with a B.S. (ACS Certified) in chemistry. After graduating from Utica, I attended
Syracuse University, where I subsequently achieved my M. Phil., and will defend my Ph.D. dissertation in December, 2021 on magnesium chelates and hypomagnesemia.

B. Positions and Honors

<table>
<thead>
<tr>
<th>Year</th>
<th>Award/Recognition</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013-2017</td>
<td>Dean’s List (4x)</td>
</tr>
<tr>
<td>2016</td>
<td>Keller’s Scholarship</td>
</tr>
<tr>
<td>2016</td>
<td>Outstanding Chemistry TA Leadership Award</td>
</tr>
<tr>
<td>2016-2017</td>
<td>TA Award (2x)</td>
</tr>
</tbody>
</table>

Invited talk at Balchem Corporation (Ogden, Utah) July 2021
Poster at 41st Northeast Regional Meeting of the American Chemical Society, Binghamton University, NY 2016

C. Contributions to Science

1. **2016-2017:** Synthesis and characterization of a series of methyl-1 indanone derivatives

   As an undergraduate student at Utica College, I partook in research concerned with synthesizing and characterizing a series of methyl-1-indanone derivatives as possible biomimetics. The majority of my contribution was focused on characterizing molecules synthesized by Kyle S. Podolak via NMR, GC/S, FT-IR and UV-Vis. Preliminary investigations indicated that product synthesis was relatively facile, and characterization provided corresponding evidence indicating product formation. These findings were reported in a poster presentation at the 41st Northeast Regional Meeting of the American Chemical Society, at Binghamton University.

   Podolak, Kyle S.; **Case, Derek R.**; Abbot, Gabrielle; Pulliam, Curtis R.; Boucher, Michelle A. From Abstracts, 41st Northeast Regional Meeting of the American Chemical Society, Binghamton, NY, United States, October 5-8 (2016), NERM-461.

2. **2017:** Evaluation of gold nanoparticle uptake in Brine Shrimp

   As an undergraduate student at Utica College, I evaluated the uptake of gold nanoparticles in brine shrimp as a means of evaluating possible heavy metal waste uptake in aquatic populations. Evaluation of gold nanoparticle uptake was carried out via atomic absorption with a gold lamp. Preliminary data indicated nanoparticle uptake in brine shrimp samples at varying concentrations.

3. **2017-2020:** Synthesis of photocatalytic rhenium (l)-naphthalimide complexes and metal diphosphonates
As a graduate research student, I started by developing synthetic approaches to novel naphthalimide small molecules to be used as photosensitizers in rhenium (I)-naphthalimide catalyst complexes. Characterization of a novel naphthalimide ligand was conducted, and the subsequent photocatalytic activity of the rhenium (I) complex was evaluated. Preliminary photocatalytic data showed photo-dependent reduction of carbon dioxide to carbon monoxide, indicating catalyst success. Metal diphosphonates were synthesized hydrothermally and characterized via SC-XRD, EA, and FT-IR.


4. 2021-Present:

As a graduate student, I worked in tandem with Balchem Corp. (Ogden, UT) to develop alkali metal/amino acid chelates as novel nutraceuticals. These studies focused on the use of magnesium, zinc and calcium salts and diglycine, triglycine and other amino acids as starting materials to form entropically favored M2+-L complexes with favorable solubility and bioavailability. Full solid-state characterization was carried out, and a subsequent litany of cell studies indicated uptake of M2+-L complexes relative to that of M2+ standard salts. These findings resulted in multiple publications and patents.


D. Additional Information: Research Support and/or Scholastic Performance

TEACHING EXPERIENCE
• Teaching Assistant, Department of Chemistry, Syracuse University (2017-2019)
  - For Introductory Chemistry lecture and lab and Forensics lab (CHE 103, 106, 107, 113, 116)
• Teaching Assistant, Department of Chemistry, Utica College (2015-2017)
  - For General Chemistry Laboratory (for majors and non-majors, CHE 211 and 212)
• Peer Tutor, Utica College (2015 – 2017)
  - General Chemistry Lab and Lecture (for majors and non-majors, CHE 211 and 212)

RESEARCH MENTORING
• Alyssa Spear – Graduate Student, Syracuse University (2019-present)
• Bria Hernandez – Undergraduate student, Northeastern University (REU 2019)
• Julian E. Minichelli – Undergraduate, Syracuse University (2018-2020)
• Alexander O. Aguirre – Undergraduate, Syracuse University (2018-2020)

EXTERNAL SERVICE
• Utica College (ACS Affiliate) Chemistry Club President (June 2016 – May 2017)
• Volunteer, Science Fair Judge, Utica College (2016)

PROFESSIONAL DEVELOPMENT
• Mentee, Resumé and CV Development Workshop (October 2016)
  - Hosted at 41st Northeast Regional Meeting of the American Chemical Society

PROFESSIONAL MEMBERSHIPS
• Member, American Association for the Advancement of Science (2020)
• Member, American Chemical Society (2015-present)
• Member, Whose Who of American Scholars (2013)