A Library of Coumarin-Enaminone Chemodosimeters for the Detection of Analytes

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A LIBRARY OF COUMARIN ENAMINOE CHEMODOSIMETERS
FOR THE DETECTION OF ANALYTES

by

Aaron Berrell Davis

A Dissertation
Submitted to the Graduate School
and the Department of Chemistry and Biochemistry
at The University of Southern Mississippi
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for the Degree of Doctor of Philosophy

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August 2016
ABSTRACT

A LIBRARY OF COUMARIN ENAMINOE CHEMODOSIMETERS

FOR THE DETECTION OF ANALYTES

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August 2016

Many anions and metal ions are of biological and environmental importance. This work describes our attempts to synthesize molecular probes to specifically target cyanide, zinc, cadmium, and mercury as these species can often result in negative effects to the environment and the human body.

The work in this dissertation, describes the synthesis of a family of coumarin-enamine chemodosimeters, in a straightforward synthetic procedure, the reaction between 7-(diethylamino)-4-hydroxycoumarin and a primary amine. The work presented in this dissertation demonstrates that these molecules can play dual roles, and therefore the work is split into two distinct parts: Part 1 describes one role of these molecular probes. In this first section three members of the coumarin-enamine family showed selectivity towards the cyanide ion over other anions studied (F\(^-\), Cl\(^-\), Br\(^-\), I\(^-\), NO\(_3\)\(^-\), OAc\(^-\), H\(_2\)PO\(_4\)\(^-\), HSO\(_4\)\(^-\), BF\(_4\)\(^-\), N\(_3\)\(^-\), SCN\(^-\), ClO\(_4\)\(^-\), and OH\(^-\)) in DMSO, with a detection limit as low as 4.2 ppb. The kinetics of the Michael addition was also investigated with a fluorescent response time calculated to be approximately (t\(_{1/2}\) = 20 s).

The second role of these molecular probes was to investigate and decimate structurally similar metal salts (Na\(^+\), Mg\(^{2+}\), Ca\(^{2+}\), Cr\(^{3+}\), Mn\(^{2+}\), Fe\(^{2+}\), Fe\(^{3+}\), Co\(^{2+}\), Ni\(^{2+}\), Cu\(^{2+}\), Zn\(^{2+}\), Pd\(^{2+}\), Ag\(^+\), Cd\(^{2+}\), Hg\(^{2+}\), and Pb\(^{2+}\)), in particular the chloride and acetate salts. A univariate approach was used. The participation of the acetate anion is key for the probe
to selectively bind the metal via the enaminone chelating motif. These molecular probes showed preference to zinc acetate salt whereby the chromophore utilized a duel signaling mechanism, inhibition of ESIPT which either quenches the fluorescence signal or shifts the band in the blue direction. Then upon the addition of the zinc(II) ion, a CHEF mechanism increases the fluorescence signal upon the coordination of the metal ion. The limit of detection was calculated to be 7.4 ppb.

This work extensively used various analytical methods to detect and monitor these analytes in particular UV-Vis and fluorescence spectroscopy. Additional techniques, for example NMR, IR, ESI-MS, and X-Ray were all utilized to help our understanding of the coordination environment of the molecular probe and the metal ions.
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# TABLE OF CONTENTS

ABSTRACT ................................................................................................................................. ii

ACKNOWLEDGMENTS ................................................................................................................ iv

LIST OF TABLES ......................................................................................................................... ix

LIST OF ILLUSTRATIONS .......................................................................................................... xi

LIST OF SCHEMES ...................................................................................................................... xix

LIST OF ABBREVIATIONS ......................................................................................................... xxiii

CHAPTER I - INTRODUCTION ................................................................................................. 1

1.1 Analytes of Interest ............................................................................................................. 2

1.1.1 Cyanide .............................................................................................................................. 2

1.1.2 Cations .............................................................................................................................. 4

1.1.2.1 Zinc ............................................................................................................................... 5

1.1.2.2 Cadmium ....................................................................................................................... 6

1.1.2.3 Mercury ......................................................................................................................... 6

1.1.2.4 Challenges of detecting the ions Zn$^{2+}$, Cd$^{2+}$, and Hg$^{2+}$ ....................................... 7

1.2 Coumarin Synthesis ........................................................................................................... 8

1.2.1 4-Hydroxycoumarin .......................................................................................................... 10

1.3 The Binding Unit: Azomethine/Enaminone ..................................................................... 12

1.4 Optical Spectroscopy ......................................................................................................... 16

1.4.1 Photoluminescence ......................................................................................................... 18
1.4.2 Photophysical Properties of Coumarin ......................................................... 20

1.5 Signaling Mechanisms ......................................................................................... 27

1.5.1 Single Channel Monitoring ........................................................................... 28

1.5.2 Hypsochromic and Bathochromic Shift Systems ......................................... 28

1.5.3 Molecular Probes that Display Hypochromic Shift ..................................... 29

1.5.4 Molecular Probes that Display Hyperchromic Shift .................................... 31

1.5.5 Dual Channel Monitoring ............................................................................. 34

1.5.6 Molecular Probes that Form Excimers and Exciplexes ............................. 35

1.5.7 Excited State Intramolecular Proton Transfer ............................................ 37

1.6 Chemodosimeters ............................................................................................. 44

1.7 Summary ........................................................................................................... 54

1.8 Hypothesis ......................................................................................................... 56

CHAPTER II – CHEMOSIMETERS FOR THE DETECTION OF NC IONS ........ 57

2.1 Introduction ....................................................................................................... 57

2.1.1 Displacement Assay ...................................................................................... 57

2.1.2 Direct Coordination to a Lewis Acid Site ................................................... 62

2.1.2.1 Direct 1,2-addition .................................................................................. 63

2.1.2.2 Direct 1,4-Addition ................................................................................. 69

2.1.3 Hypothesis .................................................................................................... 77

2.2 Experimental Data ............................................................................................ 78
2.2.1 General Synthesis of Probes **2.15** and **2.16** ................................................................. 78

2.2.2 X-Ray Studies of Compound **2.16** .................................................................................. 80

2.2.3 NMR Studies of Probes **2.15** and **2.16** ................................................................ 81

   2.2.3.1 2.16-CN adduct ...................................................................................................... 96

   2.2.3.2 NMR titrations ..................................................................................................... 107

2.2.4 Solvent Studies of Probes **2.15** and **2.16** ............................................................... 122

2.2.5 Colorimetric Response of Probe **2.15** ....................................................................... 125

2.2.6 Colorimetric Response of Probe **2.16** ....................................................................... 133

2.2.7 Fluorescence Response of Probe **2.15** .................................................................. 142

2.2.8 Fluorescence Response of Probe **2.16** .................................................................. 148

2.2.9 Additional Evidence ................................................................................................... 157

2.3 Summary .......................................................................................................................... 168

2.4 General Experimental Procedure .................................................................................... 169

CHAPTER III – THE SCREENING OF METAL IONS ............................................................ 176

3.1 Introduction .......................................................................................................................... 176

   3.1.1 Hypothesis and Rationale ......................................................................................... 185

3.2 Experimental Data ............................................................................................................. 186

   3.2.1 General Synthesis of Probes **2.15, 2.16, 3.6-3.8** and Their Metal Complexes ......... 186

   3.2.2 Solid State Studies of **2.15, 2.16, and 3.6-3.8** and Their Metal Complexes .. 188

vii
3.2.2.1 X-Ray Crystallography ................................................................. 188

3.2.2.2 Infrared (IR) Spectroscopy ............................................................. 197

3.2.3 Rationale Used in the Solution Based Experiments .......................... 204

3.2.4 NMR Studies of 2.15, 3.6, 3.7, 2.16, and 3.8 ................................. 212

3.2.5 Mass Spectrometry and Binding Ratios ........................................... 232

3.2.6 UV-Vis Response with Probes 2.15, 2.16 and 3.6-3.8 Towards MCl_x Salts ................................................................. 242

3.2.7 Fluorescence Response with Probes 2.15, 2.16 and 3.6-3.8 Towards MClx Salts ........................................................................... 251

3.2.8 UV-Vis Response from Probes 2.15, 2.16 and 3.6-3.8 Towards Metal Acetate Salts ........................................................................... 264

3.2.9 Fluorescence Response with Probes 2.15, 2.16 and 3.6-3.8 Towards MOAcx Salts ........................................................................... 277

3.2.10 Detection Limits ................................................................................. 324

3.3 Summary ............................................................................................... 328

3.4 Experimental Procedure .................................................................... 330

CHAPTER IV – CONCLUSION ................................................................... 337

REFERENCES ............................................................................................. 339
LIST OF TABLES

Table 1 Selected Properties for the Cations Mg$^2^+$, Ca$^2^+$, Zn$^2^+$, Cd$^2^+$ and Hg$^2^+$ ......................... 8
Table 2 Advantages and Disadvantages of Various Analytical Detection Methods .......... 17
Table 3 Solvent Effect on Photophysical Properties of Compounds 1.16-1.18 .................. 24
Table 4 Solvent Effect on Photophysical Properties of Compounds 1.19-1.22 .............. 26
Table 5 Photophysical Properties of Compounds 1.26-1.30 ............................................. 38
Table 6 $^1$H and $^{13}$C NMR Chemical Shifts of Compound 2.16 ................................. 87
Table 7 $^1$H and $^{13}$C NMR of Compound 2.16-CN adduct ........................................... 97
Table 8 $^1$H and $^{13}$C NMR of Compound 2.16 and 2.16-TEACN ................................. 113
Table 9 Absorption and Fluorescence Emission of Compound 2.15 in Various Solvents
.................................................................................................................................................. 123
Table 10 Absorption and Fluorescence Emission of Compound 2.16 in Various Solvents
.................................................................................................................................................. 123
Table 11 Absorbance ($\lambda_{\text{max}}$) and molar absorptivity for probes 2.15 and 2.16 ....... 125
Table 12 Selected pKa values in H$_2$O and DMSO ................................................................. 126
Table 13 Molecular Orbital Energies for the Stable 2.16 Structures .................................. 165
Table 14 Frontier Molecular Orbital Energies During C-CN Bond Formation .................. 167
Table 15 X-Ray Crystallography Data ..................................................................................... 172
Table 16 Selected $^1$H and $^{13}$C NMR chemical shifts and mass of probes 3.6-3.8 ....... 187
Table 17 Selected bond distances and angles of probes 2.15, 2.16, and 3.6-3.8 .......... 193
Table 18 Selected bond distances and angles of probe 2.15 and [Zn(2.15)$_2$] ............... 196
Table 19 The $\Delta$ABS at 330 nm by probes 2.15, 2.16, and 3.6-3.8 with metal chloride salts
.................................................................................................................................................. 250
Table 20 The $\Delta_{\text{ABS}}$ at 400 nm by probes 2.15, 2.16, and 3.6-3.8 with metal chloride salts

Table 21 The $\Delta_{\text{ABS}}$ at 330 nm by probes 2.15, 2.16, and 3.6-3.8 with metal acetate salts

Table 22 The $\Delta_{\text{ABS}}$ at 400 nm by probes 2.15, 2.16, and 3.6-3.8 with metal acetate salts

Table 23 The $\Delta_{\text{EM}}$ at 514 nm by probes 2.15, 2.16, and 3.6-3.8 with metal acetate salts
LIST OF ILLUSTRATIONS

Figure 1. The IUPAC numbering of (1.0), (1.1), and (1.2) .................................................. 9
Figure 2. Typical geometries of metal-ligand ML₂ complexes. (adapted from)⁷⁴ ........... 14
Figure 3. Rigid C(7) coumarin derivatives. (adapted from)¹¹²,¹¹³ .................................. 24
Figure 4. Flexible C(7) coumarin derivatives. (adapted from)¹¹²,¹¹³-¹¹⁴ ......................... 26
Figure 5. Structures of compounds 1.26-1.30. (adapted from)¹¹⁶,¹¹⁷ .......................... 38
Figure 6. The various isomers and tautomers of compound 2.16. ................................. 79
Figure 7. X-Ray crystal structure of compound 2.16. .................................................. 80
Figure 8. The structure and the numbering system for compound 2.15 .......................... 82
Figure 9. The ¹H NMR spectrum of compound 2.15 in CDCl₃ ........................................ 83
Figure 10. ¹H NMR stack plot of probe 2.15 (top) and compound 1.2 (bottom) in DMSO-
           d₆. ......................................................................................................................... 84
Figure 11. The ¹³C NMR spectrum of compound 2.15 in CDCl₃ ............................... 85
Figure 12. The structure and the numbering system for compound 2.16 ...................... 86
Figure 13. ¹H NMR of compound 2.16 in a mixture of DMSO-d₆:CDCl₃ (1:7) .............. 88
Figure 14. ¹³C NMR of probe 2.16 in a mixture of DMSO-d₆:CDCl₃ (1:7) .................. 90
Figure 15. ¹³C APT of probe 2.16 in a mixture of DMSO-d₆:CDCl₃ (1:7) ..................... 91
Figure 16. HSQC of compound 2.16 in a mixture of DMSO-d₆:CDCl₃ (1:7) .......... 93
Figure 17. HMBC spectrum of compound 2.16 in a mixture of DMSO-d₆:CDCl₃ (1:7). 94
Figure 18. ROESY spectrum of compound 2.16 in a mixture of DMSO-d₆:CDCl₃ (1:7).
           ......................................................................................................................... 95
Figure 19. Full ¹H NMR spectrum of the 2.16-CN⁻ adduct. ........................................ 99
Figure 20. Full ¹³C NMR spectrum of 2.16-CN⁻ adduct .............................................. 100
Figure 21. $^{13}$C APT of 2.16-CN$^-$ adduct in a mixture of DMSO-$d_6$:CDCl$_3$ (1:7) .......... 101
Figure 22. HSQC of 2.16-CN$^-$ adduct in a mixture of DMSO-$d_6$:CDCl$_3$ (1:7) .......... 103
Figure 23. Expanded HSQC of 2.16-CN$^-$ adduct in a mixture of DMSO-$d_6$:CDCl$_3$ (1:7). .............................................................. 104
Figure 24. HMBC of 2.16-CN$^-$ adduct in a mixture of DMSO-$d_6$:CDCl$_3$ (1:7) .......... 105
Figure 25. ROESY of 2.16-CN$^-$ adduct in a mixture of DMSO-$d_6$:CDCl$_3$ (1:7). .......... 106
Figure 26. $^1$H NMR titration of probe 2.16 with KCN in DMSO-$d_6$............................... 109
Figure 27. $^1$H NMR spectra of 2.16 (bottom) and 2.16-TEACN (top) in CDCl$_3$ ............. 111
Figure 28. $^{13}$C NMR spectra of 2.16 (bottom) and 2.16-TEACN (top) in CDCl$_3$ .......... 112
Figure 29. $^1$H NMR titration of probe 2.16 with TBAF in CDCl$_3$.................................. 115
Figure 30. $^1$H NMR titration of probe 2.16 with TBAOAc in CDCl$_3$............................. 117
Figure 31. $^1$H NMR of compound 2.16 with TBAF, TBAOAc, and TEACN. .................. 119
Figure 32. $^1$H NMR titration of probe 2.15 with TBAOAc in CDCl$_3$............................. 121
Figure 33. Compound 2.16 in various solvents......................................................... 124
Figure 34. UV-Vis titration with probe 2.15 and Cl$^-$ ions in DMSO. ............................ 127
Figure 35. (Top) TBAOAc (bottom) TBAF absorbance titration with probe 2.15............ 130
Figure 36. Cyanide addition to probe 2.15 UV-Vis titrations and isotherms.................. 132
Figure 37. A barchart of probe 2.15 and two equivalents of various anions.................... 133
Figure 38. UV-Vis titration with probe 2.16 and NO$_3^-$ ions in DMSO......................... 134
Figure 39. Probe 2.16 UV-Vis titration with 1. F$^-$, 2. OAc$^-$, 3. H$_2$PO$_4^-$.................... 136
Figure 40. Isotherm and absorbance titration of 2.16 with octylamine in DMSO.......... 138
Figure 41. Cyanide addition to probe 2.16 UV-Vis titrations and isotherms.................. 140
Figure 42. A barchart of probe 2.16 and two equivalents of various anions................... 141
Figure 43. Fluorescence titration with probe 2.15 and Br⁻ ions in DMSO...................... 143

Figure 44. Probe 2.15 fluorescence titration with 1. F⁻, 2. OAc⁻, 3. H₂PO₄⁻ ...................... 144

Figure 45. Isotherm and fluorescence titration of 2.15 with octylamine in DMSO...... 145

Figure 46. Cyanide addition to probe 2.15 (Left) Fl titrations (Right) isotherms........ 147

Figure 47. Fluorescence titration with probe 2.16 and Br⁻ ions in DMSO..................... 148

Figure 48. Probe 2.16 Fl. titration and isotherms with ions 1. F⁻, 2. OAc⁻, 3. H₂PO₄⁻ ... 151

Figure 49. Isotherm and fluorescence titration of 2.16 with octylamine in DMSO...... 153

Figure 50. Cyanide addition to probe 2.16 (Left) Fl titrations (Right) isotherms........ 155

Figure 51. A fluorescence bar chart of probe 2.16 with various anions....................... 156

Figure 52. Image of probe 2.16 with two equivalents of various anions. .................... 157

Figure 53. KCN Job's plots with probes 2.15 (top) and 2.16 (bottom)......................... 158

Figure 54. The 2.16 structures for the observed mass spectra shown below. ............... 159

Figure 55. ESI-MS studies of probe 2.16 under various conditions. ....................... 160

Figure 56. Mass spectrum of the 2.16-CN⁻ adduct............................................. 161

Figure 57. Cyanide detection limits with probes 2.15 and 2.16 in DMSO. ................. 163

Figure 58. The kinetics of probe 2.16 with one equivalents of KCN.......................... 164

Figure 59. Structures of cyanide adducts used for data shown in Table 2.9.............. 167

Figure 60. Molecular orbital interactions as cyanide attacks probe 2.15............... 168

Figure 61. ¹H NMR of probe 2.16 in CDCl₃ (top) and DMSO-d₆ (bottom)............... 173

Figure 62. The numbering system used for probes 2.15, 2.16, and 3.6-3.8............. 187

Figure 63. X-Ray crystal structures of probes: 2.15, 3.6, 3.7, and 3.8............... 189

Figure 64. The edge (left) and tail (right) view of the compound 2.15 crystal structure.
........................................................................................................................................ 190

xiii
Figure 65. The tail view of the compound 3.6 crystal structure…………………………… 190
Figure 66. The tail view of the deviation from planarity in the compound 3.7 crystal. . 191
Figure 67. The tail view of the deviation from planarity in the compound 3.8 crystal. . 191
Figure 68. The X-Ray crystal structure of the isolated [Zn(2.15)₂] complex……………… 194
Figure 69. The zinc hydrolyzed 3.8 complex in an O₆ geometry…………………………… 197
Figure 70. The IR spectra of the probe 2.15 and the [Zn(2.15)₂]…………………………… 198
Figure 71. The IR spectra (1900-500 cm⁻¹) of 2.15 and [Zn(2.15)₂] complex……………. 199
Figure 72. The IR spectra of 3.8 and [Zn(3.8)₂] complex……………………………………… 200
Figure 73. The IR spectra (1825-450 cm⁻¹) of 3.8 and [Zn(3.8)₂] complex………………… 201
Figure 74. The IR spectra of probe 2.16 and the [Zn(2.16)₂] complex………………………… 202
Figure 75. The IR spectra of probe 2.16 and the [Zn(2.16)] complex. …………………… 203
Figure 76. UV-Vis spectra of probe 3.8 with Zn(OAc)₂ in various solvents.……………… 205
Figure 77. The Cd(OAc)₂ fluorescence titration and binding isotherm with probe 3.8. 207
Figure 78. Zn²⁺ and Cd²⁺ acetate emission titrations and isotherms with probe 3.8….. 208
Figure 79. TBAOAc fluorescence titration and binding isotherm with probe 3.8. ……. 210
Figure 80. ZnCl₂ fluorescence titration and binding isotherm with TBAOAc and probe 3.6…………………………………………………………………………………………… 211
Figure 81. ¹H NMR spectrum of compound 3.6 in CDCl₃ (*) is the solvent peak…… 213
Figure 82. ¹³C NMR NMR spectrum of compound 3.6 in CDCl₃…………………………… 214
Figure 83. ¹H NMR spectrum of compound 3.7 in CDCl₃ (*) is the solvent peak………. 215
Figure 84. ¹³C NMR spectrum of probe 3.7 in CDCl₃………………………………………… 216
Figure 85. ¹H NMR spectrum of compound 3.8 in CDCl₃ (*) is the solvent peak………. 217
Figure 86. ¹³C NMR spectrum of compound 3.8 in CDCl₃…………………………………. 218
Figure 87. $^1$H NMR spectra of probe 2.15 and the isolated [Zn(2.15)₂] complex........ 219

Figure 88. $^{13}$C NMR spectra of probe 2.15 and the isolated [Zn(2.15)₂] complex....... 220

Figure 89. $^{13}$C NMR-APT spectra of probe 2.15 and the isolated [Zn(2.15)₂] complex. 222

Figure 90. $^1$H NMR spectra of probe 3.8 and the isolated [Zn(3.8)₂] complex.......... 223

Figure 91. $^{13}$C NMR spectra of probe 3.8 and the isolated [Zn(3.8)₂] complex.......... 224

Figure 92. $^{13}$C NMR APT spectra of probe 3.8 and the isolated [Zn(3.8)₂] complex... 225

Figure 93. The Zn(OAc)$_2$ $^1$H NMR titration with probe 2.16 with in DMSO-d$_6$........ 228

Figure 94. The Zn(OAc)$_2$ $^1$H NMR titration with probe 3.8 in DMSO-d$_6$............... 231

Figure 95. The negative mode mass spectrum of the isolated [Zn(3.8)₂] complex....... 233

Figure 96. The negative mode MS-MS of the isolated [Zn(3.8)₂] complex................. 234

Figure 97. A plausible structure for the 473 m/z signal. ........................................... 234

Figure 98. Job’s plot with probe 3.6 and Zn(OAc)$_2$ and possible coordination......... 236

Figure 99. Job’s plot between probe 3.7 and Zn(OAc)$_2$ and possible coordination...... 237

Figure 100. Job’s plot between probe 3.7 and Hg(OAc)$_2$ and possible coordination. ... 238

Figure 101. Job’s plot between probe 3.8 and Zn(OAc)$_2$ and possible coordination.... 239

Figure 102. Job’s plot between probe 2.16 and Zn(OAc)$_2$ and possible coordination... 240

Figure 103. Job’s plot between probe 2.16 and Zn$^{2+}$ ions and possible coordination.... 241

Figure 104. UV-Vis screening of metal chloride salts with probe 2.15. ..................... 243

Figure 105. UV-Vis screening of metal chloride salts with probe 2.16. ...................... 244

Figure 106. UV-Vis screening of metal chloride salts with probe 3.7. ....................... 245

Figure 107. UV-Vis screening of metal chloride salts with probe 3.6. ....................... 246

Figure 108. UV-Vis screening of metal chloride salts with probe 3.8. ....................... 247

Figure 109. ZnCl$_2$ UV-Vis titration with probe 2.15 in DMSO............................... 248
Figure 110. ZnCl$_2$ UV-Vis titration with probe 3.8 in DMSO. ........................................... 249
Figure 111. Fluorescence screening of metal chloride salts with probe 2.15 .................. 252
Figure 112. Fluorescence screening of metal chloride salts with probe 2.16 .............. 254
Figure 113. Fluorescence screening of metal chloride salts with probe 3.7 .............. 255
Figure 114. Fluorescence screening of metal chloride salts with probe 3.6 .............. 256
Figure 115. Fluorescence screening of metal chloride salts with probe 3.8 .............. 257
Figure 116. ZnCl$_2$ fluorescence titration with probe 2.15 ($\lambda_{ex}$ 408 nm) .......... 258
Figure 117. ZnCl$_2$ fluorescence titration with probe 2.16 ($\lambda_{ex}$ 408 nm) .......... 259
Figure 118. ZnCl$_2$ fluorescence titration with probe 3.7 ($\lambda_{ex}$ 408 nm) .......... 260
Figure 119. ZnCl$_2$ fluorescence titration with probe 3.6 ($\lambda_{ex}$ 408 nm) .......... 261
Figure 120. ZnCl$_2$ fluorescence titration with probe 3.8 ($\lambda_{ex}$ 408 nm) .......... 262
Figure 121. Fluorescence bar chart with all probes and metal chloride salts .......... 264
Figure 122. UV-Vis screening of metal acetate salts with probe 2.15 .................. 266
Figure 123. UV-Vis screening of metal acetate salts with probe 2.16 .............. 267
Figure 124. UV-Vis screening of metal acetate salts with probe 3.7 .............. 268
Figure 125. UV-Vis screening of metal acetate salts with probe 3.6 .............. 269
Figure 126. UV-Vis screening of metal acetate salts with probe 3.8 .............. 270
Figure 127. Zn(OAc)$_2$ isotherm and UV-Vis titration with probe 2.16 in DMSO ...... 271
Figure 128. Zn(OAc)$_2$ isotherm and UV-Vis titration with probe 3.6 in DMSO ...... 272
Figure 129. Zn(OAc)$_2$ isotherm and UV-Vis titration with probe 3.8 in DMSO ...... 274
Figure 130. Fluorescence screening of metal acetate salts with probe 2.15 ($\lambda_{ex}$ 408 nm). .......................................................................................................................... 278
Figure 131. Fluorescence screening of metal acetate salts with probe 2.16 ($\lambda_{ex}$ 408 nm).

.................................................................279

Figure 132. Fluorescence screening of metal acetate salts with probe 3.7 ($\lambda_{ex}$ 408 nm).

.................................................................280

Figure 133. Fluorescence screening of metal acetate salts with probe 3.6 ($\lambda_{ex}$ 408 nm).

.................................................................281

Figure 134. Fluorescence screening of metal acetate salts with probe 3.8 ($\lambda_{ex}$ 408 nm).

.................................................................282

Figure 135. Cu(OAc)$_2$ isotherm and fluorescence titration with probe 2.15........... 283

Figure 136. Mg(OAc)$_2$ fluorescence titration with probe 2.15. .............................. 284

Figure 137. Ca(OAc)$_2$ fluorescence titration with probe 2.15.............................. 285

Figure 138. Hg(OAc)$_2$ fluorescence titration with probe 2.15.............................. 285

Figure 139. Cd(OAc)$_2$ fluorescence titration with probe 2.15.............................. 286

Figure 140. Zn(OAc)$_2$ isotherm and fluorescence titration with probe 2.15........... 288

Figure 141. Mg(OAc)$_2$ fluorescence titration with probe 2.16. .............................. 289

Figure 142. Ca(OAc)$_2$ fluorescence titration with probe 2.16.............................. 290

Figure 143. Hg(OAc)$_2$ fluorescence titration with probe 2.16.............................. 291

Figure 144. Cd(OAc)$_2$ isotherm and fluorescence titration with probe 2.16........... 292

Figure 145. Zn(OAc)$_2$ isotherm and fluorescence titration with probe 2.16........... 294

Figure 146. Mg(OAc)$_2$ fluorescence titration with probe 3.7. .............................. 295

Figure 147. Ca(OAc)$_2$ fluorescence titration with probe 3.7.............................. 296

Figure 148. Hg(OAc)$_2$ fluorescence titration with probe 3.7.............................. 297

Figure 149. Cd(OAc)$_2$ isotherm and fluorescence titration with probe 3.7........... 298
Figure 150. Zn(OAc)$_2$ isotherm and fluorescence titration with probe 3.7 ............... 299
Figure 151. Mg(OAc)$_2$ fluorescence titration with probe 3.6. .............................. 300
Figure 152. Ca(OAc)$_2$ fluorescence titration with probe 3.6 ............................... 301
Figure 153. Hg(OAc)$_2$ isotherm and fluorescence titration with probe 3.6. ............. 302
Figure 154. Cd(OAc)$_2$ isotherm and fluorescence titration with probe 3.6 .......... 303
Figure 155. Zn(OAc)$_2$ isotherm and fluorescence titration with probe 3.6 .......... 305
Figure 156. NaOAc fluorescence titration with probe 3.8. .................................... 306
Figure 157. AgOAc fluorescence titration with probe 3.8 .................................... 307
Figure 158. Fe(OAc)$_2$ fluorescence titration with probe 3.8 ............................... 308
Figure 159. Mn(OAc)$_2$ fluorescence titration with probe 3.8 .............................. 309
Figure 160. Cu(OAc)$_2$ isotherm and fluorescence titration with probe 3.8 .......... 310
Figure 161. Co(OAc)$_2$ fluorescence titration with probe 3.8 ............................... 311
Figure 162. Ni(OAc)$_2$ isotherm and fluorescence titration with probe 3.8 .......... 312
Figure 163. Pd(OAc)$_2$ fluorescence titration with probe 3.8 ............................... 313
Figure 164. Pb(OAc)$_2$ fluorescence titration with probe 3.8 ............................... 314
Figure 165. Mg(OAc)$_2$ isotherm and fluorescence titration with probe 3.8 .......... 315
Figure 166. Ca(OAc)$_2$ isotherm and fluorescence titration with probe 3.8 .......... 316
Figure 167. Hg(OAc)$_2$ isotherm and fluorescence titration with probe 3.8 .......... 318
Figure 168. Zn(OAc)$_2$ isotherm and fluorescence titration with probe 3.8 .......... 319
Figure 169. Cd(OAc)$_2$ isotherm and fluorescence titration with probe 3.8 .......... 321
Figure 170. Probe 3.8 Zn$^{2+}$ ions detection limit. ............................................. 325
Figure 171. Probe 3.8 Cd$^{2+}$ ions detection limit. ............................................ 327
LIST OF SCHEMES

Scheme 1. Cartoon chemodosimeter of a coumarin based molecular probe. ....................... 2

Scheme 2. Electron transport pathway: 1. Uninhibited, 2. Inhibition due to NC ions
(adapted from)\textsuperscript{16} ........................................................................................................ 3

Scheme 3. The pH equilibrium between HCN and NC\textsuperscript{-} ions under different pH
conditions. (adapted from)\textsuperscript{24} .................................................................................. 4

Scheme 4. Synthesis of compounds 1.4 and 1.7. (adapted from)\textsuperscript{63,65} ....................... 10

Scheme 5. Synthesis of compounds 1.9\textsuperscript{a-c} via the Pechmann condensation reaction.
(adapted from)\textsuperscript{67} ........................................................................................................ 11

Scheme 6. Synthesis of compound 1.12 via the ZnCl\textsubscript{2}:POCl\textsubscript{3} route. (adapted from)\textsuperscript{62} .... 11

Scheme 7. Synthesis of 1.2 via the activated aryl malonate route. (adapted from)\textsuperscript{13,64,66} 12

Scheme 8. Possible modes of metal coordination. (adapted from)\textsuperscript{70,71} ......................... 13

Scheme 9. Different modes of coordination, where M\textsuperscript{8+} (n = 1, 2, 3). ......................... 15

Scheme 10. Jablonski diagram. (adapted from)\textsuperscript{102,103,105-107} ........................................ 20

Scheme 11. A directional layout of the possible shifts. ......................................................... 21

Scheme 12. ICT formation and hydrogen bonding of compound 1.15. (adapted from)\textsuperscript{117,122}
.................................................................................................................................................. 23

Scheme 13. TICT formation of coumarin derivative 1.19. (adapted from)\textsuperscript{120,122} .......... 25

Scheme 14. Cu\textsuperscript{2+} ion interaction with an ICT based probe (1.23\textsuperscript{b}). (adapted from)\textsuperscript{132} .... 29

Scheme 15. Coordination of Cu\textsuperscript{2+} ions by probes 1.23\textsuperscript{a} and 1.23\textsuperscript{b}. (adapted from)\textsuperscript{132} .... 31

Scheme 16. Photoinduced electron transfer process. (adapted from)\textsuperscript{146} ....................... 33

Scheme 17. Octahedral coordination of Zn\textsuperscript{2+} ions by probe 1.24. (adapted from)\textsuperscript{79} ....... 34

\textit{xix}
<table>
<thead>
<tr>
<th>Scheme</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>Excimer formation of probe 1.25 induced by the coordination of Al$^{3+}$ ions.</td>
<td>(adapted from) 133</td>
</tr>
<tr>
<td>19</td>
<td>Imine-enol and keto-enamine tautomer. (adapted from) 72</td>
<td>37</td>
</tr>
<tr>
<td>20</td>
<td>ESIPT process with compound 1.31. (adapted from) 148, 161</td>
<td>39</td>
</tr>
<tr>
<td>21</td>
<td>Coordination of Mg$^{2+}$ ions by compound 1.32. (adapted from) 165</td>
<td>41</td>
</tr>
<tr>
<td>22</td>
<td>Coordination of Mg$^{2+}$ ions by compound 1.33. (adapted from) 87</td>
<td>42</td>
</tr>
<tr>
<td>23</td>
<td>Coordination of Zn$^{2+}$ ions by probe 1.34. (adapted from) 139</td>
<td>43</td>
</tr>
<tr>
<td>24</td>
<td>Coordination of OAc$^{-}$ ions by compound 1.31. (adapted from) 148</td>
<td>44</td>
</tr>
<tr>
<td>25</td>
<td>Detection of Hg$^{2+}$ ions by compound 1.35. (adapted from) 134</td>
<td>46</td>
</tr>
<tr>
<td>26</td>
<td>Detection of HOCl by compound 1.37. (adapted from) 179</td>
<td>47</td>
</tr>
<tr>
<td>27</td>
<td>[Zn(1.39)$_2$]$^{2+}$ displacement assay for H$_2$PO$_4^{-}$ ions detection. (adapted from) 82</td>
<td>49</td>
</tr>
<tr>
<td>28</td>
<td>Desilylation of compound 1.40 by F$^{-}$ ions. (adapted from) 135</td>
<td>50</td>
</tr>
<tr>
<td>29</td>
<td>Desilylation of compound 1.42 by F$^{-}$ ions. (adapted from) 136</td>
<td>52</td>
</tr>
<tr>
<td>30</td>
<td>Michael addition of cysteine to compound 1.45. (adapted from) 137</td>
<td>53</td>
</tr>
<tr>
<td>31</td>
<td>Detection of N$_2$H$_4$ by compound 1.47. (adapted from) 138</td>
<td>54</td>
</tr>
<tr>
<td>32</td>
<td>Cartoon of the DA approach for the detection of cyanide.</td>
<td>58</td>
</tr>
<tr>
<td>33</td>
<td>DA approach for the detection of NC$^{-}$ ions by the complex 2.0-Cu$^{2+}$. (adapted from) 187</td>
<td>59</td>
</tr>
<tr>
<td>34</td>
<td>Cyanide detection via DA approach by the complex 2.1-Cu$^{2+}$. (adapted from) 186</td>
<td>60</td>
</tr>
<tr>
<td>35</td>
<td>NC$^{-}$ detection by complex 2.2-Cu$^{2+}$ via the DA approach. (adapted from) 12</td>
<td>61</td>
</tr>
<tr>
<td>36</td>
<td>NC$^{-}$ detection by complex 2.3-Cu$^{2+}$ via the DA approach. (adapted from) 18</td>
<td>62</td>
</tr>
</tbody>
</table>
Scheme 37. Cyanide detection by the polymer 2.4 via direct addition to a boron center. (adapted from)$^{20}$ .......................................................... 63

Scheme 38. The direct 1,2-addition of cyanide to probe 2.5. (adapted from)$^{192}$ ............... 65

Scheme 39. The 1,2-addition of NC- ions to probe 2.6. (adapted from)$^{190}$ ....................... 66

Scheme 40. Cyanide detection by the 1,2-addition to probe 2.7. (adapted from)$^{193}$ ...... 67

Scheme 41. The cyanide induced cross benzoin reaction of compound 2.8. (adapted from)$^{141}$ ........................................................................................................... 68

Scheme 42. The 1,2-addition of cyanide to compound 2.9. (adapted from)$^{15}$ ............... 69

Scheme 43. Cyanide detection from the 1,2-addition to probe 2.10. (adapted from)$^{194}$ .. 70

Scheme 44. The 1,4-addition of cyanide to compound 2.11. (adapted from)$^{21}$ ............ 71

Scheme 45. The 1,4-addition of cyanide to probe 2.12. (adapted from)$^{195}$ ..................... 73

Scheme 46. The 1,4-addition of NC- ions to the β carbon of probe 2.13. (adapted from)$^{196}$ ........................................................................................................... 74

Scheme 47. Cyanide 1,4-addition to probe 2.14. (adapted from)$^{8}$ ....................... 75

Scheme 48. The synthesis of the target probes 2.15 and 2.16. ........................................ 80

Scheme 49. The cyanide nucleophilic attack of probe 2.16. ............................................. 96

Scheme 50. Possible hydrogen bonding between 2.16 and F- ions. ......................... 114

Scheme 51. Possible hydrogen bonding between 2.16 and OAc- ions. ....................... 116

Scheme 52. Hydrogen bonding interaction between 2.15 and OAc- ions. ............... 128

Scheme 53. Hydrogen bonding between 2.15 and F- ions. ....................................... 128

Scheme 54. The Michael addition of NC- ions to the C(9) carbon of probe 2.15. .... 131

Scheme 55. Hydrogen bonding between probe 2.16 and H₂PO₄- ions. ................... 135

Scheme 56. Hydrogen bonding between 2.16 and octylamine................................. 137
Scheme 57. The ESIPT process for compound 2.16. .............................................. 166

Scheme 58. Metal ion coordination through a generic Schiff bases 3.0a and 3.0b.
(adapted from)\textsuperscript{74} ...................................................................................... 176

Scheme 59. The coordination of Cu\textsuperscript{2+} ions by compound 3.1. (adapted from)\textsuperscript{206}. 178

Scheme 60. The binding of Cd\textsuperscript{2+} and Zn\textsuperscript{2+} ions by probes 3.2a and 3.2b respectively.
(adapted from)\textsuperscript{207} ........................................................................................ 180

Scheme 61. The coordination of Zn\textsuperscript{2+} and Cd\textsuperscript{2+} ions by probe 3.3. (adapted from)\textsuperscript{208} ... 181

Scheme 62. The coordination of Zn(NO\textsubscript{3})\textsubscript{2} by compound 3.4. (adapted from)\textsuperscript{69} .......... 182

Scheme 63. The coordination of Zn\textsuperscript{2+} ions by compound 3.5. (adapted from)\textsuperscript{65} .......... 183

Scheme 64. General synthesis for probes 3.6-3.8 as well as 2.15 and 2.16....................... 186

Scheme 65. General synthesis for the isolated metal complexes................................. 188

Scheme 66. Dissociation of Zn(OAc)\textsubscript{2} in DMSO................................................................. 226

Scheme 67. The mechanism and coordination of Zn\textsuperscript{2+} ions by probe 2.16. ............ 226

Scheme 68. The mechanism and coordination of Zn\textsuperscript{2+} ions by probe 3.8. ............... 229

Scheme 69. The possible coordination of ZnCl\textsubscript{2} to probe 2.15. ................................. 248

Scheme 70. The mode of coordination between ZnCl\textsubscript{2} and probe 2.16. ....................... 253
LIST OF ABBREVIATIONS

Fl. Fluorescence
UV-Vis Ultraviolet Visible
PET Photoinduced Electron Transfer
CHEF Chelation Enhanced Fluorescence
CHEQ Chelation Enhanced Quenching
PCT Photoinduced Charge Transfer
FRET Fluorescence Resonance Energy Transfer
HOMO Highest Occupied Molecular Orbital
LUMO Lowest Unoccupied Molecular Orbital
DMSO Dimethyl Sulfoxide
IPA Isopropanol
TEOF Triethylortho Formate
DFT Density Functional Theory
TDDFT Time-dependent Density Functional Theory
ATR-IR Attenuated Total Reflectance-Infra Red
NMR Nuclear Magnetic Resonance
ESI-MS Electro Spray Ionization Mass Spectrometry
CID Collision-Induced Dissociation
COSY Correlation Spectroscopy
HMQC Heteronuclear Multiple Quantum Coherence
HMBC Heteronuclear Multiple Bond Coherence
ROESY Rotating Frame Overhauser Effect Spectroscopy
L.o.D  Limit of Detection
WHO  World Health Organization
EPA  Environmental Protection Agency
TBAF  Tetrabutyl Ammonium Fluoride
TBACl  Tetrabutyl Ammonium Chloride
TBABr  Tetrabutyl Ammonium Bromide
TBAI  Tetrabutyl Ammonium Iodide
TBANO₃  Tetrabutyl Ammonium Nitrate
TBAOAc  Tetrabutyl Ammonium Acetate
TBAH₂PO₄  Tetrabutyl Ammonium Phosphate, monobasic
TBAHSO₄  Tetrabutyl Ammonium Bisulfate
ONH₂  Octylamine
NaBF₄  Sodium Tetrafluoroborate
NaN₃  Sodium Azide
NH₄SCN  Ammonium Thiocyanate
NH₄ClO₄  Ammonium Perchlorate
NH₄OH  Ammonium Hydroxide
SH  1-pentanethiol
TEACN  Tetraethyl Ammonium Cyanide
NaCN  Sodium Cyanide
KCN  Potassium Cyanide
ppb  Parts Per Billion
ppm  Parts Per Million
<table>
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<tr>
<td>ppt</td>
<td>Parts Per Trillion</td>
</tr>
<tr>
<td>ESIPT</td>
<td>Excited State Intramolecular Proton Transfer</td>
</tr>
<tr>
<td>ESICT</td>
<td>Excited State Intramolecular Charge Transfer</td>
</tr>
<tr>
<td>TBET</td>
<td>Through Bond Energy Transfer</td>
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<tr>
<td>ex</td>
<td>Excitation</td>
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</table>
CHAPTER I - INTRODUCTION

Anions and cations are ubiquitous and found in industry, environment, and medical/biological applications. Metal ions, such as Zn$^{2+}$ ions (section 1.1.3), are essential to human life whereby any imbalances in the concentration of zinc ions can be harmful.$^1$ Many ions are found in industrial processes, such as NC$^{-}$ (section 1.1.1), Cd$^{2+}$ (section 1.1.2.2), and Hg$^{2+}$ (section 1.1.2.3). However, they are extremely toxic and are often difficult to detect and remove from the environment (section 1.1.2.4).$^{2,3}$ These concerns facilitate the necessity for a non-destructive detection methods that are accurate, reliable, inexpensive, and sensitive. One approach is to use host-guest chemistry, which has gained significant attention over the decades whereby the interaction (Scheme 1.1) between a host (molecular probes, section 1.6) and a guest (in this work cations and anions, section 1.1) results in the change of a measurable signal produced by a signaling unit (section 1.5).$^4$ In order for a molecule to generate a response a fluorophore will need to be incorporated into the molecular design, together with a binding unit (section 1.3). The fluorophore used in this work is based on the coumarin molecule (sections 1.2 and 1.4.2). Various analytical techniques have been used to monitor these signal changes that arise from the host-guest interactions (section 1.4). Of the various techniques employed this thesis will focus on ones based on optical (UV-Vis and fluorescence) spectroscopy (section 1.4.1) and NMR spectroscopy.

Of the various anions and cations that exist, this work focuses on four ions: NC$^{-}$, Zn$^{2+}$, Cd$^{2+}$, and Hg$^{2+}$ ions. The rationale for choosing these four ions was based on their biological significance and toxicity. An in depth discussion is found in section 1.1.
1.1 Analytes of Interest

1.1.1 Cyanide

Cyanide is a very toxic anion. Cyanide toxicity in humans can occur by either the absorption of cyanide through exposed skin, inhalation of gaseous cyanide, or consumption.\(^5,6\) The severity and damage depends upon the concentration and exposure time.\(^7-9\) A study by the Center for Disease Control claims that cyanide can cause toxic effects at \(0.05 \cdot \text{mg} \cdot \text{dL}^{-1}\) and death at concentrations greater than \(0.3 \cdot \text{mg} \cdot \text{dL}^{-1}\).\(^10\)

Additionally, exposure to HCN for one-hour at 110 ppm can cause life-threatening effects whereas concentrations of up to 546 ppm can cause death after only 10 minutes.\(^10\) Maximum limits have been set in place by various countries (US 200 ppb), unions (European 50 ppb), and organizations such as the WHO (49.4 ppb).\(^11-13\) While the lethality of cyanide is well-known, it is an essential ion that is used in the synthesis (plastics, fibers, and dyes)\(^14\) as well as the extraction process in gold mining.\(^9\)

The toxicity of cyanide comes from its ability to inhibit the electron transport chain in the mitochondria.\(^15-18\) Under normal conditions, electrons flow through a sequence of electron carriers, ending with electrons being transferred from cytochrome \(c\) oxidase to molecular oxygen.\(^16\) The heme group of cytochrome \(c\) facilitates this process.
and results in the oxidation of ferrous (Fe\(^{2+}\)) ions to ferric (Fe\(^{3+}\)) ions. The exact pathway in which the NC\(^-\) ion inhibits this process is still under investigation. The first suggested/accepted pathway is the NC\(^-\) ions actively compete with O\(_2\) for the active site of cytochrome (Cyt) \(a_3:Cu_B\).\(^{19-21}\) The other possible route is the direct coordination of NC\(^-\) ions to one of the \(t_{2g}\) orbitals of the ferric ion of a heme unit (typically as a low-spin complex) resulting in the displacement of O\(_2\).\(^{22,23}\) In both scenarios the end result is the same: the transfer of electrons to oxygen and the electron transfer process is inhibited (Scheme 2).\(^{16}\) This leads to hypoxia and lactate acidosis, which could lead to respiratory arrest and death.\(^3\) The names of the various cytochromes shown below are given based on the wavelength of the maximum absorbance band.\(^{16}\) The first pathway of Scheme 2 shows the electron transport that is not inhibited, while the second pathway is inhibited by NC\(^-\) ions.\(^{16}\)

![Scheme 2. Electron transport pathway: 1. Uninhibited, 2. Inhibition due to NC ions](adapted from)\(^{16}\)

NADH = nicotinamide adenine dinucleotide (reduced form), Q = coenzyme Q, Cyt \(b\) = cytochrome \(b\), Cyt \(c_1\) = cytochrome \(c_1\), Cyt \(c\) = cytochrome \(c\), and Cyt \((a + a_3)\) = cytochrome \(a\) and cytochrome \(a_3\).

The acid-base equilibrium of cyanide is very important in sensor design and therefore an understanding of the aqueous chemistry needs to be addressed (Scheme 3).\(^{24}\) The pKa of HCN is 9.14. For a solution that has a pH ≤ 7, cyanide will exist as HCN (> 99 \%), equilibrium 1 in Scheme 3). When the solution falls within the pH range of 9.3-9.5, cyanide will be in equilibrium between HCN and NC- ions (equilibrium 2 in Scheme 3)
and greater than pH 11, cyanide will exist as NC- ions (> 99 %, equilibrium 3 in Scheme 3).²⁴

Scheme 3. The pH equilibrium between HCN and NC- ions under different pH conditions. (adapted from)²⁴

The current method for trace detection of cyanide requires an extensive amount of sample pretreatment with multiple sample purification steps, including the generation of gaseous HCN.²⁵-²⁷ This is very time consuming, sensitive, and sometimes dangerous. A quick and sensitive method in which trace amounts of NC- ions can be detected with relatively little risk to the scientist would be an invaluable asset.

1.1.2 Cations

While anion recognition is now a well-established area in host-guest chemistry; cation recognition is historically initiated the field of Supramolecular chemistry. The molecular probing of metal cations specifically Zn²⁺, Cu²⁺, and Fe²⁺/³⁺, play important roles in biochemical processes. An example of a process is the biological pathway for the synthesis of neurotransmitters, such as adrenaline and dopamine, by the copper containing enzyme, tyrosinase.²⁸-³⁰ Other metals, like mercury and cadmium, are toxic to living beings and are an environmental concern. While each cation is important in its own right, this thesis focuses solely on the detection of the d-block metal ions: Zn²⁺, Cd²⁺, and Hg²⁺ ions.
1.1.2.1 Zinc. Zinc is the second most abundant metal in the human body and is used in a wide range of processes.\textsuperscript{31} It is an essential component of numerous proteins and enzymes; a few examples of metalloenzymes that employ the Zn\textsuperscript{2+} ion as a cofactor are carbonic anhydrase (converts carbon dioxide to bicarbonate), carboxy peptidase (cleaves peptide bonds at the carboxy-terminal), and superoxide dismutase (catalyzes the dismutation of the harmful O\textsubscript{2} - radical into molecular oxygen or hydrogen peroxide).\textsuperscript{23, 32} The highest concentration of zinc can be found in the brain (~0.5 mM in the “gray” matter)\textsuperscript{33} and the total concentration of zinc in tissues is around 200 µM.\textsuperscript{32, 34-36} Important biological processes utilizing Zn\textsuperscript{2+} ions include apoptosis regulation, enzyme regulation, gene expression, and neurotransmission.\textsuperscript{35, 36} However, the scientific community is currently investigating the role of zinc in possible trigger mechanisms for diseases such as Alzheimer’s disease, Parkinson’s disease, hypoxia-ischemia, and type I and II diabetes.\textsuperscript{33, 37} As zinc plays such a significant role in many biochemical processes, the ability to selectively monitor and detect Zn\textsuperscript{2+} ions is important.

Numerous research groups are currently investigating the role of the Zn\textsuperscript{2+} ion in these disorders and diseases.\textsuperscript{1, 31, 38, 39} The human body has cation-diffusion facilitator (CDF) proteins that transport metal cations from inside the cell (cytosol) to other compartments within the cell or outside of the cell (exocytosis).\textsuperscript{40} One group of CDFs that is of interest are the Zn\textsuperscript{2+} ion transporter proteins, ZnTs.\textsuperscript{40} While the most abundant ZnTs, where s = 1, 3, and 6, are being investigated as possible links to neuronal disorders and diseases,\textsuperscript{31} the ZnT8 protein is currently being studied in its role in Type II diabetes.\textsuperscript{40, 41} Diabetes is the result of a dysfunction of pancreatic β cells, whose main role in the body is to maintain metabolic homeostasis via insulin secretion based on
surrounding glucose levels. Insulin secretion occurs during exocytosis of (pro)insulin, a insulin secretory granule (ISG), which contains two Zn$^{2+}$ ions. ZnT8 regulates the uptake of Zn$^{2+}$ ions into ISGs, which contain ~70% (~10–20 mM) of the total Zn$^{2+}$ ion concentration found within pancreatic β cells. Research is still ongoing to show a direct link between mutations of the ZnT8 protein to pancreatic β cell dysfunctions and ultimately diabetes.

1.1.2.2 Cadmium. Cadmium is an extremely toxic metal used in metallurgy, electroplating, and the synthesis of pigments and quantum dots/rods. Cadmium has such a long half-life that it is essentially non-biodegradable, allowing it to accumulate in the environment and contaminate plants and drinking water. Smoking is one major source of cadmium exposure, as the tobacco plant tends to accumulate high concentrations of Cd$^{2+}$ ion (~10% of the 1.7 μg of cadmium per cigarette is inhaled). Other methods of exposure come from the ingestion of contaminated foods and water. Accumulation of the Cd$^{2+}$ ion in the human body can lead to renal dysfunction, calcium metabolism disorders, and certain types of prostate and lung cancer. Cadmium possibly causes the metabolic disorders through interfering with essential metal ions such as Zn$^{2+}$ and Ca$^{2+}$. The EPA and WHO set acceptable maximum limits of cadmium in drinking water at 5 and 3 ppb, respectively.

1.1.2.3 Mercury. The Hg$^{2+}$ ion has also drawn attention over the recent years. The EPA has mandated that the Hg$^{2+}$ ion concentration in drinking water should not exceed 2 ppb. Mercury poisoning causes brain damage, kidney failure, and various cognitive and motion disorders in humans; it also reduces the rate of photosynthesis and transpiration in plants. Mercury accumulates in the human body by absorption through
the skin, respiration, and ingestion.\textsuperscript{48, 50, 51} The toxicity of mercury is due to its high affinity for thiol groups, found in many essential proteins and enzymes.\textsuperscript{48}

1.1.2.4 Challenges of detecting the ions Zn\textsuperscript{2+}, Cd\textsuperscript{2+}, and Hg\textsuperscript{2+}. All three metals have a filled \textit{d}-orbital (\textit{d}\textsuperscript{10}), therefore many challenges must be overcome in order to monitor and screen them. The removal of the valence electrons to generate the cation results in a smaller radius than the parent atom. This decrease means that the Zn\textsuperscript{2+} ion (0.74 Å) has a similar ionic radius as Mg\textsuperscript{2+} (0.72 Å). Therefore careful analysis in spectroscopic methods to detect Zn\textsuperscript{2+} ions based on ionic size can observe false positives from Mg\textsuperscript{2+} ions. The same ionic radius problem occurs for both Cd\textsuperscript{2+} (0.95 Å) and Hg\textsuperscript{2+} (1.01 Å) with false positives arising from Ca\textsuperscript{2+} (1.0 Å). Due to their relatively similar ionic radii interferences can also arise between the ions Zn\textsuperscript{2+} and Cd\textsuperscript{2+},\textsuperscript{52} as well as Cd\textsuperscript{2+} and Hg\textsuperscript{2+}.\textsuperscript{53} A list of the ionic radii for the ions Mg\textsuperscript{2+}, Ca\textsuperscript{2+}, Zn\textsuperscript{2+}, Cd\textsuperscript{2+}, and Hg\textsuperscript{2+} are found in Table 1.\textsuperscript{23, 29, 53-55} The challenges for selective monitoring and screening of these metals can also be explained on the basis of geometry. Since zinc, cadmium, and mercury have a filled \textit{d}-orbital, these cations along with magnesium and calcium are spherical ions. The spherical nature of these cations allow for the formation of stable complexes with similar geometries (Table 1).
Table 1

Selected Properties for the Cations Mg$^{2+}$, Ca$^{2+}$, Zn$^{2+}$, Cd$^{2+}$, and Hg$^{2+}$

<table>
<thead>
<tr>
<th>M$^{2+}$ ion</th>
<th>Electronic configuration</th>
<th>Atomic radius</th>
<th>Ionic radius</th>
<th>Preferred geometry</th>
<th>HSAB classification</th>
<th>η</th>
</tr>
</thead>
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<tr>
<td>Mg$^{2+}$</td>
<td>[Ne]</td>
<td>1.45</td>
<td>0.72</td>
<td>O$_h$</td>
<td>Hard</td>
<td>32.6</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>[Ar]</td>
<td>1.94</td>
<td>1.0</td>
<td>O$_h$</td>
<td>Hard</td>
<td>19.5</td>
</tr>
<tr>
<td>Zn$^{2+}$</td>
<td>[Ar] 3$d^{10}$</td>
<td>1.42</td>
<td>0.74</td>
<td>T$_d$/O$_h$</td>
<td>Borderline</td>
<td>10.9</td>
</tr>
<tr>
<td>Cd$^{2+}$</td>
<td>[Kr] 4$d^{10}$</td>
<td>1.61</td>
<td>0.95</td>
<td>T$_d$/O$_h$</td>
<td>Soft</td>
<td>10.3</td>
</tr>
<tr>
<td>Hg$^{2+}$</td>
<td>[Xe] 4$f^{14}$ 5$d^{10}$</td>
<td>1.71</td>
<td>1.01</td>
<td>Linear/T$_d$</td>
<td>Soft</td>
<td>7.7</td>
</tr>
</tbody>
</table>

Note: Atomic and ionic radii values are in angstroms (Å, 1Å = 10$^{-10}$ M); T$_d$ = tetrahedral; O$_h$ = octahedral; absolute hardness is reported in eV (η = (I-A)/2, where I represents the ionization energy and A represents the electron affinity).$^{23, 53}$

The HSAB classification and absolute hardness values shown in Table 1 provide a separation whereby binding units can be designed to promote favorable interactions and ultimately selectivity: hard acid-hard base and soft acid-soft base.$^{23, 56}$ This concept will be implemented in Chapter III.

1.2 Coumarin Synthesis

Coumarin (1.0, Figure 1) is a natural substance found in some families of plants such as Orchidaceae and Umbelliferae.$^{57}$ Coumarin and its derivatives have been employed in the design of anti-microbial, antioxidant, antitumor, anti-inflammatory, and anti-allergic drugs.$^{58-60}$ They have also been used in optical brighteners, food additives, and cosmetics, as well as being used as commercial fluorophores (Alexa Flour® 350) (1.1, Figure 1).$^{61}$ The coumarin derivative of interest in this work is the 7-(diethylamino)-
4-hydroxycoumarin (1.2, Figure 1), based on synthesis and optical properties (section 1.4.2).

![Chemical Structures](image)

*Figure 1. The IUPAC numbering of (1.0), (1.1), and (1.2).*

The popularity of coumarin derivatives in various applications has led to the development of a significant number of pathways to synthesize coumarin and coumarin derivatives.\(^6^2-^6^7\) Coumarin is typically synthesized by an intramolecular condensation reaction. For example, the Perkin’s reaction and the Knovenagel reactions shown in Scheme 4.\(^6^3,^6^5\) If a functional group is required on the C(5) and C(8) positions, it is located on the phenol starting material. Conversely, if the group is required at the C(3) or C(4) position, the substituent will be located on the carboxylic acid. In the Perkin’s reaction shown in Scheme 4 the design of the precursor (1.3) allowed for the in situ formation of an acid bromide followed by an intramolecular cyclization, which resulted in a 3:4 fused 7-methoxycoumarin derivative (1.4).\(^6^3\) In the Knovenagel reaction (Scheme 4), the NO\(_2\) functional group is on the ester (1.6), which resulted in the NO\(_2\) substituent located at the C(3) position (1.7).\(^6^5\)
Scheme 4. Synthesis of compounds 1.4 and 1.7. (adapted from)\textsuperscript{63,65}

1.2.1 4-Hydroxycoumarin

The 7-(diethylamino)-4-hydroxycoumarin (1.2) is the coumarin derivative of interest and will serve as a common intermediate for the work presented in this thesis. Since compound 1.2 is a derivative of 4-hydroxycoumarin, this section provides three common synthetic routes to synthesize 4-hydroxycoumarin derivatives: Pechmann condensation,\textsuperscript{67} ZnCl\textsubscript{2}:POCl\textsubscript{3},\textsuperscript{62} and an activated aryl malonate\textsuperscript{13, 64, 66}.

In Scheme 5 Zhao et al. used the Pechmann condensation reaction (between compounds 1.8a-1.8c and CO(OEt)\textsubscript{2} to synthesize three different 6-substituted-4-hydroxycoumarin derivatives (1.9a-1.9c).\textsuperscript{67} This synthetic route resulted in good yields (72-85%); however, this method is air-sensitive (due to the enolate of compounds 1.8a-1.8c) and results in the evolution H\textsubscript{2}(g).
Scheme 5. Synthesis of compounds 1.9a-c via the Pechmann condensation reaction.

(adapted from)\textsuperscript{67}

Another synthetic method is the condensation between a phenol derivative (1.10) and malonic acid (1.11) in the presence of anhydrous ZnCl\textsubscript{2} and POCl\textsubscript{3} (Scheme 6).\textsuperscript{62}

This route allowed for derivatization at positions C(5) through C(8) in a ~60% yield.\textsuperscript{62}

Similar to the Pechmann reaction, this reaction is air sensitive. Also the ZnCl\textsubscript{2}:POCl\textsubscript{3} route required 30-35 hours to reach completion.\textsuperscript{62}

The example published by Ambre et al. shown in Scheme 6 is a general experimental procedure that uses the ZnCl\textsubscript{2}:POCl\textsubscript{3} method to synthesize a series of 6-substituted-4-hydroxycoumarin derivatives.\textsuperscript{62}

Scheme 6. Synthesis of compound 1.12 via the ZnCl\textsubscript{2}:POCl\textsubscript{3} route. (adapted from)\textsuperscript{62}

The approach used in this work is based on the “magic” malonate, activated arylmalonate (Scheme 7).\textsuperscript{64,66} In this method the electron withdrawing 2, 4, 6-trichlorophenol groups of 1.13 make the malonate more susceptible to nucleophilic attack from the phenol derivative 1.14. This procedure is attractive as many different functional groups can be incorporated in positions at C(5) to C(8) in a reasonable time, yields, and mild conditions (Scheme 7). Compound 1.2 is the particular coumarin derivative of
interest in this work as the reaction product is typically pure with yields ranging from 55-75\%.

\[ \text{Scheme 7. Synthesis of 1.2 via the activated aryl malonate route. (adapted from)}^{13,64,66} \]

While the C(7)-NEt\(_2\) substituent is of significant importance to the photophysical properties (section 1.4.2), the C(4)-OH substituent plays a vital role in the further derivatization of the 4-hydroxycoumarin core. An extensive number of coumarin derivatives have been reported in literature thus far. One class of coumarin derivatives that have not been explored as extensively is the coumarin-enaminones, which is the focus of this thesis.

1.3 The Binding Unit: Azomethine/Enaminone

Azomethines (aka enaminones and keto-enamines) have sparked interest as binding units to coordinate metal ions due to the N, O-donor atoms.\(^{68,69}\) The Scheme 8 provided below shows the three most commonly reported metal ion coordination.\(^{70,71}\) For simplicity R represents coumarin and R’ represents various compounds. The mixed heteroatoms of the enaminone offer a good binding motif for metal ion coordination for three major reasons.\(^{69}\)

1. The two heteroatoms can aid in the coordination of one cation over another, due to the HSAB concept.\(^{23}\)

2. The general structure of the keto-enamine offers opportunity for binding in a bidentate fashion, providing greater stability over monodentate ligands.\(^{23}\)
Upon coordination, the five- or six-membered ring (Scheme 8) formed around the metal is relatively stable.

3. Cations with larger ionic radii tend to form less stable five-membered rings than cations with a medium ionic radii. This could also aid in the coordination of one cation over another.

Scheme 8. Possible modes of metal coordination. (adapted from)\textsuperscript{70,71}

(A) The metal ion forms a six-membered ring with the electron density being evenly dispersed throughout the ring. (B) The metal ion coordinates to the oxygen of the imine-enol tautomer. (C) Enaminone zwitterion, with the metal ion coordinating to the negatively charged oxide.

The actual mode of metal ion coordination is dependent on a number of factors. For simple aromatic azomethines, the solvent polarity affects the mode of coordination.\textsuperscript{72} Solvents with a high dielectric constant, such as alcohols MeOH (32.7) and DMSO (46.7), the coordination of a metal favors the intramolecular chelate with a covalent O-M bond and a dative N\textbullet\textbullet\textbullet M bond (Scheme 8).\textsuperscript{72} In non-polar aprotic solvents (i.e. hydrocarbons and their halogen derivatives, esters, or mixtures), the metal prefers to bind to multiple enaminones through dative O\textbullet\textbullet\textbullet M bonds while in the keto-enamine tautomer (Scheme 8, 2.). Enaminones possess flexibility that allows them to coordinate to metal ions with various geometries (square planar, tetrahedral, and octahedral).\textsuperscript{72,73}

Furthermore, the R’ group will affect the chelate ring size and stereochemistry.\textsuperscript{71} In situations where azomethines compounds coordinate to the metal ion in a ML\textsubscript{2} fashion,
two enaminone ligands coordinated to one metal ion (M). The general trends of the geometry have emerged over the years (Figure 2): when M = Ni\(^{2+}\) or Cu\(^{2+}\) the geometry is typically \textit{trans} square planar, M = Pd\(^{2+}\) \textit{cis} square planar, M = Co\(^{2+}\), Zn\(^{2+}\), Cd\(^{2+}\), and Hg\(^{2+}\) commonly tetrahedral.\(^{73,74}\) While these are the most reported geometries, it has been shown that a ML\(_2\) can coordinate in an octahedral fashion as well.\(^{74}\)

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Typical geometries of metal-ligand ML\(_2\) complexes. (adapted from)\(^{74}\)}
\end{figure}

Enaminone-probes have been reported in the detection of cations such as Zn\(^{2+}\) ions,\(^{75-82}\) Cu\(^{2+}\) ions,\(^{83-86}\) Mg\(^{2+}\) ions,\(^{87,88}\) and in a number of other transition metals as well as multiple metal coordination.\(^{70,71,89-100}\) The four geometries shown in Figure 1.2 take into account the coordination of the metal ion solely in the enaminone binding site; however, this does not address the potential for additional modes of binding through other donating atoms in the coumarin molecule. A multitude of binding modes can exist that takes into account both the enaminone and the coumarin (Scheme 9). In order to improve and aid selectivity, various common binding units were chosen for the R’ group for example 1,10-phenanthroline and hydrazones (see Chapter III).
While the use of the enaminone group has been extensively investigated for coordination of metal ions, recent attention is being made toward the enaminones as anion coordination molecules. The enaminone moiety does not fall into one of the classical functional groups used for anion binding. However, due to its ability to behave as both an electrophile and a nucleophile, the enaminone serves as a potentially useful functional group in a chemodosimeter application. This key property is discussed in-depth in Chapter II.

The choice for the direct connection of the enaminone and the coumarin was based on the coordination through the enaminone coordination site will induce a significant change in the electronic properties of the coumarin derivative. These changes can be monitored and analyzed via optical spectroscopic techniques.
1.4 Optical Spectroscopy

Changes in the electronic states of compounds can be monitored by optical spectroscopic methods such as UV-Vis and fluorescence spectroscopy.\textsuperscript{102} Optical spectroscopic methods are attractive for analyte detection because the instruments are sensitive, meaning they can detect samples at low concentrations ppm to ppb ($10^{-9}$ to $10^{-12}$ M).\textsuperscript{103-105} One attractive aspect is that the sample is not destroyed and can be used. The instruments can be portable, allowing for in-field testing. A chromophore is defined as a compound or functional group that gives rise to a compound having color but does not undergo photoluminescence upon excitation. A fluorophore, on the other hand, does undergo photoluminescence upon excitation, and it may or may not have color in the visible region.\textsuperscript{103,106} The coumarin molecule is both a chromophore and fluorophore; therefore it can be analyzed by a number of optical spectroscopic methods. The advantages and disadvantages of several optical spectroscopic techniques are discussed in Table 1.2.\textsuperscript{102,104,105} Traditional techniques include atomic absorption spectrometry (AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), atomic fluorescence spectrometry (AFS), X-Ray fluorescence spectrometry (XRF). However, these are sophisticated, specialized, and expensive techniques.
### Table 2

*Advantages and Disadvantages of Various Analytical Detection Methods*

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
</table>
| **AAS** | 1. Quick analysis time  
2. Easy to use  
3. L.o.D in the ppb range | 1. Destructive method  
2. Limited to metals and metalloids  
3. Single-element technique |
| **ICP-AES** | 1. Simultaneous multiple element analysis  
2. L.o.D in the ppb range | 1. Long preparation time  
2. Destructive technique |
| **AFS** | 1. Simple to use instrumentation  
2. L.o.D in the ppb range | 1. Destructive single element technique  
2. Long preparation time  
3. Limited to metals and metalloids |
| **XRF** | 1. Nondestructive elemental analysis  
2. L.o.D. in the 10-100 ppm range  
3. Samples can be in liquid or solid state | 1. Useful for elements after boron  
2. High cost |

Note: AAS (atomic absorption spectrometry), ICP-AES (inductively coupled plasma atomic emission spectrometry), AFS (atomic fluorescence spectrometry), XRF (X-ray fluorescence spectrometry), L.o.D. (limit of detection), ppm (parts-per-million), ppb (parts-per-billion)\(^{102, 104, 108}\)

The use of fluorescence spectroscopy is used in this work for several reasons:
1. Fluorimeters have a detection limit range of $10^{-11}$ to $10^{-12}$ M, referred to the ultra low concentration range.\textsuperscript{105}

2. Fluorimeters can be made portable and less expensive than other techniques such as ESI-MS, NMR, and GC-MS.\textsuperscript{104}

3. Fluorescence is a non-destructive technique, allowing for reuse of the sample and providing a more cost-effective alternative to other methods like flame AAS.\textsuperscript{102, 104, 105}

UV-Vis spectroscopy is comparable to fluorescence spectroscopy in that both are non-destructive methods, both allow for quick analysis, and both are relatively inexpensive instrumentation.\textsuperscript{105} However, UV-Vis has a typical detection limit on the range of $10^{-4}$ to $10^{-5}$ M, which is not as sensitive as fluorescence spectroscopy.\textsuperscript{102} Also, UV-Vis spectrometers detect most organic compounds as they tend to absorb light in the ultra-violet and visible light regions. Fluorescence, on the other hand, only detects molecules that can produce a fluorescent signal, making it more specific than UV-Vis.

1.4.1 Photoluminescence

Photoluminescence spectroscopy encompasses both fluorescence and phosphorescence spectroscopy. An electron in the singlet ground state ($S_0$) absorbs energy and is promoted to the excited singlet state ($S_1$). The electron then undergoes internal conversion, a non-radiative relaxation, to the lowest level of the excited singlet state. The excited electron can undergo one of three de-excitation pathways to relax back down to ground state. The first pathway is fluorescence; here, the electron emits photons of light as it relaxes directly back to the singlet ground state. Fluorescence emission typically occurs within $10^{-10}$ to $10^{-7}$ seconds after excitation.\textsuperscript{106} The second pathway is
phosphorescence whereby the electron undergoes intersystem crossing to the excited triplet state ($T_1$), changing spin and becoming unpaired with the electron in the $S_0$ state. The spin of the electron flips again, becoming paired again, as it relaxes back to the $S_0$ state. Phosphorescence can only occur when the $S_1$ and $T_1$ states are close in energy. Due to the electron spin flips during phosphorescence, the process occurs on a slower time range than fluorescence, taking anywhere from microseconds to seconds.\textsuperscript{106} Fluorescence and phosphorescence are radiative processes, meaning they emit photons of light as during the relaxation back to the $S_0$ state. The last pathway discussed here is quenching. The process is the same as fluorescence except that the relaxation from $S_1$ to $S_0$ happens non-radiatively, for example, photoinduced electron transfer (PET) (section 1.5.4). The three pathways previously discussed are depicted in the Jablonski diagram (Scheme 10).\textsuperscript{102, 105-107}
Scheme 10. Jablonski diagram. (adapted from)\textsuperscript{102,103,105-107}

For ease of interpretation the arrows for fluorescence emission are slightly shifted. Each line in the $S_0$ ground state and $S_1$ singlet excited state represents a vibrational/rotational energy level associated with the electronic states, $S_0$, $S_1$, and $T_1$.

There are a number of transition metals, such as Hg\textsuperscript{2+} and Cd\textsuperscript{2+} induce quenching upon binding. This can be attributed to spin-orbit coupling, allowing for intersystem crossing from an exciting singlet state to an excited triplet state, resulting in the quenching of the fluorescence. The quenching is caused by the coordination of late second and third row transition metals, known as the heavy metal effect.\textsuperscript{103}

1.4.2 Photophysical Properties of Coumarin

Coumarin has an absorbance spectrum with a maxima at 350-500 nm, assigned to the $\pi-\pi^*$ transition.\textsuperscript{108} Molar absorbtivities ($\varepsilon$) are typically in the range of 18,000 to
62,000 M$^{-1}$cm$^{-1}$. The fluorescence emission coumarin derivatives are often observed between 300-600 nm. Theoretical calculations and experiments have shown that the $S_1$ emitting state is a charge transfer transition specifically internal charge transfer (ICT), and indicates that the emission of coumarin is due to a $n-\pi^*$ transition (Schemes 12 and 13). The coumarin $S_{1(\text{ICT})}$ state is influenced by the choice of solvent, which will be discussed throughout this section.

Scheme 11 is provided to introduce terminology along with the associated direction. The signal observed in UV-Vis and fluorescence spectroscopy can undergo shifts. The four major shifts are up (hyperchromic, increase), down (hypochromic, decrease), left (hypsochromic, blue), and right (bathochromic red), however, a combination of two shifts is common.

Scheme 11. A directional layout of the possible shifts.
The ICT character of the $S_1$ emitting state is a result of a change in the dipole moment of the coumarin in the excited state. Through numerous derivatizations, the 7 position of the coumarin backbone results in the largest change to the fluorescence properties of coumarin. When the substituent is electron donating in character, a bathochromic shift is seen. Increasing the electron donating character of the C(7) substituent increases the bathochromic shift. This is due to the fact that the C(7) substituent is in conjugation with the carbonyl of the lactone ring. The electron donating character of the substituent increases the “flow”, or push, of electron density into the coumarin core. This increased flow of electron density from the C(7) substituent into the coumarin core allows for an increased pull of electron density from the lactone ring and in particular the oxygen of the C(2) carbonyl group. The increased flow of electron density upon excitation increases the charge transfer character of the emitting $S_1$ state. The substitution also decrease the energy gap of the $S_1(n-\pi^*)$ and the $S_1(\pi-\pi^*)$. In cases where the C(7) substitution is either an oxygen or nitrogen atom the lowest energy level becomes the $S_1(\pi-\pi^*)$ state and, by virtue, the emitting state. By increasing the charge transfer character of the emitting state gives rise to greater response in the fluorescence emission spectrum upon changes in environment.

The C(7) substituted coumarin derivatives fall into two categories: rigid (compounds 1.15-1.18) or flexible (compounds 1.19-1.22). The flexibility or rigidity of the C(7) substituent will dictate the spectroscopic response observed from changes in environment (i.e. solvent). For rigid substituents such as the amine substituent seen in (Scheme 12, Figure 3, and Table 3) higher $\phi_F$ are observed in moderate to medium polar solvents while increasing the viscosity of the solvent reduces the $\phi_F$. An
increase in the hydrogen bonding nature of the solvent (e.g. polar protic solvents such as MeOH and EtOH) will also decrease the $\phi_{Fl}$, but the increase of the hydrogen bonding character of the solvent will induce a bathochromic shift of the fluorescence emission (Table 1.3). As the ICT state is formed more electron density is localized in the lactone ring and the oxygen atom of the C(2) carbonyl. With the increased negative charge on the oxygen atom on the lactone ring, the oxygen atom becomes a better hydrogen bond acceptor, which increases the electron “pull” and shifts the absorbance and emission to longer wavelengths (Scheme 12). The excitation results in the population of $S_1(\pi-\pi^*)$ state, which leads to the formation of the ICT conformation. In the ICT conformation the resonance of the double bonds becomes more ordered and generates a localized negative charge on the basic oxygen centers. This allows for a greater number of hydrogen bonding interactions between compound 1.15 and the solvent causing more energy to be lost and therefore a larger bathochromic shift.

![Scheme 12. ICT formation and hydrogen bonding of compound 1.15. (adapted from)](image)

A larger spectroscopic response due to changes to solvent polarity is observed in the emission spectra more than the absorbance spectra. This indicates that the excited $S_1$ state is higher in polarity than the $S_0$ ground state. This supports that the emitting state is due to a $\pi-\pi^*$ transition. Fluorescence is a slower process than absorbance, and therefore the solvent molecules have a longer time to interact with the excited
molecule. This is supported in Table 1.3 with compounds 1.17 and 1.18. When comparing the difference in the absorbance and emission maximum for 1.17 and 1.18 in EtOH and benzene the $\Delta_{\text{Abs}}(\lambda_{\text{max}})$ is 8 and 4 nm while the $\Delta_{\text{Fl}}(\lambda_{\text{max}})$ is 16 and 25 nm respectively.

![Figure 3. Rigid C(7) coumarin derivatives. (adapted from)\textsuperscript{121,122}

Table 3

**Solvent Effect on Photophysical Properties of Compounds 1.16-1.18**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Abs $\lambda_{\text{max}}$ (nm)</th>
<th>Fl $\lambda_{\text{max}}$ (nm)</th>
<th>Stoke’s shift</th>
<th>$\phi_{\text{Fl}}$</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.15</td>
<td>422</td>
<td>526</td>
<td>104</td>
<td>0.28</td>
<td>EtOH</td>
</tr>
<tr>
<td>1.16</td>
<td>388</td>
<td>470</td>
<td>82</td>
<td>0.65</td>
<td>EtOH</td>
</tr>
<tr>
<td>1.17</td>
<td>432</td>
<td>481</td>
<td>49</td>
<td>0.65</td>
<td>EtOH</td>
</tr>
<tr>
<td>1.17</td>
<td>424</td>
<td>465</td>
<td>41</td>
<td>1.00</td>
<td>Benzene</td>
</tr>
<tr>
<td>1.18</td>
<td>437</td>
<td>490</td>
<td>53</td>
<td>0.829</td>
<td>EtOH</td>
</tr>
<tr>
<td>1.18</td>
<td>433</td>
<td>465</td>
<td>32</td>
<td>0.791</td>
<td>Benzene</td>
</tr>
</tbody>
</table>

Note: Abs $\lambda_{\text{max}}$ is the wavelength, in nm, at which the maximum absorbance is located, Fl $\lambda_{\text{max}}$ is the wavelength, in nm, at which the maximum fluorescence emission intensity is located, Stoke’s shift is calculated by the following equation ($\text{Fl } \lambda_{\text{max}} - \text{Abs } \lambda_{\text{max}}$) and reported in nm, $\phi_{\text{Fl}}$ is the reported fluorescence quantum yield, and EtOH is the abbreviation for ethanol.

For functional groups that are flexible at C(7), the increase in solvent polarity increases the bathochromic and hypochromic shift seen as well as the decrease in $\phi_{\text{Fl}}$.\textsuperscript{121}

In this discussion flexible is used to describe the rotation about the heteroatom of the C(7) substituent (Scheme 13). For coumarin and the rigid C(7) coumarin derivatives, the
excitation populates the ICT emitting states $S_{1}(n-\pi^*)$ and $S_{1}(\pi-\pi^*)$ respectively. These ICT emitting states are polar and, more importantly, still planar as seen in Schemes 12 and 13.\textsuperscript{119, 130} However, for flexible C(7) substituents such as –OH, -OMe, -OEt, -NH$_2$ (compounds 1.19 and 1.20), -NHR’, and –NR’R’’ (compounds 1.21 and 1.22) a rotation or twisting about the heteroatom can occur within picoseconds of the formation of the ICT state.\textsuperscript{130} This rotation or twisting results in a non-planar, polar, twisted intramolecular charge transfer (TICT) emitting state (Scheme 13).\textsuperscript{120, 126, 127} Flexible C(7) substituents that form this TICT conformation display greater sensitivity to changes in solvent polarity (from nonpolar to polar).\textsuperscript{115, 125, 131}

![Scheme 13. TICT formation of coumarin derivative 1.19. (adapted from)\textsuperscript{120,122}](image)

Coumarin derivatives are not limited to substituents at the C(7) position; they can also have substituents at C(3) or C(4) or both.\textsuperscript{126} If the substituent at C(3) and/or C(4) is electron withdrawing in character, the pull of electron density from the lactone ring increases.\textsuperscript{110} This increases the polarity of the excited state, the change in the dipole
moment, and the ICT character of the $S_1$ emitting state, thus leading to a larger bathochromic shift with respect to coumarin, see 1.19 and 1.20 in Table 4. The opposite spectroscopic response has been shown if the substituent is electron donating in character. Comparing compounds 1.21 and 1.22 the presence of the nitrile resulted in a bathochromic shift in both UV-Vis and fluorescence spectra, but it also decreased the Stoke’s shift as well as the $\phi_{Fl}$. 

![Figure 4. Flexible C(7) coumarin derivatives. (adapted from) 120,122-124](image)

Table 4

<table>
<thead>
<tr>
<th>Compound</th>
<th>Abs $\lambda_{\text{max}}$</th>
<th>Fl $\lambda_{\text{max}}$</th>
<th>Stoke’s shift</th>
<th>$\phi_{Fl}$</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.19</td>
<td>382</td>
<td>481</td>
<td>99</td>
<td>0.53</td>
<td>EtOH</td>
</tr>
<tr>
<td>1.20</td>
<td>353</td>
<td>427</td>
<td>74</td>
<td>0.78</td>
<td>EtOH</td>
</tr>
<tr>
<td>1.21</td>
<td>355</td>
<td>395</td>
<td>40</td>
<td>0.83</td>
<td>Benzene</td>
</tr>
<tr>
<td>1.21</td>
<td>380</td>
<td>443</td>
<td>63</td>
<td>0.39</td>
<td>EtOH</td>
</tr>
<tr>
<td>1.22</td>
<td>406,422</td>
<td>445</td>
<td>39,23</td>
<td>NA</td>
<td>Benzene</td>
</tr>
<tr>
<td>1.22</td>
<td>424</td>
<td>472</td>
<td>48</td>
<td>0.034</td>
<td>EtOH</td>
</tr>
</tbody>
</table>

Note: Abs $\lambda_{\text{max}}$ is the wavelength, in nm, at which the maximum absorbance is located, Fl $\lambda_{\text{max}}$ is the wavelength, in nm, at which the maximum fluorescence emission intensity is located, Stoke’s shift is calculated by the following equation (Fl $\lambda_{\text{max}}$ - Abs $\lambda_{\text{max}}$) and reported in nm, $\phi_{Fl}$ is the reported fluorescence quantum yield, and EtOH is the abbreviation for ethanol.

Tables 3 and 4 list the photophysical properties of coumarin derivatives that exhibit the aforementioned principles. Compound 1.20 has at C(7) a flexible amino
substituent and an electron donating methyl group at C(4).\textsuperscript{122} When the methyl group is changed to an electron withdrawing group, compound \textbf{1.19} which has a trifluoromethyl substituent at C(4), the lactone ring’s electron density pulling ability is increased and the absorbance and the emission undergoes a bathochromic shift.\textsuperscript{122} The same principle is also observed in compounds \textbf{1.15} and \textbf{1.16}: leaving the C(4) substituent the same but replacing the flexible 7-amino group of \textbf{1.19} and \textbf{1.20} with a rigid 7-amino substituent causes a bathochromic shift in both the absorbance and emission spectra of \textbf{1.15} and \textbf{1.16}.\textsuperscript{122} Keeping with the rigid 7-aminocoumarin derivatives, \textbf{1.17} and \textbf{1.18} display the effects of size of the C(3) substituent on the formation of the ICT emitting state.\textsuperscript{121} In compound \textbf{1.18}, the C(3) substituent is CO\textsubscript{2}Et while the C(3) substituent of \textbf{1.17} is a CN group. The rotation that can occur in compound \textbf{1.18} (CO\textsubscript{2}Et) decreases the CT character of the lactone ring, thus resulting in a slight hypsochromic shift in the absorbance and emission spectra when compared to compound \textbf{1.18}.\textsuperscript{121} For compounds \textbf{1.17} and \textbf{1.22}, it was observed that the nitrile at the C(3) position resulted in similar absorbance and fluorescence \(\lambda_{\text{max}}\) regardless if the C(7) substituent is rigid or flexible (Tables 1.3 and 1.4).

\subsection*{1.5 Signaling Mechanisms}

In host-guest chemistry, the addition of the desired analyte of interest (guest) will result in the change of a measurable signal produced by a signaling unit of the molecular probe (host).\textsuperscript{4, 56} When the host-guest interaction is reversible in nature, the molecular probe is commonly referred to as a chemosensor. A generic layout of a chemodosimeter is shown in Scheme 1.\textsuperscript{4, 56} The molecular probes discussed in this section will focus on
reversible sensors (chemosensors); whereas for a non-reversible probe they are known as chemodosimeters (section 1.6).

Coumarin scaffolds have been incorporated into the design of many molecular probes as suitable signaling units. The spectroscopic response is observed upon the coordination of an analyte to a coumarin-based probe. These optical changes appear as a shift in the absorbance and/or emission band. The specific signaling mechanism used for analyte detection varies between molecular probes, but all of the mechanisms relevant to this work will fall into two major categories: single (Δsignal at one wavelength) or dual (Δsignal at two wavelengths, ratiometric) channel (Scheme 2.11).

1.5.1 Single Channel Monitoring

In the case of single channel monitoring, the addition and subsequent coordination of the analyte of interest will induce a shift at one wavelength (i.e. channel). The following are examples of signaling mechanisms that fall into this category.

1.5.2 Hypsochromic and Bathochromic Shift Systems

In cases when the chosen signaling mechanism is based on changes to the ICT or TICT character of the signaling unit, such as coumarin, the coordination of an analyte at the EDG portion of the molecule will induce a hypsochromic shift, and coordination of a guest at the EWG portion produces a bathochromic shift (Scheme 14). While probes can be based solely on monitoring bathochromic or hypsochromic shift, they are often accompanied with a hypochromic or hyperchromic shift as well. Compound 1.23b, synthesized by Helal et al., is provided in (Scheme 1.14) to aid in visualization.
Scheme 14. Cu$^{2+}$ ion interaction with an ICT based probe (1.23b). (adapted from)$^{132}$

(Left) The coordination of the analyte at the donor position increases the overall energy, which results in a hypsochromic shift. (Right) The coordination of the analyte at the acceptor position decreases the overall energy, which results in a bathochromic shift.$^{56, 103, 132}$

1.5.3 Molecular Probes that Display Hypochromic Shift

The “ON-OFF” or CHEQ (chelation enhancement of quenching). For this process the free probe is fluorescent or in the “ON” state. The addition of the analyte will result in a decrease of the fluorescence emission intensity (i.e. quenching). Mechanisms based on
the decrease of fluorescence emission suffer from sensitivity issues due to the fluorescent signal often becoming lost in the background noise/scattering.\textsuperscript{56} Quenching from metal ion coordination often arises due to the heavy metal effect (section 1.4.1).

This signaling mechanism has been widely used for Cu\textsuperscript{2+} ions as in the probes \textbf{1.23a} and \textbf{1.23b} synthesized by Helal et al. (Scheme 15).\textsuperscript{132} The molecular design approach for both probes is to utilize a tridentate chelating motif ONO\textsuperscript{-}, coordinating to the Cu\textsuperscript{2+} ions, which has a high affinity for such motifs (Scheme 15).\textsuperscript{132} The absorbance spectrum for \textbf{1.23a}, in DMSO-HEPES (1:3, 10 mM, pH 7.4), has an absorbance maximum at 346 nm assigned to a n-\(\pi^*\) transition. The coordination of Cu\textsuperscript{2+} ions resulted in the decrease of the absorbance band at 346 nm with an increase in the absorbance intensity at 375 nm, a 29 nm bathochromic shift. Upon excitation at 361 nm compound \textbf{1.23a} has an emission maximum at 460 nm, due to ICT from phenol and thiaazole to the coumarin, a modest hypochromic shift was seen with the addition of Cu\textsuperscript{2+} ions. No further decrease in emission intensity was observed beyond one equivalent, which indicates a 1:1 binding ratio. A 1:1 binding constant was calculated to be \(K_{11} = 4.4 \times 10^4\) M\textsuperscript{-1}. For probe \textbf{1.23b}, the addition of Cu\textsuperscript{2+} resulted in the decrease of the maximum absorbance band at 412 nm and an increase in the absorbance intensity at 460 nm, a bathochromic shift of 48 nm, with an isobestic point at 435 nm. This was also accompanied with a change in the color of the solution, from light green to yellow.\textsuperscript{132} The coordination through the C(2) carbonyl increases the electron withdrawing character of the carbonyl and increases the ICT character of \textbf{1.23a} and \textbf{1.23b}, which resulted in the observed solution color change. The excitation at 435 nm gave an emission maximum at 495 nm, which saw a hypochromic shift upon the addition of Cu\textsuperscript{2+} ions. The L.o.D. for compound \textbf{1.23b} was calculated to be
0.04 μM. High resolution fast-atom bombardment mass spectrometry (HRMS) was used to confirm a 1:1 binding ratio. A Cu^{2+} ion binding constant with probe 1.23b was calculated to be $K_{11} = 2.2 \times 10^6$ M$^{-1}$. Theoretical calculations of the 1.23b-Cu^{2+} ion molecular orbital interactions confirmed that the observed probe 1.23b emission intensity decrease is due to the photoinduced electron transfer (PET) (section 1.5.4) from the coumarin, oxide of the phenol and the nitrogen of the thioazole to the Cu^{2+} ion. Both probes 1.23a and 1.23b response towards Cu^{2+} ions in solutions of various pH were studied, which resulted in modest spectroscopic changes with the addition of Cu^{2+} ions outside the pH range 6-9. The reversibility of probe 1.23b was investigated by adding ethylene diamine tetraacetic acid (EDTA) to 1.23b-Cu^{2+}. EDTA removed the Cu^{2+} ions from compound 1.23b resulting in the recovery of 1.23b fluorescence emission intensity.

Scheme 15. Coordination of Cu^{2+} ions by probes 1.23a and 1.23b. (adapted from)

The coordination occurs in a 1:1 ratio (in DMSO-HEPES (1:3, 10 mM, pH 7.4). 1.23a - Cu^{2+} ($K_{11} = 4.4 \times 10^4$ M$^{-1}$), 1.23b - Cu^{2+} ($K_{11} = 2.2 \times 10^6$ M$^{-1}$). L.o.D. = 0.04 μM.

1.5.4 Molecular Probes that Display Hyperchromic Shift

Another type of shift is a hyperchromic shift. Probes that use this signaling mechanism exhibit either “OFF-ON” or chelation enhancement of fluorescence (CHEF). The CHEF mechanism occurs when the coordination of an analyte produces increased
fluorescence emission intensity. CHEF is commonly observed upon the binding of a $d^{10}$ metal ion, which results in the increased rigidity, planarity, and/or conjugation of the probe. CHEF can also be the result of the inhibition of another process. One such process is photo-induced electron transfer (PET).

The PET mechanism is a process (Scheme 16) in which the fluorescence emission of the initial molecular probe is quenched (i.e. “OFF”-state). When a fluorophore is excited an electron is promoted from the highest occupied molecular orbital (HOMO) ground state of the fluorophore to an excited-state lowest unoccupied molecular orbital (LUMO). As a result of the electron promotion the $E_{\text{HOMO}}$ of the fluorophore is lowered in energy. The increase in the $E_{\text{HOMO}}-E_{\text{LUMO}}$ gap of the fluorophore allows for an electron transfer from the HOMO of the binding unit to the HOMO of the fluorophore. Since the fluorophore HOMO is fully occupied, the excited electron in the LUMO can no longer relax back to the HOMO ground state of the fluorophore, which results in a quenched fluorescence emission. Typically, PET quenching is observed when the heteroatom of the binding site has a lone pair of electrons (i.e. -NH$_2$, -NHR’, -NR’R”, imines). Upon analyte coordination, the HOMO of the binding unit becomes lower in energy than the HOMO of the fluorophore. As a result of coordination the PET process is inhibited and fluorescence can occur (Scheme 16).
Sarkar et al. synthesized a coumarin-quinoline probe 1.24 that utilized a turn-on detection mechanism for the detection of Zn$^{2+}$ ions (Scheme 17). The coordination of Zn$^{2+}$ ions by probe 1.24 through the C(4)-oxide and C(3)-imine nitrogen increased the ICT character of 1.24, which resulted in a 35 nm bathochromic shift of the maximum absorbance band at 338 nm, in a CH$_3$CN-HEPES (1:1, pH 7.2) buffer. It was suggested that the lone pair of electrons on the imine nitrogen PET quenched the fluorescence emission of compound 1.24. The addition of Zn$^{2+}$ ions resulted in the inhibition of PET and an increased (22-fold enhancement) fluorescence emission intensity at 427 nm. Probe
1.24 also displayed very little interference from other metal chloride salts including CdCl$_2$, which only induced a five-fold increase. The octahedral coordination of Zn$^{2+}$ ions by 1.24 was confirmed by theoretical calculations. The $^1$H and $^{13}$C NMR spectra of free probe 1.24 and probe 1.24 with one equivalent of Zn$^{2+}$ ions was investigated: with Zn$^{2+}$ ions present the O-H signal (14.6 ppm) disappeared, the imine carbonyl carbon shifted from 182.1 to 182.8 ppm, and the δ-lactone carbonyl carbon shifted from 176.5 to 175.9 ppm. The authors did not report any binding constants or a detection limit.

Scheme 17. Octahedral coordination of Zn$^{2+}$ ions by probe 1.24. (adapted from)$^{79}$

Coordination occurs in a 1:2 (M:L) ratio, in a 1:1 CH$_3$CN-HEPES buffer (pH 7.2).

1.5.5 Dual Channel Monitoring

The addition of the analyte will induce a simultaneous change at two wavelengths (channels). Both wavelengths can be viewed as an individual single channel in the sense that each one can undergo similar shifts. However, these two channels are not independent of each other. Addition of the analyte will induce a shift in both wavelengths simultaneously. In a dual channel system, addition of the analyte results in a ratiometric change that is independent of the probe concentration. This makes it a more attractive option than single channel monitoring, which must take the probe concentration into
account.88, 139, 149 The following signaling mechanisms are classified as dual channel monitoring.

1.5.6 Molecular Probes that Form Excimers and Exciplexes

An excimer/exciplex is a ratiometric signaling mechanism in which a non-radiative energy transfer occurs through a dipole-dipole interaction between the \( \pi \)-orbital of the excited donor fluorophore and the \( \pi \)-orbital of an acceptor fluorophore in the ground state.\(^{150}\) When the donor and acceptor fluorophore are the same, the mechanism is referred to as excimers; this is most commonly seen with pyrene signaling units and has been previously used in the Wallace research group.\(^{151, 152}\) When the donor and acceptor signaling units are different, the mechanism is referred to as an exciplex.\(^{150}\) The excimer/exciplex emission signal always occurs at longer wavelengths due to energy lost during the dipole-dipole interaction. The broadness observed by this emission is a result of the lack of vibrational structure.\(^{150}\)

When the analyte induces the formation of an excimer/exciplex, the coordination of the analyte brings the two fluorophores together in close proximity to each other.\(^{153}\) As a result, the emission of the monomer decreases as the two fluorophores come closer together and the emission of the excimer/exciplex increases. When the analyte inhibits the excimer/exciplex formation, the coordination of the analyte results in the separation of the two fluorophores and the decrease of the excimer/exciplex emission with a simultaneous increase in the monomer emission.\(^{142, 154}\)

Lin et al. used compound 1.25, an ethylene diamine linked bis-coumarin probe, to monitor the excimer formation between the \( \pi-\pi^* \) stacking of the two coumarin moieties upon the coordination of Al\(^{3+}\) ions (Scheme 18).\(^{133}\) In CH\(_2\)Cl\(_2\) (an apolar solvent), probe
1.25 exhibited both monomer and excimer emission bands, 395 and 500 nm respectively. The solvent system of CH$_3$OH:CH$_2$Cl$_2$:H$_2$O (2:3:0.01 ratio) is used as it was experimentally determined to be the solvent system that resulted in the largest decrease of excimer emission in the free state. In this solvent system, the addition of Al$^{3+}$ ions resulted in a 7 nm bathochromic shift of the absorbance maximum at 327 nm with an isobestic point at 340 nm. The coordination of Al$^{3+}$ ions through the two nitrogen atoms of the ethylene diamine spacer reduced their electron donating character, leading to the inhibition of PET quenching. When excited at 340 nm the addition of Al$^{3+}$ ions resulted in a hyperchromic shift of the emission at 395 nm (16-fold increase) and at 500 nm (57-fold increase). A Job’s plot confirmed the binding ratio of 1:1 [Al(1.25)$^{3+}$] with a binding constant calculated to be $K_{11} = 1.2 \times 10^5$ M$^{-1}$. The mode of coordination through the two nitrogen atoms was confirmed through $^1$H NMR. The addition of Al$^{3+}$ caused a broadening and downfield shift of the protons N-H, C(4)H, and the methyl linkers. The addition of Fe$^{3+}$ ions also showed a modest hyperchromic shift of the emission and the formation of the excimer, but in a 1:12:12 ratio of 1.25-Al$^{3+}$-Fe$^{3+}$ the observed emission spectrum was significantly increased with respect to Fe$^{3+}$, indicating selectivity towards Al$^{3+}$ over Fe$^{3+}$ ions. However, no limit of detection was reported. In Scheme 18, the forward arrow shows the coordination of the Al$^{3+}$ ion, which induces the formation of the coumarin excimer. The reverse arrow in the scheme below shows the reversibility of 1.25 with the addition of the bidentate chelator ethylene diamine, EN, which removes the Al$^{3+}$ ion from 1.25. The authors did not report a detection limit.
Scheme 18. Excimer formation of probe 1.25 induced by the coordination of Al$^{3+}$ ions.

(adapted from)$^{133}$

Binding occurs in a 1:1 ratio ($K_{11} = 1.2 \times 10^5 \text{ M}^{-1}$). Reversibility was shown by the addition of EN.

1.5.7 Excited State Intramolecular Proton Transfer

Excited-state intramolecular proton transfer (ESIPT) is a mechanism in which a proton is transferred between two heteroatoms while in the excited-state. The ESIPT mechanism is commonly observed with imine-enol and keto-enamine tautomers, the proton transfer and tautomerization is shown in Scheme 19.$^{155, 156}$ ESIPT is stabilized by a strong intramolecular hydrogen bond and resonance assisted hydrogen bonding (RAHB)$^{157}$ It is well known that the intramolecular hydrogen bonding interaction is typically very short ($\leq 2.00 \text{ Å}$) and the hydrogen bond forms a pseudo six member ring.$^{148, 158}$

Scheme 19. Imine-enol and keto-enamine tautomer. (adapted from)$^{72}$

Although this process occurs very rapidly in the excited-state, energy is lost during the proton transfer and often results in large Stoke’s shift (Table 1.5)$^{156}$ The large Stoke’s shift is an attractive property for molecular probes because it allows for the inner filter effect to be avoided.$^{156, 159}$ Shown in Figure 1.5 and Table 1.5 are a selected number
of compounds 1.26-1.30 that undergo the ESIPT mechanism (Scheme 20). The compounds were chosen to provide insight to the effects on the Stoke’s shift due to the substituent location and the electron donating or electron withdrawing character of the substituent.

Figure 5. Structures of compounds 1.26-1.30. (adapted from)

Table 5

Photophysical Properties of Compounds 1.26-1.30

<table>
<thead>
<tr>
<th>Compound</th>
<th>Abs $\lambda_{\text{max}}$ (nm)</th>
<th>Fl $\lambda_{\text{max}}$ (nm)</th>
<th>Stoke’s shift</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.26a</td>
<td>339</td>
<td>556</td>
<td>217</td>
<td>cyclohexane</td>
</tr>
<tr>
<td>1.27a</td>
<td>348</td>
<td>573</td>
<td>225</td>
<td>cyclohexane</td>
</tr>
<tr>
<td>1.26b</td>
<td>365</td>
<td>547</td>
<td>182</td>
<td>cyclohexane</td>
</tr>
<tr>
<td>1.27b</td>
<td>384</td>
<td>437/575</td>
<td>53/191</td>
<td>cyclohexane</td>
</tr>
<tr>
<td>1.28a</td>
<td>373</td>
<td>627</td>
<td>254</td>
<td>cyclohexane</td>
</tr>
<tr>
<td>1.28b</td>
<td>381</td>
<td>655</td>
<td>274</td>
<td>cyclohexane</td>
</tr>
<tr>
<td>1.29(o/p)</td>
<td>430/397</td>
<td>648/635</td>
<td>218/238</td>
<td>cyclohexane</td>
</tr>
<tr>
<td>1.30(o/p)</td>
<td>389/368</td>
<td>605/600</td>
<td>216/232</td>
<td>cyclohexane</td>
</tr>
</tbody>
</table>

Note: Abs $\lambda_{\text{max}}$ is the wavelength, in nm, at which the maximum absorbance is located, Fl $\lambda_{\text{max}}$ is the wavelength, in nm, at which the maximum fluorescence emission intensity is located, Stoke’s shift is calculated by the following equation (Fl $\lambda_{\text{max}}$ - Abs $\lambda_{\text{max}}$) and values are reported in nm. Entries with two values denote imine-enol/keto-enamine were observed in the spectrum.
The ESIPT process is illustrated in Scheme 20, compound 1.31 will be used as the example. The lowest energy and thereby the most stable tautomer, that is commonly reported, in the ground state is the imine-enol tautomer. Upon excitation an electron is promoted from the $S_0$(imine-enol) to the excited singlet state, $S_1$(imine-enol). During the internal conversion process the proton of the enol O-H is transferred to the nitrogen of the imine, double bonds are rotated to yield the keto-enamine tautomer. As the excited singlet $S_1$(keto-enamine) state is populated, energy is lost. This energy loss will in turn result in a larger Stoke’s shift. Once the $S_1$(keto-enamine) is fully populated, the electron will relax back to the keto-enamine ground state, $S_0$(keto-enamine). As previously stated, the lowest ground state energy tautomer is the imine-enol therefore in the $S_0$(keto-enamine) another proton transfer occurs to generate the imine-enol tautomer again.

Scheme 20. ESIPT process with compound 1.31.(adapted from) 

$E_0$ is the imine-enol tautomer in the ground state. $E'$ is the imine-enol tautomer in the excited singlet state (arrows are provided to show proton transfer). $K'$ is the keto-enamine tautomer in the excited singlet state. $K_0$ is the keto-enamine tautomer in the ground state (arrows are provided to show proton transfer).
The ESIPT signaling mechanism can also be influenced by the choice of solvent. In polar protic solvents such as MeOH and EtOH, the enol O-H can participate in hydrogen bonding with the solvent. In situations like the one just mentioned, the ESIPT process is inhibited, and the emission spectrum will be a result of the $S_0$(imine-enol) to $S_1$(imine-enol) transition (Scheme 21).\textsuperscript{75, 155, 159, 165}

Since the enaminone functional group contains both an EDG in the amine and an EWG in the carbonyl, a push-pull of electron density occurs. This allows for the enaminone to act as both an electrophile and a nucleophile.\textsuperscript{166} A common application of the ESIPT signaling mechanism is the analyte-induced inhibition of the ESIPT process.\textsuperscript{167} In doing so the emission spectra often undergoes a hypsochromic shift with a concomitant hyperchromic shift.\textsuperscript{168} The inhibition can occur through multiple pathways. In the first pathway, metal coordination, the hydrogen atom is replaced by the metal (Schemes 21 and 22).\textsuperscript{87, 139, 165, 167} The second effect is due to anion binding, whereby hydrogen bonding interrupts the ESIPT mechanism, by deprotonation.\textsuperscript{101, 148}

Liu et al. synthesized probe 1.32 which showed selectively towards Mg\textsuperscript{2+} ions, via the inhibition of an $E/Z$ C=N isomerization, in an alcoholic environment (Scheme 21).\textsuperscript{165} The absorption spectrum shows a 54 nm bathochromic shift (354 to 408 nm) upon the binding of Mg\textsuperscript{2+} ions. In the protic solvents MeOH and/or EtOH, a hydrogen bond was formed with the enol O-H of compound 1.32 and the solvent molecule (Scheme 21), which allowed for the $E/Z$ C=N isomerization and a decrease of the fluorescence emission intensity ($\phi_{Fl} = 0.003$). The emission spectrum of compound 1.32 displayed the emission bands from both tautomers: imine-enol at 458 nm and keto-enamine at 520 nm ($\lambda_{ex}$ 408 nm). Unlike the other probes based on the inhibition of ESIPT, compound 1.32
did not display the same spectroscopic responses. Generally, the addition of the analyte would induce a hyperchromic shift of the emission at 458 nm but at the expense of the keto-enamine emission at 520 nm. For probe 1.32 a hypochromic shift of the emission band at 520 nm was not observed. Therefore, the increase in the emission band at 458 nm due to coordinating Mg$^{2+}$ ions was a result of the inhibition of the $E/Z\ C=N$ isomerization and not the inhibition of the ESIPT process. A Job’s plot confirmed that the binding ratio of 1.32-Mg$^{2+}$ occurs in a 1:1 ratio with a calculated binding constant $K = 1.3 \times 10^5 \ M^{-1}$. It was noted that the addition of both Zn$^{2+}$ and Ca$^{2+}$ ions did induce a small hyperchromic shift but not to any appreciable extent as the Mg$^{2+}$ ions (148-fold enhancement, $\phi_{Fl} = 0.55$). A detection limit (in EtOH) was calculated to be $2.3 \times 10^{-8} \ M$. It was also reported that compound 1.32 was able to detect Mg$^{2+}$ ions in EtOH-HEPES (99:1, 10 mM, pH range 6-8).

![Scheme 21. Coordination of Mg$^{2+}$ ions by compound 1.32. (adapted from)\textsuperscript{165}](image)

Coordination occurs in a 1:1 (in EtOH). Mg$^{2+}$ ions increased the $\phi_{Fl}$ from 0.0032 to 0.55. L.o.D. = $2.3 \times 10^{-8} \ M \ (K = 1.3 \times 10^5 \ M^{-1})$.\textsuperscript{165}

Qin et al. designed probe 1.33 that utilized both ESIPT and PET processes to detect Mg$^{2+}$ ions (Scheme 22).\textsuperscript{87} Due to the hydrazone linker that is present in compound 41
1.33 both the keto-enamine and the imine-enol tautomers exhibited very little to no fluorescence emission at 445 nm when excited at 398 nm. The addition of Mg\(^{2+}\) ions resulted in the inhibition of the PET and a hyperchromic shift in the fluorescence emission intensity. In the absence of Mg\(^{2+}\) ions 1.33 has an absorbance spectrum with local maxima at 310, 317, 357, and 362 nm due to the \(\pi-\pi^*\) transitions of the major and minor isomers (Scheme 22). In the presence of Mg\(^{2+}\) ions the 310 and 317 nm absorbance bands merged into one band and the same occurred for the 357 and 362 nm absorbance bands. The addition of Mg\(^{2+}\) ions gave rise to isobestic points at 325 and 365 nm with an increase in absorbance at 420 nm. A 1:1 1.33-Mg\(^{2+}\) binding constant \(K_{11}\) was calculated to be \(3.5 \times 10^4\) M\(^{-1}\). A detection limit, in CH\(_3\)CN, was calculated at 0.68 ppm. A \(^1\)H NMR study was carried out to confirm the mode of coordination shown below. The addition of Mg\(^{2+}\) ions to 1.33 resulted in the slight disappearance of the enol O-H signal of both isomers as well as the amide N-H of the major isomer. The imine proton, major isomer, shifted upfield with the addition of Mg\(^{2+}\) ions. The involvement of the quinoline nitrogen is supported by the observed downfield shift of the C-H proton on both isomers.\(^{87}\)

![Scheme 22. Coordination of Mg\(^{2+}\) ions by compound 1.33. (adapted from)\(^{87}\)](image)

Studies were carried out in CH\(_3\)CN. Coordination occurs in a 1:1 ratio, L.o.D. = 0.68 ppm, and \(K_{11} = 3.5 \times 10^4\) M\(^{-1}\).\(^{87}\)
The dipodal benzimidazole based probe 1.34 reported by Kim et al. detected Zn$^{2+}$ ions by the inhibition ESIPT (Scheme 23).$^{139}$ Compound 1.34 was screened towards various metal nitrate salts, in a 1:9 DMSO-CH$_3$CN solution. The individual addition of Cu$^{2+}$, Fe$^{3+}$, Co$^{2+}$, and Ni$^{2+}$ ions resulted in a decrease in the emission band at 443 nm, which is attributed to PET quenching as a result of the electron transfer from 1.34 to the unfilled d-orbital of these metal ions. The addition of Zn$^{2+}$ ions induced changes of the charge transfer, CT, band of 1.34 resulting in a 46 nm hypsochromic shift of the maximum emission band, 443 to 397 nm, and a ~20-fold enhancement of the emission intensity at 397 nm. A 1:1 binding ratio was determined by a Job’s plot and a binding constant $K_{11} = 1.4 \times 10^4$ M$^{-1}$ was calculated via Benesi-Hildebrand plot. A detection limit was calculated to be 0.3 μM.$^{139}$

![Scheme 23. Coordination of Zn$^{2+}$ ions by probe 1.34. (adapted from) $^{139}$](image)

Coordination occurs in a 1:1 ratio (1:9 DMSO-CH$_3$CN solution). L.o.D. = 0.3 μM ($K_{11} = 1.4 \times 10^4$ M$^{-1}$).$^{139}$

ESIPT was also reported, by Goswami et al., as an effective signaling mechanism for the detection of OAc$^-$ ions (Scheme 24).$^{148}$ The addition of OAc$^-$ ions to probe 1.31 resulted in a decrease in the absorbance bands at 322 and 368 nm and an increase at 440 nm with an isobestic point at 395 nm. A visual solution color change from colorless to green-yellow was observed. To account for the absorption band appearing at 440 nm it
was suggested by Goswami et al. that the 1:1 1.31-OAc$^-$ ion interaction resulted in the deprotonation of the naphthalene O-H. The deprotonation then leads to an enhancement of $\pi$ delocalization and a reduction of the $\pi$-$\pi^*$ transition.$^{148}$ A detection limit (UV-Vis) was calculated to be 2.0 $\mu$M with a $K_{\text{assoc.}} = 2.6 \times 10^4$ M$^{-1}$. Compound 1.31 owed its weak fluorescence intensity ($\lambda_{\text{ex}}$ 368 nm) to the emission of the enol form and 1.31 not undergoing ESIPT. The addition of OAc$^-$ ions induced the turn on of the ESIPT mechanism, which resulted in a $\sim$ 13-fold increase of the fluorescence intensity. A detection limit was calculated to be 4.4 $\mu$M with a $K_{\text{assoc.}} = 4.0 \times 10^4$ M$^{-1}$. A small hyperchromic shift of the fluorescence intensity was observed upon the addition of benzoate, due to the same carboxylate motif, but to no appreciable amount with respect to the OAc$^-$ ions.$^{148}$

Scheme 24. Coordination of OAc$^-$ ions by compound 1.31. (adapted from)$^{148}$

Interaction occurred in a 1:1 ratio (100% CH$_3$CN). L.o.D. = 4.4 $\mu$M (fluorescence) 2.0 $\mu$M (UV-Vis) ($K_{\text{assoc.}} = 4.0 \times 10^4$ M$^{-1}$).$^{148}$

1.6 Chemodosimeters

Chemodosimeters differ from chemosensors in that the probe undergoes an irreversible chemical change.$^4, 22, 56, 146, 169-171$ However, the process, while usually irreversible, has been shown to be reversible in a few cases.$^7, 172-174$ Chemodosimeters are commonly used in anion detection (Schemes 26-31) but can be used for the detection of cations as well (Scheme 25).$^{48, 175}$ Some chemodosimeters, upon the addition of analyte, have a stoichiometric probe-analyte ratio. For others, the addition of a catalytic amount of
analyte either directly induces a chemical reaction or orientates a cascade reaction, resulting in the regeneration of the analyte and irreversibly altering the probe. The latter of the two methods takes much longer to occur but allows for higher sensitivity.\textsuperscript{56}

Chemodosimeters can also be used to initiate a signaling mechanism. One example is the analyte causes an irreversible reaction that results in a through bond energy transfer (TBET) mechanism. TBET occurs when two fluorophores that have no spectral overlap are linked together through a conjugated spacer.\textsuperscript{150,176-178} With respect to chemodosimeters that use the TBET sensing mechanism the two fluorophores are linked together through a non-conjugated spacer. The addition of the analyte induces a reaction that results in the spacer becoming conjugated allowing for the TBET process to occur.\textsuperscript{150} The analyte induced TBET process allows makes this a dual channel signaling mechanism. In the TBET “OFF-state” the absorbance and emission spectrum is due to the donor moiety. In the TBET “ON-state” the excitation of the donor moiety results in the transfer, decrease in emission intensity, of energy to the acceptor moiety, increase in emission intensity.\textsuperscript{150} Gong et al. synthesized molecule 1.35, a rhodamine-coumarin ensemble, for the selective detection of the Hg\textsuperscript{2+} ions by utilizing the TBET mechanism (Scheme 25).\textsuperscript{134} In a solution of THF-HEPES (1:1, 0.01M, pH 7.2), probe 1.35 has an initial absorbance maximum at 420 nm, which is assigned to the absorbance of the coumarin. The addition of HgCl\textsubscript{2} resulted in a 30 nm bathochromic shift of the absorbance band at 420 nm with the concomitant hyperchromic shift of a new absorbance band at 567 nm, due to the ring opening of the rhodamine. In the absence of Hg\textsuperscript{2+} ions the excitation of compound 1.35, at 420 nm, resulted in an emission band centered at 470 nm, solely due to the fluorescence of coumarin donor moiety. The addition of Hg\textsuperscript{2+} ions
resulted in a decrease the emission intensity at 470 nm and a hyperchromic shift of the new emission band at 580 nm, a Stoke’s shift of 110 nm. The thiosemicarbazide functional group of 1.35 is known to undergo desulfurization and cyclization to a 1,3,4-oxadiazole. Upon the desulfurization the product (compound 1.36) is now fully conjugated and therefore the emission at 580 nm is the result of TBET. To confirm the TBET signaling mechanism, H⁺ ions were added to a solution of 1.35 to induce a ring opening of the rhodamine moiety. This resulted in the increase of emission band at 470 nm and a small increase in the emission intensity at 580 nm. Compound 1.36 was isolated and the structure was confirmed by the analysis of ¹H NMR and mass spectrometry. A limit of detection was calculated to be 7 nM. This design approach was applied to detect Hg²⁺ ions in Hela cells.¹³⁴

Scheme 25. Detection of Hg²⁺ ions by compound 1.35. (adapted from)¹³⁴

Lo.D. = 7 nM in THF-HEPES (1:1, 0.01M, pH 7.2). The desulfurization and subsequent cyclization of the thiosemicarbazide of 1.35 to a 1,3,4-oxadiazole by the addition of the Hg²⁺ ions in a 1:1 solution. The Hg²⁺ ions detection limit was calculated at 7 nM.¹³⁴

Zhang et al. used a similar approach for 1.37 in the detection of HOCl by TBET signaling mechanism (Scheme 26).¹⁷⁹ In an EtOH-Na₂HPO₄ buffer (3:7, 50 mM, pH 6)
solution, the free probe 1.37 has an initial absorbance at 350 nm and has a fluorescence emission maximum at 440 nm ($\lambda_{\text{ex}}$ 350 nm). The absorbance and emission spectra of 1.37 is due solely to the naphthalene moiety. No significant changes were observed to the 350 nm absorbance band upon the addition of HOCl; however, the absorbance of the ring opening of the rhodamine moiety resulted in a new absorbance band at 570 nm. The addition of HOCl induced a decrease of the emission intensity at 440 nm (energy being transferred from the naphthalene moiety) and an increase in the emission intensity at 585 nm (energy being accepted by the now open rhodamine moiety), a bathochromic shift in the maximum fluorescence emission intensity of 145 nm. This transfer of energy from the naphthalene moiety to the rhodamine moiety occurs in ratiometric fashion upon the addition of HOCl. The conversion of 1.37 to 1.38 went to completion in under 50 seconds, no rate constant was reported. A HOCl limit of detection was calculated to be 0.1 $\mu$M. $^{179}$ This approach was used to investigate the endogenous HOCl concentrations in murine RAW264.7 cells, which produce HOCl in the presence of lipopolysaccharides. $^{179}$

![Scheme 26](image)

**Scheme 26. Detection of HOCl by compound 1.37.** (adapted from) $^{179}$

*Studies carried out in EtOH-Na$_2$HPO$_4$ buffer (3:7 50 mM, pH 6). The conversion of 1.37 to 1.38 went to completion within 50 seconds with the L.o.D. = 0.1 $\mu$M. $^{179}$*

Another type of chemodosimeter design approach is a displacement. Zhang et al. synthesized a naphthalene-enaminone (1.39)-Zn$^{2+}$ ensemble to selectively detect H$_2$PO$_4^-$.
ions (Scheme 27). The 1.39-Zn$^{2+}$ complex detects H$_2$PO$_4^-$ ions via an “OFF-ON-OFF” signaling mechanism. Prior to the addition of Zn$^{2+}$ ions the absorbance spectrum of the probe has two bands, at 238 and 310 nm, that is associated with the $\pi-\pi^*$ transitions and the band at 400 and 418 nm are due to the C=N isomerization. It was observed that the free probe 1.39 displays a very weak emission band at 451 nm when excited at 400 nm. The addition of Zn$^{2+}$ ions resulted in the inhibition of the C=N isomerization and the merger of the two absorbance signals at 400 and 418 nm as well as a hyperchromic shift of the absorbance at 238 nm. The addition of Zn$^{2+}$ ions also resulted in a 3 nm hypsochromic shift and a large hyperchromic shift of the emission intensity. Probe 1.39 also showed selectivity towards Zn$^{2+}$ ions over Cd$^{2+}$ ions in organic solvents (MeOH or CH$_3$CN) 1.39:Zn$^{2+}$:Cd$^{2+}$ equivalence ratio of 1.0:0.5:1.0 respectively. When the same experiment as just stated was repeated in the presence of the ions Cu$^{2+}$ and Fe$^{3+}$ little to no enhancement was observed. The decreased selectivity towards Zn$^{2+}$ ions in the presence of the ions Cu$^{2+}$ and Fe$^{3+}$ is due to the heavy metal effect. The decreased selectivity is observed in both solvent systems. The detection limit of Zn$^{2+}$ ions was calculated to be 2.5 $\mu$M with a log $K_{\text{assoc.}}$ = 3.58, and a binding ratio of 1:2 [Zn(1.39)$_2$]$^{2+}$ was confirmed by a Job’s plot. It should be noted that upon coordination of Zn$^{2+}$ ions the $^1$H NMR showed no deprotonation of the phenolic proton. The fluorescence spectroscopic response of the isolated and in situ formed [Zn(1.39)$_2$]$^{2+}$ complex were both screened against various anions: Cl$^-$ and OAc$^-$ (increased emission intensity); F$^-$, Br$^-$, I$^-$, NO$_3^-$, SO$_4^{2-}$, HPO$_4^{2-}$, PO$_4^{3-}$, and P$_2$O$_7^{4-}$ (small intensity decrease); H$_2$PO$_4^-$ (quenched). A H$_2$PO$_4^-$ ion detection limit was calculated to be in the 0.5-4.5 $\mu$M range.
Scheme 27. \([\text{Zn(1.39)}_2]^{2+}\) displacement assay for \(\text{H}_2\text{PO}_4^-\) ions detection. (adapted from)\(^{82}\)

Compound 1.39 coordinates \(\text{Zn}^{2+}\) ions in a 2:1 (L:M) ratio (L.o.D. = 2.5 \(\mu\)M, log \(K_{\text{assoc.}} = 3.58\)). \(\text{H}_2\text{PO}_4^-\) ions displace the \(\text{Zn}^{2+}\) ions (L.o.D. in the 0.5-4.5 \(\mu\)M range).\(^{45}\)

As stated previously various properties can be exploited when designing chemodosimeters (Schemes 28 and 29).\(^{135, 136}\) Compounds 1.40 and 1.42 are two chemodosimeters synthesized for the selective binding of \(\text{F}^-\) ions. Fluoride is known to form strong dative covalent bonds with boron and silicon forming Lewis acid-base adducts. These moieties are often used in sensor design for fluoride recognition. Using Si-O and Si-F typical bond strengths (368 and 565 kJmol\(^{-1}\) respectively)\(^{53}\), it can easily be deduced if the target analyte is the \(\text{F}^-\) ion then one should incorporate a Si-O bond in the chemodosimeter, known as desilylation.\(^{135, 136}\)

Cao et al. synthesized a conjugated coumarin-BODIPY ratiometric chemodosimeter 1.40, which selectively detected the \(\text{F}^-\) ions via desilylation (Scheme 28).\(^{135}\) Upon the addition of \(\text{F}^-\) ions, the Si-O bond is cleaved resulting in the decrease of the absorbance bands at 398 and 592 nm and an increase in the absorbance intensity at ~ 450 and ~ 690 nm (three isobestic points observed at 420, 510, and 612 nm).\(^{135}\) Compound 1.40 has an initial maximum fluorescence emission at 606 nm (\(\lambda_{\text{ex}}\) 420 nm), which decreased upon the addition of \(\text{F}^-\) ions and an increase in the emission intensity at
472 nm. The response observed in both the absorbance spectrum and fluorescence emission spectrum is due to changes in the ICT character of 1.40 and 1.41. Theoretical calculations and molecular modeling were investigated to explain the large hypsochromic shift of the fluorescence emission. It was concluded that the desilylation of compound 1.40 resulted in the coumarin rotating orthogonally (planarity broken) to the BODIPY unit, which increases the $E_{HOMO}$ and lowering the HOMO-LUMO energy gap difference resulting in the emission at a lower wavelength. The ICT character of coumarin derivatives with substituents at C(7) and C(3) has been previously discussed (section 1.4.2). BODIPY also has ICT character when substitutions are located at the C(3’) position. The conversion of Si-O to O-H changes the electron donating character of the coumarin C(7) substituent and therefore changes the overall ICT character of 1.41, which results in the large observed spectroscopic response. Compound 1.40 has a F$^-$ ions detection limit of 0.12 μM. While this a relatively low detection limit, this work was carried out in 100% DMSO.

Scheme 28. Desilylation of compound 1.40 by F$^-$ ions. (adapted from)
Sokkalingam et al. used a similar approach for compound 1.42, which selectively detected F⁻ ions through desilylation via an “OFF-ON” signaling mechanism (Scheme 29). Upon the addition of F⁻ ions the Si-O bond of 1.42 is cleaved resulting in a 104 nm bathochromic shift of the absorbance from 330 nm to 434 nm with an isobestic point at 363 nm. The excitation of 1.42 at 410 nm resulted in a turn on and an increase of the fluorescence emission band at 500 nm. The absorbance and fluorescence isotherms indicate the reaction between 1.42 and the F⁻ ion occurs in a one-to-one fashion. The large bathochromic shift due to the addition of F⁻ ions, along with the observed changes in the color of the solution from colorless to yellow and pH, is attributed to the formation of the chromenolate anion (1.43). To confirm that the formation of the 1.43 ion is the reason for the large bathochromic shift in absorbance spectrum, the absorbance of 1.44 was obtained over the pH range 3-10. When the pH of the solution is ≤ 7 the absorbance maximum is at 335 nm, which is due to the protonated 1.44; however, when the pH of the solution is > 8 the maximum absorbance band shifted to 410 nm. ¹H NMR was used to investigate that the addition of F⁻ ions induced the cleavage of the Si-O bond (in CD₃CN). It was observed that the desilylation induced an upfield shift of the signals produced by the protons located on the coumarin core, C-H (positions 5, 6, 8, and 3). The fluoride detection limit was calculated to be ~50 nM (in CH₃CN) and ~ 0.3 ppm in HEPES buffer (pH 7.4, 0.5% CH₃CN). The kinetics of 1.42 was also tested in various organic-aqueous solvent ratios to determine the effects of water to the desilylation. In organic media, the desilylation of 1.42 went to completion in under 10 seconds; however, in the presence of water the reaction required an increase in time, by a factor of ~450, to reach completion.
Scheme 29. Desilylation of compound 1.42 by F⁻ ions. (adapted from)¹³⁶

In 100% CH₃CN the desilylation leads to compound 1.43 and a L.o.D. = ~50 nM. In HEPES buffer (pH 7.4, 0.5% CH₃CN) the desilylation leads to compound 1.44 and a L.o.D. = ~3 ppm.¹³⁶

Chemodosimeters have also been employed for the detection of thiols (R-SH). The coumarin derivative 1.45, synthesized by Jung et al., contained a doubly activated (α,β-unsaturated diester) Michael carbon that detected biologically relevant thiols through the inhibition of ICT resulting in an “OFF-ON” signaling mechanism (Scheme 30).¹³⁷

Compound 1.45 was screened against various amino acids in PBS buffer (10 mM, pH 7.4, 10% DMSO). A spectroscopic response was observed upon the addition of cysteine, homocysteine, and glutathione. The largest spectroscopic response was observed upon the addition of cysteine (Scheme 30).¹³⁷ The addition of cysteine resulted in the decrease of the maximum absorbance at 491 nm and an increase in the absorbance at 429 nm. The addition of cysteine also resulted in the increase of the fluorescence emission intensity at 502 nm. Mass spectrometry was investigated to confirm the addition of cysteine to probe 1.45. The observed mass difference of compound 1.45 and compound 1.46 was 122 m/z, which is the mass of cysteine.¹³⁷ ¹H NMR was investigated and confirmed that cysteine is adding in a 1,4 fashion by observing the upfield shift of the C-H₁ signal from ~7.7 (1.45) to ~4.4 ppm (1.46) as well as the new C-H₄ signal at ~4.3 ppm.¹³⁷ A detection limit was calculated to be 30 nM. It was hypothesized that the selectivity of compound 1.45 towards cysteine, pKₐ = 8.3, was aided by the chosen pH of 7.4, which suggested that in
the solvent system the cysteine thiol existed predominately as the more nucleophilic thiolate anion. To confirm this hypothesis, the spectroscopic response of probe 1.45 upon the addition of cysteine was investigated over the pH range 6-11. As the pH of the solution was made more basic, up to 11, the addition of cysteine resulted in larger enhancements of fluorescence emission intensity, thus supporting the reactive nucleophile is the thiolate anion.\textsuperscript{137} This is an attractive approach for the detection of biologically relevant thiols; however, the observed spectroscopic response required the addition of 100 equivalents of the thiol. It should be addressed that the authors did not screen other anions that can potentially undergo similar 1,4-additions like NC\textsuperscript{-} ions. The investigation of a similar doubly activated coumarin chemodosimeter will be discussed in Chapter II.

\begin{center}
\begin{tikzpicture}
\node [draw,thick] (1) {1.45};
\node [draw,thick, right=2cm of 1] (2) {1.46};
\node [draw,thick, below=1cm of 1] (3) {1.45};
\node [draw,thick, below=1cm of 2] (4) {1.46};
\draw [->] (1) -- (2);
\end{tikzpicture}
\end{center}

Scheme 30. Michael addition of cysteine to compound 1.45. (adapted from)\textsuperscript{137}

\textit{L.o.D. = 30 nM in PBS buffer (10 mM, pH 7.4, 10% DMSO).}\textsuperscript{137}

Goswami et al. reported a coumarin probe (1.47) for the selective detection of N\textsubscript{2}H\textsubscript{4} (Scheme 31).\textsuperscript{138} This drastic change in electronics resulted in a colorimetric change from orange-yellow to light green.\textsuperscript{138} The absorption spectra, in CH\textsubscript{3}CN, saw a decrease of the maximum absorbance at 485 nm and an increase in the absorbance band at 395 nm. Upon the addition of N\textsubscript{2}H\textsubscript{4} two isobestic points were observed at 273 and 335 nm. This supports that the interaction between probe 1.47 and N\textsubscript{2}H\textsubscript{4} results in a new species,
compound 1.48, which has a different absorbance spectrum. The addition of N₂H₄ also resulted in the decrease of the maximum emission band at 545 nm with a new maximum emission band at 500 nm. The observed spectroscopic response is due to the inhibition of ICT as a result of the formation of the pyrazole of compound 1.48. Since the product 1.48 of the reaction between compound 1.47 and N₂H₄ results in change in mass, mass spectrometry was investigated to confirm this mass difference between compounds 1.47 and 1.48, 356 and 352 m/z respectively (values reported as [M+H]⁺).¹³⁸ ¹H NMR was also used to confirm that the reaction between probe 1.47 and N₂H₄ resulted in compound 1.48 by the disappearance of the O-H signal in the probe 1.47 spectrum at 8.6 ppm and the new N-H and pyrazole C-H signals at 7.9 and 6.8 ppm respectively.¹³⁸ A detection limit was calculated to be 3.4 × 10⁻⁶ M with the reaction completed within 180 seconds. A rate constant k, was calculated at 20 × 10⁻³ s⁻¹. Excess of various diamines were tested with no significant spectroscopic changes reported.¹³⁸

Scheme 31. Detection of N₂H₄ by compound 1.47. (adapted from)¹³⁸

₁.⁷ Summary

In summary this general introduction discussed the industrial applications, biological significance, and the difficulty of detecting the NC⁻, Zn²⁺, Cd³⁺, and Hg²⁺ ions. The synthetic routes and photophysical properties of coumarin and its derivatives were
reviewed. The role of azomethines (enaminones) as suitable anion and cation binding units were mentioned. Various signaling mechanisms based on optical spectroscopic techniques were discussed, and examples for each mechanism were given. ESIPT, commonly exhibited by enaminones, has many desirable qualities as a signaling mechanism for molecular probes. The advantages of chemodosimeters were also highlighted.
1.8 Hypothesis

The information provided in this chapter serves as a basis for the research that will be shown and discussed in Chapters II and III. The data collected answer the following three hypotheses:

1. The synthesis of a coumarin-enaminone probe will take advantage of the electrophilic nature of enaminones and serve as a suitable site for a nucleophilic attack from NC⁻ ions.

2. The synthesis of coumarin-enaminone probes will use the nucleophilic bidentate enaminone to coordinate a $d^{10}$ metal ion (Zn²⁺, Cd²⁺, Hg²⁺).

3. The synthesis of a coumarin-enaminone probe can serve as an ion-pair sensor.
CHAPTER II – CHEMODOSIMETERS FOR THE DETECTION OF NC IONS

2.1 Introduction

This chapter focuses solely on the detection of cyanide using chemodosimeters (section 1.6). In literature there are three common chemodosimeter motifs: 3, 18, 20, 182-184

1. Displacement assay (DA)
2. Direct addition to a Lewis acid site
   2.1 1,2-addition
   2.2 1,4-addition

2.1.1 Displacement Assay

Many of the chemodosimeters that employ the DA motif contain a transition metal ion, for example Cu$^{2+}$ ions, coordinated to a binding unit. In this approach, the coordination of the Cu$^{2+}$ ion, a 3$d^9$, typically results in a decrease in the fluorescence emission intensity. As NC$^-$ ions are added to the probe-Cu$^{2+}$ complex, the NC$^-$ ions will coordinate to and eventually displace the copper ions and, due to the synergistic effect, form a stable complex as [Cu(CN)$_x$]$^{n-}$, where n = 2 or 4. This displacement results in the recovery of the initial uncoordinated probe’s fluorescence emission signal. For this design to work, however, the $K_{assoc}$ for the probe-Cu$^{2+}$ complex cannot exceed the formation constant of [Cu(CN)$_x$]$^{n-}$. The process from probe-Cu$^{2+}$ to the displacement of the stable [Cu(CN)$_x$]$^{n-}$ complex is an “OFF-ON” signaling mechanism. 8, 12, 18, 20, 172, 183, 185-187

Scheme 32 depicts a cartoon representation of a chemodosimeter that uses the DA approach. The top portion of the scheme represents the coordination of a transition metal ion to a molecular probe. The bottom portion of the scheme depicts the displacement of
that transition metal ion from the addition of the NC\(^-\) ion and the formation of the metal-cyanide complex. This chemodosimeter displacement motif has drawn attention in the detection of the NC\(^-\) ion, as shown in Schemes 33-36.\(^{12, 18, 186, 187}\)

Scheme 32. Cartoon of the DA approach for the detection of cyanide.

*(Top) Shows the coordination of the molecular probe with the transition metal. (Bottom) Shows the transition metal-probe complex and the displacement of the metal by addition of NC\(^-\) ions.*

Wang et al. published a pyrene-Cu\(^{2+}\) based probe 2.0 for the detection of NC\(^-\) ions that employed the DA approach (Scheme 33).\(^{187}\) The detection mechanism for this work is an “ON-OFF-ON” system. The pyrene probe was initially fluorescent (i.e. “ON”). The coordination of Cu\(^{2+}\) ions to the probe quenched the fluorescence emission through PET (i.e. “OFF”). Upon the addition of NC\(^-\) ions, the Cu\(^{2+}\) ion was stripped out from probe 2.0, forming the [Cu(CN)\(_x\)]\(^{n-}\) complex. However, the system requires 20 equivalences of NC\(^-\) ions to reestablish the fluorescence signal back to its original intensity. The limit of
detection was calculated to be $1.2 \times 10^{-6}$ M in a H$_2$O-CH$_3$CN solution (80:20 ratio). While the 2.0-Cu$^{2+}$ complex was able to work in an aqueous solution coordination, a large and potentially harmful amount of cyanide was necessary to displace the copper ion and recover the original fluorescence intensity.

Scheme 33. DA approach for the detection of NC$^{-}$ ions by the complex 2.0-Cu$^{2+}$.

(adapted from)

Jung et al. synthesized a coumarin-Cu$^{2+}$ probe (2.1) to selectively detect NC$^{-}$ ions using the DA approach in 10 mM PBS buffer, pH 7.4, 1.0% DMSO (Scheme 34).

Probe 2.1 initially had an absorbance maximum at 521 nm, which shifted in the blue direction upon the addition of NC$^{-}$ ions. As a consequence of the MLCT, the coordination of the Cu$^{2+}$ ions resulted in a weak fluorescence emission band ($\phi_{Fl2.1} = 0.02$) at 514 nm ($\lambda_{ex} = 479$ nm). When NC$^{-}$ ions were added, the fluorescence emission intensity at 514 nm increased slightly due to the displacement of the Cu$^{2+}$ ions as [Cu(CN)$_x$]$^{n-}$. The resulting coumarin-imine intermediate was then hydrolyzed to produce the coumarin-aldehyde product. This caused the fluorescence emission intensity to increase dramatically ($\phi_{Fl\text{aldehyde}} = 0.65$). The hydrolysis was confirmed by ESI-MS and $^1$H NMR spectra. The mass observed (270 m/z) and the splitting pattern were identical to a pure sample of the coumarin-aldehyde. A detection limit was calculated to be in the range of $10^{-8}$ M.

Since cyanide often accumulates in the liver, this design approach was applied to detect
NC\textsuperscript{−} ions in HepG2 cells. The ability to selectively detect NC\textsuperscript{−} ions in almost pure aqueous solution is highly desirable. However, the full fluorescence emission enhancement required 20 minutes. Also the formation of the [Cu(CN)\textsubscript{3}]\textsuperscript{n−} complex and \(\sigma\)-aminophenol is undesirable.

Scheme 34. Cyanide detection via DA approach by the complex \(\text{2.1-Cu}^{2+}\). (adapted from)\textsuperscript{186}

\textit{The Cu}^{2+} ion displacement required 16 equivalents of NC\textsuperscript{−} ions (10 mM PBS buffer pH 7.4 with 1% DMSO). The displacement is followed by an imine hydrolysis. L.o.D. in the 10\textsuperscript{−8} M range.\textsuperscript{196}

Unlike the previous molecular probes that utilize the Cu\textsuperscript{2+} ion DA approach, the molecular probe \(\text{2.2}\), synthesized by Jung et al., works in 100% aqueous HEPES buffer (10 mM, pH 7.4) (Scheme 35).\textsuperscript{12} Probe \(\text{2.2}\) initially exhibited an absorbance maximum at 460 nm. The addition of Cu\textsuperscript{2+} ions resulted in a 12 nm red shift of the signal at 460 nm as well as a hyperchromic shift of the absorbance at 472 nm. Additionally, the fluorescence emission intensity at 525 nm decreased. The \(\text{2.2-Cu}^{2+}\) complex showed only a spectroscopic response upon the addition of NC\textsuperscript{−} ions: a hypsochromic shift in the absorbance spectrum and a hyperchromic shift of the emission at 525 nm, essentially reverting the absorbance and emission spectra back to that of free probe \(\text{2.2}\).\textsuperscript{12} Twenty equivalents of KCN was required to achieve complete displacement of the Cu\textsuperscript{2+} ion as [Cu(CN)\textsubscript{3}]\textsuperscript{n−}. A limit of detection was calculated to be 724 nM.\textsuperscript{12}
Scheme 35. NC detection by complex 2.2-Cu$^{2+}$ via the DA approach. (adapted from)\textsuperscript{12}  

The displacement of Cu$^{2+}$ ions from the complex required the addition of 20 equivalents of NC ions, (HEPES buffer 10 mM pH 7.4).  

$L.o.D. = 724 \text{ nM.}^{12}$  

In the Cu$^{2+}$ ion DA approach, the “OFF-ON” sensing mechanism commonly refers to the reestablishment of a fluorescence signal, but this approach can be applied for other optical phenomena as well. Probe 2.3, published by Reddy et al., utilized the Cu$^{2+}$ ion DA approach to monitor the reestablishment of the phosphorescence emission of probe 2.3 (Scheme 36).\textsuperscript{18} Phosphorescence is typically observed with lanthanide ions; however, Ir$^{3+}$ ion complexes such as compound 2.3 favor intersystem crossing from the $S_1^*$ state to the $T_1^*$ state.\textsuperscript{18} It was observed that the addition of Cu$^{2+}$ ions, in CH$_3$CN-HEPES buffer (0.4:99.6, 10 mM, pH 7.6), caused no significant changes in the absorbance and fluorescence spectra of probe 2.3 ($\lambda_{ex} 380 \text{ nm, } \lambda_{em} 583 \text{ nm}$).\textsuperscript{18} It did, however, result in a decrease in phosphorescence emission intensity of probe 2.3 ($\phi_{Fl(2.3)} = 0.038$, $\phi_{Fl(2.3-Cu)} = 0.005$). The addition of 40 equivalents of various anions resulted in no changes in the fluorescence emission band of 2.3-Cu complex. Upon investigation of the phosphorescence emission response, the addition of NC$^-$ ions induced a turn-on and reestablishment of the phosphorescence emission signal, due to the displacement of the Cu$^{2+}$ ion. A detection limit was calculated to be 0.38 ppm. The design approach of
compound 2.3 was used in two different biological applications: to detect the cellular uptake of NC\textsuperscript{-} ions in Hela cells (detection limit calculated to be 0.2 ppm) and to monitor the \textit{in situ} release of NC\textsuperscript{-} ions from the decomposition of the cyanohydrin mandelonitrile by the enzyme hydroxynitrile lyase\textsuperscript{18}.

![Scheme 36. NC\textsuperscript{-} detection by complex 2.3-Cu\textsuperscript{2+} via the DA approach. (adapted from)\textsuperscript{18}](image)

\textit{Studies carried out in CH\textsubscript{3}CN-HEPES buffer (0.4:99.6, 10 mM, pH 7.6). The displacement required 20 equivalents of NC\textsuperscript{-} ions. L.o.D. = 0.38 ppm.}\textsuperscript{18}

\textbf{2.1.2 Direct Coordination to a Lewis Acid Site}

An alternative to the displacement assay approach is direct coordination to the molecular probe (Lewis acid-base adduct). The advantage of this method is that it avoids forming the harmful [Cu(CN)\textsubscript{x}]\textsuperscript{10-} in solution.

One example of a Lewis acid site is the incorporation of an electron deficient boron center. The probe 2.4, synthesized by Chakraborty et al., is an example (Scheme 37) of a fluorescent polymer backbone, which included an electron deficient BMes\textsubscript{2} center for the detection of NC\textsuperscript{-} ions through an “ON-OFF” signaling mechanism\textsuperscript{20}. Of the various anions tested (in CH\textsubscript{2}Cl\textsubscript{2}) only the ions F\textsuperscript{-} and NC\textsuperscript{-} resulted in a spectroscopic change, both anions reacting in a 1:1 ratio with each boron center. The new covalent bonds, B-F and B-CN, resulted in small shifts in the probe 2.4 absorbance $\lambda_{\text{max}}$ at 350 nm spectrum: 13 nm and 8 nm bathochromic shift for F\textsuperscript{-} and NC\textsuperscript{-} ions respectively.\textsuperscript{20} Along
with the bathochromic shift the addition of NC\textsuperscript{-} ions also increased the absorbance intensity at 358 nm inducing a visual solution color change from colorless to yellow\textsuperscript{20}. The authors suggested that the colorimetric response is due to the B-F and B-CN bonds converting the boron center from electron deficient to electron rich. Upon excitation at 360 nm the fluorescence emission band of probe \textit{2.4} is centered at 409 nm. The addition of F\textsuperscript{-} ions resulted in a ~ 2-fold decrease in the 409 nm emission intensity, while the NC\textsuperscript{-} ions induced a 30-fold decrease in emission intensity. A detection limit for NC\textsuperscript{-} ions was calculated to be ~ 0.5 \( \mu \text{M} \textsuperscript{20} \). While this design approach avoids forming the [Cu(CN)\textsubscript{x}]\textsuperscript{n-} complex, the entirety of the work was carried out in 100\% organic solvent. Furthermore, the use of electron deficient boron centers for detecting NC\textsuperscript{-} ions often suffer from false positives from F\textsuperscript{-} ions.

Scheme 37. Cyanide detection by the polymer \textit{2.4} via direct addition to a boron center. (adapted from)\textsuperscript{20}

\textit{The fluorescence arises from the polymeric backbone probe. The cyanide addition occurs in a 1:1 ratio with each boron center. L.o.D. = \(~ 0.5 \mu \text{M in } CH\textsubscript{2}CL\textsubscript{2}\textsuperscript{20} \).}

\textit{2.1.2.1 Direct 1,2-addition.} Although an electron deficient boron species serves as a viable Lewis acid site for a NC\textsuperscript{-} ion attack\textsuperscript{20,183,188} the false positives from the fluoride...
ion have made this a rarely used design approach. Another common approach is to incorporate an electrophilic $sp^2$ hybridized carbon that serves as the Lewis acidic site. Lin et al. demonstrated that with a highly electrophilic dicyanovinyl functional group, probe 2.5 was able to selectively detect NC$^-\text{ions}$ in THF-HEPES (0.01 M, pH 7.3, 80:20 ratio) (Scheme 38). The addition of NC$^-\text{ions}$ led to a colorimetric response from orange to colorless, which resulted from a decrease in the absorbance $\lambda_{\text{max}}$ at 330 nm and 472 nm and an increase in the absorbance intensity at 376 nm. The kinetics of the cyanide reaction was studied. After 15 minutes (used 20 equivalents of NC$^-\text{ions}$) the reaction went completion, with a pseudo-first order rate constant calculated to be $k = 0.4$ min$^{-1}$. Upon excitation at 418 nm, probe 2.5 had a fluorescence emission $\lambda_{\text{max}}$ at 626 nm, due to ICT. The addition of NC$^-\text{ions}$ resulted in a breaking of the conjugation at the dicyanovinyl group, inhibiting the ICT and causing a 75-fold decrease of the emission band at 626 nm and a 103 nm blue shift. The new maximum emission band at 523 nm is due to the ESIPT process, which was unaffected by the addition of NC$^-\text{ions}$. A detection limit was calculated to be 2.4 $\mu\text{M}$. Probe 2.5 serves as a good example of an electrophilic carbon serving as a chemodosimeter for NC$^-\text{ions}$; however, the detection limit fails to meet the acceptable maximum level in drinking water (1.9 $\mu\text{M}$). Furthermore, the selectivity of probe 2.5 towards CN$^-\text{ions}$ was only investigated with the cyanide and the various anions being tested in a 1:1 ratio (1:20:20 2.5-CN-various mono anions).
Scheme 38. The direct 1,2-addition of cyanide to probe 2.5. (adapted from)\textsuperscript{192}

$L.o.D. = 2.4 \mu M \text{ in THF-HEPES buffer (0.01 M, pH 7.3, 80:20 ratio). A pseudo first order rate constant } k = 0.355 \text{ min}^{-1}$.\textsuperscript{192}

Noh et al. synthesized probe 2.6 that utilized a simple imine as the electrophilic site for NC\textsuperscript{-} ions (Scheme 39).\textsuperscript{190} Compound 2.6 displayed spectroscopic responses towards the ions F\textsuperscript{-}, OAc\textsuperscript{-}, and NC\textsuperscript{-} in 100% CH\textsubscript{3}CN. However, in a 10\% bis-tris/THF (0.01 M, pH 7.0) solution, only the NC\textsuperscript{-} ion produced a spectroscopic response. The selectivity gained by switching solvents is due to the ability of compound 2.6 to undergo a proton transfer.\textsuperscript{190} The addition of NC\textsuperscript{-} ions induced a hypochromic shift of the absorbance maximum at 354 nm and a concomitant hyperchromic shift in the absorbance at 426 nm with an isobestic point at 371 nm. This led to a visible color change of the solution from colorless to orange.\textsuperscript{190} The addition of NC\textsuperscript{-} ions also caused a 102-fold hyperchromic shift of the fluorescence emission intensity at 492 nm ($\lambda_{\text{ex}}$ 320 nm). Probe 2.6 showed selectivity towards the NC\textsuperscript{-} ion in the presence of other anions (1:1 ratio). A limit of detection was calculated to be $6.0 \times 10^{-8} M$.\textsuperscript{190}
Scheme 39. The 1,2-addition of NC- ions to probe 2.6. (adapted from)\textsuperscript{190}

Reaction occurs in a 1:1 ratio, 10% bis-tris/THF (0.01 M, pH 7.0). L.o.D. = 6.0 × 10\textsuperscript{-8} M.\textsuperscript{190}

The benzothiazole derivative 2.7 synthesized by Goswami et al. employed an electrophilic carbonyl carbon for the ratiometric detection of NC- ions (Scheme 40).\textsuperscript{193} In a MeCN-HEPES buffer (pH 7.5, 1:1 ratio), probe 2.7 had a maximum absorbance band at 346 nm, which decreased with the addition of NC- ions as the absorbance band at 438 nm increased. An isobestic point at 375 nm was observed. Also, the color of the solution changed from colorless to bright green.\textsuperscript{193} With the addition of two equivalents of NC- ions, the conversion of probe 2.7 to the 2.7- NC- adduct was complete in under five minutes. The fluorescence emission band at 521 nm decreased with the addition of NC- ions while the emission band at 436 nm increased (\(\lambda_{ex} 344\) nm). The fluorescence response observed is attributed to the inhibition of the ESIPT process.\textsuperscript{193} The formation of the 2.7-CN adduct was confirmed by \(^1\)H NMR: the aldehyde proton of compound 2.7 (~13 ppm) is shifted significantly upfield to ~5.6 ppm upon the addition of NC- ions. A detection limit was calculated to be 1.6 \(\mu\)M.\textsuperscript{193}
Scheme 40. Cyanide detection by the 1,2-addition to probe 2.7. (adapted from)\textsuperscript{193}

(Left) The ESIPT mechanism of 2.7 without cyanide. (Right) ESIPT inhibition due to NC\textsuperscript{-} ions. L.o.D. = 1.6 μM, in MeCN-HEPES buffer (pH 7.5, 1:1 ratio).\textsuperscript{193}

Lee et al. synthesized a chemodosimeter (probe 2.8) that utilized an intramolecular cross-benzoin reaction to selectively detect NC\textsuperscript{-} ions.\textsuperscript{141} The mechanism in which the NC\textsuperscript{-} ion induced the reaction is shown in Scheme 41 and was confirmed by \textsuperscript{1}H NMR analysis.\textsuperscript{141} In the presence of ten equivalents of KCN, the reaction was completed within ten seconds. Probe 2.8 displayed a selective spectroscopic response towards the NC\textsuperscript{-} ion in 1% DMSO PBS buffer (10 mM, pH 7.4). The absorbance spectrum exhibited a 20-fold hyperchromic shift upon the addition of the NC\textsuperscript{-} ion, with a visual color change of the solution from pale orange to pink. The fluorescence spectrum displayed an increase in the emission intensity at 595 nm (λ_{ex} 565 nm) upon the addition of cyanide with the limit of detection calculated to be 4 nM.\textsuperscript{141} This design approach was used to detect KCN in Hela and A549 cells at a 50 nM concentration in blood serum.\textsuperscript{141}
The chemodosimeter 2.9 synthesized by Ali et al. displayed selectivity towards NC\(^{-}\) ions (50 equivalents) over various anions tested (60 equivalents) in THF-HEPES buffer (10 mM, pH 7.0, 80:20 ratio) (Scheme 42).\(^{15}\) The addition of TBACN resulted in the hypochromic shift of the absorbance \(\lambda_{\text{max}}\) at 376 nm and an increase in the absorbance intensity at both 315 and 396 nm with isobestic points at 335 and 410 nm. The absorbance was accompanied by a visual color change of the solution from light-yellow to a red-yellow.\(^{15}\) Prior to the addition of the NC\(^{-}\) ion, the excitation of probe 2.9 at 376 nm resulted in a weak dark-green emission band at 528 nm (\(\phi_{\text{Fl}} = 0.01\)).\(^{15}\) This emission band was the result of ESIPT and was predominately the emission of the keto-enamine tautomer. The presence of the NC\(^{-}\) ion resulted in the enhancement of the emission band at 528 nm (~8-fold increase) as well as a ~14-fold increase of the emission band at 494 nm (\(\phi_{\text{Fl}} = 0.121\)). A Job’s plot confirmed the 1:1 interaction between compound 2.9 and the NC\(^{-}\) ion. \(^{1}H\) NMR and X-Ray crystallography confirmed the reaction mechanism and
product shown in Scheme 2.11. The detection limit was calculated to be ~15 ppb. This is significantly lower than the WHO limit (1.9 μM). Also the ability of 2.9 to work in buffered media would make this motif an attractive method. However, during the cyclization to form the new oxazole ring, the NC⁻ ion is displaced from compound 2.9, leaving free NC⁻ ions in solution.

Scheme 42. The 1,2-addition of cyanide to compound 2.9. (adapted from)

Cyanide induced a deprotonation, cyclization, and finally the displacement of the NC⁻ ions. L.o.D. = ~15.34 ppb in a THF-HEPES buffer (10 mM, pH 7.0, 80:20 ratio).

2.1.2.2 Direct 1,4-Addition. The detection of cyanide by a direct 1,2-addition avoids forming the toxic [Cu(CN)₃]⁻ complex, unlike DA approach, and false positives from F⁻ ions, unlike using boron as a Lewis acid site. However, the 1,2-addition is not the only site at which cyanide can directly attack. The direct 1,4-addition (thermodynamic/Michael addition) also uses an electrophilic carbon as a Lewis acid site.
for nucleophilic attack from NC\textsuperscript{-} ions. Compound 2.10, published by Park et al., is an example of a Michael addition by NC\textsuperscript{-} ions (Scheme 43).\textsuperscript{194} Upon the attack of the Michael carbon by the NC\textsuperscript{-} ion, probe 2.10 shifted to the keto-enol tautomer, which resulted in a [1,3] sigmatropic rearrangement (the bolded proton in Scheme 43 is the hydrogen moving in the rearrangement).\textsuperscript{194} Investigation of the pH effect revealed that probe 2.10 could detect NC\textsuperscript{-} ions in DMSO-H\textsubscript{2}O (9:1 ratio) within the pH range of 4-11. Only the colorimetric response of probe 2.10 was studied: the solution turned from colorless to violet upon the addition of NC\textsuperscript{-} ions, and the absorbance spectrum showed a 170 nm bathochromic shift of the signal at 390 nm.\textsuperscript{194} A detection limit was calculated to be 1.27 mM.\textsuperscript{194} The NC\textsuperscript{-} ion addition went to completion within five hours with a second-order rate constant \( k_2 = 6.5 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}.\textsuperscript{194} \) While visual confirmation of NC\textsuperscript{-} ions present in a sample is an attractive quality, the previous chemodosimeters (probes 2.0-2.9)\textsuperscript{12, 15, 18, 20, 141, 186, 187, 190, 192, 193} for NC\textsuperscript{-} ions detection have displayed lower detection limits and faster detection rates.

![Scheme 43. Cyanide detection from the 1,2-addition to probe 2.10. (adapted from)\textsuperscript{194}](image)

*Cyanide induced [1,3]-sigmatropic shift of compound 2.10 in DMSO-H\textsubscript{2}O (90:10 ratio, pH range of 4-11). L.o.D. = 1.27 mM.*\textsuperscript{194}
Kim et al. designed an activated coumarin derivative (probe 2.11) for the detection of NC\(^-\) ions (Scheme 44).\(^\text{21}\) The presence and position of the electron withdrawing group NO\(_2\) resulted in the nucleophilic attack from the NC\(^-\) ions at the available C(4) site of the coumarin core (Scheme 44).\(^\text{21}\) While the kinetic addition carbon is open for a NC\(^-\) ion attack, the C(4) carbon is also a Michael carbon to the δ-lactone making the C(4) carbon doubly “activated”. The addition of the NC\(^-\) ion resulted in the decrease of the absorbance maximum at 468 nm and an increase in absorbance intensities at 300 and 550 nm. The significant change observed in the absorbance spectrum of probe 2.11 was also visually detected from the solution color change from orange to pink.\(^\text{21}\)

When the NC\(^-\) ion added to the C(4) carbon, the lactone ring gained more electron density withdrawing character, giving the compound a larger ICT character and resulting in the hyperchromic shift of the emission band at 468 nm. A detection limit was calculated to be ~3 \(\mu\)M.\(^\text{21}\) Unfortunately, this high detection limit does not meet WHO standards (1.9 \(\mu\)M)\(^\text{11, 12}\) and was carried out in pure organic media. It should be noted that the authors did not discuss the exact mechanism for the hydride removal.

Scheme 44. The 1,4-addition of cyanide to compound 2.11. (adapted from)\(^\text{21}\)

*The addition occurs at the C(4) carbon of the coumarin core. L.o.D. = ~3 \(\mu\)M.*\(^\text{21}\)
The probe 2.12, synthesized by Park et al., serves as another example of a chemodosimeter motif where the detection occurs from cyanide attacking a Michael carbon (Scheme 45).\textsuperscript{195} The intramolecular hydrogen bonding between the $\omega$-hydroxyl and carbonyl functional group results in the electrophilic character of the vinylic carbon. In 100% MeCN, only cyanide resulted in spectroscopic changes to the absorbance spectrum: a decrease of the absorbance $\lambda_{\text{max}}$ at 430 nm, an increase of the absorbance band at 260 nm, and an isobestic point 272 nm, indicating the conversion of probe 2.12 to the 2.12-NC$^-$/adduct. The solution’s color change from yellow to colorless signified that the conjugation of compound 2.12 was broken.\textsuperscript{195} The excitation of probe 2.12 at 272 nm resulted in a weak fluorescence emission band at 469 nm, which saw a 1,300-fold enhancement upon the addition of 100 equivalents of NC$^-$ ions. A Job’s plot confirmed the interaction between probe 2.12 and the NC$^-$ ion in a 1:1 ratio.\textsuperscript{195} The deconjugation of compound 2.12 was confirmed by investigating the $^1$H and $^{13}$C NMR spectra of both probe 2.12 and the 2.12-NC$^-$ adduct. After 10 hours, the addition of one equivalent of NC$^-$ ions resulted in the disappearance of the $sp^2$ vinylic proton signals at 8.3 and 7.5 ppm and the appearance of new $sp^3$ chemical shifts at 4.4 and ~3.5 ppm.\textsuperscript{195} This supports that the solution de-colorization is due to the deconjugation of compound 2.12. The new $sp^3$ hybridized carbons were observed in the $^{13}$C NMR spectrum with chemical shifts values of 41 and 33 ppm.\textsuperscript{195} Upon the investigation of the kinetics of the NC$^-$ ion attack, a pseudo first order rate constant $k$ was calculated to be $8.2 \times 10^{-5}$ s$^{-1}$; a limit of detection was calculated to be 1.7 ppm.\textsuperscript{195} While probe 2.12 can detect NC$^-$ ions below the WHO limit, the 10 hours required for observing full conversion to the adduct is undesirable.
Scheme 45. The 1,4-addition of cyanide to probe 2.12. (adapted from)\textsuperscript{195}

The addition occurs in a 1:1 ratio, in 100\% MeCN. L.o.D. = 1.7 ppm with a pseudo first order rate constant $k = 8.2 \times 10^{-5}$ s\textsuperscript{-1}.\textsuperscript{195}

The coumarin-meldrum’s acid conjugate 2.13 synthesized by Li et al. utilized a doubly activated electrophilic Michael carbon for the detection of NC\textsuperscript{-} ions (Scheme 46).\textsuperscript{196} The addition of NC\textsuperscript{-} ions to compound 2.13 in a DMSO:Tris-HCl buffer (10 mM, pH 7.4, 90:10 ratio) resulted in the decrease of the maximum absorbance band at 551 nm and an increase in the absorbance band at 415 nm, a hypsochromic shift of 136 nm. A solution color change from amaranth to pale yellow was also observed.\textsuperscript{196} The excitation of probe 2.13 at 451 nm displayed two weak emission bands: one at 641 nm ($\phi_{Fl} = 0.078$) and the other at 491 nm ($\phi_{Fl} = 0.083$). Upon the addition of NC\textsuperscript{-} ions, the emission band at 641 nm decreased while the emission intensity at 491 nm increased by 432-fold ($\phi_{Fl} = 0.555$).\textsuperscript{196} The kinetics of probe 2.13 were investigated, and a pseudo first order rate constant $k$ was calculated at $5.81 \times 10^{-2}$ s\textsuperscript{-1}. A detection limit was calculated to be 27 nM.\textsuperscript{196} A Job’s plot confirmed the interaction between probe 2.13 and the NC\textsuperscript{-} ion occurred in a 1:1 ratio. $^1$H NMR confirmed that the site of the NC\textsuperscript{-} ion attack occurred at the $\beta$ carbon by monitoring the $\beta$, $\gamma$, and $\delta$ proton signals of compound 2.13 before the addition of NC\textsuperscript{-} ions (8.09, 8.43, and 7.6 ppm respectively) and after the addition (4.54, 6.50, 6.40 ppm respectively).\textsuperscript{196}
Scheme 46. The 1,4-addition of NC\(^{−}\) ions to the β carbon of probe 2.13. (adapted from)\(^{196}\)

The reaction occurs in a 1:1 ratio in a DMSO-Tris-HCl buffer (10 mM, pH 7.4, 90:10 ratio). L.o.D. = 27 nM with a pseudo first order rate constant \(k = 5.81 \times 10^{-2} \text{s}^{-1}\).\(^{196}\)

A similar design motif, seen in Scheme 47, was studied by Zhou et al. The chemodosimeter 2.14 has a coumarin-indanedione conjugate with a doubly activated Michael carbon that serves as an electrophilic site of attack for NC\(^{−}\) ions (Scheme 2.16).\(^{8}\) \(^1\)H NMR investigation confirmed the NC\(^{−}\) ion attack occurs at the C-H carbon by monitoring the chemical shift of H before and after the addition of NC\(^{−}\) ions (9.92 to 4.71 ppm respectively).\(^{8}\) The initial absorbance \(\lambda_{\text{max}}\) at 548 nm, in 100% MeCN, decreased with the addition of NC\(^{−}\) ions while a new absorbance band appeared at 390 nm. The presence of NC\(^{−}\) ions induced a solution color change from purple to colorless.\(^{8}\) The excitation of probe 2.14 at 440 nm resulted in two emission bands at 609 and 485 nm. After the addition of the NC\(^{−}\) ions, the emission band at 609 nm sharply decreased while the emission band at 485 nm enhanced by ~6.5-fold. Shown in Scheme 2.16, the attack of the NC\(^{−}\) ion lead to the deconjugation of probe 2.14, which inhibited the ICT and caused the decrease of the emission band at 609 nm.\(^{8}\) A detection limit was calculated to be 0.18 \(\mu\)M. The 1:1 reaction ratio was confirmed by ESI-MS, where the predicted [2.14-CN]\(^{−}\) adduct was observed at 399 m/z.\(^{8}\) A rate constant was not reported by Zhou et al., but the reaction went to completion under 20 seconds.\(^{8}\)
Scheme 47. Cyanide 1,4-addition to probe 2.14. (adapted from)\textsuperscript{8}

Studies were carried out in 100% MeCN. L.o.D. = 0.18 μM. The 1:1 reaction ratio was confirmed by the [2.14-CN\textsuperscript{−}] adduct observed at 399 m/z.\textsuperscript{8}

This literature review of NC\textsuperscript{−} chemodosimeters aims to provide a look into the various design motifs currently published; however, each design motif has an advantage and disadvantage over another. The displacement assay motif provides for a more sensitive “OFF-ON” signaling mechanism (section 1.5.4), but it results in the formation of a toxic metal cyanide salt, such as [Cu(CN)\textsubscript{x}]\textsuperscript{n−}, in solution. On the other hand, the design motif based on the direct addition of cyanide to a Lewis acid center such as boron or a carbonyl carbon avoids the formation of a toxic metal cyanide salt. This motif often requires less equivalents of cyanide and offers faster kinetics over the displacement approach. However, chemodosimeters based on cyanide addition on a boron center suffer from false positives from fluoride ions. The implementation of a Michael carbon for a 1,4-addition of cyanide avoids both the formation of a toxic metal cyanide salt and false positives from F\textsuperscript{−} ions. Furthermore, the direct 1,4-addition motif forms a more thermodynamically stable adduct over the 1,2-addition to a carbonyl carbon.

From these cyanide chemodosimeter examples shown in (Schemes 32-47)\textsuperscript{8, 12, 15, 18, 20, 141, 186, 187, 190, 192, 193} improvements in sensitivity, selectivity, kinetics, and ability to work in aqueous solutions are needed. Therefore, a goal was set to synthesize a simple
coumarin-enaminone chemodosimeter to improve upon the sensitivity, selectivity, and kinetics of previously published chemodosimeters that use the direct 1,4-addition.
2.1.3 Hypothesis

The main purpose of this study was to synthesize a simple coumarin-enaminone probe that serves as a selective and sensitive chemodosimeter for cyanide. A coumarin-enaminone sensor design was chosen for three main reasons:

1. Ease of synthesis, resulting in high yields (80% for target probes 2.15 and 2.16).
2. The coumarin core allowed for the investigation of both absorbance and fluorescence spectroscopic responses.
3. The enaminone provided an electrophilic site for a 1,4-addition (Michael addition) and not a 1,2-addition.

With these reasons in mind, probes 2.15 and 2.16 were designed and synthesized as a means to gain a fundamental understanding of the coumarin-enaminone system upon which other probes would be designed.
2.2 Experimental Data

2.2.1 General Synthesis of Probes 2.15 and 2.16

Compounds 2.15 and 2.16 (Scheme 48) are very similar in structure. However, the 4-aminopyridine of compound 2.16 offers a change in the basicity of the nitrogen of the enaminone. This change would facilitate a faster NC\(^-\) ion attack along with an increased selectivity towards cyanide. Therefore, compound 2.15 serves as a model for probe 2.16.

Due to the tautomerization and isomerization that can occur with enaminones, the molecular probes 2.15 and 2.16 can exist in a number of various tautomers and isomers of each tautomer. Shown in Figure 6 are the possible tautomers and isomers of compound 2.16. The structures A-D, I, and J are the possible structures of the imine-enol tautomer. The structures E-H are of compound 2.16 in the keto-enamine tautomer. See section 1.5.7 for the discussion on compounds that undergo ESIPT and see section 2.2.9 for the investigation on the molecular modeling of compound 2.16.
The synthesis of probes 2.15 and 2.16 requires multiple steps to isolate the two desired probes (Scheme 48). The first step is the synthesis of 1.14. This is achieved by refluxing compounds 1.11 and 2.17 for three hours in phosphorus (V) oxychloride under anhydrous conditions. The reaction is quenched with cold DI-H$_2$O and neutralized with a saturated NaHCO$_3$ solution, which gave a 95% yield. The solid isolated is confirmed as compound 1.14 by the observation of a singlet at 7.53 ppm (Ar-H) in the $^1$H NMR spectrum and the signal at 168.8 ppm (C=O) in the $^{13}$C NMR, which matches literature values. The second step is the isolation of 1.2. Compound 1.2 is synthesized by refluxing compounds 1.13 and 1.14 in anhydrous toluene for three hours. The reaction is cooled to room temperature, and the solid is isolated as pure compound 1.2 with a 75% yield. The solid isolated is confirmed by the observation of singlets at 11.91 ppm (O-H) and 5.25 ppm (C(3)H) in the $^1$H NMR spectrum and the signal at 166.9 ppm (C=O) in the $^{13}$C NMR.
80 ppm (C(2)=O) in the $^{13}$C NMR, which agreed with literature values. The final step in the synthesis is the addition the appropriate amine to a solution of compound 1.2 and triethyl orthoformate (TEOF) in 2-propanol. aniline for compound 2.15 and 4-aminopyridine for compound 2.16. The reaction is allowed to reflux for three hours during which a yellow or yellow-orange precipitate forms. The solid is isolated from vacuum filtration as the desired probe in a pure form. A detailed synthesis procedure is provided in the experimental section 2.4.

Scheme 48. The synthesis of the target probes 2.15 and 2.16.

2.2.2 X-Ray Studies of Compound 2.16

The structure of compound 2.16 was investigated by single crystal X-Ray crystallography (Figure 7). It was shown that the structure of probe 2.16 was planar (H:M symbol –P1, triclinic crystal packing). The numbering system for compound 2.16 is found in Figure 8. The C(9)-N bond distance (1.33 Å) is slightly longer than the 1.295 Å reported for a similar compound in the imine-enol tautomer, indicating that probe 2.16 is in the keto-enamine tautomer. The C(4)=O···H-N distance is 1.95 Å, which is longer than the other coumarin-enaminones that will be discussed in section 3.2.1. The increased
intramolecular hydrogen bond distance is due to the electron withdrawing character of the para substituted pyridine ring. This explanation is also supported by the decreased N-H bond distance (0.86 Å), which is shorter than the distances reported in section 3.2.1, and literature values are in the range of 0.90-0.94 Å. The longer C(9)-N (1.33 Å) and C(4)=O•••H-N bond distances and along with the short N-H bond distance suggest that the C(9) carbon will serve as a viable site for nucleophilic addition.

Figure 7. X-Ray crystal structure of compound 2.16.

2.2.3 NMR Studies of Probes 2.15 and 2.16

^1H and ^13C NMR spectrometry were investigated to confirm the structures of both probes 2.15 and 2.16. The numbering system for probe 2.15 that is used in the NMR spectra is provided in Figure 8. Based on literature, coumarin-enaminones will exist in solution primarily as the E/Z isomers of the keto-enamine tautomer. From the values published by both Traven and Wolfbeis et al., the N-H ^1H NMR chemical shift appear as a pair of doublets (E) ~13 ppm and (Z) ~11 ppm. The ^1H NMR spectrum of probe 2.15 (Figure 9) shows that the expected doublets are present ((E) 13.65 and (Z) 11.69 ppm) and are in the range expected by coumarin-enaminones. The downfield shift of the (E) N-H with respect to the (Z) N-H is due to the stronger
intramolecular hydrogen bond between the C(4)=O···H-N than the C(2)=O···H-N. This is because the C(4)=O is more electronegative than the C(2)=O. When the enamine is in the Z isomer, the C(9)H is closer to the C(4)=O. As such, it is more de-shielded and therefore appears further downfield than the C(9)H in the E isomer. The Z isomer also results in a stronger hydrogen bond interaction between C(4)=O and C(5)H, so the doublet for C(5)H appears shifted downfield for the Z isomer. The NEt₂ protons are not labeled; however, the CH₃ and the CH₂ appear at 1.24 and 3.45 ppm respectively.

![Figure 8](image.png)

*Figure 8.* The structure and the numbering system for compound 2.15.

To also confirm the structure of probe 2.15, the ¹H NMR spectrum was compared to the ¹H NMR spectrum of compound 1.2 (Figure 10). As the stack plot shows, both the C(3)H signal at 5.25 ppm and the O-H at 11.91 ppm in the spectrum for compound 1.2 (bottom) are absent in the spectrum of probe 2.15 (top).

The ¹³C NMR spectrum of compound 2.15 (Figure 11) has a new carbonyl carbon chemical shift at 181.0 ppm. Based on literature values, this new signal is assigned to the C(4) carbon of compound 2.15.¹⁹⁸ The C(4) signal is further downfield than the C(2) signal due to the intramolecular hydrogen bonding of the keto-enamine.
Figure 9. The $^1$H NMR spectrum of compound 2.15 in CDCl$_3$.

*represents solvent signal
Figure 10. \(^1\)H NMR stack plot of probe 2.15 (top) and compound 1.2 (bottom) in DMSO-\(d_6\).
Figure 11. The $^{13}$C NMR spectrum of compound 2.15 in CDCl$_3$.

*represents the solvent signal.
Using the same literature values and the $^1$H NMR spectrum of 2.15, the presence of the enaminone of 2.16 was confirmed by the N-H doublets at 13.47 and 11.52 ppm, for the $E$ and $Z$ isomers respectively (Figure 13). The numbering system used for 2.16 is shown in Figure 12. In the $^{13}$C NMR (Figure 14), the presence of the C(4) signal at 180.2 ppm also confirms the structure of 2.16.

Figure 12. The structure and the numbering system for compound 2.16.

To gain an understanding of the changes that occur upon the nucleophilic attack from cyanide a series of 1D and 2D experiments were carried out. The cyanide salt chosen for these experiments was KCN because this salt produced the largest optical spectroscopic response from probes 2.15 and 2.16 (see sections 2.2.5-2.2.8). However, due to solubility, a 1:7 mixture of DMSO-$d_6$:CDCl$_3$ was used. DMSO-$d_6$ was required to dissolve the KCN salt while CDCl$_3$ was used to dissolve probe 2.16.

From the $^1$H NMR spectrum shown in Figure 13, $E/Z$ isomers of the keto-enamine tautomer are observed for N-H and C(9)H. This was expected as the isomer chemical shifts were observed in Figure 61. Table 6 provides numerical values for the $^1$H NMR (Figure 13) and the $^{13}$C NMR (Figure 14) chemical shifts of 2.16. From the spectrum in Figure 13, it is observed that the $E$ isomer is more predominant over the $Z$ isomer in
solution. Using the integration values of the $E/Z$ chemical shifts of the N-H signal, the percentage of both isomers can be calculated: $E/Z$ isomer ratios are 93.2:6.82 in CDCl$_3$, 75.8:24.2 in DMSO-$d_6$, and 80.2:19.8 in 1:7 DMSO-$d_6$:CDCl$_3$.

Table 6

$^1$H and $^{13}$C NMR Chemical Shifts of Compound 2.16

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</tbody>
</table>

Note. Proton NMR signals are reported in parts per million (ppm, Hz) and Carbon signals are reported in parts per million.

The isomerization of the keto-enamine also gives rise to a double set of aromatic signals. Shown in the inset of Figure 13, the aromatic protons closest to the enaminone (C(5)H and C(11/11’)H are the most affected by the isomerization.
Figure 13. $^1$H NMR of compound 2.16 in a mixture of DMSO-$d_6$:CDCl$_3$ (1:7).

* and + are the solvent and water signals respectively. Chemical shift values are provided in Table 6.
As a result of using DMSO-\textsubscript{d6}, the percentage of the Z isomer in solution is increased. It is reasonable to believe that as a hydrogen bonding acceptor DMSO stabilizes the Z isomer, whereas CHCl\textsubscript{3} does not and shows very little to no Z isomer in the NMR spectra. This gives rise to two \textsuperscript{13}C chemical shifts for the carbons that are the most affected by the isomerization. The \textsuperscript{13}C NMR spectrum of probe \textbf{2.16} is shown in Figure 14. The top-most inset shows the \textit{E/Z} two chemical shift signals for C(3), C(11/11’), and C(5) while the second inset shows the \textit{E/Z} carbon signals for C(10) and C(8). It should be noted that the two regions needed to be expanded to easily distinguish the \textit{E/Z} carbon signals; however, the two isomer signals for C(2) and C(4) are clearly distinguishable with no expansion required. Intramolecular hydrogen bonding results in an increased deshielding of the C(4) carbon in the \textit{E} isomer but not the Z isomer, causing it to appear further downfield than its Z isomer counterpart. In the Z isomer, however, the C(2) carbon signal is shifted downfield due to the intramolecular hydrogen bonding.

In order to correctly assign the \textsuperscript{13}C signals, a \textsuperscript{13}C APT experiment was investigated (Figure 15). Unlike the \textsuperscript{13}C NMR spectrum, the \textsuperscript{13}C APT spectrum allowed for unambiguous assignment of the carbon chemical shifts. For CH and CH\textsubscript{3} carbons, a positive chemical shift was observed while CH\textsubscript{2} and quaternary carbons gave rise to a negative chemical shift. This experiment aided in assigning all of the quaternary carbons: C(2), C(3), C(4), C(4a), C(7), C(8a), and C(10).
Figure 14. $^{13}$C NMR of probe 2.16 in a mixture of DMSO-$d_6$:CDCl$_3$ (1:7).
Figure 15. $^{13}$C APT of probe 2.16 in a mixture of DMSO-d$_6$:CDCl$_3$ (1:7).
After assigning the chemical shifts in the 1D NMR experiments (\(^1\)H and \(^{13}\)C NMR), a series of 2D NMR experiments were investigated. The first experiment performed was Heteronuclear Single Quantum Coherence (HSQC) spectroscopy, which is a \(^1\)H-\(^{13}\)C-NMR experiment (Figure 16). Each carbon on probe 2.16 that has a proton attached to it will give rise to a cross peak. Since the C(13) and C(14) carbon and proton signals were easily assigned, their cross peaks are omitted. The HSQC aided in identifying the \(^1\)H and \(^{13}\)C NMR assignments for C(5), C(6), C(8), C(9), C(11/11’), and C(12/12’).

The last 2D NMR experiment performed to ultimately confirm and fully assign probe 2.16 was a Heteronuclear Multi-Bond Correlation (HMBC) spectroscopy, shown in Figure 17. This through bond technique allowed for the identification of the quaternary carbons.

The Rotating frame nuclear Overhauser Effect (ROESY) spectroscopy experiment is shown in Figure 18: ROESY shows through space correlations between protons that are less than 5 Å away from each other. However, no useful information was obtained from the experiment and is provided strictly for comparison with Figure 25.
Figure 16. HSQC of compound 2.16 in a mixture of DMSO-\textit{d}_6:CDCl_3 (1:7).

Each cross peak represents a specific C-H bond.
Figure 17. HMBC spectrum of compound 2.16 in a mixture of DMSO-$d_6$:CDCl$_3$ (1:7).
Figure 18. ROESY spectrum of compound 2.16 in a mixture of DMSO-$d_6$:CDCl$_3$ (1:7).
2.2.3.1 2.16-CN adduct. To the sample of compound 2.16 used for the NMR experiments shown in the above figures was added 1.2 equivalents of KCN, in DMSO-$d_6$, to ensure completion. The full range of NMR experiments were carried out (Figures 19-25) and the chemical shift assignments are provided in Table 7. The previous numbering system as seen in Figure 12 was used to label the chemical shifts in the spectra and table below.

The nucleophilic attack by NC$^-$ ions to the C(9) leads to a change of hybridization ($sp^2$ to $sp^3$) and therefore a change in geometry (trigonal planar to tetrahedral). Based on the results of the NMR experiments on the subsequent pages as well as the molecular modeling (section 2.2.9), the mechanism for the NC$^-$ ions addition at C(9) is shown in Scheme 49.

Scheme 49. The cyanide nucleophilic attack of probe 2.16.

The addition occurs at the $sp^3$ hybridized C(9) by NC$^-$ ions and the resulting tetrahedral $sp^3$ hybridized C(9).
Table 7

$^1$H and $^{13}$C NMR of Compound 2.16-CN adduct

<table>
<thead>
<tr>
<th>Label</th>
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<th>Carbon</th>
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<td>163.6</td>
</tr>
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<td>3</td>
<td>NA</td>
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<td>4a</td>
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<td>149.3</td>
</tr>
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<td>7.65 (1H, d, $J = 8.8$)</td>
<td>125.7</td>
</tr>
<tr>
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<td>6.49 (1H, d, $J = 8.7$)</td>
<td>106.8</td>
</tr>
<tr>
<td>7</td>
<td>NA</td>
<td>110.8</td>
</tr>
<tr>
<td>8</td>
<td>6.32 (1H, s)</td>
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</tr>
<tr>
<td>8a</td>
<td>NA</td>
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</tr>
<tr>
<td>9</td>
<td>5.75 (1H, d, $J = 8.5$)</td>
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</tr>
<tr>
<td>10</td>
<td>NA</td>
<td>151.5</td>
</tr>
<tr>
<td>11/11’</td>
<td>6.70 (2H, d, $J = 5.50$)</td>
<td>107.7</td>
</tr>
<tr>
<td>12/12’</td>
<td>8.11 (2H, d, $J = 5.60$)</td>
<td>149.1</td>
</tr>
<tr>
<td>13</td>
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<tr>
<td>14</td>
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<tr>
<td>-CN</td>
<td>NA</td>
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</tr>
<tr>
<td>-NH</td>
<td>6.95 (1H, d, $J = 8.8$)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Note. Proton NMR signals are reported in ppm, Hz. Carbon NMR signals are reported as ppm. Coupling constants ($J$) are in Hz. #H represents the number of protons responsible for the chemical shift. S stands for singlet, d stands for doublet, and t stands for triplet.

The addition of NC$^-$ ions resulted in the loss of the keto-enamine $E/Z$ isomerization in the $^1$H NMR spectrum (Figure 19) and only a single compound present in the sample. The single product is the result of the negative charge residing on the C(4) oxide (more electronegative) rather than a C(2) oxide. $^{198, 200}$ The adduct is also stabilized by the intramolecular hydrogen bonding shown in Scheme 49. The addition of NC$^-$ ions to the C(9) carbon resulted in a substantial upfield shifts for the following $^1$H signals: N-H from 13.47 to 6.95 ppm and C(9)H from 8.85 to 5.75 ppm. The upfield shift of the N-H signal is due to the decrease in intramolecular hydrogen bonding, while the shift of the C(9)H signal is due to the change in hybridization.
The inset of the $^1$H NMR found in Figure 19 is provided to show a more detailed view of the aromatic region. Observed in the inset was the C(9)H and N-H both appeared as doublets due to the splitting between each other.

In the $^{13}$C NMR spectrum (Figure 20) of the 2.16-CN$^-$ adduct, the signal for C(3) at 99.2 ppm was shifted upfield to 88 ppm, the C(9) ($sp^2$) signal at 151.8 ppm was shifted upfield to 39.7 ppm ($sp^3$), the C(10) signal at 144.4 ppm was shifted downfield to 151.5 ppm. The new carbon signal at 119.5 ppm was assigned to the carbon of cyanide, which is supported by literature values. From the $^{13}$C NMR spectrum shown in Figure 2.15 the assignment of the C(9) signal was difficult to assign therefore a $^{13}$C APT experiment was performed.

The results of the $^{13}$C APT experiment (Figure 21) was not beneficial in the assignment of the C(9) signal therefore a HSQC was performed (Figures 22 and 23). While the $^{13}$C APT did not aid in the assignment of the C(9) signal, it did confirm that the new $^{13}$C chemical shift at 119.5 ppm is due to the cyanide carbon.
Figure 19. Full $^1$H NMR spectrum of the 2.16-CN$^-$ adduct.

Experiment carried out in a mixture of DMSO-$d_6$:CDCl$_3$ (1:7) (* ) and (+) are due to the solvent and water respectively.
Figure 20. Full $^{13}$C NMR spectrum of 2.16-CN$^+$ adduct.

Experiment carried out in a mixture of DMSO-$d_6$/CDCl$_3$ (1:7) C(3) 99.2 to 88 ppm, C(10) 144.4 to 151.5 ppm, CN appeared at 119.5 ppm, and C(9) 151.8 to 39.7 ppm.
Figure 21. $^{13}$C APT of 2.16-CN$^-$ adduct in a mixture of DMSO-$d_6$:CDCl$_3$ (1:7).

Carbon of CN confirmed at 119.5 ppm and C(10) confirmed at 151.5 ppm.
The $^{13}$C NMR and $^{13}$C APT experiments left some ambiguity in regards to the assignment of the C(9) signal. The $^1$H-$^{13}$C HSQC experiment shown in Figure 2.17 has a cross peak between the C(9)H signal at 5.75 ppm and the carbon signal at 39.7 ppm. This indicates that the C(9) carbon signal is located under the DMSO-$d_6$ signal. The full HSQC spectrum in Figure 22 also shows the cross peaks for C(13) and C(14). The cross peak at ~ 2.5 ppm and ~ 40 ppm is assigned to the solvent. The expanded HSQC spectrum shown in Figure 23 aided in identifying the C(8), C(6), C(11/11’), C(5), and C(12/12’) carbon signals. The lack of a cross peak at 6.95 ppm supported that this signal is due to the N-H proton.

After the HSQC was carried out, a HMBC was performed (Figure 24). This through bond technique confirmed that the cyanide added to the C(9) carbon from the cross peak between the C(9)H (5.75 ppm) and the cyanide carbon signal at (119.5 ppm).

ROESY was the next experiment performed; the full spectrum is shown in Figure 25 (Figure 18 for the free probe 2.16). Since this is a through space experiment, less than 5 Å separation, $^1$H-$^1$H cross peaks of significance are the N-H:C(11/11’)H (labeled b) and the C(9)H:C(11/11’)H (labeled a).
Figure 22. HSQC of 2.16-CN⁻ adduct in a mixture of DMSO-d₆:CDCl₃ (1:7).
Figure 23. Expanded HSQC of 2,16-CN\textsuperscript{−} adduct in a mixture of DMSO-\textit{d}_6:CDCl\textsubscript{3} (1:7).
Figure 24. HMBC of 2.16-CN⁻ adduct in a mixture of DMSO-d₆:CDCl₃ (1:7).
Figure 25. ROESY of 2.16-CN⁻ adduct in a mixture of DMSO-d₆:CDCl₃ (1:7).

Circled cross peaks are the N-H and C(11/11'H) (b) and the C(9)H and C(11/11'H) (a).
2.2.3.2 NMR titrations. To obtain a further understanding of the interaction between probe 2.16 and NC\textsuperscript{-} ions a \textsuperscript{1}H NMR titration with KCN in DMSO-\textit{d}\textsubscript{6} was investigated (Figure 26). With increased presence of NC\textsuperscript{-} ions, compound 2.16 is converted into the cyanide adduct (Scheme 49). This means that as NC\textsuperscript{-} ions are added less of free 2.16 and the \textsuperscript{1}H chemical shifts associated with free probe 2.16 are present in solution. As the concentration of free probe 2.16 decreased, the concentration of the 2.16-NC\textsuperscript{-} adduct increased as well as the \textsuperscript{1}H chemical shifts associated with the 2.16-CN\textsuperscript{-} adduct. In Figure 26 is a stack plot of the \textsuperscript{1}H NMR spectra obtained after the following additions of KCN, 0.0-1.2, 1.5, and 2.0 equivalents. From the analysis and interpretation of Figure 26 the conversion continues with each addition up to 1.2 equivalents whereby all 2.16 is converted into the adduct, as there are no further changes in the spectrum after. The KCN \textsuperscript{1}H NMR titration indicated that the interaction between probe 2.16 and NC\textsuperscript{-} ions occurred in a 1:1 ratio, since there are no observable changes after the addition of 1.2 equivalents of KCN. The 1:1 reaction ratio will be confirmed in section 2.2.9. For the C(13)H and the C(14)H signals no shifts were observed and therefore omitted from Figure 26.

As 2.16 is converted to the 2.16-CN\textsuperscript{-} adduct, the protons located on C(5), C(6), and C(8) become slightly more shielded on the adduct and appear upfield with respect to their chemical shifts on 2.16. The addition of NC\textsuperscript{-} ions induced a larger upfield shift for C(11/11’’)H and C(12/12’’)H proton signals. The larger upfield shift for the pyridine signals is due to the breaking of conjugation at resulting in more electron density (shielding) going into the pyridine ring.
The addition of NC⁻ ions resulted in significant changes to the \(^1\)H NMR spectrum, therefore symbols were used as labels in the \(^1\)H NMR titrations shown in Figure 26: * = the \(E/Z\) isomers of the N-H; # = the \(E/Z\) isomers of C(9)H; @ = C(12/12')H; $ = the \(E/Z\) isomers of the C(5)H; % = C(11/11')H; & = C(6)H; and + = C(8)H.

From the NMR titration it is observed that the reaction between probe 2.16 and NC⁻ ions is not reversible (lack of free 2.16 \(^1\)H signals after 1.2 equivalents). This indicated that probe 2.16 is a true chemodosimeter.
Figure 26. $^1$H NMR titration of probe 2.16 with KCN in DMSO-$d_6$.

Equivalents used: 0.0-1.2 by 0.1 increments, 1.5, and 2.0.
The $^1$H NMR full titrations with probe 2.16 and TBAF (Figure 29) and TBAOAc (Figure 30) were carried out in CDCl$_3$. For a direct comparison to the response observed with the ions F$^-$ and OAc$^-$, a NC$^-$ ion experiment in CDCl$_3$ was needed. To perform this experiment the tetraethylammonium cyanide salt (TEACN) was chosen. To ensure that the NC$^-$ ions reaction went to completion, 1.3 equivalents of TEACN was added. Shown in Figure 27 is the stack plot of 2.16 (bottom) and 2.16 with 1.3 equivalents TEACN (top). The inset is provided in order to aid in the visualization and interpretation of the $^1$H NMR spectra. In comparing the TEACN adduct in Figure 27 and KCN adduct in Figure 19 similar shifts (all free 2.16 signals downfield of 5 ppm shifted upfield) were observed (Table 8).

A $^{13}$C NMR was also performed for both 2.16 (bottom) and 2.16-CN adduct (top) (Figure 28). Since the experiment was carried out in CDCl$_3$ (100%) the $sp^3$ C(9) carbon signal is easily identified at 40.3 ppm. All of the other shifts observed (values provided in Table 8) are identical to the shifts observed with KCN (Figure 20). The spectra shown in Figures 20 and 28 indicated that the response to cyanide is independent of the salt used.
Figure 27. $^1$H NMR spectra of 2.16 (bottom) and 2.16-TEACN (top) in CDCl$_3$.

* = N-H; # = C(9)H; @ = C(12/12')H; S = C(5)H; % = C(11/11')H; & = C(6)H; and + = C(8)H.
Figure 28. $^{13}$C NMR spectra of 2.16 (bottom) and 2.16-TEACN (top) in CDCl$_3$.

$+$ = CDCl$_3$. * represents the CH$_3$ of TEA and ** represents the CH$_2$ of TEA.
Table 8

$^1$H and $^{13}$C NMR of Compound 2.16 and 2.16-TEACN

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<th>$^1$H w/</th>
<th>$^{13}$C w/o</th>
<th>$^{13}$C w/</th>
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Note: $^1$H and $^{13}$C NMR chemical shifts (δ) are reported in ppm. All values have been rounded to the nearest tenth. For any observed $E/Z$ isomer the $Z$ isomer chemical shifts are reported in parenthesis.
As shown in Table 12, the F⁻ ions are stronger conjugate bases than NC⁻ ions and could undergo a similar nucleophilic addition; however, the fluoride ion is more electronegative than the cyanide ion and therefore will deprotonate over addition. With this in mind a full ¹H NMR titration with TBAF in CDCl₃ was investigated (Figure 29). The addition of F⁻ ions resulted in the disappearance of the N-H signal at 13.47 ppm with no appearance of a new chemical shift at 6.95 ppm. The C(9)H chemical shift slightly broadened out with a small downfield shift. The broad C(9)H signal and the downfield shift is attributed to C(9)H-F hydrogen bonding (Scheme 50). All signals below 5 ppm belong to C(13)H, C(14)H, and TBA. The new ¹H signal at 10.20 ppm towards the end of the titration is due to the formation of the HC(9)=N imine.

Scheme 50. Possible hydrogen bonding between 2.16 and F⁻ ions.
Figure 29. $^1$H NMR titration of probe 2.16 with TBAF in CDCl$_3$.

Equivalents used: 0.0-1.5 by 0.1 increments, 2.0, 2.5, and 5.0. Proton labeling: * = N-H; # = C(9)H; @ = C (12/12')H; $ = C(5)H; \% = C(11/11')H; & = C(6)H; and + = C(8)H.
To fully rule out that F\textsuperscript{-} ions are undergoing a nucleophilic addition, a \textsuperscript{1}H NMR titration was carried out with TBAOAc (Figure 30). Acetate was chosen as it is a weaker conjugate base than both ions NC\textsuperscript{-} and F\textsuperscript{-} therefore most likely lead to deprotonation and a similar titration as seen in (Figure 29). Scheme 51 depicts the possible hydrogen bonding that can occur between the OAc\textsuperscript{-} ions and 2.16.

![Scheme 51. Possible hydrogen bonding between 2.16 and OAc\textsuperscript{-} ions.](image)

The addition of OAc\textsuperscript{-} ions resulted in the disappearance of the N-H signal at 13.47 ppm (Figure 30). The appearance of the new \textsuperscript{1}H signal at 10.23 ppm is assigned to the HC(9)=N imine. The two similar \textsuperscript{1}H NMR titrations confirm that both F\textsuperscript{-} and OAc\textsuperscript{-} ions induce deprotonation and not addition.
Figure 30. $^1$H NMR titration of probe 2.16 with TBAOAc in CDCl$_3$.

Equivalents used: 0.0-1.5 by 0.1 increments, 2.0, 2.5, and 5.0. Proton labeling: * = N-H; # = C(9)H; @ = C(12/12')H; $ = C(5)H; % = C(11/11')H; & = C(6)H; and + = C(8)H.
Finally, to put the three $^1$H NMR titrations in perspective, a stack plot of 1.3 equivalents of each anion with probe 2.16 is shown in Figure 31. From this stack plot it is clear that the NC$^-$ ions interaction with 2.16 is significantly different than F$^-$ and OAc$^-$ ions.
Figure 31. $^1$H NMR of compound 2.16 with TBAF, TBAOAc, and TEACN.

1.3 equivalents of anions used (in CDCl$_3$) * = N-H; # = C(9)H; @ = C(12/12')H; $S$ = C(5)H; % = C(11/11')H; & = C(6)H; and + = C(8)H.
To support the results shown in Figure 30, a full $^1$H NMR titration with TBAOAc was investigated with 2.15 (Figure 32). Analogous to the response observed in Figure 30 the addition of OAc$^-$ ions to 2.15 resulted in the deprotonation of the N-H; however, the addition of OAc$^-$ ions did not induce the formation of an imine (Scheme 52). In Figure 32 the addition of OAc$^-$ ions to 2.15 resulted in the deprotonation followed by the coordination of the acetic acid O-H. The hydrogen bonding of the hydroxyl proton is assigned to the broad band being shifted downfield from 2.0 to 4.0 ppm. An arrow is provided to show the acetic acid O-H signal.
Figure 32. $^1$H NMR titration of probe 2.15 with TBAOAc in CDCl$_3$.

Equivalents used: 0.0-1.5 by 0.1 increments, 2.0, 2.5, and 5.0. Proton labeling: * = N-H; # = C(9)H; @ = C(12/12')H; $ = C(5)H; % = C(11/11')H; & = C(6)H; and + = C(8)H.
2.2.4 Solvent Studies of Probes 2.15 and 2.16

The chosen solvent used in the NMR experiments has either been DMSO or CHCl₃, (or a combination of the two) to aid in the solubility of the molecular probe 2.16 and the cyanide salt. It is well established that the fluorescence exhibited by coumarin derivatives are greatly affected to changes in the environment, since the S₁ state is more polar than the S₀ state (section 1.4.2).¹²³,¹²⁸,²⁰² Therefore the effect of various solvents on the absorbance and emission spectra of 2.15 and 2.16 were investigated. The solvents used in this experiment were chosen in attempt to observe the effect of changes in polarity and the effect of aprotic vs. protic solvents. The increased solvent polarity resulted in a larger Stoke’s shift indicating the emission of 2.15 and 2.16 are the result of a π-π* transition. The effects of the solvents are summarized in Tables 9 and 10. From Tables 9 and 10 it is observed that probes 2.15 and 2.16 exhibit larger Stoke shifts in solvents with higher dielectric constants. In solvents with high dielectric constants (more polar) the stability of the S₁ state increases. The increased stability leads to more energy being lost and the fluorescence emission to occur at longer wavelengths (lower energy). The polarity factor (ΔF) allowed for a method for analyzing the spectroscopic response of compounds 2.15 and 2.16 in various environments. In Figure 33 is a picture of probe 2.16 in various solvents: (top) naked eye and (bottom) under UV light.
Table 9

Absorption and Fluorescence Emission of Compound 2.15 in Various Solvents

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<th>Fl. ( \lambda_{\text{max}} )</th>
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<td>114</td>
<td>0.309</td>
</tr>
<tr>
<td>EtOH</td>
<td>24.5</td>
<td>399</td>
<td>516</td>
<td>117</td>
<td>0.289</td>
</tr>
<tr>
<td>Ace</td>
<td>20.7</td>
<td>394</td>
<td>511</td>
<td>117</td>
<td>0.284</td>
</tr>
<tr>
<td>THF</td>
<td>7.6</td>
<td>394</td>
<td>484</td>
<td>90</td>
<td>0.210</td>
</tr>
<tr>
<td>EtOAc</td>
<td>6.0</td>
<td>392</td>
<td>480</td>
<td>88</td>
<td>0.199</td>
</tr>
<tr>
<td>CHCl₃</td>
<td>4.8</td>
<td>399</td>
<td>460</td>
<td>61</td>
<td>0.148</td>
</tr>
<tr>
<td>CH₂Cl₂</td>
<td>8.9</td>
<td>398</td>
<td>480</td>
<td>82</td>
<td>0.217</td>
</tr>
<tr>
<td>Tol</td>
<td>2.4</td>
<td>395</td>
<td>449</td>
<td>54</td>
<td>0.015</td>
</tr>
<tr>
<td>Bz</td>
<td>2.3</td>
<td>396</td>
<td>457</td>
<td>61</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Note. The excitation wavelength used for each solvent was the absorbance \( \lambda_{\text{max}} \).

Table 10

Absorption and Fluorescence Emission of Compound 2.16 in Various Solvents

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Dielectric constant</th>
<th>Abs. ( \lambda_{\text{max}} )</th>
<th>Fl. ( \lambda_{\text{max}} )</th>
<th>Stoke’s Shift</th>
<th>( \Delta F )</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>46.7</td>
<td>401</td>
<td>574</td>
<td>173</td>
<td>0.263</td>
</tr>
<tr>
<td>MeCN</td>
<td>37.5</td>
<td>401</td>
<td>561</td>
<td>160</td>
<td>0.305</td>
</tr>
<tr>
<td>DMF</td>
<td>36.7</td>
<td>402</td>
<td>577</td>
<td>175</td>
<td>0.274</td>
</tr>
<tr>
<td>MeOH</td>
<td>32.7</td>
<td>406</td>
<td>573</td>
<td>167</td>
<td>0.309</td>
</tr>
<tr>
<td>EtOH</td>
<td>24.5</td>
<td>343</td>
<td>385</td>
<td>42</td>
<td>0.289</td>
</tr>
<tr>
<td>Ace</td>
<td>20.7</td>
<td>401</td>
<td>549</td>
<td>148</td>
<td>0.284</td>
</tr>
<tr>
<td>THF</td>
<td>7.6</td>
<td>401</td>
<td>520</td>
<td>119</td>
<td>0.210</td>
</tr>
<tr>
<td>EtOAc</td>
<td>6.0</td>
<td>400</td>
<td>520</td>
<td>120</td>
<td>0.199</td>
</tr>
<tr>
<td>CHCl₃</td>
<td>4.8</td>
<td>415</td>
<td>491</td>
<td>76</td>
<td>0.148</td>
</tr>
<tr>
<td>CH₂Cl₂</td>
<td>8.9</td>
<td>411</td>
<td>510</td>
<td>99</td>
<td>0.217</td>
</tr>
<tr>
<td>Tol</td>
<td>2.4</td>
<td>406</td>
<td>473</td>
<td>67</td>
<td>0.015</td>
</tr>
<tr>
<td>Bz</td>
<td>2.3</td>
<td>406</td>
<td>484</td>
<td>78</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Note. The excitation wavelength used for each solvent was the absorbance \( \lambda_{\text{max}} \).
Figure 33. Compound 2.16 in various solvents.

From the results of the solvent studies three solvents were chosen (CHCl₃, EtOH, and DMSO) to investigate the molar absorptivity (ε) of probes 2.15 and 2.16 (Table 11). The data reported in Table 11 shows that the λ_max of probe 2.15 is relatively unaffected by changing the solvent, even in the presence of H₂O; however, the absorbance λ_max of probe 2.16 is significantly influenced by changes in solvents. Upon a literature search it was discovered that probes that undergo ESIPT could have a max absorbance at two different wavelengths based on the solvent of choice. The absorbance at ~ 400 nm is due to the absorbance of the keto-enamine, while the absorbance at ~ 340 nm is due to the imine-enol tautomer. The imine-enol tautomer is only observed with probe 2.16, which is
attributed to the more acidic N-H hydrogen bonding with the EtOH and H$_2$O. Probe 2.15 possess an overall higher $\varepsilon$ than probe 2.16 in all of the solvents investigated.

Table 11

*Absorbance ($\lambda_{\text{max}}$) and molar absorptivity for probes 2.15 and 2.16*

<table>
<thead>
<tr>
<th>Compound</th>
<th>CHCl$<em>3$ $\lambda</em>{\text{max}}$ ($\varepsilon$)*</th>
<th>EtOH $\lambda_{\text{max}}$ ($\varepsilon$)*</th>
<th>DMSO $\lambda_{\text{max}}$ ($\varepsilon$)*</th>
<th>DMSO-H$<em>2$O $\lambda</em>{\text{max}}$ ($\varepsilon$)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.16</td>
<td>415 (38,723)</td>
<td>342 (43,654)</td>
<td>404 (44,387)</td>
<td>348 (25,599)</td>
</tr>
<tr>
<td>2.15</td>
<td>402 (50,460)</td>
<td>400 (62,485)</td>
<td>398 (51,963)</td>
<td>398 (57,150)</td>
</tr>
</tbody>
</table>

Note. Maximum absorbance ($\lambda_{\text{max}}$) is reported in nm; Molar absorptivity ($\varepsilon$) is reported in Lmol$^{-1}$cm$^{-1}$. ($\lambda_{\text{max}}$ in nm; $\varepsilon$ in Lmol$^{-1}$cm$^{-1}$)

Based on the discussions and results shown so far, the solvent chosen to investigate the colorimetric and fluorescence responses towards various anions was DMSO. The choice of the polar aprotic solvent DMSO was based on the desire to eliminate the possibility of hydrogen bonding between the anions and the solvent.\textsuperscript{14}

Therefore the strength of each anion as a nucleophile (Lewis base) is based on the basicity and the electronegativity of the atom in which the negative charge is located (Table 12). Lastly both probes exhibit relatively large Stoke’s shifts in DMSO: 2.15 (144 nm) and 2.16 (173 nm).

2.2.5 *Colorimetric Response of Probe 2.15*

To determine if both compounds were in fact selective for cyanide, various anions were tested: TBA salts of F$^-$, Cl$^-$, Br$^-$, I$^-$, NO$_3^-$, OAc$^-$, H$_2$PO$_4^-$, HSO$_4^-$, NaBF$_4$, NaN$_3$, NH$_4$SCN, NH$_4$ClO$_4$, NH$_3$OH, octylamine, TEACN, KCN, and NaCN. The rational for examining these anions are:
1. OH\textsuperscript{-}, SCN\textsuperscript{-}, and F\textsuperscript{-} ions as well as octylamine can undergo a Michael addition.

2. The anions test if the spectroscopic response is influenced more by the basicity or geometry (or both) of each anion.

3. Examine if the spectroscopic response is dependent on the cyanide salt used.

4. Ultimately the selectivity of probes 2.15 and 2.16.

Table 12

\textit{Selected pKa values in H\textsubscript{2}O and DMSO.}

<table>
<thead>
<tr>
<th>Anions</th>
<th>H\textsubscript{2}O</th>
<th>DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>F\textsuperscript{-}</td>
<td>3.17</td>
<td>15</td>
</tr>
<tr>
<td>Cl\textsuperscript{-}</td>
<td>-7</td>
<td>1.8</td>
</tr>
<tr>
<td>Br\textsuperscript{-}</td>
<td>-8</td>
<td>0.9</td>
</tr>
<tr>
<td>I\textsuperscript{-}</td>
<td>-9</td>
<td>NA</td>
</tr>
<tr>
<td>NO\textsubscript{3}\textsuperscript{-}</td>
<td>-1.3</td>
<td>NA</td>
</tr>
<tr>
<td>OAc\textsuperscript{-}</td>
<td>4.76</td>
<td>12.3</td>
</tr>
<tr>
<td>H\textsubbox{2}PO\textsubscript{4}\textsuperscript{-}</td>
<td>2.12</td>
<td>NA</td>
</tr>
<tr>
<td>HSO\textsubscript{4}\textsuperscript{-}</td>
<td>-10</td>
<td>NA</td>
</tr>
<tr>
<td>BF\textsubscript{4}\textsuperscript{-}</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>N\textsubscript{3}\textsuperscript{-}</td>
<td>4.72</td>
<td>7.9</td>
</tr>
<tr>
<td>SCN\textsuperscript{-}</td>
<td>4</td>
<td>NA</td>
</tr>
<tr>
<td>ClO\textsubscript{4}\textsuperscript{-}</td>
<td>-10</td>
<td>NA</td>
</tr>
<tr>
<td>OH\textsuperscript{-}</td>
<td>15.7</td>
<td>32</td>
</tr>
<tr>
<td>NC\textsuperscript{-}</td>
<td>9.4</td>
<td>12.9</td>
</tr>
</tbody>
</table>

Note: The pKa values of selected monoanionic species in H\textsubscript{2}O and DMSO. The values were obtained from multiple sources\textsuperscript{53, 105, 204} NA stands for not available.

The colorimetric response of 2.15 towards various anions. From the \textsuperscript{1}H NMR titration (Figure 26) the interaction between cyanide and each probe will be in a 1:1 ratio. Therefore, two equivalents were chosen as the end point since all additions or deprotonations should have occurred by this equivalence. The majority of the anions tested resulted in very small changes in the absorption spectrum of 2.15. A single titration
(Cl⁻ ions) is provided as a representation for the anions that resulted in no significant changes in the absorbance spectrum of 2.15 (Figure 34).

![UV-Vis titration](image)

**Figure 34.** UV-Vis titration with probe 2.15 and Cl⁻ ions in DMSO. 

[2.15] = 1.56 × 10⁻⁵ M [TBACl] = 3.11 × 10⁻⁴ M (equivalences used: 0.0, 0.5, 1.0, 1.5, and 2.0). Other anions include: Br⁻, I⁻, NO₃⁻, H₂PO₄⁻, HSO₄⁻, BF₄⁻, N₃⁻, ClO₄⁻, SCN⁻, OH⁻, and octylamine.

Not shown in Figure 34 is the colorimetric response from the ions F⁻, OAc⁻, and NC⁻. This is due to these anions produced a significant spectroscopic response from 2.15.

From the NMR titration shown in Figure 32, the addition of the OAc⁻ ions will deprotonate the enamine N-H and will induce a colorimetric response. Shown in Scheme 52 is the hydrogen bonding interaction between OAc⁻ ions and probe 2.15.
Upon the addition of the ions OAc\(^-\) and F\(^-\) a deprotonation occurs resulting in the breaking of conjugation in probe 2.15 inducing a blue shift of the maximum absorbance band along with a decolorization of the solution. However, the absorbance titrations in Figure 35 do not support this claim. This is due to a hydrogen bonding interaction between the anion and the N-H (or O-H) as well as the deprotonation followed by the coordination of the acid. The possible hydrogen bonding interaction between probe 2.15 and the anions OAc\(^-\) and F\(^-\) are shown in Schemes 52 and 53.

Scheme 53. Hydrogen bonding between 2.15 and F\(^-\) ions.
The addition of TBAF induced a larger hypochromic shift of the maximum absorbance than the previously tested anions. Fluoride also induced a small increase in the absorbance intensity at 335 nm. A similar response was observed upon the addition of OAc\(^-\) ions shown in Figure 35. The addition of both anions resulted in a pseudo isobestic point at 368 and 366 nm, F\(^-\) and OAc\(^-\) ions respectively. The pseudo isobestic point along with the increase and decrease at 335 and 405 nm respectively indicates that less of the free 2.15 and more of the hydrogen bonding complex is present in solution.
Figure 35. (Top) TBAOAc (bottom) TBAF absorbance titration with probe 2.15.

Carried out in DMSO. [2.15] = 1.56 × 10⁻⁵ M, [TBAOAc] = 3.11 × 10⁻⁴ M, [TBAF] = 3.11 × 10⁻⁴ M. Equivalences used: 0.0, 0.5, 1.0, 1.5, and 2.0.

With all of the anions examined the investigation turned to the three cyanide salts.

The NC⁻ ions produce a larger decrease in the absorbance intensity at 405 nm and an
increase in the absorbance intensity at 335 nm with an isobestic point at 342 nm (Figure 36). The ratiometric response observed indicates that the addition of the NC$^-$ ions results in the formation of another absorbing species in solution (2.15-CN$^-$ adduct). As the conversion occurs less of free probe 2.15 is present in solution and a decreased absorbance at 405 nm is observed. Likewise as probe 2.15 is converted to the adduct (more of 2.15-CN is present in solution) an increase in the absorbance at 335 nm is observed. In Scheme 54 a mechanism is provided to aid in the visualization of the NC$^-$ ions attack.

Scheme 54. The Michael addition of NC$^-$ ions to the C(9) carbon of probe 2.15.
Figure 36. Cyanide addition to probe 2.15 UV-Vis titrations and isotherms.

Carried out in DMSO. 1. TEACN, 2. NaCN, 3. KCN. [2.15] = 1.56 × 10^{-5} M [NC] = 3.11 × 10^{-4} M. Equivalences used: 0.0-2.0 by 0.1 increments. Titrations are on the left and the isotherms are on the right.
In Figure 37 is a ratiometric bar chart that summarizes all of the colorimetric responses observed from probe 2.15 with two equivalents of the various anions. The bar chart clearly shows that NC⁻ ions produced the largest ratiometric response.

Figure 37. A barchart of probe 2.15 and two equivalents of various anions.

Carried out in DMSO. [2.15] = 1.56 × 10⁻⁵ M, [Anion] = 3.11 × 10⁻⁵ M.

2.2.6 Colorimetric Response of Probe 2.16

As previously stated, the probe 2.16 will provide larger spectroscopic changes, with respect to probe 2.15. This is due to the increased electron withdrawing character of pyridine compared to the benzene of aniline. The increase results in an increase in acidity of the enamine N-H, which makes it more susceptible to strong conjugate bases. To be consistent a full titration, up to two equivalents, was performed with the ions F⁻, OAc⁻, and NC⁻ (Figures 39 and 41). The screening of the other various anions with probe 2.16 all resulted in a small decrease in the absorbance band at 405 nm and a small increase at 335 nm. Since all of the anions induced the same colorimetric response, the NO₃⁻ titration...
is shown in Figure 38. Upon investigating the response to the addition of the various ions, a significant change in the absorbance spectrum was observed when \textbf{2.16} is in the presence of H$_2$PO$_4^-$ ions and octylamine. Therefore full titrations were carried out and are shown in Figures 39 and 40.

\textbf{Figure 38}. UV-Vis titration with probe \textbf{2.16} and NO$_3^-$ ions in DMSO.

\[ [\text{TBA}_3\text{NO}_3] = 3.11 \times 10^{-4} \, \text{M}. \]  
Equivalences used: 0.0, 0.5, 1.0, 1.5, and 2.0). Anions that produce the same response: Cl$^-$, Br$^-$, I$^-$, HSO$_4^-$, BF$_4^-$, N$_3^-$, ClO$_4^-$, SCN$^-$, and OH$^-$.  

The addition of F$^-$ ions to probe \textbf{2.16} (Scheme 50) resulted in a decrease of the absorbance band at 405 nm and an increase in the band at 335 nm, with an isobestic point at 342 nm (Figure 39 # 1). The isotherm depicts the changes at these two wavelengths as the F$^-$ ions concentration increases. As the equivalents added approached two equivalents the amount of change at both wavelengths decreased. This is indicative of an equilibrium being reached.
Upon the addition of OAc\(^-\) ions to **2.16**, the observed response (Figure 39 # 2) was a slight decrease in the absorbance band at 335 nm and a slight increase in the absorbance at 405 nm up to 1.1 equivalents. The isotherm shows that at higher concentrations of OAc\(^-\) ions present will result in the decrease of the 405 nm absorbance band. The nearly 1:1 coordination ratio indicates that hydrogen bonding occurs up to one equivalents and deprotonation of the enamine N-H occurs after one equivalent. The possible hydrogen bonding interactions between probe **2.16** and acetate was shown in Scheme 51.

Upon the addition of H\(_2\)PO\(_4\)^- ions (Figure 39 # 3) probe **2.16** displayed a colorimetric response similar to that of F\(^-\) ions but to a lesser extent: the absorbance intensity at 405 nm decreased and increased at 335 nm with a pseudo isobestic point at 342 nm. The possible hydrogen bonding interaction between probe **2.16** and H\(_2\)PO\(_4\)^- ions is shown in Scheme 55.

![Scheme 55. Hydrogen bonding between probe **2.16** and H\(_2\)PO\(_4\)^- ions.](image-url)
Figure 39. Probe 2.16 UV-Vis titration with 1. F\(^-\), 2. OAc\(^-\), 3. H\(_2\)PO\(_4\)\(^-\).

Carried out in DMSO. (Left) titration (Right) isotherm. [2.16] = 1.56 × 10\(^{-5}\) M, [Anions] = 3.11 × 10\(^{-4}\) M. Equivalences used: 0.0-2.0 by 0.1 increments.
Unlike the previous three anion titrations provided above, the addition of octylamine to probe 2.16 resulted in little to no change of the absorbance band at 335 nm (Figure 40); however, octylamine induced a decrease of the absorbance band at 405 nm with a small increase in the absorbance intensity at 361 nm and a pseudo isobestic point at 375 nm. The different spectroscopic response observed with the addition of octylamine, compared to the previous three anions (Figure 39) is due to hydrogen bonding between the amine of octylamine and the enamine N-H as well as the ketone at C(4) (Scheme 56). The unique response from the addition of octylamine indicates that the observed changes to the absorbance spectrum is due to an acid-base reaction.

Scheme 56. Hydrogen bonding between 2.16 and octylamine.
Figure 40. Isotherm and absorbance titration of 2.16 with octylamine in DMSO.

\[ [2.16] = 1.56 \times 10^{-5} \text{ M} \ [\text{octylamine}] = 3.11 \times 10^{-4} \text{ M} \]. Equivalences used: 0.0-2.0 by 0.1 increments.

The addition of NC' ions to probe 2.16 resulted in a similar ratiometric response as probe 2.15: a decrease in the absorbance band at 405 nm and an increase in the intensity at 335 nm with an isobestic point at 342 nm (Figure 41). The decrease in the absorbance at 405 nm indicates that as NC' ions are added the presence of free 2.16
decreases. This is attributed to the conversion of 2.16 to 2.16-CN⁻ adduct, Scheme 49. As more of the adduct is formed the absorbance at 335 nm increases, until roughly one equivalence of NC⁻ ions are added. Once 2.16 is fully converted to the adduct, the addition of more NC⁻ ions resulted in the decrease of the absorbance band at 335 nm. This is shown with the isotherms provided for the titration with each cyanide salt, (Figure 41). The maximum absorbance intensity at 335 nm occurred close to one equivalence of NC⁻ ions. The 1:1 reaction ratio between probe 2.16 and cyanide supports the KCN ¹H NMR titration (Figure 26). The Job’s plot provided in Figure 53, vide infra, provides further evidence that one to one is the reaction ratio.
Figure 41. Cyanide addition to probe 2.16 UV-Vis titrations and isotherms.

Carried out in DMSO. 1. TEACN, 2. NaCN, 3. KCN. [2.16] = 1.56 × 10⁻⁵ M [NC⁻] = 3.11 × 10⁻⁴ M. Equivalences used: 0.0-2.0 by 0.1 increments. Titrations are on the left and the isotherms are on the right.
The isotherms found in Figures 41 shows that the response of 2.16 towards the cyanide salts is ratiometric. Therefore to compare the colorimetric response from the various anions (two equivalents) a ratio of the absorbance intensity at 335 and 405 nm was calculated and is shown in Figure 42.

![Graph showing absorbance ratio (335 nm / 405 nm) for various anions.]

Figure 42. A bar chart of probe 2.16 and two equivalents of various anions. Carried out in DMSO. [2.15] = 1.56 × 10^{-5} M, [Anion] = 3.11 × 10^{-5} M.

From the observed spectroscopic response of 2.15 and 2.16 towards the anions screened it is apparent that changing the basicity of the enaminone N-H does in fact change the response of the compound. As with the model compound 2.15, 2.16 also displayed a ratiometric response towards NC\(^-\) ions with an isobestic point at 340 nm, but 2.16 showed a similar response to both ions F\(^-\) and H\(_2\)PO\(_4\)^-. As stated previously a ratiometric response is the result of the change in concentration of two adsorbing/emitting species. When compared to the response observed with NC\(^-\) ions the initial maximum absorbance at 405 nm is completely gone indicating little to none of the initial absorbing
species is present in the cuvette; however, this is not the case for the ions F\(^-\) and H\(_2\)PO\(_4\)\(^-\). Octylamine also gave what resembled a ratiometric response but not to the extent produced by NC\(^-\) ions. The response observed toward OAc\(^-\) ions indicated that the most likely cause was the deprotonation of the N-H.

2.2.7 Fluorescence Response of Probe 2.15

To get a better understanding of the spectroscopic response of 2.15 towards various anions, a series of screenings were investigated. For the anions screened (Cl\(^-\), I\(^-\), NO\(_3\)\(^-\), H\(_2\)PO\(_4\)\(^-\), HSO\(_4\)\(^-\), BF\(_4\)\(^-\), N\(_3\)\(^-\), ClO\(_4\)\(^-\), SCN\(^-\), and OH\(^-\)) that resulted in little to no spectroscopic changes to the emission spectrum of 2.15 the titration with Br\(^-\) ions is provided in Figure 43. The excitation wavelength chosen was 339 nm, but under normal circumstances the excitation wavelength chosen is the wavelength at which the compound has the highest absorbance intensity to populate the singlet excited state. In the case of probes 2.15 and 2.16, vide infra, 339 nm the maximum absorbance of 1.2 was used. As previously stated (section 1.5.7) the imine-enol tautomer is the most commonly reported ground state for a compound that undergoes ESIPT. Therefore, exciting at a wavelength close the absorbance maximum of 1.2 would ensure that the emission was solely due to the keto-enamine tautomer (\(\lambda\)\(\text{em}\) 535 nm). The evidence thus far has indicated that the NC\(^-\) ions attack occurs at C(9) carbon of the keto-enamine tautomer, therefore it is crucial that the emission comes from strictly this tautomer. Provided in Scheme 40 is the ESIPT process of probe 2.7 before and after the addition of NC\(^-\) to help illustrate this.
Figure 43. Fluorescence titration with probe 2.15 and Br⁻ ions in DMSO.

\( \lambda_{ex} = 339 \text{ nm.} \ \text{[2.15]} = 1.56 \times 10^{-4} \text{ M} \ \text{[TBABr]} = 3.11 \times 10^{-3} \text{ M.} \) Equivalences used: 0.0, 0.5, 1.0, 1.5, and 2.0. Other anions include: Cl⁻, I⁻, NO₃⁻, HSO₄⁻, BF₄⁻, N₃⁻, ClO₄⁻, SCN⁻, and OH⁻.

The addition of each one of these ions F⁻, OAc⁻, and H₂PO₄⁻ to 2.15 resulted in a decrease of the fluorescence emission intensity at 535 nm (Figure 44). The amount of decrease in intensity observed at 535 nm is based on the relative strength of the individual anion (Table 12). To put the intensity decreases in perspective the addition of F⁻ ions produced a \( \Delta (F_l(\text{final}) - F_l(\text{initial})) \) of -0.89, while the ions OAc⁻ and H₂PO₄⁻ resulted in \( \Delta \) values of -0.72 and -0.23 respectively. Meaning that H₂PO₄⁻ ions are most likely hydrogen bonding (similar to Scheme 55) with little to no deprotonation occurring. The OAc⁻ ions are hydrogen bonding and deprotonating (Scheme 52), while F⁻ ions are deprotonating and potentially PET quenching (section 1.5.4).
Figure 44. Probe 2.15 fluorescence titration with 1. F\(^-\), 2. OAc\(^-\), 3. H\(_2\)PO\(_4\)^-.

Carried out in DMSO (\(\lambda_{ex} 339\) nm). (Left) titration (Right) isotherm. [2.16] = 1.56 \times 10^{-4} M, [Anions] = 3.11 \times 10^{-3} M. Equivalences used: 0.0-2.0 by 0.1 increments.
The addition of octylamine (Figure 45) resulted in the decrease of the 2.15 keto-enamine tautomer emission intensity at 535 nm similar to the previous three anions; however, the decrease observed was not continuous as in Figure 44. This is due to octylamine participating in hydrogen bonding with the enaminone (similar to Scheme 56) and inducing a slight perturbation of the ESIPT process. The result of this is a new emission band appearing at 456 nm.

*Figure 45. Isotherm and fluorescence titration of 2.15 with octylamine in DMSO.*

($\lambda_{ex}$ 339 nm). [2.15] = $1.56 \times 10^{-4}$ M [octylamine] = $3.11 \times 10^{-3}$ M. Equivalences used: 0.0, 0.5, 1.0, 1.5, and 2.0.

The spectroscopic response of 2.15 towards the three cyanide salts was investigated next. The addition of NC$^-$ ions resulted in an observed ratiometric response
whereby the presence of NC\(^-\) decreased the 2.15 keto-enamine tautomer emission at 575 nm and an increase of the new emission band at 356 nm with an isoemissive point at 440 nm. All three cyanide salts produced a similar response indicating that the response was independent of the counter cation. In Figure 36 each cyanide salt reached a plateau prior to the addition of two equivalents; however, this was not observed in the fluorescence titrations shown in Figure 46. The constant increase of the emission intensity at 356 nm with the addition of NC\(^-\) ions after one equivalence is due to the cation stabilizing the oxide and the kinetics, which will be discussed in section 2.2.9.
Figure 46. Cyanide addition to probe 2.15 (Left) Fl titrations (Right) isotherms.

Carried out in DMSO ($\lambda_{ex}$ 339 nm). 1. TEACN, 2. NaCN, 3. KCN. [2.15] = 1.56 x 10$^{-4}$ M [NC$^-$] = 3.11 x 10$^{-3}$ M. Equivalences used: 0.0-2.0 by 0.1 increments.
2.2.8 Fluorescence Response of Probe 2.16

The majority of the anions screened (Cl\(^-\), Br\(^-\), I\(^-\), NO\(_3\)^-, HSO\(_4\)^-, BF\(_4\)^-, N\(_3\)^-, ClO\(_4\)^-, SCN\(^-\), and OH\(^-\)) with probe 2.16 produced a slight decrease in the fluorescence emission intensity at 575 nm. In Figure 47 is the titration with probe 2.16 and Br\(^-\) ions.

![Figure 47. Fluorescence titration with probe 2.16 and Br\(^-\) ions in DMSO.](image)

\(\lambda_{ex} = 339\text{ nm}, [2.16] = 1.56 \times 10^{-4} \text{ M} [\text{TBABr}] = 3.11 \times 10^{-3} \text{ M}\). Equivalences used: 0.0, 0.5, 1.0, 1.5, and 2.0. Other anions include: Cl\(^-\), I\(^-\), NO\(_3\)^-, HSO\(_4\)^-, BF\(_4\)^-, N\(_3\)^-, ClO\(_4\)^-, SCN\(^-\), and OH\(^-\).

Since 2.16 produced a colorimetric response upon the addition of H\(_2\)PO\(_4\)^- ions, the half equivalences screening will be discussed briefly. Similar to the titration spectrum shown in Figure 47, the addition of H\(_2\)PO\(_4\)^- ions resulted in a slight decrease of the emission band at 575 nm (Figure 48); however, the addition of H\(_2\)PO\(_4\)^- ions also produced a new emission signal appearing at 356 nm, due to the partial inhibition of the ESIPT process (emission of the imine-enol). The maximum intensity of this new emission band was observed upon the addition of 0.5 equivalents indicating that the interaction is due to
hydrogen bonding (Scheme 55), occurs in a two 2.16 probes to one H$_2$PO$_4^-$ ion ratio. Subsequent additions induced a decrease in emission intensity at 356 and 575 nm.

The investigation of the spectroscopic response of 2.16 towards the addition of OAc$^-$ ions resulted in a continuous decrease of the emission band at 575 nm with a new emission band appearing at 356 nm (Figure 48). The emission intensity at 356 nm increased up to the addition of 0.3 equivalents and additions there after resulted in in a decreased fluorescence signal. The initial increase at 356 nm is due to ESIPT inhibition from hydrogen bonding (Scheme 51) resulting in the imine-enol emission. The enol O-H is more acidic than the enamine N-H making it more susceptible to deprotonation, which results in a decrease of the emission signal. The overall decrease in the emission spectrum of 2.16 upon the addition of OAc$^-$ ions can also be attributed to the $E/Z$ isomerization of the imine. The new emission band at 402 nm is due to the coordination of the acetic acid O-H proton to the deprotonated probe 2.16.

In comparison to the partial inhibition of the ESIPT process from the ions H$_2$PO$_4^-$ and OAc$^-$, the addition of F$^-$ ions to probe 2.16 results in a significant decrease of the keto-enamine tautomer emission band at 575 nm (Figure 48). Similar to OAc$^-$ ions, the F$^-$ ion produced new emission bands at 356 and 397 nm. The hydrogen bonding between probe 2.16 and F$^-$ ions is shown in Scheme 50. The rationale for the spectroscopic response produced by F$^-$ ions is the same as OAc$^-$ ions. The only difference is the amount of intensity decrease, which is attributed to the F$^-$ ions are a stronger conjugate base.

To determine if the emission intensity decrease from the ions F$^-$ and OAc$^-$, the $^1$H NMR titrations shown in Figures 29 and 30 respectively were revisited. Upon analyzing the $^1$H NMR titration between probe 2.16 and OAc$^-$ ions, the disappearance of the N-H
proton signal confirms that a deprotonation is occurring. From the addition of 0.5 equivalents upward there is a new proton signal appearing at 10.23 ppm, which is assigned to the imine HC=N proton. The appearance of this new signal indicates that the emission intensity decrease is due to the E/Z isomerization of the imine. For the fluoride $^1$H NMR titration (Figure 29), the imine HC=N signal is not observed up to one equivalents. The lack of this signal indicates that as the enamine N-H or enol O-H is being deprotonated and the negative charge on one of the heteroatoms results in PET quenching, which induces a decrease in emission intensity.
**Figure 48.** Probe 2.16 Fl. titration and isotherms with ions 1. F⁻, 2. OAc⁻, 3. H₂PO₄⁻.

Carried out in DMSO (λₑₓ 339 nm). (Left) titration (Right) isotherm. [2.16] = 1.56 × 10⁻⁴ M, [Anions] = 3.11 × 10⁻³ M. Equivalences used for H₂PO₄⁻: 0.0, 0.5, 1.0, 1.5, and 2.0. Equivalences used for F⁻ and OAc⁻ ions: 0.0-2.0 by 0.1 increments.
Up to this point the various anions screened have induced either partial inhibition of ESIPT (Figure 48 # 1) or complete inhibition of ESIPT due to deprotonation, (Figure 48 # 2 and 3). With the addition of octylamine to probe 2.16 (Figure 49) the maximum emission band at 575 nm was shifted to 480 nm. The new band at 480 nm also experienced an increase in fluorescence emission intensity with successive additions of octylamine. This unique signal is attributed to an acid-base reaction.
Figure 49. Isotherm and fluorescence titration of **2.16** with octylamine in DMSO. 

$\lambda_{ex}$ 339 nm. $[\text{2.16}] = 1.56 \times 10^{-4}$ M [octylamine] = 3.11 \times 10^{-3}$ M. Equivalences used: 0.0-2.0 by 0.1 increments.

After screening probe **2.16** with the anions above, the fluorescence response of probe **2.16** towards cyanide was investigated (Figure 50). All three cyanide salts produced a similar response and therefore will be discussed together as one. The addition of NC$^-$ ions resulted in the disappearance of emission band at 575 nm. It was shown in
section 2.2.3 that the NC\textsuperscript{-} ions undergo a nucleophilic addition at the C(9) carbon of \textbf{2.16}. In doing so the keto-enamine tautomerization is lost and since \textbf{2.16} can no longer undergo tautomerization the ESIPT process no longer occurs (i.e. $\lambda_{\text{ex}}$ 575 nm disappears). The mechanism of the cyanide attack of probe \textbf{2.16} (Scheme 49) results in the breaking of conjugation leaving the only fluorescence that can occur is coming from the coumarin core of \textbf{2.16}. In regards to optical spectroscopy the \textbf{2.16} adduct structure is the C(4) oxide version of compound \textbf{1.2}. This implies that the adduct will emit close to the emission wavelength of \textbf{1.2} and provides an explanation for the new emission band at 356 nm. An isoemissive point was observed at 475 nm, which supports the addition of cyanide is attacking the free \textbf{2.16} in solution and converting it to the \textbf{2.16-CN\textsuperscript{-}} adduct (i.e. two emitting species in solution). The isotherms in Figure 50 show the increase in emission intensity at 356 nm reaches a plateau at one equivalent, which supports the 1:1 reaction ratio.
Figure 50. Cyanide addition to probe 2.16 (Left) Fl titrations (Right) isotherms.

Carried out in DMSO ($\lambda_{ex}$ 339 nm). 1. TEACN, 2. NaCN, 3. KCN. [2.16] = $1.56 \times 10^{-4}$ M [NC] = $3.11 \times 10^{-3}$ M. Equivalences used: 0.0-2.0 by 0.1 increments.
To compare the spectroscopic changes observed for the various anions, a bar chart was generated (Figure 51). The chart shows the ratio of the fluorescence emission intensity at 356 and 575 nm with the various anions. The three cyanide salts produced a similar response and therefore KCN was chosen to represent them in the bar chart.

![Bar chart showing fluorescence intensity ratios](image)

**Figure 51.** A fluorescence bar chart of probe 2.16 with various anions.


In order to fully grasp the colorimetric (top) and fluorescence (bottom) response of 2.16, two photographs are provided below in Figure 52. For both the top and bottom photograph the order of the cuvettes is the following: (A) 2.16 blank, two equivalences of (B) KCN, (C) F⁻, (D) H₂PO₄⁻, (E) Br⁻, (F) Cl⁻, (G) I⁻, (H) NO₃⁻, (I) BF₄⁻, (J) N₃⁻, (K) HSO₄⁻, (L) SCN⁻, (M) ClO₄⁻, (N) OAc⁻. In regards to changes to the solution color (i.e. colorimetric response), only the addition of NC⁻ ions resulted in complete decolorization, F⁻ and OAc⁻ ions resulted in a lightening of the yellow solution. The same samples that were used in the top image were subjected to a UV-lamp. In the presence of the ions F⁻
and OAc\(^-\) the fluorescence emission is quenched, while the H\(_2\)PO\(_4\)\(^-\) ions produced a lesser emission intensity decrease. The addition of NC\(^-\) ions also resulted in a blue emission.

![Image of probe 2.16 with two equivalents of various anions.](image)

**Figure 52.** Image of probe 2.16 with two equivalents of various anions. 
(Top) Colorimetric response via naked eye in DMSO (Bottom) under the UV-lamp. [2.16] = 0.2 moldm\(^{-3}\) [Anions] = 0.3 moldm\(^{-3}\)).

Order: (A) 2.16 (B) KCN (C) TBAF (D) TBAH\(_2\)PO\(_4\) (E) TBABr (F) TBACl (G) TBAI (H) TBANO\(_3\) (I) NaBF\(_4\) (J) NaN\(_3\) (K) TBAHSO\(_4\) (L) NH\(_4\)SCN (M) NH\(_4\)ClO\(_4\) (N) TBAOAc.

### 2.2.9 Additional Evidence

A Job’s plot was investigated to determine the reaction ratio between probe 2.15 and KCN (top Figure 53). The intersection of the two linear lines at a mole fraction (χ) of 0.5 indicates that the interaction between probe 2.15 and NC\(^-\) ions occurs in a 1:1 fashion. A Job’s plot was also investigated with probe 2.16 and KCN (bottom Figure 53). While the intersection of the two linear lines fell slightly above a mole fraction of 0.5, it can be inferred that a 1:1 between probe 2.16 and NC\(^-\) ions is occurring.
Figure 53. KCN Job’s plots with probes 2.15 (top) and 2.16 (bottom).

To further confirm the NMR and X-Ray crystal structure, the mass of 2.16 was obtained using electro-spray ionization mass spectrometry, ESI-MS. The predicted mass of [2.16+H]^+ is 338 m/z, which is confirmed in Figure 55 (row 1 left). From this result, a series of experiments were investigated to confirm the data shown in sections 2.2.3, 2.2.6,
and 2.2.8. In Figure 55 (row 1 right) F⁻ ions were added to a solution of 2.16, the parent signal is observed at 336 m/z [2.16-H⁺]. In Figure 55 (row 2 left) OAc⁻ ions were added to a solution of 2.16. In this mass spectrum the [2.16-H⁺]⁻ at 336 m/z is observed, as well as the sodium adduct at 360 m/z. In Figure 55 (row 2 right) octylamine was added to a solution of 2.16, the parent signal is observed at 336 m/z [2.16-H⁻]. In Figure 54 below are the structures that correspond to the observed parent peaks in Figures 55 and 56.

![Figure 54. The 2.16 structures for the observed mass spectra shown below.](image)

**Figure 54.** The 2.16 structures for the observed mass spectra shown below.
To confirm all of the cyanide data presented thus far, KCN was added to 2.16 and the mass spectrum was recorded (Figure 56). From the predicted [2.16+CN-H]⁻ adduct structure a molecular weight of 363 g mol⁻¹ was calculated, which is the parent m/z peak observed in Figure 56. This also further confirms that the interaction between 2.16 and NC⁻ ions result in the Michael addition.

Figure 55. ESI-MS studies of probe 2.16 under various conditions.

(Row 1 left) The mass spectrum of 2.16 (2.16+H⁺ (338 m/z)). (Row 1 right) The mass spectrum of 2.16 + F⁻ (2.16-H⁺ (336 m/z)). (Row 2 left) The mass spectrum of 2.16 + Octylamine⁻ (2.16-H⁺ (336 m/z)). (Row 2 right) The mass spectrum of 2.16 + octylamine (2.16-H⁺ (336 m/z)).
**Figure 56.** Mass spectrum of the **2.16-CN**$^-$ adduct.

The adduct appears at 363 m/z [**2.16-CN**$^-$]. Probe **2.16** appears at 336 m/z [**2.16-H**]
Upon designing probes 2.15 and 2.16 for the detection NC<sup>-</sup> ions one goal was to detect below the 1.9 μM (49.4 ppb) maximum limit set by WHO. For this reason the sensitivity of both probes were investigated. The detection limit was first investigated with the model probe 2.15. Using the UV-Vis titration with KCN (Figure 36) a limit of detection (Figure 57) was calculated (confidence level 95%, t-value 2.145, df 12) to be 22.2 ppb (y<sub>B</sub> + 3S<sub>B</sub>), where y<sub>B</sub> = intensity of the blank and 3S<sub>B</sub> = three times the standard deviation. Next, the detection limit using UV-Vis spectroscopy was investigated with probe 2.16 and NaCN (titration shown in Figure 41). Applying the same methods as above the parameters used were confidence level 95%, t-value 2.131, df 13 and the detection limit was calculated to be 36.4 ppb (Figure 57). Finally, the detection limit with 2.16 and KCN was investigated using fluorescence spectroscopy, which was calculated to be 4.2 ppb (Figure 57). The deconjugation and the conversion into a completely different absorbing species (the adduct) gave rise to both probes 2.15 and 2.16 detecting cyanide concentrations in the ppb range through UV-Vis spectroscopy.
Figure 57. Cyanide detection limits with probes 2.15 and 2.16 in DMSO.

1. Probe 2.15 with KCN (UV-Vis, 22.2 ppb). 2. Probe 2.16 with NaCN (UV-Vis, 36.4 ppb). 3. Probe 2.16 with KCN (fluorescence λₐₓ 339 nm, 4.2 ppb).
Another issue with chemodosimeters for the detection of NC$^-$ ions is that the reaction often requires long periods of time to reach completion. Therefore the rate of cyanide attack of probe 2.16 was investigated using UV-Vis spectroscopy. In order to calculate the reaction kinetics, the change in absorbance intensity at 332 nm was monitored for three minutes after the addition of one equivalent of KCN (Figure 58). A pseudo first order rate constant $k$ was calculated to be 0.03 s$^{-1}$, which is faster than the kinetics of probes (2.5, 2.10, and 2.13)$^{192, 195, 196}$ (equation used $k = \ln(A_F \div (A_F - A_T))$ where $A_F =$ the final absorbance intensity at 332 nm and $A_T =$ the absorbance at the time interval). Using this rate constant a half life ($t_{1/2} = \ln(2 \div k)$) was calculated to be 23 seconds.

![Figure 58](image)

**Figure 58.** The kinetics of probe 2.16 with one equivalents of KCN.

Carried out in DMSO (UV-Vis) (Left) A pseudo-first order kinetic plot of 2.16 with one equivalent of KCN. (Right) The increase in absorbance of 2.16 at 332 nm.

Refer back section 1.5.7 for the discussion on ESIPT. Theoretical calculations of the HOMO and LUMO energy levels of probe 2.16 in the ground state and the first excited-state were investigated. All calculations were carried out by using Spartan ’14.$^{205}$

In Figure 6 are the possible conformers of compound 2.16. Using the values in Table 13...
along with these conformers, an ESIPT process for probe 2.16 can be constructed (Scheme 57). The conformer with the lowest S$_0$ HOMO energy level is structure (B) (imine-enol, -5.85 eV). This is in agreement with the literature reported ground-state tautomer.$^{11,160,161}$ Upon excitation at 339 nm the electrons in the HOMO of (B) are promoted to the S$_1$ α-LUMO (-1.061 eV). In this state, ESIPT occurs and results in the conformer (E) in the S$_1$ α-LUMO state (keto-enamine, -1.195 eV). While other conformers have lower α-LUMO energies, the α-HOMO of conformer is the lowest in energy and therefore the most stable in the S$_1$ state. In the S$_1$ α-LUMO state of conformer (E) the electron relaxes back down to the S$_0$ HOMO of conformer (E) (-5.672 eV). Here a reverse ground state proton transfer occurs to relax conformer (E) to conformer (B), which is the most stable conformer.

Table 13

**Molecular Orbital Energies for the Stable 2.16 Structures**

<table>
<thead>
<tr>
<th>Compound</th>
<th>HOMO (eV)</th>
<th>LUMO (eV)</th>
<th>α-HOMO (eV)</th>
<th>α-LUMO (eV)</th>
<th>β-HOMO (eV)</th>
<th>β-LUMO (eV)</th>
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<tr>
<td>B</td>
<td>-5.850</td>
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<tr>
<td>E</td>
<td>-5.672</td>
<td>-2.307</td>
<td>-3.342</td>
<td>-1.195</td>
<td>-5.860</td>
<td>-4.878</td>
</tr>
<tr>
<td>F</td>
<td>-5.641</td>
<td>-2.147</td>
<td>-3.208</td>
<td>-1.039</td>
<td>-5.726</td>
<td>-4.812</td>
</tr>
<tr>
<td>G</td>
<td>-5.745</td>
<td>-2.270</td>
<td>-3.140</td>
<td>-1.308</td>
<td>-5.617</td>
<td>-5.061</td>
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</table>

Note: The theoretical calculation values provided were calculated using Spartan ’14.$^{205}$
Scheme 57. The ESIPT process for compound 2.16.

Values obtained from Table 13 and conformers are found in Figure 6. The excitation and emission wavelengths were the values used and experimentally observed.

Molecular modeling was investigated to show (Figure 60) the changes to the frontier molecular orbitals (values in Table 14) as the new C-CN bond is forming. The geometries used for the modeling are provided in Figure 59. In Table 12 the starting distance of 3.15 Å was chosen because at this distance there were no interactions between the NC⁻ ions and the probes, and the distance of 1.48 Å was calculated, as the most stable C-CN bond length therefore was the end point.
Figure 59. Structures of cyanide adducts used for data shown in Table 2.9.

Representations of structures used to generate input geometries for MMFF94/PM6 calculations (X = N or CH).

Table 14

Frontier Molecular Orbital Energies During C-CN Bond Formation

<table>
<thead>
<tr>
<th>Orbital energy (eV)</th>
<th>C–CN distance (Å)</th>
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<tr>
<td></td>
<td>2.147</td>
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<td>X=N</td>
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<td>LUMO</td>
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<td>HOMO</td>
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<td>X=CH</td>
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<td>1.93</td>
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<tr>
<td>HOMO</td>
<td>-2.81</td>
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Note: Values for X=N refer to probe 2.16 and when X=CH it is referring to probe 2.15.

Shown in Figure 60 is the model of the NC\(^-\) ions attack of the C(9) carbon of 2.15: (left) the structures of the HOMOs (right). Going from top to bottom the NC\(^-\) ion is 3.15 Å away from probe 2.15. At this distance it is observed that the NC\(^-\) ion does not perturb the planar structure of 2.15. Next the NC\(^-\) ion is now 2.81 Å away from probe 2.15 and at this distance 2.15 begins to bend in preparation for the nucleophilic attack. This observed until the two-most bottom structures. In these structures the C-CN bond (1.48 Å) is fully formed and the planarity is permanently lost.
Figure 60. Molecular orbital interactions as cyanide attacks probe 2.15.

2.3 Summary

The coumarin-enaminone probes 2.15 and 2.16 were synthesized (in 80% yield, section 2.4) as a novel approach that was used for the detection of NC⁻ ions. Of the other anions screened a majority of them produced no significant spectroscopic changes (Figures 34, 37, 38, 42, 43, 47, and 51); however, the ions F⁻, OAc⁻, and H₂PO₄⁻ as well as the neutral compound octylamine produced a deprotonation of the enamine N-H. This was confirmed by NMR experiments (Figures 29, 30, and 32) and ESI-MS (Figure 55). The presence of cyanide (determined to be independent of salt used) both probes 2.15 and
2.16 resulted in numerous optical spectroscopic changes: a hypsochromic shift of the maximum absorbance band (isobestic point at 342 nm) (Figures 36 and 42), the decolorization of the probe solution (Figure 52), and the decrease of the 545/575 nm (2.15/2.16 respectively) keto-enamine emission band and the increase of the emission band at 356 nm (isoemissive point at 440/475 nm 2.15/2.16 respectively) (Figures 46 and 50). The colorimetric response is due to the deconjugation of the probes, which was confirmed by the upfield shifts of the N-H and C(9)H signals (Figures 19, 26, 27, and 31) and molecular modeling (Figure 60). The 1:1 reaction ratio between the probes and cyanide was confirmed in Figure 53. The limit of detections calculated for probes 2.15 and 2.16 (Figure 57) all fall below the maximum limit set by WHO and the detection limits reported with probes 2.0 (except 2. in Figure 57), 2.3 (except 2. in Figure 57), 2.5, 2.7, 2.10, 2.11, 2.12, and 2.14. The ability of detecting cyanide at low concentrations is promising and can be built upon in the future with different probes.

The work presented in Chapter II provides the framework for making improvements in the detection of cyanide in aqueous solutions as well as investigating the effect of substituents on the aniline ring in terms of sensitivity and reaction rate.

2.4 General Experimental Procedure

Following the literature procedures, the commercially available reagents 2,4,6-trichlorophenol (2.17) and malonic acid (1.11) were refluxed for three hours in phosphorus (V) oxychloride under anhydrous conditions. The reaction was then allowed to cool to room temperature and was quenched with cold deionized water then neutralized with saturated sodium bicarbonate solution. The resulting solid bis-(2,4,6-trichlorophenyl)malonate (1.14) was collected by vacuum filtration and recrystallized
from ethyl acetate in a 90 % yield, (Scheme 48). The product was confirmed by the singlet that represented the four aromatic protons at δ (ppm) 7.53 and the singlet 3.85 (s, 2H, CH₂); ¹³C NMR δ (ppm): 168.8, 149.0, 128.6, 123.8, 123.5 (DMSO-d₆).

**1.14** (9.26 g, 20.0 mmol) and 3-diethylaminophenol (**1.13**) (3.30 g, 20.0 mmol), and anhydrous toluene (50 mL) were refluxed for three hours. The reaction was allowed to cool to room temperature. The precipitate, 7-(diethylamino)-4-hydroxycoumarin (**1.2**) was collected by vacuum filtration and washed with toluene (3.50 g, 75 % yield) (Scheme 48). ¹H NMR (300 K, DMSO-d₆, 400 MHz): δ (ppm) 11.91 (s, 1H, OH), 7.55 (d, 1H, J = 9.0 Hz, ArH), 6.65 (dd, 1H, J = 9.0 and 2.2 Hz, ArH), 6.45 (d, 1H, J = 2.2 Hz, ArH), 5.25 (s, 1H, CH), 3.42 (dq, 4H, J = 14.0 and 7.0 Hz, CH₂), 1.11 (t, 6H, J = 7.0, CH₃); ¹³C NMR (300 K, DMSO-d₆, 100 MHz): δ (ppm) 166.9, 163.2, 156.6, 151.3, 124.6, 108.6, 103.9, 98.9, 86.6, 44.4, 12.8.

**1.2** (233.3 mg, 1.0 mmol), and aniline (91.1 μL, 0.9997 mmol), and triethyl orthoformate (250 μL, 1.5 mmol) were refluxed in 2-propanol (5 mL) for two hours (Scheme 48). The reaction was allowed to cool to room temperature and the resulting solid (**2.15**) was collected by vacuum filtration then washed with 2-propanol, which gave a yield of 268 mg (0.98 mmol, 80 % yield); ¹H NMR (300 K, CDCl₃, 400 MHz): δ (ppm) 13.65 (d, 1H, J = 12.5 Hz, NH), 8.82 (d, 1H, J = 13.3 Hz, CH_enamine), 7.87 (d, 1H, J = 9.0 Hz, CH₄coumarin), 7.45 (t, 2H, J = 7.9 Hz, CH_aromatic), 7.30 (dt, 3H, J = 15.9 and 7.5 Hz, CH_aromatic), 6.59 (dd, 1H, J = 9.0 and 2.4 Hz, CH₄coumarin), 6.38 (d, 1H, J = 2.3 Hz, CH₄coumarin), 3.45 (q, 4H, J = 7.1 Hz, CH₂), 1.15 (t, 6H, J = 7.1 Hz, CH₃); ¹³C NMR (300 K, CDCl₃, 100 MHz): δ (ppm) 181.0, 164.5, 157.2, 153.5, 152.9, 138.2, 130.8, 127.3, 126.6, 118.0, 108.8, 108.4, 98.1, 97.2, 44.9, 12.9; ESI-MS m/z [M+H]^+ = 337.0; IR
(ATR solid); (cm$^{-1}$) 3239 (w) $\nu_{\text{NH}}$, 3059 (w) $\nu_{\text{C(ename)}}$, 2970 (w) $\nu_{\text{CH}}$, 1716 (s) $\nu_{\text{CO}}$ ($\delta$ lactone), 1571 $\nu_{\text{CO}}$ (ketone); HRMS observed for $\text{C}_{20}\text{H}_{20}\text{N}_{2}\text{O}_{3} = 336.1484$; Calculated for $\text{C}_{19}\text{H}_{19}\text{N}_{3}\text{O}_{3} = 336.1474$.

1.2 (466.5 mg, 2.0 mmol), and 4-aminopyridine (188.1 mg, 2.0 mmol), and triethyl orthoformate (500 μL, 3.0 mmol) were refluxed in 2-propanol (10 mL) overnight (Scheme 48). The reaction was allowed to cool to room temperature and the resulting solid (2.16) was collected by vacuum filtration then washed with 2-propanol, which gave a yield of 553.7 mg (1.64 mmol, 82 % yield); $^1$H NMR (300 K, DMSO-$d_6$, 400 MHz): $\delta$ (ppm) 13.25 ($d$, $J = 12.8$ Hz, 1H), 11.46 ($d$, $J = 13.9$ Hz, 1H), 8.88 ($t$, $J = 11.3$ Hz, 1H), 8.57 ($d$, $J = 5.5$ Hz, 2H), 7.74 ($t$, $J = 7.7$ Hz, 1H), 7.62 ($t$, $J = 7.7$ Hz, 2H), 6.69 ($d$, $J = 7.2$ Hz, 1H), 6.43 ($d$, $J = 17.9$ Hz, 1H), 3.45 ($dd$, $J = 13.7$, 6.7 Hz, 4H), 1.14 ($t$, $J = 6.9$ Hz, 6H); $^{13}$C NMR (300 K, CDCl$_3$, 100 MHz): $\delta$ (ppm) 181.1, 163.9, 157.3, 153.4, 152.2, 151.6, 145.0, 127.7, 111.8, 108.7, 108.6, 100.1, 97.2, 44.9, 12.5; ESI-MS; m/z for [M+H]$^+$ = 338.2; IR (ATR solid); (cm$^{-1}$) 3059 (w) $\nu_{\text{C(ename)}}$, 2970 (w) $\nu_{\text{CH}}$, 1716 (s) $\nu_{\text{CO}}$ ($\delta$ lactone), 1571 $\nu_{\text{CO}}$ (ketone) cm$^{-1}$; HRMS observed for $\text{C}_{19}\text{H}_{19}\text{N}_{3}\text{O}_{3} = 337.1430$; Calculated for $\text{C}_{19}\text{H}_{19}\text{N}_{3}\text{O}_{3} = 337.1426$.

In Table 13 is the crystallographic data obtained from the X-Ray crystal structure of probe 2.16 (Figure 7), which was grown by the slow evaporation of DMSO.
Table 15

**X-Ray Crystallography Data**

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<td>Z = 2</td>
</tr>
<tr>
<td>$M_r = 337.37$</td>
<td>$F(000) = 356$</td>
</tr>
<tr>
<td>Triclinic, P1</td>
<td>$D_x = 1.397 \text{Mg m}^{-3}$</td>
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<td>Hall symbol: -P 1</td>
<td>Cu Kα radiation, $\lambda = 1.54184 \text{Å}$</td>
</tr>
<tr>
<td>$a = 5.0790 \ (4) \text{ Å}$</td>
<td>Cell parameters from 3404 reflections</td>
</tr>
<tr>
<td>$b = 12.8141 \ (10) \text{ Å}$</td>
<td>$\theta = 3.6-63.6^\circ$</td>
</tr>
<tr>
<td>$c = 13.2671 \ (9) \text{ Å}$</td>
<td>$\mu = 0.79 \text{ mm}^{-1}$</td>
</tr>
<tr>
<td>$\alpha = 70.308 \ (5)^\circ$</td>
<td>$T = 100 \text{ K}$</td>
</tr>
<tr>
<td>$\beta = 80.767 \ (5)^\circ$</td>
<td>Lath, yellow</td>
</tr>
<tr>
<td>$\gamma = 88.005 \ (5)^\circ$</td>
<td>0.42 x 0.09 x 0.02 mm</td>
</tr>
<tr>
<td>$V = 70.308 \ (10) \text{ Å}^3$</td>
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</tr>
</tbody>
</table>

Note: $a$, $b$, and $c$ are the lengths of the crystal unit cell. $\alpha$, $\beta$, and $\gamma$ are the unit cell angles between them.
Figure 61. $^1$H NMR of probe 2.16 in CDCl$_3$ (top) and DMSO-$d_6$ (bottom).

Stock solutions of compounds 2.15 and 2.16 as well as the various anions ([3.1 mmoldm$^{-3}$]) were prepared in 100% DMSO. For all UV-Vis studies, 5 μL of the stock solution of 2.15 and 2.16 were diluted to 1 mL for a final working concentration of 16 μM. Before use an aliquot of the various anion was diluted to 311 μM whereby 5 μL of the anion = 0.1 equivalent. For all fluorescence titrations, 100 μL of the stock solution of
2.15 and 2.16 were diluted to 2 mL for a final working concentration of 16 μM. The addition of 10 μL ([Anions] = 311 μM) = 0.1 equivalents.

For the computational data discussed in section 2.2.9, structure A was constructed using the ‘Build’ option in Spartan ‘14. Analysis of 144 conformers resulted in two groups being retained: a cluster of 14 very similar structures within 7.6 kJmol⁻¹ of the lowest energy conformer a second cluster of 17 structures related to structure B with energies between 58.7 and 67.3 kJmol⁻¹ of the lowest energy conformer. The equilibrium geometry of A was refined using molecular mechanics energy minimization methods (MMFF94) within Spartan ‘14 and used as the starting geometry for gas phase DFT calculations (B3LYP/6-311G*). The same method was applied to structure E. Analysis of 72 conformers again resulted in two groups being retained: a cluster of 14 very similar structures within 7.9 kJmol⁻¹ of the lowest energy conformer a second cluster of 15 structures related to structure F with energies between 63.0 and 71.3 kJmol⁻¹ of the lowest energy conformer. The same method was applied to structure G. Analysis of 72 conformers again resulted in two groups being retained: a cluster of 14 very similar structures within 8.0 kJmol⁻¹ of the lowest energy conformer a second cluster of 16 structures related to structure H with energies between 57.2 and 65.4 kJmol⁻¹ of the lowest energy conformer. MMFF94-derived structures were used as inputs for DFT calculations as before. First excited (triplet) states were computed as above but using a time-dependent density functional (TDDFT) model as implemented in Spartan ‘14, also using B3LYP/6-311G* in a vacuum.

For the molecular modeling of the cyanide attack a sequence of structures was generated in which NC⁻ ions approached the target carbon down a vector. A reaction
trajectory was attempted using DFT/B3LYP/6-311G* but simulations resulted in proton abstraction rather than cyanide addition so a simpler method was employed. A trial semi-empirical simulation indicated that there were no orbital interactions between NC− ions and the target at distances exceeding 3.147 Å for both reactions. This was taken as a starting point and the C-CN distance was reduced in steps of 0.333 Å down to 1.480 Å, which had been identified as the optimum C-CN bond distance from a DFT/B3LYP/6-311G* calculation (Table 2.10). At each step, the C-CN distance was constrained by freezing the positions of both carbon atoms and the C-CN angle was constrained to 180°. With these constraints, geometry optimization was undertaken using molecular mechanics (MMFF94). To account for the transition between the initial system in which NC− ions had no interaction with the neutral target to that in which cyanide is covalently linked and the negative charge has migrated to the phenol, three models were used (Figure 59). In the first, both oxygen atoms were given a formal double bond (A); in the last, all bond migrations had occurred (C) and in the intermediate four structures, delocalized bonds were set (B). The resulting structures were used to generate molecular orbitals at a semi-empirical (PM6) level.
CHAPTER III – THE SCREENING OF METAL IONS

3.1 Introduction

The importance and toxicity of the \( d^{10} \) metal ions (Zn\(^{2+}\), Cd\(^{2+}\), and Hg\(^{2+}\)) has been previously discussed in Chapter I. This work will focus on the coordination and detection of metal ions via an enaminone binding unit, that is incorporated into a molecular probe scaffold, see section 1.3.

The azomethine moiety (enaminones) is a common functional group used to bind to metal ions.\(^{71}\) The coordination of a metal ion with an azomethine is analogous to the more commonly reported salicylaldehyde (3.0a) and 2-hydroxy-1-napthaldehyde (3.0b) Schiff bases (Scheme 57).\(^{74}\) The salicylaldehyde Schiff base is commonly focus in the imine-enol tautomer, while the 2-hydroxy-1-napthaldehyde Schiff bases prefers the keto-enamine tautomer.\(^{74}\) The preference of the keto-enamine tautomer has been attributed to the stability provided by the fused aromatic ring of the naphthalene unit.\(^{74}\)

![Scheme 58. Metal ion coordination through a generic Schiff bases 3.0a and 3.0b.](adapted from)\(^{74}\)
Free enaminones still possess the enamine N-H and are unable to coordinate metal ions through the azomethine binding site. Therefore this hydrogen atom has to be removed prior to metal ion coordination. With a larger ionic radius the metal ions will induce significant changes to the binding unit.\textsuperscript{53, 72-74}

1. The redistribution of π-electrons will give rise to changes in the bond (C-N, C-C, and C-O) distances of the azomethine moiety: C-N > C=N, C-O > C=O, C-C > C=C.

2. Bond angles increase to accommodate the coordination (amount increased is dependent on metal ion). Also changes in hybridization results in changes in bond angles.

3. Changes in the orientation of the amino substituent. This is dependent on the steric hindrance of the substituent as well as the metal ion being coordinated.

4. The coordination of the larger metal ion results in an increased separation between the O•••N atoms (on average 2.59 to 2.79 Å).

Kumbhar et al. synthesized compound 3.1 to selectively detect Cu\textsuperscript{2+} ions, via CHEF, through thio-β enaminone PET inhibition mechanism (Scheme 59).\textsuperscript{206} Upon investigating the colorimetric response of probe 3.1, in MeCN-HEPES (9:1, pH 7, 10 mM), towards various metal ions, no significant changes were observed. However, the addition of Cu\textsuperscript{2+} ions resulted in a ~20-fold enhancement of the fluorescence emission intensity at 567 nm along with a 10 nm red shift. The coordination of Cu\textsuperscript{2+} ions resulted in PET inhibition, which increased the $\phi_{Fl}$ of compound 3.1 by a factor of 18.\textsuperscript{206} Job’s plot confirmed the 1:1 coordination to form the tetrahedral [Cu(3.1)ClH\textsubscript{2}O] complex shown in Scheme 58. This was further confirmed by mass spectrometry analysis, where a
489 m/z signal is seen and assigned to the Cu$^{2+}$ complex. The binding constant ($K_{\text{assoc.}}$) was calculated to be $1.1 \times 10^4$ M$^{-1}$, and the limit of detection was calculated to be $8.2 \times 10^{-7}$ M. The selectivity of probe 3.1 was investigated and showed little to no interference from any other metal ions tested, equivalence ratio of 1:10:10 3.1:Cu$^{2+}$:other metal ion. $^1$H NMR studies showed that with the addition of five equivalences of Cu$^{2+}$ ions the N-H signal at 14.69 ppm disappeared as well as the downfield shift of the enamine C-H from 7.11 to 7.64-7.86 ppm (authors reported as a range). It is suggested that a ligand-to-metal charge transfer process from compound 3.1 to the Cu$^{2+}$ ion is the major contributor to the optical response and complex stability, which was confirmed by theoretical calculations. This design approach is an example of CHEF by the inhibition of PET (section 1.5.4).

Scheme 59. The coordination of Cu$^{2+}$ ions by compound 3.1. (adapted from)

Studies were carried out in MeCN-HEPES (9:1, pH 7, 10 mM). Coordination occurs in a 1:1 ratio, $K_{\text{assoc.}} = 1.1 \times 10^4$ M$^{-1}$, L.o.D. = $8.2 \times 10^{-7}$ M.

The benzoxazole moiety has particularly common ESIPT based molecular probe used for cation detection. Xu et al. took advantage of this mechanism by synthesizing two benzoxazole based probes 3.2a and 3.2b for the selective detection of Cd$^{2+}$ and Zn$^{2+}$ ions respectively by the inhibition of the ESIPT process (Scheme 60). Compound 3.2a shows a maximum absorbance at 325 nm (in 1:1 MeCN:HEPES buffer (pH 7.4)) and
upon the addition of Cd\(^{2+}\) ions the band hypochromically shifts, with the appearance of a hyperchromic band at 370 nm, through an isobestic point at 350 nm. Upon the excitation of compound 3.2a at 360 nm a fluorescent emission band is seen at 468 nm, assigned to the keto-enamine tautomer. Aliquots of Cd\(^{2+}\) ions increased in the fluorescence intensity at 468 nm. The calculated binding constant (\(K_{11}\)) was \(2.0 \times 10^4\) M\(^{-1}\) with a detection limit calculated at \(1.3 \times 10^{-7}\) M.\(^{207}\) NMR spectroscopy was used to prove that the metal ion was bound within the chelating motif. The presence of Cd\(^{2+}\) ions induced an upfield shift of the O-H\(_1\) signal, from 11.10 to 11.07 ppm, and a downfield shift of the O-H\(_2\) signal, from 9.56 to 9.60 ppm. It is shown that when the hydrogen atom on O-H\(_2\) was replaced with a methyl group, as in the case with molecular probe 3.2b, the probe shows selectivity towards Zn\(^{2+}\) ions. Compound 3.2b has a maximum absorbance centered at 325 nm and upon the addition of Zn\(^{2+}\) ions this absorbance band decreased which was accompanied by an increase in the absorbance intensity at ~370 nm with an isobestic point at 340 nm. The excitation of 3.2b at 360 nm resulted in a maximum emission band centered at 455 nm and the addition of Zn\(^{2+}\) ions induced an increase in the fluorescence intensity at 455 nm. Unlike compound 3.2a, when probe 3.2b is excited at 360 nm an emission band is observed at 455 nm and a near infrared (NIR) emission band at 880 nm, assigned to the ESIPT band.\(^{207}\) The 880 nm emission band increased with the addition of Zn\(^{2+}\) ions. The [Zn(3.2b)] coordination ratio of 1:1 was confirmed by a Job’s plot and a binding constant, \(K_{11}\), was calculated at \(6.5 \times 10^4\) M\(^{-1}\).\(^{207}\) A detection limit was calculated at \(1.6 \times 10^{-8}\) M.\(^{207}\) The selectivity of both probes was investigated. In the case of probe 3.2b the presence of Cu\(^{2+}\) ions resulted in interference with Cd\(^{2+}\) ions detection; however, probe 3.2a was able to selectively bind Zn\(^{2+}\) ions with no false positives from other metal
ions, in a 1:20:20 $3.2b$:Zn$^{2+}$:X ratio (X = Li$^+$, Na$^+$, K$^+$, Mg$^{2+}$, Ca$^{2+}$, Ba$^{2+}$, Cr$^{2+}$, Co$^{2+}$, Cu$^{2+}$, Mn$^{2+}$, Ni$^{2+}$, Pb$^{2+}$, Cd$^{2+}$, Hg$^{2+}$, Fe$^{2+}$, Fe$^{3+}$, Al$^{3+}$, and Ce$^{3+}$). The disappearance of the O-H signal in the $^1$H NMR spectrum of probe $3.2b$ upon the addition of Zn$^{2+}$ ions indicates that the oxygen atom is involved in the coordination of the Zn$^{2+}$ ion.\textsuperscript{207}

Scheme 60. The binding of Cd$^{2+}$ and Zn$^{2+}$ ions by probes $3.2a$ and $3.2b$ respectively.

(adapted from)\textsuperscript{207}

Studies carried out in MeCN:HEPES buffer (1:1, pH 7.4). (Top) $3.2a$ coordination of Cd$^{2+}$ ions in a 1:1 ratio, $K_{11} = 1.99 \times 10^4$ M$^{-1}$, L.o.D. = 1.33 \times 10^{-7}$ M. (Bottom) $3.2b$ coordination of Zn$^{2+}$ ions in a 1:1 ratio, $K_{11} = 6.51 \times 10^4$ M$^{-1}$, L.o.D. = 1.63 \times 10^{-8}$ M.\textsuperscript{207}

Ma et al. synthesized a coumarin-Schiff base (3.3) that is used to detect Zn$^{2+}$ ions (Scheme 61).\textsuperscript{208} In a 5:45:50 DMF-MeCN-H$_2$O (TRIS 10 mM) solution, compound 3.3 has a fluorescence emission maximum centered at 620 nm. Upon excitation at 500 nm when Zn$^{2+}$ (as the perchlorate salt) ions were added the emission maximum was increased by a 35-fold enhancement.\textsuperscript{208} The increased emission intensity was accompanied by a 50 nm blue shift. To confirm the hypsochromic shift is due to coordination and not hydrolysis the emission spectrum of the aldehyde precursor was recorded. A Job’s plot was investigated to confirm the 3.3-Zn$^{2+}$ ion 1:1 interaction. The
1:1 interaction was also confirmed through mass spectrometry by observing the predicted [Zn(3.3)]\(^+\) mass at 503 m/z. A \(K_{11}\) binding constant was calculated at \(2.2 \times 10^6\) M\(^{-1}\). A \(^1\)H NMR experiment was investigated to confirm the proposed coordination shown in Scheme 3.4. With the addition of Zn\(^{2+}\) ions the N-H proton signal of probe 3.3 at 11.2 ppm disappeared. The same trend is seen in the presence of Cd\(^{2+}\) ions. The Zn\(^{2+}\) ion selectivity of probe 3.3 was investigated. For all of the metal ions studied an emission increase in the presence of Zn\(^{2+}\) was observed (3.3:Zn\(^{2+}\):X 1:2:2), except in the case of Cu\(^{2+}\) ions which resulted in complete quenching of the fluorescence emission (X = Li\(^+\), Na\(^+\), K\(^+\), Mg\(^{2+}\), Ca\(^{2+}\), Co\(^{2+}\), Cu\(^{2+}\), Fe\(^{2+}\), Ni\(^{2+}\), Pb\(^{2+}\), Cd\(^{2+}\), and Hg\(^{2+}\)). The sensitivity of 3.3 was investigated, which resulted in a detection limit ranging from 0.5-5.0 \(\mu\)M.

![Scheme 61. The coordination of Zn\(^{2+}\) and Cd\(^{2+}\) ions by probe 3.3. (adapted from)\(^{208}\)](image)

*Studies were carried out in a 5:45:50 DMF-MeCN-H\(_2\)O (TRIS 10 mM) solution. L.o.D. = 0.5-5.0 \(\mu\)M range (\(K_{11}\) = 2.21 \(\times\) 10\(^6\) M\(^{-1}\)).\(^{208}\)

Kumar et al. designed another coumarin-Schiff base (3.4) for the detection of Zn\(^{2+}\) ions (Scheme 62).\(^{69}\) In a 1:1 EtOH-H\(_2\)O solution compound 3.4 has a maximum absorbance located at 498 nm. Upon the addition of Zn\(^{2+}\) ions (as the nitrate salt) the maximum absorbance band decreased with the simultaneous increase of the absorbance intensity at 475 nm, with an isobestic point seen at 487 nm. The C=N isomerization of probe 3.4 resulted in a weak emission band at 530 nm (\(\lambda_{ex}\) 470 nm). The presence of the Zn(NO\(_3\))\(_2\) inhibited the isomerization, which gave rise to a 20 nm blue shift and an enhancement of the fluorescence emission intensity. A similar spectroscopic response
was observed with the ZnCl$_2$ and Zn(OAc)$_2$ salts. From a Job’s plot the interaction between probe 3.4 and the Zn$^{2+}$ ion was determined as a 1:1. A $K_{11}$ binding constant was calculated at $1.89 \times 10^7$ M$^{-1}$. By investigating the changes to the $^1$H NMR spectrum of compound 3.4, due to the addition of two equivalents of Zn(NO$_3$)$_2$, it was determined that the disappearance of the N-H proton signal is the result of enolization. The $^1$H NMR results were supported by the crystal structure of the [Zn(3.4)NO$_3$] complex as well as shifts observed in the IR spectrum. The C(2) oxygen atom was shifted from 1684 to 1669 cm$^{-1}$ while the C=N imine stretch at 1615 moved to 1610 cm$^{-1}$, for the free compound 3.4 and [Zn(3.4)NO$_3$] complex respectively. A detection limit was calculated at $3.26 \times 10^{-9}$ M.$^{69}$ The extremely low detection limit was attributed to changes in the ICT character of 3.4 as well as CHEF (as a result of inhibition of C=N isomerization). However, in a different solvent system for example 1:10:10 3.4:Zn$^{2+}$:Cu$^{2+}$ ratio the emission intensity of compound 3.4 did not increase indicating that the Cu$^{2+}$ ion results in the interference of detecting the Zn$^{2+}$ ion due to quenching.$^{69}$

![Scheme 62. The coordination of Zn(NO$_3$)$_2$ by compound 3.4. (adapted from)$^{69}$](image)

Studies were carried out in a 1:1 EtOH-H$_2$O solution. L.o.D. = $3.26 \times 10^{-9}$ M ($K_{11} = 1.89 \times 10^7$ M$^{-1}$).$^{69}$
Ray et al. synthesized a coumarin Schiff base (3.5) that is able to detect Zn\(^{2+}\) ions (in 100% MeCN) by the inhibition of ESIPT (Scheme 63).\(^{65}\) Compound 3.5 has a maximum absorbance band at 445 nm, and upon the addition of Zn\(^{2+}\) ions the maximum absorbance undergoes a 75 nm bathochromic shift (from 445 to 520 nm) going through an isobestic point at 460 nm. Upon excitation at 445 nm, compound 3.5 has a weak fluorescence emission band at 535 nm, due to ESIPT. The coordination of the Zn\(^{2+}\) ion induced a significant increase in the emission intensity as well as a 15 nm red shift. The inhibition of the ESIPT process does not typically induce a bathochromic shift. In the case of compound 3.5, the coordination site involves the C(2)=O (Scheme 3.6), which in turn creates more electron pull from the C(2)=O. The larger the pull of electron density will result in the S\(_1\) excited state of probe 3.5 to posses more TICT character, which will reduce the energy level of the emitting state causing an emission shifted further in the red direction (section 1.4.2). The data collected from the techniques, absorbance and fluorescence titrations, a binding constant \(K_{\text{assoc.}}\) was calculated to be \(8.2 \times 10^4\) M\(^{-1}\).\(^{65}\)

Scheme 63. The coordination of Zn\(^{2+}\) ions by compound 3.5. (adapted from)\(^{65}\)

*Studies carried out in MeCN (100%). Binding occurs in a 1:1 ratio \(K_{\text{assoc.}} = 8.2 \times 10^4\) M\(^{-1}\).*

From the examples shown in this section and discussion of enaminones The use of enaminones in the coordination of metal ions is well established (section 1.3). The incorporation of this motif, as the binding site, on a molecular probe has been used to coordinate Zn\(^{2+}\) ions (Schemes 17, 23, 27, and 63).
Previously it was shown that strong conjugate bases such as F\(^-\) and OAc\(^-\) will quench the fluorescence emission by deprotonation (see Chapter II). It became obvious that upon the removal of the hydrogen atom an ideal chelating motif is formed, whereby metal coordination can occur. The investigation was turned to studying molecular probes for sensing metal ions by manipulating the inhibition of ESIPT (section 1.5.7) and CHEF (section 1.5.4) mechanisms with a family of coumarin enamine molecular probes.
3.1.1 Hypothesis and Rationale

Previously reported enaminone probes have not addressed all of the factors that affect metal ion coordination: substituents, solvent, and metal ion salt. This is especially true for coumarin-enaminone molecular probes. To address the lack of research a small library of coumarin-enaminone molecular probes were synthesized.

1. The synthesis of coumarin-enaminone probes will use the nucleophilic bidentate enaminone to coordinate a $d^{10}$ metal ion ($\text{Zn}^{2+}$, $\text{Cd}^{2+}$, $\text{Hg}^{2+}$).

2. The synthesis of a coumarin-enaminone probe can serve as an ion-pair sensor.

To address the first hypothesis a series of coumarin-enaminone probes were synthesized with the only variance being the absence/presence of a heteroatom in the aromatic ring, number of heteroatoms, and the location of the heteroatom(s). The family of probes (2.15, 2.16, and 3.6-3.8) examined the effects of the heteroatom in metal ion coordination. This is achieved by keeping the solvent (DMSO) and the metal salt consistent.

The second hypothesis is addressed by investigating different metal ion salts to determine if the ion-pair factors into the spectroscopic response.
3.2 Experimental Data

3.2.1 General Synthesis of Probes 2.15, 2.16, 3.6-3.8 and Their Metal Complexes

The synthetic procedure to isolate the probes 3.6, 3.7, and 3.8 (Scheme 64) is analogous to the one described in Chapter II (Scheme 48). The appropriate primary amine was added to a 2-propanol solution of compound 1.2 and triethyl orthoformate. The yellow-orange solid was isolated and fully characterized (section 3.4).64

Scheme 64. General synthesis for probes 3.6-3.8 as well as 2.15 and 2.16.

The structure of probes 3.6, 3.7, and 3.8 were confirmed by the appearance of the E/Z isomers of the N-H proton signal at ~13.50 and ~11.60 ppm respectively, see Table 16 for characterization. These chemical shifts are in agreement with previously reported N-H $^1$H NMR signals.198,209 The percent ratio of isomers present for each probe is also reported in Table 16. The $^{13}$C NMR shows the chemical shift at ~181.0 ppm assigned to the C(4)=O in a keto-enamine.209 Also provided in Table 16 are the experimental values of the molecular weight of the probes, which is in agreement with the predicted values (not shown). The $^1$H and $^{13}$C NMR spectra for these probes are discussed in depth in section 3.2.4. For full characterization of the molecular probes, see the experimental section, 3.4.
Figure 62. The numbering system used for probes 2.15, 2.16, and 3.6-3.8.

Table 16

*Selected* $^1$H and $^{13}$C NMR chemical shifts and mass of probes 3.6-3.8

<table>
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<tr>
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</table>

Note: The $E/Z$ columns refer to the $^1$H NMR of the keto-enamine tautomer for each probe. The NMR values are given in ppm. The NMR samples were carried out in CDCl$_3$. The reported mass is the most abundant signal on the mass spectrum for each probe. $E/Z$ percent ratio is calculated from the integration values (iv) of the N-H $^1$H NMR signal (Equation used: (iv $E$ ÷ (iv $E$ + iv $Z$)) × 100%).

The synthesis of the metal complexes (Scheme 65) was adapted from the procedure described by Hökelek et al. For the desired metal complex, the appropriate M(OAc)$_x$ salt (x = 2 or 3) was added to a boiling methanol solution and allowed to react for three hours. After which time the solid was collected by vacuum filtration as a pure metal complex. The synthesis in Scheme 65 shows only the 1:2 [M(probe)$_2$] complex since this is the most commonly reported binding ratio for the isolated complexes.
Scheme 65. General synthesis for the isolated metal complexes.

The identification of the complexes were confirmed by the disappearance of the N-H signal in the $^1$H NMR spectrum along with the presence of the imine C(9)H signal. The structure was also confirmed by the downfield shift (12 and ~10 ppm) of the $^{13}$C NMR C(9) and C(10) carbon signals respectively. Both $^1$H and $^{13}$C NMR support the coordination induces the formation of the imine. The appearance of a Zn-O stretch at 557 cm$^{-1}$ also supports the metal binding is occurring in the manner shown in Scheme 65. The mode of coordination is also supported by the solid state studies, which is discussed below in section 3.2.2. Furthermore the $^1$H and $^{13}$C NMR spectra for these probes will be discussed in further detail in section 3.2.4, full characterization is highlighted at the end of the chapter (section 3.4).

3.2.2 Solid State Studies of 2.15, 2.16, and 3.6-3.8 and Their Metal Complexes

3.2.2.1 X-Ray Crystallography. To confirm that the solid isolated from the synthesis of the probes are in agreement with the predicted structures crystals were grown for single crystal X-Ray analysis. The crystal structures of probe 2.15 and 3.6-3.8 are shown in Figure 63. Upon examining the four crystal structures below, it is observed that
each molecular probe is in the thermodynamically favorable $E$ isomer of the keto-enamine tautomer.

$\textbf{Figure 63.}$ X-Ray crystal structures of probes: 2.15, 3.6, 3.7, and 3.8.

For compound 2.15 the crystal parameter data is as follows: formula $C_{20}H_{20}N_2O_3$ (F.W. 336.38), monoclinic crystal system, $P2(1)/n$ space group. The compound 2.15 crystal is planar across the coumarin core. The planarity deviates slightly at the $\text{C(3)=C(9)}$ bond due to the hybridization (Figure 64). In the coumarin core all of the carbon atoms are $sp^2$ hybridized, while this holds true for the $\text{C(9)}$ carbon, the enamine nitrogen is trigonal pyramidal (ideally $107.3^\circ$ bond angles). This causes a deviation from planarity in the $\text{N-H}$ bond as well as the aniline ring from the coumarin core. Figure
64 shows the deviation of compound 2.15 in two ways: (left) edge view and (right) tail view.

Figure 64. The edge (left) and tail (right) view of the compound 2.15 crystal structure.
All bonds are black, all oxygen atoms are red, all nitrogen atoms are blue, and the hydrogen atoms have been omitted for clarity purposes.

The crystal structure for compound 3.6 is: C_{19}H_{19}N_{3}O_{3} (F.W. 337.37), monoclinic crystal system, C2/c space group. While the same hybridization exists in compound 3.6 (compared to compound 2.15) the crystal structure is planar across the coumarin core and the enamine, with the deviation arising at the pyridine ring (Figure 65). The pyridine nitrogen is deviating in a counterclockwise fashion in the figure below.

Figure 65. The tail view of the compound 3.6 crystal structure.
All bonds are black, all oxygen atoms are red, all nitrogen atoms are blue, and the hydrogen atoms have been omitted for clarity purposes.
For compound 3.7 the crystal parameter data is as follows: formula $\text{C}_{19}\text{H}_{19}\text{N}_{3}\text{O}_{3}$ (F.W. 337.37), orthorhombic crystal system, Pbca space group. Analogous to compound 3.6, the compound 3.7 crystal only shows a tail view of the deviation from planarity at the pyridine ring (Figure 66). The pyridine nitrogen is deviating in a clockwise fashion in the figure below.

**Figure 66.** The tail view of the deviation from planarity in the compound 3.7 crystal. All bonds are black, all oxygen atoms are red, all nitrogen atoms are blue, and the hydrogen atoms have been omitted for clarity purposes.

For compound 3.8 the crystal parameter data is as follows: formula $\text{C}_{18}\text{H}_{18}\text{N}_{4}\text{O}_{3}$ (F.W. 338.36), monoclinic crystal system, C2/c space group. As in the case with compound 3.6 and 3.7 the crystal of compound 3.8 has a slight deviation from planarity at the pyrimidine ring (Figure 67).

**Figure 67.** The tail view of the deviation from planarity in the compound 3.8 crystal. All bonds are black, all oxygen atoms are red, all nitrogen atoms are blue, and the hydrogen atoms have been omitted for clarity purposes.
In Table 17 the values of selected bond distances and angles from the crystal structures in Figure 63 are given. Using an average C=O bond distance of 1.23 Å, the C(4)=O values reported in Table 17 are in good agreement, which indicates that in the solid state the most thermodynamically stable tautomer is the keto-enamine. With the exception of compound 3.7 the N-H bond distances are shorter than the average N-H distance of 1.01 Å. The slightly longer 1.02 Å N-H bond distance and shorter intramolecular O•••H bond distance (1.78 Å) indicates that the N-H proton will be less shielded, compared to the other probes. This is supported by the further downfield shift of the E N-H signal at 13.71 ppm. For comparison purposes the previously discussed compound 2.16 crystal structure values are provided in Table 17.

For the bonds O=C(4)-C(3), C(4)-C(3)=C(9), and C(3)=C(9)-N expected 122° bond angle is observed. In the case of the C(9)-N-H bond angle, the expected 107.3° trigonal pyramidal bond angle is more obtuse for all five probes. This is due to the nitrogen atom of the enamine is connected to two trigonal planar carbon atoms, which will have a bond angle closer to 122°. In order to accommodate the 122° bond angle, the C(9)-N-H bond angle must deviate from the expected 107.3° bond angle.
Table 17

Selected bond distances and angles of probes 2.15, 2.16, and 3.6-3.8

<table>
<thead>
<tr>
<th>Bond Length</th>
<th>2.15</th>
<th>3.6</th>
<th>3.7</th>
<th>2.16</th>
<th>3.8</th>
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<tr>
<td>N-H⋯O</td>
<td>1.83</td>
<td>1.84</td>
<td>1.78</td>
<td>1.95</td>
<td>1.82</td>
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<tr>
<td>N-H</td>
<td>0.93</td>
<td>0.92</td>
<td>1.02</td>
<td>0.86</td>
<td>0.90</td>
</tr>
<tr>
<td>C(4)=O⋯N</td>
<td>2.62</td>
<td>2.61</td>
<td>2.66</td>
<td>2.64</td>
<td>2.62</td>
</tr>
<tr>
<td>C(4)=O</td>
<td>1.25</td>
<td>1.25</td>
<td>1.26</td>
<td>1.25</td>
<td>1.25</td>
</tr>
<tr>
<td>C(9)-N</td>
<td>1.32</td>
<td>1.32</td>
<td>1.30</td>
<td>1.33</td>
<td>1.32</td>
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<tr>
<td>C(3)=C(9)</td>
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<td>1.38</td>
<td>1.40</td>
<td>1.38</td>
<td>1.39</td>
</tr>
<tr>
<td>C(3)-C(4)</td>
<td>1.45</td>
<td>1.45</td>
<td>1.43</td>
<td>1.45</td>
<td>1.45</td>
</tr>
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<table>
<thead>
<tr>
<th>Bond Angles</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>O=C(4)-C(3)</td>
<td>122.2</td>
<td>122.4</td>
<td>122.7</td>
<td>122.2</td>
<td>122.7</td>
</tr>
<tr>
<td>C(4)-C(3)=C(9)</td>
<td>122.0</td>
<td>121.1</td>
<td>122.1</td>
<td>122.5</td>
<td>121.4</td>
</tr>
<tr>
<td>C(3)=C(9)-N</td>
<td>122.1</td>
<td>122.5</td>
<td>123.0</td>
<td>122.6</td>
<td>122.3</td>
</tr>
<tr>
<td>C(9)-N-H</td>
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<td>113.0</td>
<td>109.4</td>
<td>115.4</td>
<td>108.3</td>
</tr>
<tr>
<td>N-H⋯O</td>
<td>141.2</td>
<td>139.6</td>
<td>142.3</td>
<td>137.0</td>
<td>146.5</td>
</tr>
</tbody>
</table>

Note: The numbering system used in the table follows Figure 62. The bonds selected are bolded and are reported in Å (1Å = 10^{-10}m). The selected bond angles are reported in degrees (°).

Again to confirm that the solid isolated from the synthesis of the metal complexes are in agreement with the predicted structures, crystals were grown for single crystal X-Ray analysis. From literature the coordination of a metal ion to a keto-enamine will induce significant changes to bond distances and angles. This is due to the larger ionic radius of the metal ion, with respect to a hydrogen atom. In the model probe 2.15 the only
possible coordination will occur at the keto-enamine site and should be easily comparable to previously reported keto-enamine metal complexes. The probe 2.15 zinc complex crystal structure was solved (Figure 68). The crystal structure shows the zinc atom is in a distorted Td geometry between the enaminone of two 2.15 probes. The structure also shows that during the reaction to isolate the complex a deprotonation of the N-H occurred.

*Figure 68.* The X-Ray crystal structure of the isolated [Zn(2.15)2] complex.

For complex [Zn(2.15)2] the crystal structure parameters data is as follows:

- **formula** C_{40}H_{38}N_{4}O_{6}Zn (F.W. 736.13), monoclinic crystal system, P-1 space group. The coordination of the zinc atom resulted in an increased distance between C(4)O-N: free compound 2.15 2.62 Å, for the Zn(2.15)2 complex 2.15 #1 (2.93 Å) and 2.15 #2 (2.92 Å).

The reported O-Zn and N-Zn bond distances for [Zn(2.15)2] is shorter than similar enaminone-zinc complexes: O-Zn (2.0392\textsuperscript{211} and 1.999 Å\textsuperscript{78}) and N-Zn (2.2316 and 2.038 Å).\textsuperscript{78, 211} The shorter metal-heteroatom bond distance indicates that the 2.15-Zn interaction is stronger than the two similar enaminone-zinc complexes.
The coordination of the zinc atom also induces larger bond angles: C(3)-C(9)-N, C(3)-C(4)-O, and C(4)-O-Zn (comparable to C(4)-O-H). The increased N-O bond distance and the previously mentioned larger bond angles resulted in the O-Zn-N having a smaller bond angle (96.7°) than the N-H•••O (141.2°). In the general synthesis section it was mentioned that the \(^1\)H NMR spectrum of the complex showed a singlet ~ 9.0 ppm which was assigned to an imine C-H (Figure 87) in agreement with the crystal structure (Figure 68). In the crystal structure of compound \(2.15\) the C(9)-N-H bond angle is 111.8° while on the [Zn\((2.15)_2\)] the C(9)=N-Zn bond angle is 119.4/5°, which is closer to the expected 122°. A selected list of bond distances and angles for probe 2.15 and [Zn\((2.15)_2\)] is provided in Table 18.
Table 18

**Selected bond distances and angles of probe 2.15 and [Zn(2.15)₂].**

<table>
<thead>
<tr>
<th>Bond Distances</th>
<th>2.15</th>
<th>[Zn(2.15)₂] Complex</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>2.15 # 1</td>
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<tr>
<td>Bond Lengths</td>
<td>NA</td>
<td>1.92</td>
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<tr>
<td>O-Zn</td>
<td>NA</td>
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</tr>
<tr>
<td>N-Zn</td>
<td>NA</td>
<td>2.0</td>
</tr>
<tr>
<td>C(4)O•••N</td>
<td>2.62</td>
<td>2.93</td>
</tr>
<tr>
<td>C(4)O</td>
<td>1.25</td>
<td>1.28</td>
</tr>
<tr>
<td>C(9)N</td>
<td>1.32</td>
<td>1.31</td>
</tr>
<tr>
<td>C(3)(C(9)</td>
<td>1.38</td>
<td>1.41</td>
</tr>
<tr>
<td>C(3)-C(4)</td>
<td>1.45</td>
<td>1.42</td>
</tr>
<tr>
<td>Bond Angles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OC(4)C(3)</td>
<td>122.2</td>
<td>124.1</td>
</tr>
<tr>
<td>C(4)C(3)(C(9)</td>
<td>122.0</td>
<td>126.2</td>
</tr>
<tr>
<td>C(3)(C(9)N</td>
<td>122.1</td>
<td>127.6</td>
</tr>
<tr>
<td>C(9)-N-H /C(9)NZn</td>
<td>111.8</td>
<td>119.4</td>
</tr>
<tr>
<td>N-H•••O /NZnO</td>
<td>141.2</td>
<td>96.7</td>
</tr>
<tr>
<td>C(4)=O•••H / C(4)OZn</td>
<td>99.8</td>
<td>125.6</td>
</tr>
</tbody>
</table>

Note: The numbering system used in the table follows Figure 62. The bonds selected are bolded and are reported in Å (1 Å = 10⁻¹⁰ m). The angles are reported in degrees (°).

In the attempt to grow crystals of the [Zn(3.8)₂] complex, which required several months, hydrolysis occurred (Figure 69). The Zn²⁺ ions coordinate to probe 3.8 in a 1:2 (M:L) ratio. The molecular geometry around the zinc is a near perfect octahedral geometry. Two water molecules are bound in the axial positions and the two ligands are occupying the equatorial positions. To determine if the hydrolysis is in fact due to the crystal growing conditions or the reaction conditions NMR experiments were investigated, see section 3.2.4.
Figure 69. The zinc hydrolyzed 3.8 complex in an O_h geometry.

Grown by slow evaporation in DMSO.

3.2.2.2 Infrared (IR) Spectroscopy. To further investigate the structure of the complexes in the solid state, a series of IR experiments were performed. Since the [Zn(2.15)_2] crystal contains the aniline still coordinated, the first set of IR experiments focused on the [Zn(2.15)_2] complex. The IR spectra of the probe 2.15 and [Zn(2.15)_2] complex is provided in Figure 70. The most significant observance from the full IR spectra is the disappearance, in the complex, of the enamine N-H at 3239 cm\(^{-1}\). The loss of the N-H stretching signal is in agreement with the crystal structure (Figure 68) and the \(^1\)H NMR spectrum (Figure 87). No other significant changes were observed in the two IR spectra between 3,500-1,900 cm\(^{-1}\).
Figure 70. The IR spectra of the probe 2.15 and the [Zn(2.15)₂].

Since there is significant changes to the molecular probe upon coordination an expanded $sp^2$ and fingerprint region spectrum is provided in Figure 71. The most significant piece of information shown in Figure 71 is the new IR signal at 557 cm⁻¹, which is assigned to the Zn-O bond. This confirms that the solid isolated from the reaction (Scheme 65) is in fact the [Zn(2.15)₂] complex. The coordination of the Zn²⁺ ions also resulted in a 15 cm⁻¹ of the $\delta$ lactone and a new C(9)=N stretch at 1601 cm⁻¹.
Figure 71. The IR spectra (1900-500 cm\(^{-1}\)) of 2.15 and \([\text{Zn}(2.15)_2]\) complex.

Next the IR spectrum of the \([\text{Zn}(3.8)_2]\) complex was investigated (Figure 72). The enamine N-H appears to be absent from the complex IR spectrum indicating that the nitrogen atom is participating in the coordination. The structure of probe 3.8 as well as plausible structures of the complex are also provided in Figure 72 to aid in the interpretation of the \([\text{Zn}(3.8)_2]\) complex IR spectrum.
Figure 72. The IR spectra of 3.8 and \([\text{Zn}(3.8)_2]\) complex.

Using the wavenumbers found in the \([\text{Zn}(2.15)_2]\) complex, the assignments of the IR signals for the \([\text{Zn}(3.8)_2]\) complex were made (Figure 73). The Zn-O stretch appeared at 478 cm\(^{-1}\). The coordination of the Zn\(^{2+}\) ions resulted in a 7 cm\(^{-1}\) shift of the \(\delta_{\text{lactone}}\), imine C=N stretches at 1614 and 1599 cm\(^{-1}\), and C=C stretches at 1581, 1566, and 1535 cm\(^{-1}\).
Figure 73. The IR spectra (1825-450 cm\(^{-1}\)) of 3.8 and [Zn(3.8)\(_2\)] complex.

In the [Zn(2.16)\(_2\)] complex full IR spectrum shown in (Figure 74) the enamine C-H is still present at 3060 cm\(^{-1}\). This suggests that the coordination is occurring at the pyridine nitrogen. The large shift of the \(\delta_{\text{lactone}}\) (29 cm\(^{-1}\)) along with a new C-O stretch at 1211 cm\(^{-1}\) and Zn-O bond stretching at 478 cm\(^{-1}\) the IR spectrum indicates that coordination is solely through two pyridine nitrogen atoms is highly improbable due to the new C-O and Zn-O IR signals; however, this does not completely rule out that one pyridine nitrogen could be participating in coordination.
Figure 74. The IR spectra of probe 2.16 and the [Zn(2.16)₂] complex.

(Top) the full IR spectra of probe 2.16 and the [Zn(2.16)₂] complex. (Bottom) the IR spectra (1750-450 cm⁻¹) of probe 2.16 and the Zn(2.16)₂ complex.

The IR spectrum of the [Zn(2.16)] complex (Figure 75) shows the disappearance of the C(9)-H stretch at 3060 cm⁻¹. Investigating the 1750-450 cm⁻¹ range of the IR
spectra, it was observed that no further shift of the $\delta_{\text{lactone}}$ occurred as well as no new C-O or Zn-O bond signals.

*Figure 75.* The IR spectra of probe 2.16 and the [Zn(2.16)] complex. (Top) the full IR spectra of probe 2.16 and the Zn(2.16) complex. (Bottom) the IR spectra (1750-450 cm$^{-1}$) of probe 2.16 and the Zn(2.16) complex.
The results of the solid state experiments shown in this section were extremely beneficial. The crystallographic data of the probes 2.15 and 3.6-3.8 confirmed their structures. The $T_d$ geometry of the $[\text{Zn(2.15)}_2]$ complex is in good agreement with other Zn$^{2+}$ ion coordination compounds. The $[\text{Zn(2.15)}_2]$ complex crystal structure supported the IR studies. The IR spectrum of $[\text{Zn(2.15)}_2]$ complex was used as a model and aided in interpreting the IR spectra for the $[\text{Zn(3.8)}_2]$, $[\text{Zn(2.16)}_2]$, and $[\text{Zn(2.16)}]$. 

To analyze the zinc complexes, the investigation moved from solid state techniques to solution based techniques. In the next section the mass spectrometry and NMR spectroscopy experiments further confirm the complex structures; however, the rationale for the rest of this chapter needs to be discussed.

3.2.3 Rationale Used in the Solution Based Experiments

The studies discussed in section 2.2.4 showed that the spectroscopic response is dependent on the solvochromatic nature of the solution. Most of the studies were carried out in DMSO, but the optical properties were first investigated in EtOH. Since polar protic solvents like EtOH could potentially take part in hydrogen bonding with the molecular probe, which would interfere with the optical response from the probe, it was eliminated as the chosen solvent. Another possible candidate for the solvent is MeCN. Acetonitrile, as an aprotic solvent, avoids forming hydrogen bonds with the molecular probe; however, preliminary colorimetric results show that the responses observed in MeCN are not as large as seen in DMSO. The absorbance spectrum of probe 3.8 was recorded DMSO (black), EtOH (blue), and MeCN (red) with and without one equivalent of Zn(OAc)$_2$ (Figure 76).
The results discussed in Chapter II can be used to explain why the largest optical response is observed in DMSO. When the metal acetate salt is dissolved in DMSO, the dissociated acetate forms a solvation shell, and with DMSO being an aprotic solvent the basicity of acetate increases (Table 12, Scheme 66). Once the metal solution is added to the probe solution, the more basic acetate will deprotonate the enaminone, which will then result in metal ion coordination. In the sections to come it will be clear that the choice of metal salt used (i.e. anion) plays a crucial role in the spectroscopic response observed.

To further investigate the changes to the spectroscopic response produced by the choice of solvent, a titration with Cd(OAc)$_2$ and probe 3.8 was carried out in two different solvents (Figure 77). Both titrations (one in DMSO and one in MeOH) along with their respective isotherms are presented side-by-side for easy direct comparison. The
excitation wavelength of 339 nm was chosen to be consistent with Chapter II. In the
titration carried out in DMSO the excitation at 339 nm resulted in an emission spectrum
of solely the keto-enamine. Upon the addition of Cd$^{2+}$ ions the ESIPT process is inhibited
and a fluorescence emission intensity increase along with a 52 nm blue shift is observed
($K_{11} = 2.8 \times 10^4$ M$^{-1}$). When the same titration was carried out in MeOH, the emission
spectrum of probe 3.8 displayed a fluorescence signal for both the imine-enol (373 nm)
and the keto-enamine (559 nm). Both of the tautomer emission bands increased as Cd$^{2+}$
ions were added. From the MeOH titration data a binding constant was calculated to be
$K_{11} = 3.8 \times 10^3$ M$^{-1}$. 
Figure 77. The Cd(OAc)\(_2\) fluorescence titration and binding isotherm with probe 3.8.

\(\lambda_{\text{ex}} 339\) nm \(3.8\) = \(1.56 \times 10^{-5}\) M \(\text{[Cd(OAc)\(_2\)]}\) = \(3.11 \times 10^{-4}\) M. (Left) Titration was carried out in DMSO. (Right) Titration was carried out in MeOH (equivalences used: 0.0-2.0 by increments of 0.1, 2.5, 3.0, 4.0, and 5.0).

Since the ESIPT process results in the emission of the keto-enamine (confirmed in Chapter II), the investigation turned to exciting the molecular probes at the keto-enamine absorbance maximum (408 nm). In Figure 78 shows the spectroscopic response of probe 3.8 with Zn(OAc)\(_2\) in MeCN and Cd(OAc)\(_2\) in MeOH. The titrations in DMSO are shown in Figures 168 and 169 respectively. Probe 3.8 in MeCN has a fluorescence emission intensity maximum at 557 nm, which was shifted 54 nm in the blue direction along with an intensity increase in the presence of Zn\(^{2+}\) ions \(K_{11} = 1.7 \times 10^5\) M\(^{-1}\). The maximum fluorescence emission band in MeOH for probe 3.8 is seen at 563 nm \(\lambda_{\text{ex}} 408\) nm. This
fluorescence signal undergoes a 49 nm blue shift and an emission intensity increase with the addition of Cd$^{2+}$ ions ($K_{11} = 6.3 \times 10^3$ M$^{-1}$).

Figure 78. Zn$^{2+}$ and Cd$^{2+}$ acetate emission titrations and isotherms with probe 3.8.

While the trend in the spectroscopic response (blue shift accompanied by an intensity increase) from exciting at 339 and 408 nm is nearly identical, 408 nm was used as the excitation wavelength in this chapter. This is based on all five probes discussed have a maximum absorbance, due to the keto-enamine, at 408 nm. Also the excitation at this wavelength (408 nm) will ensure that more electrons will be promoted into the S$_1$ state, when compared to exciting at 339 nm.
Since the acetate ion significantly enhances spectroscopic response towards the various metal ions, the response of the anion itself was investigated. Probe 3.8 was used to investigate the spectroscopic changes of TBAOAc (Figure 79) (DMSO, $\lambda_{ex}$ 408 nm). When OAc$^-$ ions are added to probe 3.8 the fluorescence emission band is blue shifted and the intensities at 490 and 557 nm are increased. The emission intensity increase at 490 nm is relatively similar to the addition of a metal acetate salt, but the observed increase is not as significant as shown in Figures 77 and 78. However, the response from TBAOAc indicates that initially the OAc$^-$ ion is forming a hydrogen bond interaction between the keto-enamine, partially inhibiting the ESIPT process due to the equilibrium. When the acetate ion is in the presence of a suitable metal ion, the inhibition is increased due to the C(4)=O-metal ion interaction weakening the intramolecular hydrogen bond between the ketone and the enamine. Once the ketone is no longer participating in the tautomerization the acetate ion deprotonates the N-H, which induces the imine formation and ultimately a new covalent metal-oxygen bond (Schemes 67 and 68). When this new covalent bond is formed, the ESIPT process is fully inhibited and a large blue shift and emission intensity increase is observed ($K_{11} = 1.6 \times 10^5 \text{ M}^{-1}$).
Figure 79. TBAOAc fluorescence titration and binding isotherm with probe 3.8.

Titration was carried out in DMSO (λex 408 nm) [3.8] = 1.56 × 10⁻⁵ M [TBAOAc] = 3.11 × 10⁻⁴ M (equivalences used: 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0, 4.0, and 5.0).

To further confirm this process is correct two equivalents of TBAOAc was added to a solution of probe 3.6 then a full ZnCl₂ titration was investigated in DMSO (λex 408 nm) (Figure 80). The initial ZnCl₂ titration is shown in Figure 118. Analogous to the
probe 3.6 Zn(OAc)$_2$ titration (Figure 154) the addition of acetate ions followed by the subsequent coordination of ZnCl$_2$ resulted in the inhibition of ESIPT. ZnCl$_2$ produced a 64 nm blue shift along with an increase in the emission intensity ($\lambda_{em}$ 487 nm). The blue shift is nearly identical to the one induced by Zn(OAc)$_2$, 63 nm ($K_{11} = 1.4 \times 10^5$ M$^{-1}$).

![Figure 80. ZnCl$_2$ fluorescence titration and binding isotherm with TBAOAc and probe 3.6.](image)

Titrations were carried out in DMSO ($\lambda_{ex}$ 408 nm) with two equivalents of TBAOAc. [3.6] = 1.56 $\times$ 10$^{-5}$ M [ZnCl$_2$] = 3.11 $\times$ 10$^{-4}$ M [TBAOAc] = 3.11 $\times$ 10$^{-3}$ M (equivalences of ZnCl$_2$ used: 0.0-2.0 by increments of 0.1, 2.5, 3.0, 4.0, and 5.0)
The spectroscopic response shown in Figure 79 will be used in analyzing the optical signal changes observed in the sections below. From Figure 80 it is indicated that the metal acetate responses can be mimicked with the use of TBAOAc and another metal salt.

3.2.4 NMR Studies of 2.15, 3.6, 3.7, 2.16, and 3.8

While the solid state shows the probes in the $E$ isomer of the keto-enamine tautomer, the NMR experiments discussed in Chapter II clearly shows that when the probes are in solution they can exist in both the $E/Z$ isomers of the keto-enamine tautomer. The $^1$H and $^{13}$C NMR spectra of the free probes will be discussed followed by NMR spectroscopy experiments on the metal complexes and lastly $^1$H NMR titrations. The assignments of the chemical shifts shown below are agreement with previously reported values. Since the ethyl substituents are far away from the binding site, no changes to the chemical shifts were observed for the CH$_2$ and CH$_3$ groups in the NMR spectrum, therefore they will not be discussed any further. For simplicity the assignments of these chemical shifts are left out intentionally: for $^1$H NMR N-CH$_2$ appears at roughly 3.4 ppm while the N-CH$_2$-CH$_3$ appears at roughly 1.1 ppm, for $^{13}$C NMR ~ 44 and ~12 ppm respectively.

In regards to compound 3.6 it is expected that the $^1$H NMR spectrum should contain two signals one for each the $E/Z$ isomer for the following protons N-H (13.57 and 11.71 ppm), C(9)H (9.60 and 9.51 ppm), and C(5)H (7.95 and 7.85 ppm), which is observed in (Figure 81). From the $^1$H NMR spectrum it is apparent that one isomer is more prevalent than the other in solution ($E/Z$ isomer ratio 9:1). Based upon the data discussed in Chapter II and reported values in literature the $E$ isomer of the keto-enamine
is the most predominant. As such, taking the integration value for the enamine N-H $E$ and $Z$ isomer a percentage in solution ratio can be calculated, which resulted in 9 to 1 respectively. Assignments of the pyridine protons for the $E/Z$ isomers lie on top of each other and were difficult to assign, except in the case of C(11)H (7.10 and 7.04 ppm respectively). Since the C(11)H proton is the closest to the enaminone, isomerization produces two distinguishable doublets.

![Chemical structure](image)

**Figure 81.** $^1$H NMR spectrum of compound 3.6 in CDCl$_3$ (*) is the solvent peak.

The $E/Z$ isomerization of the keto-enamine tautomer of compound 3.6 is also observed in the $^{13}$C NMR spectrum (Figure 82). Analogous to the $^1$H NMR the predominant isomer is the $E$ isomer. In Figure 3.21 the C(9) carbon signal is shifted further downfield upon isomerization to the $Z$ isomer, 152.8 to 153.0 ppm ($E$ to $Z$). Downfield shifts are also observed for the C(2) 164.2 to 165.9 ppm, C(3) 99.3 to 99.6 ppm, and C(5) 127.5 to 128.3 ppm, $E$ to $Z$ respectively.
Figure 82. $^{13}$C NMR NMR spectrum of compound 3.6 in CDCl$_3$.

The $^1$H NMR spectrum of compound 3.7 (Figure 83) again shows the $E/Z$ isomers of the keto-enamine in solution. Again the $E$ isomer is the more predominant isomer in solution with an $E/Z$ percentage ratio in solution, based on the integration values of the N-H, of 9 to 1 respectively.
Figure 83. $^1$H NMR spectrum of compound 3.7 in CDCl$_3$ (*) is the solvent peak.

The $^{13}$C NMR spectrum of compound 3.7 shows only one significant distinguishable carbon signal for both the E and Z isomers, assigned to the C(5) (Figure 84). The isomerization to the Z isomer induces a downfield shift of the C(5) signal from 127.5 to 128.2 ppm. The other Z isomer signals were not strong enough to be seen in the $^{13}$C NMR spectrum. The top right insert is included for the assignments of the C(6) and C(7) carbon signals.
The same trend is seen for compound 3.8. The $^1$H NMR spectrum (Figure 85) shows $E$ and $Z$ isomer proton signals for the enamine N-H, C(9)H, and the C(5)H. Due to the two nitrogen atoms in the pyrimidine ring, the isomerization does not produce any extra splitting of the C(11/11’)H and C(12)H proton signals. Using the integration value of both $E$ and $Z$ N-H isomers, a percentage ratio in solution was calculated at 93% to 7%, $E$ to $Z$ respectively.
Figure 85. $^1$H NMR spectrum of compound 3.8 in CDCl$_3$ (* is the solvent peak.

Similar to the $^{13}$C NMR spectrum of compound 3.7, the spectrum for compound 3.8 (Figure 86) has no observable carbon signals for the Z isomer. Unique to compound 3.8, the C(10) carbon signal is further down field than the C(9) signal. This is most likely due to the two nitrogen atoms in both ortho positions to the C(10) carbon.
Figure 86. $^{13}$C NMR spectrum of compound 3.8 in CDCl$_3$.

Using the $^1$H and $^{13}$C NMR assignments of the free probes the NMR spectra of the [Zn(2.15)$_2$] and the [Zn(3.8)$_2$] complexes were less challenging to interpret as only one isomer is formed. The [Zn(2.15)$_2$] crystal structure in (Figure 68) shows the coordination is 2:1 (L:M) ratio with zinc in a T$_d$ geometry. As stated before the coordination of zinc results in the formation of a C(9)=N imine bond. For ease of comparison (Figure 87) contains the $^1$H NMR spectra of compound 2.15 and the [Zn(2.15)$_2$] complex. The coordination of the Zn$^{2+}$ ion results in the disappearance of the N-H signal as well as the downfield shift of the C(9)H signal from 8.82 to 9.04 ppm. This is in agreement with the crystal structure (Figure 68) and the IR spectrum (Figures 70 and 71). The chemical shifts in the complex spectrum marked with (!) show that some of the free compound 2.15 in solution. This is either due to residual 2.15 left over from the
complex synthesis or the small amount of water in the CDCl$_3$ solvent resulted in the displacement of one of the coordinated 2.15 probes.

![Image](image_url)

**Figure 87.** $^1$H NMR spectra of probe 2.15 and the isolated [Zn(2.15)$_2$] complex. (Bottom) probe 2.15 (Top) the complex. The NMR experiments were carried out in CDCl$_3$. (*) is the solvent peak. (!) is residual probe 2.15 in solution.

The $^1$H NMR spectrum shows few changes to the proton chemical shifts of the free 2.15 ligand with the exception of the N-H proton; however, the coordination of the Zn$^{2+}$ ion induced a significant change to the $^{13}$C NMR spectrum. For ease of comparison (Figure 88) contains the $^{13}$C NMR spectra of compound 2.15 and the [Zn(2.15)$_2$] complex. The $sp^2$ hybridization remains unchanged for the C(9) carbon in the compound 2.15 upon coordination, but the change in the electronegativity of the atoms that the C(9) carbon is double bonded to induced a downfield shift from 153.5 ppm (C(9)$_{enamine}$) to 165.7 ppm (C(9)$_{imine}$). This shift is increased as the imine nitrogen is donating electron density.
to aid in the stability of the coordinated Zn$^{2+}$ ion. While the connectivity to the C(10) carbon remained unchanged upon coordination, the imine nitrogen atom involved in coordination results in a significant downfield shift of the C(10) signal from 138.2 to 149.0 ppm. The formation of the new covalent Zn-O bond in the complex results in less electron density being pulled from the C(4) carbon, which induced an upfield shift in the signal from 181.0 to 178.3 ppm. The upfield shift is also due to the new C(4)=C(3) double bond.

*Figure 88.* $^{13}$C NMR spectra of probe 2.15 and the isolated [Zn(2.15)$_2$] complex.

(Bottom) probe 2.15 (Top) the complex. The NMR experiments were carried out in CDCl$_3$. (*) is the solvent peak. (!) is residual probe 2.15 in solution.

In order to confidently assign the $^{13}$C NMR chemical shifts shown in Figure 88, a $^{13}$C NMR APT experiment was carried out on both compound 2.15 and the complex (Figure 89). Observed in the $^{13}$C NMR spectra shown in Figure 88, the C(7) and C(6)
carbon signals are very close to each other. The $^{13}$C NMR APT experiment allowed for a method to distinguish the two signals. As the C(7) carbon is a quaternary carbon and therefore a negative signal is seen whereas the C(6) carbon has a proton attached to it and therefore a positive signal is expected (Figure 89). With only small changes to the chemical shift of C(4a), C(5), C(8a), C(11/11’), C(12/12’), and C(13) the assignment of these signals were based on the $^{13}$C NMR of compound 2.15 in Figure 11. The $^{13}$C APT spectra shown below in Figure 89 was used to determine where the C(2), C(9), and C(10) carbon signals shifted to upon coordination. As previously stated the C(9) and C(10) carbon signals were expected to shift downfield upon coordination. Since C(9) has a proton attached, the new positive signal at 165.7 ppm was assigned to C(9). With the odds that the C(2) carbon signal would shift upfield from 164.5 to 149.0 ppm being extremely low and highly improbable the new negative signal at 149.0 ppm in the complex was assigned to C(10). This leaves the new negative signal at 165.8 ppm due to C(2).
As seen in the [Zn(2.15)₂] complex ¹H NMR spectrum (Figure 87) the isolated [Zn(3.8)₂] complex ¹H NMR spectrum (Figure 90) has no enamine N-H signals. This indicates that again the coordination resulted in the formation of a C(9)=N imine bond. By comparison, the 3.8 complex imine C(9) signal is significantly further downfield than the 2.15 complex. This again indicates that the coordination of the Zn²⁺ ion results in the deprotonation of the N-H, which was observed in Figure 87. With compound 2.15 the imine formation and coordination resulted in a very small downfield shift of the C(9)H signal; however, in the case of compound 3.8 the imine C(9)H signal is significantly shifted downfield.
Figure 90. $^1$H NMR spectra of probe 3.8 and the isolated [Zn(3.8)$_2$] complex.

(Bottom) probe 3.8 (Top) the complex. The NMR experiments were carried out in CDCl$_3$. (*) is the solvent peak.

The stacked $^{13}$C NMR spectrum of 3.8 and the [Zn(3.8)$_2$] complex located in (Figure 91) shows that upon coordination the carbon signals for C(4), C(12), and C(3) all experience an upfield shift.
Figure 91. $^{13}$C NMR spectra of probe 3.8 and the isolated [Zn(3.8)$_2$] complex.

(Bottom) probe 3.8 (Top) the complex. The NMR experiments were carried out in CDCl$_3$. (*) is the solvent peak.

To confirm the $^{13}$C NMR signal assignments, a $^{13}$C NMR APT experiment was performed on both compound 3.8 and the complex (Figure 92). The negative signal for C(7) allowed for distinguishing from the C(6) signal. With only a small upfield shift observed for the C(11/11’)H signal in Figure 90 it was anticipated that the C(11/11’) carbon signal would also experience a slight upfield shift. This aided in assigning the new positive signal in the complex at 164.9 ppm is due to the C(9) carbon. For the carbon signals attributed to C(4a), C(8a), and C(2) only small upfield shifts were observed. The remaining negative signal in the complex spectrum, at 165.8 ppm, is assigned to C(10).
Figure 92. $^{13}$C NMR APT spectra of probe 3.8 and the isolated [Zn(3.8)$_2$] complex. (Bottom) probe 3.8 (Top) the complex. The NMR experiments were carried out in CDCl$_3$. (*) is the solvent peak.

Since compound 3.8 possess two nitrogen atoms in the ortho positions to the enamine nitrogen, any participation of one of the pyrimidine nitrogen atoms would result in a splitting of the pyrimidine proton signals: C(11/11’)H and C(12)H. This is not observed in Figure 90 and suggests that the pyrimidine nitrogen atoms are not involved in coordination.

In order to confirm where the coordination of a metal ion would occur, a model NMR experiment was carried out. The $^1$H NMR titration with Zn(OAc)$_2$ and probe 2.16 was investigated (Figure 93). The choice of the metal acetate salt is based upon the fact that upon dissolving the salt (Scheme 66) free OAc$^-$ ions will be in solution allowing for similar deprotonation that is observed in Figure 30.
Scheme 66. Dissociation of Zn(OAc)$_2$ in DMSO.

In Scheme 67 the coordination of the Zn$^{2+}$ ion by probe 2.16 is shown. When the Zn(OAc)$_2$ solution is added to the probe 2.16 sample the acetate anion will deprotonate the enamine N-H, resulting in a negative charge on the enamine nitrogen. The extra lone pair of electrons on the enamine nitrogen will then resonate to form an imine, a C(3)=C(4), and ultimately result in the formation of the C(4) oxide. This resonance structure, with the negative charge on the C(4) oxygen atom, is more stable than the former due to the increased electronegativity of the oxygen atom over nitrogen atom, 3.5 and 3.0 respectively.\(^{53}\) Once the stable resonance structure is formed then the C(4) oxide forms a new Zn-O covalent bond, which is stabilized by the dative bond between Zn-N=N=C(9) as shown in Scheme 67. A possible alternative coordination route is provided at the bottom of Scheme 67.

Scheme 67. The mechanism and coordination of Zn$^{2+}$ ions by probe 2.16.
The initial addition of Zn(OAc)$_2$ to probe 2.16 in DMSO-$d_6$ resulted in a gradual disappearance of the N-H and C(9)H (Figure 93). This indicates that the first step in coordination of the Zn$^{2+}$ ion involves the deprotonation of the enamine N-H. Along with the observed deprotonation the presence of the Zn$^{2+}$ ion induced a broadening and upfield shift of the signals produced by the C(11/11’) and C(12/12’) protons. As the titration is continued and more of the Zn$^{2+}$ ion is present in the sample, the signal produced by the C(12/12’) protons became sharp again and remained slightly shifted upfield, 8.57 to 8.52 ppm (Figure 92). The C(11/11’) signal initially at 7.61 ppm us shifted to 7.29 ppm with the addition of the Zn$^{2+}$ ion. As higher equivalents were added the new broad signal at 7.29 ppm became sharp again and slowly shifted back downfield to 7.44 ppm (Figure 93). The new signal at 8.65 ppm is attributed to the newly formed imine proton, C(9)H. The results of this titration indicates that the coordination of Zn(OAc)$_2$ occurs though the deprotonation of the enamine N-H followed by the formation of the imine and the negatively charged oxide on C(4). This leads to the coordination of the Zn$^{2+}$ ion through the oxide and a dative bond between the imine nitrogen and the Zn$^{2+}$ ion, as depicted in Scheme 67.
Figure 93. The Zn(OAc)$_2$ $^1$H NMR titration with probe 2.16 with in DMSO-$_d_6$.

Equivalents used: 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 2.0, 2.5, and 5.0.
After the $^1$H NMR titration with probe 2.16, a Zn(OAc)$_2$ $^1$H NMR titration was investigated with probe 3.8. A similar mode of coordination between 3.8 and the Zn$^{2+}$ ion is provided in Scheme 68. The reasoning discussed for Scheme 66 and 67 apply to Scheme 68. The major difference between Scheme 67 and 68 is that 3.8 has a nitrogen atom in the pyrimidine ring that can possibly stabilize the coordination of the Zn$^{2+}$ ion by forming another Zn-N dative bond.

Scheme 68. The mechanism and coordination of Zn$^{2+}$ ions by probe 3.8.

The addition of Zn(OAc)$_2$ to probe 3.8 resulted in a disappearance of the N-H and the C(9)H signals (Figure 3.33). As the E/Z C(9)H chemical shifts at 9.17 and 9.32 ppm, respectively, disappeared there are two new signals that appeared at 9.97 and 9.78 ppm, which is assigned to the imine C(9)H proton. Since the pyrimidine has two nitrogen atoms that can participate in the coordination of the Zn$^{2+}$ ions it is observed that until one equivalence of Zn(OAc)$_2$ added there are two distinct C(11/11’)H proton signals (Figure 3.33). As compound 3.8 coordinates at one binding site a nitrogen atom in the pyrimidine ring is closer to the coordination, while the other is not resulting in two different C(11)H and C(11’)H signals (Scheme 68). After the addition of one equivalents the Zn$^{2+}$ ion
coordinates to the second binding site whereby causing both C(11) and C(11’) to become identical with the same chemical shift value of 8.82 ppm.
Figure 94. The Zn(OAc)$_2$ $^1$H NMR titration with probe 3.8 in DMSO-$d_6$.

Equivalents used: 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 2.0, 2.5, and 5.0.
The $^1$H and $^{13}$C NMR spectra of compounds 3.6-3.8 shown in Figures 81–86 are in agreement with their respective crystal structures (Figure 63). The IR spectra (Figures 70–73) and NMR spectra (Figures 87–92) of both the [Zn(2.15)$_2$] and [Zn(3.8)$_2$] complex are in agreement with each other, and these results support that the isolated [Zn(3.8)$_2$] complex is the structure shown in Figure 90.

Full Zn(OAc)$_2$ $^1$H NMR titrations were carried out with probes 2.16 (Figure 93) and 3.8 (Figure 94). Both experiments show that the initial addition of Zn$^{2+}$ ions produces different species in solution, but in the presence of one equivalent of the metal ion, the various speciation decreases and the most stable coordination became the predominant chemical shifts.

3.2.5 Mass Spectrometry and Binding Ratios

With the masses of the compounds 3.6-3.8 previously reported (Table 16), mass spectrometry was used to investigate the coordination complex. The [Zn(3.8)$_2$] complex was studied to determine if the solid is the predicted complex or the hydrolyzed complex (Figure 69). While determining what complex was isolated the mass spectrum would provide an insight to the specific coordination ratio. A MeOH-DMSO solvent system was used due to solubility. Initially the sample was analyzed without a charging agent to determine if the isolated complex is a salt. This resulted in no signal in both the positive and negative mode, and with no signals being observed a charging agent (NaCl) was used. The [Zn(3.8)$_2$] complex with NaCl produced no signal in the positive mode; however, a signal was observed in the negative mode (Figure 95). The bottom spectrum in (Figure 95) is the theoretical isotopic distribution mass spectrum.
Figure 95. The negative mode mass spectrum of the isolated \([\text{Zn}(3.8)_2]\) complex.

Experiment carried out in a MeOH-DMSO solvent system. NaCl was used as a charging agent. (Bottom) Theoretical isotopic distribution mass spectrum \((\text{[Zn}(3.8)_2]^{-})\).

Once the conditions were established, a collision-induced dissociation (CID) mass spectrometry experiment (MS-MS) was investigated (Figure 96). Selecting 473 \(m/z\) as the parent ion to be analyzed. The fragmentation of this signal shows the predominant daughter signal is the mass of the free \textbf{3.8} (338 \(m/z\)). The second most abundant signal
appears at 472 m/z, the loss of a proton. With relatively low abundance the last significant daughter signal to mention appears at 436 m/z, which is the loss of a chloride.

Figure 96. The negative mode MS-MS of the isolated [Zn(3.8)2] complex.

MS-MS on 472 m/z. Experiment carried out in a MeOH-DMSO solvent system. NaCl was used as a charging agent.

Based on the mass spectrum of the isolated complex shown in (Figure 95) and the results of the MS-MS experiment in (Figure 96) a plausible structure for the 473 m/z signal is provided in Figure 97.

Figure 97. A plausible structure for the 473 m/z signal.
Since the low resolution mass spectrometry showed only a 1:1 ratio complex, the [Zn(3.8)₂] complex was sent off for high resolution mass spectrometry. The results of the study showed the predominant parent peak at 738.19 m/z, which is the predicted mass of the [Zn(3.8)₂] complex. The complex was further investigated via hydrogen, carbon, and nitrogen elemental analysis. The theoretical values were calculated to be C (58.42), H (4.63), and N (15.14), while the actual values were found to be C (57.48), H (4.60), and N (14.96).

The difference in the binding ratio between the various salts, solvents, and excitation wavelengths led to the investigation of a series of Job’s plots. In Figure 98 the binding ratio between probe 3.6 and Zn(OAc)₂ was determined to be 1:2 (L:M), in DMSO (λ<sub>ex</sub> 408 nm). Under the same conditions the same ratio was determined for the binding ratio between probe 3.7 and Zn(OAc)₂ (Figure 99). Hg(OAc)₂ resulted in a 1:1 binding ratio with probe 3.7 (Figure 100). This is most likely structure due to the ionic radius of Hg<sup>2+</sup> preventing a 1:2 (L:M) as seen in Figures 98 and 99. Possible metal ion coordination is provided with their respective Job’s plot.
Figure 98. Job’s plot with probe 3.6 and Zn(OAc)$_2$ and possible coordination.
Figure 99. Job’s plot between probe 3.7 and Zn(OAc)$_2$ and possible coordination.
Figure 100. Job’s plot between probe 3.7 and Hg(OAc)$_2$ and possible coordination.

Upon investigating the binding ratio between probe 3.8 and Zn(OAc)$_2$ the
concentration used in the two Job’s plots shown above resulted in a maximum at 0.8 $\chi$ of
Zn(OAc)$_2$. In order to overcome this problem, the concentration was reduced by a factor
of 10. This resulted in a maximum at 0.5, which indicates a 1:1 binding ratio between
probe 3.8 and Zn(OAc)$_2$ (Figure 101).
Figure 101. Job’s plot between probe 3.8 and Zn(OAc)$_2$ and possible coordination.

In the case of probe 2.16 and Zn(OAc)$_2$ the maximum was centered at a Zn(OAc)$_2$ \( \chi \) of 0.66 (Figure 102). This is indicative of a 1:2 (L:M) binding ratio. This falls in line with the Zn(OAc)$_2$ Job’s plot with probes 3.6 and 3.7.
Next the binding ratio was investigated for the interaction between probe 2.16 and Zn(ClO$_4$)$_2$, Figure 3.42 (top, DMSO, $\lambda_{ex}$ 339 nm). The upside down parabola with a maximum centered at 0.5 indicates the interaction occurs at a 1:1 ratio. Under these same conditions the same binding ratio was determined for the coordination of Zn(BF$_4$)$_2$ with probe 2.16 (bottom Figure 103).
Figure 103. Job’s plot between probe 2.16 and Zn$^{2+}$ ions and possible coordination.

Experiment carried out in 100% DMSO (λ$_{ex}$ 339 nm). Results show a 1:1 (L:M) binding ratio. (Top) Zn(ClO$_4$)$_2$ (Bottom) Zn(BF$_4$)$_2$
3.2.6 UV-Vis Response with Probes 2.15, 2.16 and 3.6-3.8 Towards MCl, Salts

In order to get an understanding of the optical properties of the molecular probes, various metal salts (two equivalents in DMSO), were investigated. First the compounds were screened with an array of metal chlorides (Mg$^{2+}$, Ca$^{2+}$, Pb$^{2+}$, Pd$^{2+}$, Fe$^{2+}$, Fe$^{3+}$, Ni$^{2+}$, Co$^{2+}$, Cu$^{2+}$, Cr$^{3+}$, Hg$^{2+}$, Cd$^{2+}$, and Zn$^{2+}$).

No significant optical change in the absorbance band at 401 nm for probe 2.15 is seen. However, upon the addition of FeCl$_2$, FeCl$_3$, PdCl$_2$, and CuCl$_2$ a broad shoulder appears at 362 nm (Figure 104). The absorbance intensity increase of probe 2.15 between 290-362 nm with these metal ions is due to one of two reasons. The first explanation is that since these metal ions absorb in the UV-Vis region the observed spectroscopic response observed below is due to the spectrum of the metal alone and not due to a probe 2.15 metal ion interaction. The other explanation for this observed response is the coordination of the metal ion results in a higher presence of the imine-enol tautomer in solution. However, since the maximum absorbance at 401 nm does not decrease, to signify that energy is being loss, the most likely reason for the increase observed for these metal ions is the metal ions alone are responsible for the spectra.
Figure 104. UV-Vis screening of metal chloride salts with probe 2.15.

Screening carried out in DMSO. [2.15] = 1.56 \times 10^{-5} \text{M} [\text{MCl}_x] = 3.11 \times 10^{-5} \text{M}. All other MCl\textsubscript{x} salts (Mg\textsuperscript{2+}, Pb\textsuperscript{2+}, Co\textsuperscript{2+}, Ni\textsuperscript{2+}, Hg\textsuperscript{2+}, Cd\textsuperscript{2+}, Zn\textsuperscript{2+}, and Cr\textsuperscript{3+}) screened produced no significant response.

The same screening experiment was investigated with probe 2.16 (Figure 105).

Similar to compound 2.15, the absorbance intensity of probe 2.16 increased between 290-360 nm in the presence of FeCl\textsubscript{2}, FeCl\textsubscript{3}, PdCl\textsubscript{2}, and CuCl\textsubscript{2}. The addition of the Fe\textsuperscript{2+}, Fe\textsuperscript{3+}, and Cu\textsuperscript{2+} ions also produced an increase in the absorbance intensity of probe 2.16 at 406 nm ($\lambda_{\text{max}}$). The presence of Pd\textsuperscript{2+} ions induced a 6 nm bathochromic shift of the maximum absorbance band as well as an increase in the absorbance intensity at 460 nm. The addition of Cd\textsuperscript{2+} ions resulted in a 16 nm hypsochromic shift of the maximum absorbance band, while inducing a decrease in the absorbance intensity between 290-340 nm.
Tests were carried out in DMSO. \([2.16] = 1.56 \times 10^{-5} \text{M} \) \([\text{MCl}_x] = 3.11 \times 10^{-5} \text{M}\). All other \(\text{MCl}_x\) salts (\(\text{Mg}^{2+}\), \(\text{Pb}^{2+}\), \(\text{Co}^{2+}\), \(\text{Ni}^{2+}\), \(\text{Hg}^{2+}\), \(\text{Zn}^{2+}\), and \(\text{Cr}^{3+}\)) screened produced no significant response.

Probe 3.7 displayed an analogous colorimetric response (Figure 106) as observed with compound 2.15 (Figure 104). This indicates that the location of the nitrogen atom is an important factor in the coordination of the metal ions. In comparing the observed colorimetric response from compound 2.16 (Figure 105) and probe 3.7 (Figure 106), the nitrogen atom of the pyridine ring in the meta position does not significantly influence the coordination of the metal chloride salts. The addition of \(\text{FeCl}_2\), \(\text{FeCl}_3\), \(\text{PdCl}_2\), and \(\text{CuCl}_2\) induced the absorbance intensity increase between 290-360 nm (330 nm for \(\text{Cu}^{2+}\) ions) and a negligible increase of probe 3.7 absorbance \(\lambda_{\text{max}}\) at 400 nm.
Figure 106. UV-Vis screening of metal chloride salts with probe 3.7.

Tests were carried out in DMSO. \[ [3.7] = 1.56 \times 10^{-5} \text{M} \quad [\text{MCl}_x] = 3.11 \times 10^{-5} \text{M} \]. All other \( \text{MCl}_x \) salts (\( \text{Mg}^{2+}, \text{Pb}^{2+}, \text{Co}^{2+}, \text{Ni}^{2+}, \text{Hg}^{2+}, \text{Cd}^{2+}, \text{Zn}^{2+}, \) and \( \text{Cr}^{3+} \)) screened produced no significant response.

A further investigation was carried out to see the effect of the nitrogen atom in the pyridine ring has with the coordination of metal ions. Following the same trend the nitrogen is moved one position closer (ortho position) in probe 3.6 (Figure 107). With the addition of \( \text{Cu}^{2+} \) ions to probe 3.6 a small shoulder appeared as seen in Figures 104-106. The \( \text{Pd}^{2+} \) ions produced a similar to the response from probe 3.6 as seen in Figure 105, small red shift and an increase in absorbance intensity at 330 nm.
Figure 107. UV-Vis screening of metal chloride salts with probe 3.6.

Tests were carried out in DMSO. $[3.6] = 1.56 \times 10^{-5} \text{M}$ $[\text{MCl}] = 3.11 \times 10^{-5} \text{M}$. All other MCl salts (Mg$^{2+}$, Pb$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Hg$^{2+}$, Cd$^{2+}$, Zn$^{2+}$, and Cr$^{3+}$) screened produced no significant response.

Finally the same experiment was investigated with probe 3.8 (Figure 108). Again only the metal ions Cu$^{2+}$, Fe$^{2+}$/Fe$^{3+}$, and Pd$^{2+}$ induced a significant spectroscopic response. For probe 3.8, the increased absorbance intensity at 330 nm produced by the addition of Pd$^{2+}$ ions is nearly identical to the intensity observed at 400 nm. This response is larger than what is seen with probes 2.16 and 3.6 (Figures 105 and 107 respectively), which indicates the second nitrogen atom in the ortho position of the heterocycle makes the Pd$^{2+}$ ions and probe interaction more favorable.
Tests were carried out in DMSO. \([3.8] = 1.56 \times 10^{-5} \text{M} [\text{MCl}_x] = 3.11 \times 10^{-5} \text{M}\). All other MCl\(_x\) salts (Mg\(^{2+}\), Pb\(^{2+}\), Co\(^{2+}\), Ni\(^{2+}\), Hg\(^{2+}\), Cd\(^{2+}\), Zn\(^{2+}\), and Cr\(^{3+}\)) screened produced no significant response.

From the results of the fluorescence screening, which will be discussed in section 3.2.8, a full UV-Vis titration with ZnCl\(_2\) was investigated with probes 2.15 and 3.8, Figures 109 and 110 respectively.

The addition of ZnCl\(_2\) to a solution of probe 2.15 resulted in the decrease of the maximum absorbance band centered at 401 nm (Figure 109). The spectroscopic response observed in the UV-Vis spectrum below is analogous to the response observed upon the addition of TBACl (Figure 34) in Chapter II. The colorimetric response shown below indicates that the coordination of the Zn\(^{2+}\) ion to probe 2.15 is aided by hydrogen bonding between the N-H and the Cl\(^-\) ion. From a Benesi-Hildebrand plot a \(K_{11}\) binding constant was calculated to be \(4.9 \times 10^3 \text{M}^{-1}\). A possible mode of coordination is shown in Scheme 69.
Scheme 69. The possible coordination of ZnCl$_2$ to probe 2.15.

Figure 109. ZnCl$_2$ UV-Vis titration with probe 2.15 in DMSO.

$[2.15] = 1.56 \times 10^{-5}$M $[ZnCl_2] = 3.11 \times 10^{-4}$M

As the model probe, the response shown above was expected. To determine if the response is unique to compound 2.15 or not, the same experiment was investigated with probe 3.8 (Figure 110). Alone the UV-Vis spectrum of probe 3.8 has an absorbance band
at 335 and 411 nm. The two observed absorbance bands are due to the imine-enol and keto-enamine tautomers respectively. The coordination of ZnCl₂ resulted in the decrease of both absorbance bands. From a Benesi-Hildebrand plot a $K_{11}$ binding constant was calculated to be $8.2 \times 10^4$ M$^{-1}$. The binding constant calculated is a magnitude of ten larger than the constant calculated for probe 2.15. This indicates that the pyrimidine nitrogen atoms aid in coordination stability.

Figure 110. ZnCl₂ UV-Vis titration with probe 3.8 in DMSO.

$[3.8] = 1.56 \times 10^{-3}$ M $[\text{ZnCl}_2] = 3.11 \times 10^{-4}$ M

The analysis of the colorimetric response observed from the five probes towards the various metal chloride salts (two equivalences) revealed that any changes to the absorbance spectrum of the compound occurred at 330 and 400 nm. Two tables are provided with the numerical values of the changes in the absorbance intensity at 330 and 400 nm (Table 19 and 20 respectively). For all five probes the absorbance intensity at 330 nm increased in the presence of Pd$^{2+}$, Cu$^{2+}$, Fe$^{2+}$, and Fe$^{3+}$ ions (Table 19). When
examining the responses at 400 nm the Zn\(^{2+}\), Cd\(^{2+}\), and Hg\(^{2+}\) ions produced an increase in the absorbance intensity for probes 2.15 and 3.8 (Table 20).

Table 19

*The Δ\text{ABS} at 330 nm by probes 2.15, 2.16, and 3.6-3.8 with metal chloride salts*

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<th>Probes</th>
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<th>2.16</th>
<th>3.6</th>
<th>3.7</th>
<th>3.8</th>
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<td>-0.003</td>
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<td>0.050</td>
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<td>0.141</td>
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<td>-0.001</td>
<td>-0.002</td>
<td>-0.020</td>
</tr>
<tr>
<td>Co(^{2+})</td>
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<td>-0.006</td>
<td>-0.016</td>
<td>-0.006</td>
<td>-0.035</td>
</tr>
<tr>
<td>Ni(^{2+})</td>
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<td>-0.019</td>
<td>-0.009</td>
<td>-0.047</td>
</tr>
<tr>
<td>Cu(^{2+})</td>
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<td>0.007</td>
<td>0.050</td>
<td>0.057</td>
<td>0.046</td>
</tr>
<tr>
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<td>0.190</td>
<td>0.488</td>
<td>0.463</td>
<td>0.528</td>
</tr>
<tr>
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<td>-0.005</td>
<td>-0.007</td>
<td>-0.009</td>
</tr>
<tr>
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<td>0.001</td>
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<td>-0.005</td>
</tr>
<tr>
<td>Hg(^{2+})</td>
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<td>-0.001</td>
<td>-0.022</td>
</tr>
<tr>
<td>Cd(^{2+})</td>
<td>0.002</td>
<td>-0.002</td>
<td>-0.054</td>
<td>-0.002</td>
<td>-0.021</td>
</tr>
<tr>
<td>Zn(^{2+})</td>
<td>0.000</td>
<td>-0.007</td>
<td>-0.053</td>
<td>-0.008</td>
<td>-0.026</td>
</tr>
</tbody>
</table>

Note: Numerical values are from the two equivalence screenings shown in Figures 3.45 - 3.49. The change in absorbance intensity at 330 nm is calculated from the following equation: (absorbance intensity at 330 nm with metal ion - absorbance intensity at 330 nm without metal ion).
### Table 20

*The $\Delta_{\text{ABS}}$ at 400 nm by probes 2.15, 2.16, and 3.6-3.8 with metal chloride salts*

<table>
<thead>
<tr>
<th>Probes</th>
<th>2.15</th>
<th>2.16</th>
<th>3.6</th>
<th>3.7</th>
<th>3.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr$^{3+}$</td>
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<td>-0.003</td>
<td>0.018</td>
<td>-0.008</td>
<td>0.029</td>
</tr>
<tr>
<td>Fe$^{3+}$</td>
<td>0.041</td>
<td>0.021</td>
<td>0.053</td>
<td>0.028</td>
<td>0.076</td>
</tr>
<tr>
<td>Fe$^{2+}$</td>
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<td>0.015</td>
<td>0.052</td>
<td>0.027</td>
<td>0.066</td>
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<tr>
<td>Pb$^{2+}$</td>
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<td>0.003</td>
<td>0.023</td>
<td>-0.008</td>
<td>0.030</td>
</tr>
<tr>
<td>Co$^{2+}$</td>
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<td>-0.007</td>
<td>-0.002</td>
<td>-0.018</td>
<td>0.022</td>
</tr>
<tr>
<td>Ni$^{2+}$</td>
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<td>-0.017</td>
<td>-0.019</td>
<td>-0.023</td>
<td>0.012</td>
</tr>
<tr>
<td>Cu$^{2+}$</td>
<td>0.018</td>
<td>0.014</td>
<td>0.019</td>
<td>-0.008</td>
<td>0.041</td>
</tr>
<tr>
<td>Pd$^{2+}$</td>
<td>0.047</td>
<td>0.017</td>
<td>0.012</td>
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<td>0.084</td>
</tr>
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<td>Mg$^{2+}$</td>
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<td>0.004</td>
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<td>0.013</td>
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<td>Ca$^{2+}$</td>
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<td>0.006</td>
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<td>0.007</td>
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<td>Hg$^{2+}$</td>
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<td>0.000</td>
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<td>0.046</td>
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<td>Cd$^{2+}$</td>
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<td>-0.207</td>
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<tr>
<td>Zn$^{2+}$</td>
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<td>-0.005</td>
<td>-0.001</td>
<td>-0.001</td>
<td>0.043</td>
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</table>

Note: Numerical values are from the two equivalence screenings shown in Figures 3.45 - 3.49. The change in absorbance intensity at 400 nm is calculated from the following equation: (absorbance intensity at 400 nm with metal ion - absorbance intensity at 400 nm without metal ion).

#### 3.2.7 Fluorescence Response with Probes 2.15, 2.16 and 3.6-3.8 Towards MClx Salts

After screening the colorimetric response of the probes towards the various metal chloride salts, the fluorescence responses were investigated. Initially each probe was...
screened against two equivalences of the various metal chloride salts. As the model probe 2.15 will be discussed first (Figure 111). The excitation at 408 nm would ensure that the emission spectrum observed would be mainly the keto-enamine tautomer. With this in mind and the fact that all of the metal chloride salts screened produced the same spectroscopic response, slight decrease, it inferred that the coordination is occurring in a similar manner. Furthermore, the decrease in the emission intensity, at 533 nm, as a result of coordination indicates that the coordination is slightly disturbing the ESIPT process but not inhibiting the process. The inhibition of the ESIPT process will be apparent in the metal acetate salts section 3.2.9.

![Figure 111. Fluorescence screening of metal chloride salts with probe 2.15.](image)

Experiment carried out in DMSO $\lambda_{ex}$ 408 nm. [2.15] = 1.56 $\times$ 10^{-5} M $[\text{MCl}_x]$ = 3.11 $\times$ 10^{-5} M.

The same experiment was repeated with probe 2.16 (Figure 112). Upon the addition of PdCl$_2$ the maximum emission band centered at 564 nm decreased with roughly half of the original emission intensity as the blank. In the presence of ZnCl$_2$ the
emission spectrum of probe 2.16 slightly increased along with a hypsochromic shift (Figure 112). Also the addition of ZnCl₂ resulted in the increase of the fluorescence emission intensity at 482 nm. Since this was not observed in the response displayed by compound 2.15, then the presence of the nitrogen atom of the pyridine is involved in the coordination or influencing the coordination through the keto-enamine. In Scheme 70 the possible mode of coordination between ZnCl₂ and probe 2.16 is provided. Also the response shown towards ZnCl₂ is representative of ESIPT inhibition. All other metal chloride salts screened produced no significant response.

Scheme 70. The mode of coordination between ZnCl₂ and probe 2.16.
Figure 112. Fluorescence screening of metal chloride salts with probe 2.16.

Experiment carried out in DMSO $\lambda_{ex}$ 408 nm. $[\text{2.16}] = 1.56 \times 10^{-5}\text{M}$, $[\text{MCl}_x] = 3.11 \times 10^{-5}\text{M}$.

Next the response from probe 3.7 towards the metal chloride salts was investigated (Figure 113). The presence of MgCl$_2$ resulted in the largest increase in fluorescence emission intensity at 552 nm. However, increased intensity was also induced by the coordination of PdCl$_2$, FeCl$_2$, FeCl$_3$, NiCl$_2$, HgCl$_2$, and ZnCl$_2$. Out of the metal ions that increased the fluorescence emission intensity at 552 nm, ZnCl$_2$ is the only ion that also induced an increase of the fluorescence emission intensity at 460 nm.
Figure 113. Fluorescence screening of metal chloride salts with probe 3.7.

Experiment carried out in DMSO $\lambda_{ex}$ 408 nm. $[3.7] = 1.56 \times 10^{-5}$ M $[MCl] = 3.11 \times 10^{-5}$ M.

The screening with probe 3.6 (Figure 114) produced similar responses to that observed with compound 2.16 (Figure 112). The addition of ZnCl$_2$ induced the largest increase of the fluorescence emission intensity at 549 nm. All of the other metal chloride salts screened resulted in a decrease in emission intensity, with the coordination of PdCl$_2$ resulting in the largest decrease.
Finally the screening experiment was investigated with probe 3.8 (Figure 115).

The coordination of ZnCl$_2$ resulted in an increase in emission intensity, at 541 nm, as well as a hypsochromic shift from 577 to 541 nm. When comparing the responses of probes 2.16, 3.6, and 3.8 towards ZnCl$_2$ the probe 3.8 produced the most pronounced signal change, largest increase in fluorescence emission intensity and hypsochromic shift, of the three probes. The presence of CdCl$_2$ produced the next largest spectroscopic change to the emission spectrum of probe 3.8. The response observed is similar to that of ZnCl$_2$ but not as significant. This indicates that the ionic radius of the ion is a factor in the probe 3.8 coordination of the metal. This is further confirmed with an observed response from CaCl$_2$. While CaCl$_2$ did not produce a significant change to the maximum emission band of compound 3.8 it did induce an increase in the emission intensity 460 nm similar to ZnCl$_2$ and CdCl$_2$. 

**Figure 114.** Fluorescence screening of metal chloride salts with probe 3.6. Experiment carried out in DMSO $\lambda_{ex}$ 408 nm. $[3.6] = 1.56 \times 10^{-5}$ M $[\text{MCl}_x] = 3.11 \times 10^{-5}$ M.
Figure 115. Fluorescence screening of metal chloride salts with probe 3.8.

Experiment carried out in DMSO $\lambda_{ex}$ 408 nm. $[3.8] = 1.56 \times 10^{-5}$ M $[\text{MCl}] = 3.11 \times 10^{-5}$ M.

Out of the metal chloride salts screened ZnCl$_2$ induced the most significant changes to the fluorescence emission spectrum for all of the probes, excluding probe 2.15. From these results a full titration with ZnCl$_2$ was investigated with all of the probes.

The addition of ZnCl$_2$ to probe 2.15 resulted in a steady decrease of the fluorescence emission intensity at 553 nm (Figure 116). The lack of any significant spectroscopic response supports coordination solely to the C(4) oxygen atom.
Figure 116. ZnCl$_2$ fluorescence titration with probe 2.15 ($\lambda_{ex}$ 408 nm).

Titration carried out in 100% DMSO $[2.15] = 1.56 \times 10^{-5}$ M $[ZnCl_2] = 3.11 \times 10^{-4}$ M.

When the same titration was repeated with probe 2.16 a slight increase at the initial maximum fluorescence emission at 555 nm is observed (Figure 117). While the change is inconsistent at 555 nm, the successive additions of ZnCl$_2$ at 500 nm resulted in a steady near linear increase. This is shown in the isotherm located in Figure 3.55. From a Benesi-Hildebrand plot, using the fluorescence emission intensity at 500 nm, a $K_{11}$ binding constant was calculated to be $8.0 \times 10^4$ M$^{-1}$. 
Figure 117. ZnCl$_2$ fluorescence titration with probe 2.16 ($\lambda_{ex}$ 408 nm).

Titration carried out in 100% DMSO $[2.16] = 1.56 \times 10^{-5}$ M $[\text{ZnCl}_2] = 3.11 \times 10^{-4}$ M.

The addition of ZnCl$_2$ resulted in an increase of the initial maximum fluorescence emission intensity of probe 3.7 at 546 nm (Figure 118). The response from probe 3.7 is unique in the fact that it is the only probe to show only an increase in the initial maximum emission band with no blue shift.
Figure 118. ZnCl$_2$ fluorescence titration with probe 3.7 ($\lambda_{ex}$ 408 nm).

Titration carried out in 100% DMSO [3.7] = 1.56 $\times$ 10$^{-5}$ M [ZnCl$_2$] = 3.11 $\times$ 10$^{-4}$ M.

The addition of ZnCl$_2$ to probe 3.6 resulted in the increase of the fluorescence emission intensity at 539 nm, which was inconsistent; however, the increase observed at 490 nm was close to linear as the concentration of ZnCl$_2$ in the probe 3.6 sample increased (Figure 119).
Figure 119. ZnCl\(_2\) fluorescence titration with probe 3.6 (\(\lambda_{ex}\) 408 nm).

Titration carried out in 100% DMSO \([3.6] = 1.56 \times 10^{-5} \text{ M} \ [\text{ZnCl}_2] = 3.11 \times 10^{-4} \text{ M} \).

With the addition of ZnCl\(_2\) to probe 3.8, the emission spectrum maximum underwent a gradual hypsochromic shift from 576 to 536 nm with an increase in the emission intensity (Figure 120).
Figure 120. ZnCl$_2$ fluorescence titration with probe 3.8 ($\lambda_{\text{ex}}$ 408 nm).

Titration carried out in 100% DMSO $[3.8] = 1.56 \times 10^{-5} \text{ M}$ $[\text{ZnCl}_2] = 3.11 \times 10^{-4} \text{ M}$.

From both colorimetric and fluorescence screenings and full titrations investigated a trend is observed.

1. The largest spectroscopic response is observed when the nitrogen atom in the pyridine ring is located in the ortho or para position.

2. For probes 3.6 and 3.8 the nitrogen atom(s) in the ortho position gives rise to a spectroscopic response in the presence of Cd$^{2+}$ ions over other metal ions.

3. The probes 2.16, 3.6, and 3.8 showed a red shift (UV-Vis, Figures 105, 107, and 108 respectively) upon the addition of Pd$^{2+}$ ions, which suggests that the metal ion altered the ICT character (section 1.5.2) of the probes. The quenching of the fluorescence emission intensity (Figures 112, 114, and 115...
respectively) upon the coordination of Pd\textsuperscript{2+} ions is attributed to the heavy metal effect.

The bar chart below (Figure 121) summarizes the observed fluorescence responses from the screening experiments discussed above. In regards to detecting Zn\textsuperscript{2+} ions, a larger response is observed when the nitrogen atom(s) is located in either the \textit{para} or \textit{ortho} positions. However, when in the nitrogen atom(s) are in the \textit{ortho} position(s) a, spectroscopic response is also observed with the addition of Cd\textsuperscript{2+} ions, which was previously discussed in Chapter I as a common interfering ion for Zn\textsuperscript{2+} metals. The response towards Cd\textsuperscript{2+} ions is increased when both \textit{ortho} positions are nitrogen atoms, as is the case with probe 3.8. When the nitrogen atom of the pyridine ring is located in the \textit{meta} position (probe 3.7) the addition of Hg\textsuperscript{2+} ions induced an increase in emission intensity. Since the Hg\textsuperscript{2+} ion is relatively larger than the Zn\textsuperscript{2+} and Cd\textsuperscript{2+} ions (Table 1), the response from probe 3.7 is attributed to coordination solely through the pyridine nitrogen. This indicates that probe 3.7 could serve as a probe for the detection of Hg\textsuperscript{2+} ions; however, from the bar chart below numerous metal ions induced a larger increase in the probe 3.7 fluorescence emission intensity. All positive values in Figure 121 indicate a fluorescence emission intensity increase, while a negative value shows a decreased emission signal.
Figure 121. Fluorescence bar chart with all probes and metal chloride salts.

(λ_ex 408 nm).ΔEM = (emission intensity with MCl, salt) – (emission intensity without MCl, salt). Positive values represent an intensity increase while a negative value indicates an intensity decrease. [probe] = 1.56 × 10^{-5} M [MCl,] = 3.11 × 10^{-5} M.

There was very little spectroscopic response seen with the addition of the metal chloride salts. This indicated that the properties responsible for making the molecular probe fluoresce were not changed. However, previously when the anions were investigated with molecular probe 2.16 (see Chapter II) it was noticed that the F⁻ and OAc⁻ ions visually affected the spectroscopic response (Figure 48). Therefore the investigation turned to the spectroscopic response from metal acetate. Unfortunately, many metal fluorides are insoluble and were not investigated.

3.2.8 UV-Vis Response from Probes 2.15, 2.16 and 3.6-3.8 Towards Metal Acetate Salts

From the colorimetric and fluorescence responses observed from the probes with the addition of metal chloride salts, the spectroscopic responses towards a number of
metal acetate salts were investigated: Na\(^+\), Ag\(^+\), Mg\(^{2+}\), Ca\(^{2+}\), Mn\(^{2+}\), Fe\(^{2+}\), Co\(^{2+}\), Ni\(^{2+}\), Cu\(^{2+}\), Pb\(^{2+}\), Pd\(^{2+}\), Hg\(^{2+}\), Cd\(^{2+}\), and Zn\(^{2+}\) ions. First, the colorimetric responses of the probes towards the various metal acetate salts (two equivalences) were screened.

The metal acetate salts were first screened with the model probe 2.15 to establish a baseline for comparison to the responses observed from the other probes. It is observed (Figure 122) that with the change of the metal salt used a larger response is induced. Out of the various metal acetate salts screened, the addition of Cu\(^{2+}\), Co\(^{2+}\), and Ni\(^{2+}\) ions induced the largest decrease in the maximum absorbance band at 406 nm. These particular metal ions also resulted in a hypsochromic shift of the maximum absorbance band from 406 nm to 388 (Cu\(^{2+}\) ions) and 380 nm (Co\(^{2+}\) and Ni\(^{2+}\) ions). Also, the addition of the Co\(^{2+}\) and Ni\(^{2+}\) ions induced a decrease in the maximum absorbance intensity, while the presence of the Cu\(^{2+}\) ion caused an increase in intensity.
Figure 122. UV-Vis screening of metal acetate salts with probe 2.15.

Tests were carried out in DMSO. [2.15] = 1.56 × 10^{-5} M [MOAc_x] = 3.11 × 10^{-5} M. All other MOAc_x salts (Na^{+}, Ag^{+}, Ca^{2+}, Mg^{2+}, Fe^{2+}, Pb^{2+}, Mn^{2+}, Hg^{2+}, Cd^{2+}, and Zn^{2+}) screened produced no significant response.

Upon screening the responses from probe 2.16 towards the various metal acetate salts (Figure 123) numerous responses were observed. Probe 2.16 alone has a maximum absorbance at 408 nm (keto-enamine) and a local maxima at 339 nm (imine-enol). The addition of Zn^{2+} ions induced a decrease at 339 nm with an increase at 408 nm. For the Cd^{2+} and Hg^{2+} ions a smaller increase was observed at 408 nm with a similar decrease at 339 nm. However, these two d^{10} metal ions induced a small increase in the absorbance at 350 nm. With the addition of Ni^{2+} ions a blue shift, 24 nm, was observed along with an increase in absorbance intensity at 384 nm. The Ni^{2+} ion also induced a decrease in the absorbance intensity at 339 nm. The presence of Co^{2+} ions also induced a blue shift, 20 nm, along with decreases at both 408 and 339 nm. In the case of Mg^{2+} and Ca^{2+} ions the maximum absorbance was shifted from 408 to 380 and 377 nm respectively. Unlike the
previous metal ions discussed the Mg$^{2+}$ and Ca$^{2+}$ ions induced an increase in the absorbance at 339 nm.

![UV-Vis screening of metal acetate salts with probe 2.16.](image)

*Figure 123. UV-Vis screening of metal acetate salts with probe 2.16.*

Tests were carried out in DMSO. $[2.16] = 1.56 \times 10^{-5}$ M $[\text{MOAc}_x] = 3.11 \times 10^{-5}$ M. All other MOAc$_x$ salts (Ag$^+$, Fe$^{2+}$, Pb$^{2+}$, Pd$^{2+}$, Mn$^{2+}$, Cu$^{2+}$, Hg$^{2+}$, Cd$^{2+}$, and Zn$^{2+}$) screened produced no significant response.

The screening for probe 3.7 is shown in Figure 124. Both Ca$^{2+}$ and Na$^+$ ions resulted in a decrease in the maximum absorbance along with a slight blue shift and an overall broadening of the absorbance band. The addition of Co$^{2+}$ and Ni$^{2+}$ ions produced a blue shift of the maximum absorbance band and a decrease in the intensity at 330 nm. The presence of Cu$^{2+}$ ions resulted in the same degree in the blue shift as the Na$^+$ and Ca$^{2+}$ ions, but the copper cation increased the maximum absorbance intensity. The Cu$^{2+}$ ions also produced a larger decrease in the absorbance intensity at 330 nm.
Tests were carried out in DMSO. \([3.7] = 1.56 \times 10^{-5} \text{M} \) \([\text{MOAc}] = 3.11 \times 10^{-5} \text{M}\). All other MOAc salts (Ag\(^{+}\), Mg\(^{2+}\), Fe\(^{2+}\), Pb\(^{2+}\), Pd\(^{2+}\), Mn\(^{2+}\), Hg\(^{2+}\), Cd\(^{2+}\), and Zn\(^{2+}\)) screened produced no significant response.

The screening investigated with probe 3.6 is shown in Figure 125. The addition of Mg\(^{2+}\) and Ca\(^{2+}\) ions resulted in the decrease of the maximum absorbance band at 405 nm with an increase in the absorbance intensity at 339 nm. With the addition of Cd\(^{2+}\) ions the maximum absorbance underwent a slight hypsochromic shift with a small increase in intensity. The addition of Zn\(^{2+}\) ions resulted in a bathochromic shift of the maximum absorbance along with a slight increase in intensity, while the addition of Hg\(^{2+}\) ions produced a larger bathochromic shift and increase in maximum absorbance. The largest hypsochromic shift was observed with the addition of the Ni\(^{2+}\) ion. The presence of Pd\(^{2+}\) ions resulted in a slight decrease and red shift of the maximum absorbance band as well as an increase in the absorbance intensity at 470 nm.
Figure 125. UV-Vis screening of metal acetate salts with probe 3.6.

Tests were carried out in DMSO. [3.6] = 1.56 × 10^{-5} M [MOAc] = 3.11 × 10^{-5} M. All other MOAc, salts (Na^{+}, Ca^{2+}, Mg^{2+}, Fe^{2+}, Pb^{2+}, Mn^{2+}, Cu^{2+}, Co^{2+}, Cd^{2+}, and Zn^{2+}) screened produced no significant response.

Upon screening the metal acetate salts with probe 3.8 the majority of the cations produced a colorimetric response (Figure 126). Only the ions Na^{+}, Mg^{2+}, Ca^{2+}, Mn^{2+}, and Fe^{2+} produced an increase in the absorbance intensity at 334 nm and a decrease at 410 nm (absorbance maximum of probe 3.8) except for Fe^{2+} ions, which resulted in no change of the band at 410 nm. The remaining metal ions screened resulted in a decrease in the absorbance intensity at 334 nm and a bathochromic shift (ranging from 2 to 18 nm) of the 410 nm absorbance band. The only exception to the bathochromic shift is the Ni^{2+} ions, which produced a hypsochromic shift of 12 nm.
Figure 126. UV-Vis screening of metal acetate salts with probe 3.8.

Tests were carried out in DMSO. [3.8] = 1.56 × 10⁻⁵ M [MOAc₄] = 3.11 × 10⁻⁵ M. All other MOAc salts (Ag⁺ and Pb²⁺) screened produced no significant response.

After screening the metal acetate salts, full Zn(OAc)₂ titrations were investigated with probes 2.16 (Figure 127), 3.6 (Figure 128), and 3.8 (Figure 129). The addition of Zn(OAc)₂ to probe 2.16 resulted in the decrease of the maximum absorbance band at 405 nm along with a slight blue shift (Figure 127). The Zn²⁺ ion also induced a decrease in the absorbance intensity at 336 nm. The isotherm provided at the top of Figure 127 shows the changes in intensity at these two wavelengths. From a Benesi-Hildebrand plot a $K_{11}$ binding constant was calculated to be $1.2 \times 10^4$ M⁻¹.
Figure 127. Zn(OAc)$_2$ isotherm and UV-Vis titration with probe 2.16 in DMSO.

$[2.16] = 1.56 \times 10^{-5}$ M, $[\text{Zn(OAc)}_2] = 3.11 \times 10^{-4}$ M.

When the Zn(OAc)$_2$ absorbance response was investigated with probe 3.6 (Figure 128) the band at 336 nm decreased as the concentration of Zn$^{2+}$ ions increased. This response is similar to compound 2.16; however, the addition of Zn$^{2+}$ ions resulted in an increase along with a 5 nm bathochromic shift in the maximum absorbance band of probe
3.6 at 402 nm. The isotherm located in Figure 128 shows the changes in absorbance intensities at 336 and 407 nm as the Zn$^{2+}$ ion concentration increases. From a Benesi-Hildebrand plot a $K_{11}$ binding constant was calculated to be $1.6 \times 10^4$ M$^{-1}$.

![Graph](image)

*Figure 128. Zn(OAc)$_2$ isotherm and UV-Vis titration with probe 3.6 in DMSO.*

$[3.6] = 1.56 \times 10^{-3}$ M $[\text{Zn(OAc)}_2] = 3.11 \times 10^{-4}$ M.
When the Zn(OAc)$_2$ absorbance response was investigated with probe 3.8 (Figure 129) the band at 332 nm decreased as the concentration of Zn$^{2+}$ ions increased. The presence of Zn$^{2+}$ ions also increased the initial maximum absorbance band at 408 nm along with a 14 nm bathochromic shift. The isotherm located in Figure 3.68 shows the changes in absorbance intensities at 332 and 422 nm as the Zn$^{2+}$ ion concentration increases. From a Benesi-Hildebrand plot a $K_{11}$ binding constant was calculated to be $2.5 \times 10^4$ M$^{-1}$. 

Figure 129. Zn(OAc)$_2$ isotherm and UV-Vis titration with probe 3.8 in DMSO.

$[3.8] = 1.56 \times 10^{-3} \text{M} \quad [\text{Zn(OAc)}_2] = 3.11 \times 10^{-4} \text{M}.$

To summarize all of the colorimetric responses observed two tables were prepared (Tables 21 and 22). The values provided in each table give the numerical change in the absorbance intensity at 330 nm (Table 21) and 400 nm (Table 22). These two wavelengths were chosen because 330 nm is the wavelength of the imine-enol, while 400 nm is assigned to the keto-enamine tautomer.
Table 21

The $\Delta_{ABS}$ at 330 nm by probes 2.15, 2.16, and 3.6-3.8 with metal acetate salts

<table>
<thead>
<tr>
<th>Probes</th>
<th>2.15</th>
<th>2.16</th>
<th>3.6</th>
<th>3.7</th>
<th>3.8</th>
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<td>Na$^+$</td>
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<td>Pb$^{2+}$</td>
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<tr>
<td>Pd$^{2+}$</td>
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<td>Fe$^{2+}$</td>
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<td>-0.054</td>
<td>-0.162</td>
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<td>-0.172</td>
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<td>Hg$^{2+}$</td>
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<tr>
<td>Cd$^{2+}$</td>
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<td>-0.132</td>
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<tr>
<td>Zn$^{2+}$</td>
<td>0.030</td>
<td>-0.023</td>
<td>-0.078</td>
<td>-0.030</td>
<td>-0.109</td>
</tr>
</tbody>
</table>

Note: Numerical values are from the two equivalence screenings shown in Figures 3.63 - 3.67. The change in absorbance intensity at 330 nm is calculated from the following equation: (absorbance intensity at 330 nm with metal ion - absorbance intensity at 330 nm without metal ion).
Table 22

The $\Delta_{\text{ABS}}$ at 400 nm by probes 2.15, 2.16, and 3.6-3.8 with metal acetate salts

<table>
<thead>
<tr>
<th>Probes</th>
<th>2.15</th>
<th>2.16</th>
<th>3.6</th>
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Note: Numerical values are from the two equivalence screenings shown in Figures 3.63 - 3.67. The change in absorbance intensity at 400 nm is calculated from the following equation: (absorbance intensity at 400 nm with metal ion - absorbance intensity at 400 nm without metal ion).

In section 3.2.7 the metal chloride salts produced very little changes to the ESIPT emission signal of the probes. The results in section 3.2.3 confirmed that the acetate ion is
required to observe a large fluorescence response. Therefore the fluorescence responses produced by the metal acetates were investigated with probes 2.15, 2.16 and 3.6-3.8.

3.2.9 Fluorescence Response with Probes 2.15, 2.16 and 3.6-3.8 Towards MOAcx Salts

First the various metal acetate salts (two equivalents) were screened with the five molecular probes. The same screening process shown in the previous three sections was followed in this section. Probe 2.15 has a fluorescence emission maximum at 533 nm (DMSO, λ<sub>ex</sub> 408 nm). Unlike the metal chloride salts (Figure 111), the acetate salts produced a larger spectroscopic response (Figure 130). A slight increase in the emission band at 533 nm was observed upon the addition of Pb<sup>2+</sup> ions. In the presence of Zn<sup>2+</sup> ions a small intensity decrease (at 533 nm) was observed as well as an increase in the emission intensity at 450 nm. The increase at 450 nm is a unique response and only observed in the presence of Zn<sup>2+</sup> ions. The cations Pd<sup>2+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, and Cu<sup>2+</sup> produced a significant decrease of the maximum emission band at 533 nm. The remaining metal ions screened produced no significant response.
Figure 130. Fluorescence screening of metal acetate salts with probe 2.15 ($\lambda_{ex}$ 408 nm).

Tests were carried out in DMSO. [2.15] = 1.56 × 10^{-5} M [MOAc] = 3.11 × 10^{-5} M. All other MOAc salts (Na+, Ag+, Ca2+, Mg2+, Fe2+, Mn2+, Hg2+, and Cd2+) screened produced no significant response.

The metal acetate salts were then screened with probe 2.16 (Figure 131) and the expected spectroscopic response from the inhibition of the ESIPT process is observed with Zn2+ ions and Cd2+ ions. Probe 2.16 has a maximum fluorescence emission band at 574 nm. The addition of Zn2+ ions produced a 65 nm blue shift of the maximum emission, while the addition of Cd2+ ions induced an 84 nm blue shift. It should be noted that Hg2+ ions did result in a slight increase in the emission intensity at 509 nm; however, this increase is not as significant as seen with the Zn2+ and Cd2+ ions. In the presence of Co2+, Cu2+, Ni2+, and Pd2+ ions the fluorescence emission of probe 2.16 is completely quenched. The inset found in Figure 131 is provided to observe the degree in which these metal ions quench the emission signal.
Tests were carried out in DMSO. $[2.16] = 1.56 \times 10^{-5} \text{ M}$ $[\text{MOAc}] = 3.11 \times 10^{-5} \text{ M}$. All other MOAc salts (Na$^+$, Ag$^+$, Ca$^{2+}$, Fe$^{2+}$, and Mn$^{2+}$) screened produced no significant response.

In the case of probe 3.7, the Zn$^{2+}$ and Cd$^{2+}$ ions are the only ions to induce an emission increase at 460 nm (Figure 132). The addition of Na$^+$ ions induced the largest increase. In the presence of Pd$^{2+}$, Co$^{2+}$, and Cu$^{2+}$ ions a decrease in the emission intensity is observed.
**Figure 132.** Fluorescence screening of metal acetate salts with probe 3.7 ($\lambda_{ex}$ 408 nm).

Tests were carried out in DMSO. $[3.7] = 1.56 \times 10^{-5}$ M $[\text{MOAc}] = 3.11 \times 10^{-5}$ M. All other MOAc$_x$ salts (Ag$^+$, Cu$^{2+}$, Mg$^{2+}$, Pb$^{2+}$, Fe$^{2+}$, Ni$^{2+}$, and Mn$^{2+}$) screened produced no significant response.

In the screening of the metal acetate salts with probe 3.6 (Figure 133) an inhibition of the ESIPT process is observed. The ions that induced the inhibition are the $d^{10}$ metals. It was observed that with the decrease in ionic radii of the $d^{10}$ metals (Table 1) resulted in a more pronounced ESIPT inhibition, larger increase in fluorescence emission intensity as well as larger blue shift. In the presence of Ni$^{2+}$, Co$^{2+}$, and Cu$^{2+}$ ions the fluorescence emission of probe 3.6 was decreased.
Figure 133. Fluorescence screening of metal acetate salts with probe 3.6 ($\lambda_{ex}$ 408 nm). Tests were carried out in DMSO. [3.6] = 1.56 × 10^{-5} M [MOAc], = 3.11 × 10^{-5} M. All other MOAc salts (Na+, Ag+, Ca^{2+}, Mg^{2+}, Pb^{2+}, Fe^{2+}, and Mn^{2+}) screened produced no significant response.

In the case of probe 3.8 there are numerous spectroscopic responses observed (Figure 134). The maximum emission band of probe 3.8 is blue shifted the most in the presence of Ca^{2+} ions, along with an increase in intensity. Unlike the response shown with the other probes, the ESIPT process of probe 3.8 is most inhibited in the presence of Cd^{2+} ions, followed by Zn^{2+} then Hg^{2+} and to a small extent Pb^{2+} ions. Previously shown with probes 2.16 and 3.6, the degree of ESIPT inhibition resulted in both a larger emission intensity increase and blue shift; however, with probe 3.8 the blue shift for the Cd^{2+} and Zn^{2+} ions are identical and only vary in emission intensity. This indicates that the coordination of the larger Cd^{2+} ion results in a more rigid complex than Zn^{2+} ion.
Tests were carried out in DMSO. $[3.8] = 1.56 \times 10^{-5}$ M [MOAc] = $3.11 \times 10^{-5}$ M. All other MOAc salts (Na$, Ag$, Fe$^{2+}$, and Mn$^{2+}$) screened produced no significant response.

From the screenings a trend emerged. When the nitrogen atom of the heterocycle is located in the ortho or para position the ability of a metal ion to induce inhibition of the ESIPT process is increased. When the probe contains one nitrogen atom in either the ortho or para position, the Zn$^{2+}$ ions will give rise to a larger response over Cd$^{2+}$ ions. However, for probe 3.8 the two nitrogen atoms in both ortho positions displayed a larger spectroscopic response towards Cd$^{2+}$ ions.

To get a further understanding of the spectroscopic responses observed from the screenings, full titrations were investigated with the five probes for the metal ions (Mg$^{2+}$, Ca$^{2+}$, Hg$^{2+}$, Cd$^{2+}$, and Zn$^{2+}$) that produced a change to the emission signal for the most number of probes.

The presence of the Cu$^{2+}$ ion resulted in a quenching of the fluorescence emission intensity for all five probes. To show this response the Cu(OAc)$_2$ titration with probe 2.15

**Figure 134.** Fluorescence screening of metal acetate salts with probe 3.8 ($\lambda_{ex}$ 408 nm).
is shown in Figure 3.74. Since no significant changes to the emission spectrum of probe 2.15 after the addition of one equivalent of Cu$^{2+}$ ions, the isotherm shown in Figure 135 below indicates that the binding ratio is 1:1.

**Figure 135.** Cu(OAc)$_2$ isotherm and fluorescence titration with probe 2.15.

$\lambda_{ex}$ 408 nm) Titration carried out in 100% DMSO. [2.15] = 1.56 × 10$^{-5}$ M [Cu(OAc)$_2$] = 3.11 × 10$^{-4}$ M (equivalences used: 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0, 4.0, and 5.0).
For the full titrations investigated with probe 2.15 the general spectroscopic response was a decrease in the maximum emission intensity at 537 nm (Figures 136-138). For the metal ions Mg$^{2+}$ (Figure 136), Ca$^{2+}$ (Figure 137), and Hg$^{2+}$ (Figure 138) the decrease observed was very small, which indicated that interaction between probe 2.15 and these ions are not favorable. The addition of Mg$^{2+}$ and Hg$^{2+}$ ions did not produce enough of a signal change to be able to calculate a binding constant; however, a binding constant was calculated from a Benesi-Hildebrand plot for the Ca$^{2+}$ ions titration ($K_{11} = 4.0 \times 10^4$ M$^{-1}$).

![Figure 136. Mg(OAc)$_2$ fluorescence titration with probe 2.15.](image)

$\lambda_{ex}$ 408 nm) Titration carried out in 100% DMSO. [2.15] $= 1.56 \times 10^{-5}$ M [Mg(OAc)$_2$] $= 3.11 \times 10^{-4}$ M (equivalences used: 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0, 4.0, and 5.0).
Figure 137. Ca(OAc)$_2$ fluorescence titration with probe 2.15.

(λ$_{ex}$ 408 nm) Titration carried out in 100% DMSO. [2.15] = 1.56 × 10$^{-5}$M [Ca(OAc)$_2$] = 3.11 × 10$^{-4}$M (equivalences used: 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0, 4.0, and 5.0).

Figure 138. Hg(OAc)$_2$ fluorescence titration with probe 2.15.

(λ$_{ex}$ 408 nm) Titration carried out in 100% DMSO. [2.15] = 1.56 × 10$^{-5}$M [Hg(OAc)$_2$] = 3.11 × 10$^{-4}$M (equivalences used: 0.0-2.0 by increments of 0.1, 2.5, 3.0, 4.0, and 5.0).
The addition of the smaller Cd\(^{2+}\) ions, compared to the ionic radius of Hg\(^{2+}\) ions, a larger decrease in the emission band of probe 2.15 was observed (Figure 139). The increased response with the addition of Cd\(^{2+}\) ions indicates that the observed change in emission intensity is due to the preferred geometry of Cd\(^{2+}\) or the absolute hardness along with ionic radii (Table 1). Alone using the metal ion radius is not sufficient enough to explain the observed response from Cd\(^{2+}\), since the smaller Mg\(^{2+}\) ion induced very little response. From a Benesi-Hildebrand plot a \(K_{11}\) binding constant was calculated to be 3.7 \(\times\) 10\(^4\) M\(^{-1}\).

\[\text{Figure 139. Cd(OAc)}_2\text{ fluorescence titration with probe 2.15.}\]

(\(\lambda_{\text{ex}}\) 408 nm) Titration carried out in 100% DMSO. [2.15] = 1.56 \(\times\) 10\(^{-4}\) M [Cd(OAc)] = 3.11 \(\times\) 10\(^{-4}\) M (equivalences used: 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0, 4.0, and 5.0).

Unlike the previous full titrations with probe 2.15, the addition of Zn(OAc)\(_2\) resulted in a decrease in the emission intensity at 537 nm along with a slight blue shift (Figure 140). The change to the emission signal at 537 nm was accompanied by a
simultaneous increase in the emission intensity at 460 nm. A pseudo isoemissive point
was observed at 485 nm. The degree of decrease observed due to the addition of Zn$^{2+}$
ions was relatively similar to that observed from Cd$^{2+}$ ions. This indicated that either the
preferred geometry or absolute hardness was the principle component to explain the
decreased emission intensity. With both the Zn$^{2+}$ and Cd$^{2+}$ ions having similar preferred
geometries and absolute hardness, the increase in intensity observed with Zn$^{2+}$ ions is
attributed to the smaller ionic radius. From a Benesi-Hildebrand plot a $K_{11}$ binding
constant was calculated to be $3.8 \times 10^4$ M$^{-1}$. To confirm the binding constant value
HypSpec was used to analyze the data. From HypSpec a log $K_{11}$ was calculated to be 5.1,
which is in good agreement with the constant calculated from the double-reciprocal plot.
Figure 140. Zn(OAc)$_2$ isotherm and fluorescence titration with probe 2.15.

(λ$_{ex}$ 408 nm) Titration carried out in 100% DMSO. [2.15] = 1.56 × 10$^{-5}$ M [Zn(OAc)$_2$] = 3.11 × 10$^{-4}$ M (equivalences used: 0.0-2.0 by increments of 0.1, 2.5, 3.0, 4.0, and 5.0).

With the model compound 2.15 metal acetate salt titrations complete, the investigation turned to the spectroscopic responses observed from probe 2.16. The first
metal ion investigated with probe 2.16 was the Mg\(^{2+}\) ions (Figure 141). With the addition of Mg(OAc)\(_2\) the maximum emission band of probe 2.16 at 572 nm decreased and a pseudo isoemissive point was observed at 483 nm. Unlike the response observed from compound 2.15, the Mg\(^{2+}\) ions also induced an increase in the emission intensity at 460 nm. From a Benesi-Hildebrand plot a \(K_{11}\) binding constant was calculated to be \(3.9 \times 10^4\) M\(^{-1}\), which is in good agreement with the Hypspec log \(K_{11}\) calculated to be 5.3.

![Figure 141. Mg(OAc)\(_2\) fluorescence titration with probe 2.16.](image)

\(\lambda_{\text{ex}} 408\) nm Titration carried out in 100% DMSO, [2.16] = 1.56 \times 10^{-5} M [Mg(OAc)\(_2\)] = 3.11 \times 10^{-4} M (equivalences used: 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0, 4.0, and 5.0).

Upon the addition of Ca\(^{2+}\) ions the maximum emission band of probe 2.16 at 562 nm decreased along with an increase in the emission intensity at 460 nm (Figure 142). The spectroscopic response observed from the presence of Ca\(^{2+}\) ions is similar to that of Mg\(^{2+}\) ions. However, the larger Ca\(^{2+}\) ionic radius shifted the pseudo isoemissive point from 483 nm (Mg\(^{2+}\) ions) to 506 nm. From a Benesi-Hildebrand plot a \(K_{11}\) binding
constant was calculated to be $6.7 \times 10^4 \text{M}^{-1}$ and with the Hypspec program a log $K_{11}$ was calculated to be 4.5.

Figure 142. Ca(OAc)$_2$ fluorescence titration with probe 2.16.

($\lambda_{ex} \, 408 \text{ nm}$) Titration carried out in 100% DMSO. [2.16] = 1.56 $\times$ $10^{-4} \text{M}$ [Ca(OAc)$_2$] = 3.11 $\times$ $10^{-4} \text{M}$ (equivalences used: 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0, 4.0, and 5.0).

With the slightly larger metal ion Hg$^{2+}$, a smaller change to the emission spectrum of probe 2.16 was observed (Figure 143). The decrease of the maximum emission band at 570 nm induced upon the addition of Hg$^{2+}$ ions was also accompanied by a 23 nm blue shift. While a binding constant could not be calculated from a Benesi-Hildebrand plot, through HypSpec a log $K_{11}$ = 4.7.
Figure 143. Hg(OAc)$_2$ fluorescence titration with probe 2.16.

(λ$_{ex}$ 408 nm) Titration carried out in 100% DMSO. [2.16] = 1.56 × 10$^{-5}$ M [Hg(OAc)$_2$] = 3.11 × 10$^{-4}$ M (equivalences used: 0.0-2.0 by increments of 0.1, 2.5, 3.0, 4.0, and 5.0).

The spectroscopic response observed in the emission spectrum of probe 2.16 upon the addition of Cd$^{2+}$ ions is unique (Figure 144). As shown with the three previous metal ions the addition of Cd$^{2+}$ ions induced a decrease of the maximum emission band at 571 nm. However, the Cd$^{2+}$ ion induced a significant emission intensity increase at 490 nm, giving rise to a ratiometric response as seen in the isotherm provided in Figure 144. A pseudo isoemissive point is observed at 540 nm. From a Benesi-Hildebrand plot a $K_{11}$ binding constant was calculated to be 2.1 × 10$^4$ M$^{-1}$, while the HypSpec log $K_{11}$ constant was calculated to be 5.0.
Figure 144. Cd(OAc)$_2$ isotherm and fluorescence titration with probe 2.16. 

(λ$_{ex}$ 408 nm) Titration carried out in 100% DMSO. [2.16] = 1.56 × 10$^{-5}$ M [Cd(OAc)$_2$] = 3.11 × 10$^{-4}$ M (equivalences used: 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0, 4.0, and 5.0).

Upon the addition of Zn(OAc)$_2$ the ESIPT process of probe 2.16 is inhibited (Figure 145). As expected with metal coordinated ESIPT inhibition, the addition of Zn$^{2+}$
ions induced a 64 nm blue shift and a significant increase in the emission intensity. With this degree of ESIPT inhibition no isoemissive point or ratiometric response was observed. The sigmoidal binding isotherm seen in Figure 3.84 indicates that probe 2.16 cooperatively coordinates the Zn\(^{2+}\) ions, specifically in a 2:1 (M:L) ratio as seen in Figure 102. From a Benesi-Hildebrand plot a \(K_{11}\) binding constant was calculated to be \(1.7 \times 10^4\) M\(^{-1}\). Upon analyzing the binding constant calculated from HypSpec multiple possible binding constants were calculated: \(\log K_{11} = 4.6\), \(\log K_{12} = 11.5\), and \(\log K_{21} = 8.6\).
Figure 145. Zn(OAc)$_2$ isotherm and fluorescence titration with probe 2.16.

(λ$_{ex}$ 408 nm) Titration carried out in 100% DMSO. [2.16] = 1.56 × 10^{-5} M [Zn(OAc)$_2$] = 3.11 × 10^{-4} M (equivalences used: 0.0-2.0 by increments of 0.1, 2.5, 3.0, 4.0, and 5.0).

Moving the pyridine nitrogen atom from the para to the meta position, as is the case with probe 3.7, the observed spectroscopic responses mirror those observed from
probe 2.15. With the addition of the Mg$^{2+}$ ions (Figure 146) a decrease is observed in the maximum emission band of probe 3.7 at 556 nm. A $K_{11}$ binding constant was calculated to be $2.6 \times 10^4$ M$^{-1}$ (HypSpec log $K_{11} = 4.9$).

![Figure 146. Mg(OAc)$_2$ fluorescence titration with probe 3.7.](image)

*O$_{ex}$ 408 nm* Titration carried out in 100% DMSO. [3.7] = $1.56 \times 10^{-5}$ M [Mg(OAc)$_2$] = $3.11 \times 10^{-4}$ M (equivalences used: 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0, 4.0, and 5.0).

The addition of Ca$^{2+}$ ions induced a similar response in the emission spectrum of probe 3.7 (Figure 147). From a Benesi-Hildebrand plot a $K_{11}$ binding constant was calculated to be $4.9 \times 10^4$ M$^{-1}$ (HypSpec log $K_{11} = 5.0$).
Figure 147. Ca(OAc)$_2$ fluorescence titration with probe 3.7. 

(λ$_{ex}$ 408 nm) Titration carried out in 100% DMSO. [3.7] = 1.56 × 10$^{-5}$ M [Ca(OAc)$_2$] = 3.11 × 10$^{-4}$ M (equivalences used: 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0, 4.0, and 5.0).

The addition of the Hg$^{2+}$ ions induced a smaller decrease in the maximum emission band of probe 3.7 at 555 nm (Figure 148). However, unlike the response observed from the addition of Mg$^{2+}$ ions and Ca$^{2+}$ ions, the presence of Hg$^{2+}$ ions induced a small increase in the emission intensity at 460 nm. From a Benesi-Hildebrand plot a $K_{11}$ binding constant was calculated to be 1.7 × 10$^4$ M$^{-1}$ (HypSpec log $K_{11}$ = 4.3).
**Figure 148.** Hg(OAc)$_2$ fluorescence titration with probe 3.7.

(λ$_{ex}$ 408 nm) Titration carried out in 100% DMSO. [3.7] = 1.56 × 10$^{-5}$ M [Hg(OAc)$_2$] = 3.11 × 10$^{-4}$ M (equivalences used: 0.0-2.0 by increments of 0.1, 2.5, 3.0, 4.0, and 5.0).

The addition of Cd$^{2+}$ ions induced a larger decrease in the emission spectrum of probe 3.7 at 554 nm and a more pronounced increase in the emission intensity at 450 nm (Figure 149). A pseudo isoemissive point is observed at 468 nm. From a Benesi-Hildebrand plot a log $K_{11}$ binding constant was calculated to be 6.4 × 10$^4$ M$^{-1}$ (HypSpec log $K_{11}$ = 5.5).
Figure 149. Cd(OAc)$_2$ isotherm and fluorescence titration with probe 3.7.

$\lambda_{\text{ex}}$ 408 nm) Titration carried out in 100% DMSO. [3.7] = 1.56 x 10^{-5} M [Cd(OAc)$_2$] = 3.11 x 10^{-4} M (equivalences used: 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0, 4.0, and 5.0).

The spectroscopic response observed upon the addition of Zn$^{2+}$ ions to probe 3.7 is shown in Figure 150. The Zn$^{2+}$ ions resulted in a slight decrease of the initial emission maximum with a significant increase in the emission intensity at 491 nm. From a Benesi-
Hildebrand plot a $K_{11}$ binding constant was calculated to be $1.9 \times 10^4$ M$^{-1}$ (HypSpec log $K_{11} = 4.7$).

Figure 150. Zn(OAc)$_2$ isotherm and fluorescence titration with probe 3.7.

(λ$_{ex}$ 408 nm) Titration carried out in 100% DMSO. [3.7] = 1.56 × 10$^{-5}$ M [Zn(OAc)$_2$] = 3.11 × 10$^{-4}$ M (equivalences used: 0.0-2.0 by increments of 0.1, 2.5, 3.0, 4.0, and 5.0).
Next the spectroscopic response towards these metal ions was investigated with probe 3.6. The addition of Mg(OAc)$_2$ resulted in a small decrease in the probe 3.6 maximum emission band at 554 nm (Figure 151). Due to the lack of a significant response with the addition of Mg$^{2+}$ ions a binding constant could not be calculated.

![Figure 151. Mg(OAc)$_2$ fluorescence titration with probe 3.6.](image)

$\lambda_{ex}$ 408 nm) Titration carried out in 100% DMSO. [3.6] = $1.56 \times 10^{-5}$ M [Mg(OAc)$_2$] = $3.11 \times 10^{-4}$ M (equivalences used: 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0, 4.0, and 5.0).

Similarly, the addition of Ca$^{2+}$ ions resulted in little spectroscopic changes in the emission spectrum of probe 3.6 (Figure 152). A $K_{11}$ binding constant, Benesi-Hildebrand plot, was calculated to be $1.3 \times 10^4$ M$^{-1}$. 
Figure 152. Ca(OAc)$_2$ fluorescence titration with probe 3.6.

(λ$_{ex}$ 408 nm) Titration carried out in 100% DMSO. [3.6] = 1.56 × 10$^{-5}$ M [Ca(OAc)$_2$] = 3.11 × 10$^{-4}$ M (equivalences used: 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0, 4.0, and 5.0).

Upon the addition of Hg$^{2+}$ ions the spectroscopic response observed from probe 3.6 (Figure 153) is similar to that observed with compound 2.16 (Figure 143). The Hg$^{2+}$ ion induced an increase in the emission intensity at 480 nm as well as a decrease in the maximum emission band centered at 550 nm. The decrease in emission intensity at 550 nm was accompanied by a 24 nm blue shift. Again the sigmoidal binding isotherm observed at 480 nm indicates that probe 3.6 coordinates Hg$^{2+}$ ions in a cooperative fashion. This binding curve is attributed to the free acetate ions in solution aiding in the coordination of Hg$^{2+}$ ions. From a Benesi-Hildebrand plot a $K_{11}$ binding constant was calculated to be 2.4 × 10$^4$ M$^{-1}$ (HypSpec log $K_{11}$ = 5.5).
Figure 153. Hg(OAc)$_2$ isotherm and fluorescence titration with probe 3.6.

(λ$_{ex}$ 408 nm) Titration carried out in 100% DMSO. [3.6] = 1.56 × 10$^{-5}$ M [Hg(OAc)$_2$] = 3.11 × 10$^{-4}$ M (equivalences used: 0.0-2.0 by increments of 0.1, 2.5, 3.0, 4.0, and 5.0).

The addition of the Cd$^{2+}$ ions resulted in a 63 nm blue shift of the maximum fluorescence emission intensity (Figure 154). The decrease in emission intensity at 553 nm is accompanied by an increase in the emission intensity at 490 nm, going through a pseudo isoemissive point at 532 nm. From a Benesi-Hildebrand plot a $K_{11}$ binding
constant was calculated to be $4.2 \times 10^4 \text{ M}^{-1}$ (HypSpec log $K_{11} = 4.8$). The investigation of the binding constant in HypSpec also gave rise to a log $K_{21}$ binding constant (Cd(3.6)$_2$) calculated to be 8.9.

Figure 154. Cd(OAc)$_2$ isotherm and fluorescence titration with probe 3.6.

($\lambda_{ex}$ 408 nm) Titration carried out in 100% DMSO. [3.6] = 1.56 x 10$^{-5}$ M [Cd(OAc)$_2$] = 3.11 x 10$^{-4}$ M (equivalences used: 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0, 4.0, and 5.0).
The addition of Zn(OAc)$_2$ resulted in the probe 3.6 ESIPT inhibition (Figure 155). The significant increase observed in the emission intensity, at 488 nm, as a result of the addition of Zn$^{2+}$ ions was accompanied by a 63 nm blue shift, from 551 to 488 nm. Unlike the response shown in the compound 2.16 emission spectrum, a pseudo isoemissive point is observed at 583 nm. From a Benesi-Hildebrand plot a $K_{11}$ binding constant was calculated to be $2.4 \times 10^4$ M$^{-1}$ (HypSpec log $K_{11} = 5.0$). The investigation of the binding constant in HypSpec also gave rise to a log $K_{21}$ binding constant (Zn(3.6)$_2$) calculated to be 8.9.
Figure 155. Zn(OAc)$_2$ isotherm and fluorescence titration with probe 3.6.

($
\lambda_{ex}$ 408 nm) Titration carried out in 100% DMSO, [3.6] = 1.56 $\times$ 10$^{-5}$ M [Zn(OAc)$_2$] = 3.11 $\times$ 10$^{-4}$ M (equivalences used: 0.0-2.0 by increments of 0.1, 2.5, and 3.0).

Out of the five probes investigated, probe 3.8 produced a fluorescence response (Figure 134) towards most of the metal acetate salts. For this reason a titration with each metal acetate salt was investigated with probe 3.8.

305
The addition of NaOAc induced a slight increase in the probe 3.8 fluorescence emission intensity at 575 and 470 nm (Figure 156). Due to the lack of a significant response with the addition of Na$^+$ ions a binding constant could not be calculated.

Figure 156. NaOAc fluorescence titration with probe 3.8.

($\lambda_{ex}$ 408 nm) Titration carried out in 100% DMSO. [3.8] = 1.56 × $10^{-5}$ M [NaOAc] = 3.11 × $10^{-4}$ M (equivalences used: 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0, 4.0, and 5.0).

Next probe 3.8 was investigated with AgOAc (Figure 157). The addition of Ag$^+$ ions induced a slight increase of the fluorescence emission intensity at 470 nm, which was accompanied by a decrease of the probe 3.8 maximum emission band at 564 nm.
Figure 157. AgOAc fluorescence titration with probe 3.8.

(λ<sub>ex</sub> 408 nm) Titration carried out in 100% DMSO. [3.8] = 1.56 × 10<sup>-5</sup> M [AgOAc] = 3.11 × 10<sup>-4</sup> M (equivalences used: 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0, 4.0, and 5.0).

With the addition of Fe(OAc)<sub>2</sub>, probe 3.8 produced no significant changes to the fluorescence emission spectrum, which did not allow for a binding constant to be calculated (Figure 158).
Figure 158. Fe(OAc)$_2$ fluorescence titration with probe 3.8.

$\lambda_{ex}$ 408 nm) Titration carried out in 100% DMSO. [3.8] = 1.56 \times 10^{-5}$M [Fe(OAc)$_2$] = 3.11 \times 10^{-4}$M (equivalences used: 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0, 4.0, and 5.0).

The addition of Mn(OAc)$_2$ resulted a slight increase of the fluorescence emission intensity at 470 nm and a slight decrease of the probe 3.8 emission band at 571 nm (Figure 159). The small changes observed from the addition of Mn$^{2+}$ ions were not significant enough to calculate a binding constant.
Figure 159. Mn(OAc)$_2$ fluorescence titration with probe 3.8.

(λ$_{ex}$ 408 nm) Titration carried out in 100% DMSO. [3.8] = 1.56 × 10$^{-5}$ M [Mn(OAc)$_2$] = 3.11 × 10$^{-4}$ M (equivalences used: 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0, 4.0, and 5.0).

The addition of Cu(OAc)$_2$ resulted in the decrease of emission intensity at 575 nm (Figure 160). The decrease observed followed a linear path until two equivalents, the isotherm is provided in Figure 159. From a Benesi-Hildebrand plot a $K_{11}$ binding constant was calculated to be 1.6 × 10$^4$ M$^{-1}$. 
Figure 160. Cu(OAc)$_2$ isotherm and fluorescence titration with probe 3.8.

$\lambda_{\text{ex}}$ 408 nm) Titration carried out in 100% DMSO. [3.8] = $1.56 \times 10^{-5}$ M [Cd(OAc)$_2$] = $3.11 \times 10^{-4}$ M (equivalences used: 0.0-2.0 by increments of 0.1, 2.5, 3.0, 4.0, and 5.0).

The addition of Co(OAc)$_2$ resulted in a decrease of the probe 3.8 emission band at 575 nm (Figure 161). From a Benesi-Hildebrand plot a $K_{11}$ binding constant was calculated to be $1.9 \times 10^4$ M$^{-1}$. 

310
Figure 161. Co(OAc)$_2$ fluorescence titration with probe 3.8.

($
\lambda_{ex}$ 408 nm) Titration carried out in 100% DMSO. [3.8] = 1.56 × 10$^{-5}$ M [Co(OAc)$_2$] = 3.11 × 10$^{-4}$ M (equivalences used: 0.0-2.0 by increments of 0.1, 2.5, 3.0, 4.0, and 5.0).

Unlike the observed response from the addition of Cu(OAc)$_2$ and Co(OAc)$_2$, the addition of Ni(OAc)$_2$ resulted in the decrease of the emission band at 575 nm along with a slight increase in the emission intensity at 470 nm (Figure 162). A pseudo isoemissive point is observed at 500 nm. From a Benesi-Hildebrand plot a log $K_{11}$ binding constant was calculated to be 4.5.
Figure 162. Ni(OAc)\(_2\) isotherm and fluorescence titration with probe 3.8.

(\(\lambda_{ex}\) 408 nm) Titration carried out in 100% DMSO. [3.8] = 1.56 × 10\(^{-5}\) M [Ni(OAc)\(_2\)] = 3.11 × 10\(^{-4}\) M (equivalences used: 0.0-2.0 by increments of 0.1, 2.5, 3.0, 4.0, and 5.0).

The only response observed from probe 3.8 with addition of Pd(OAc)\(_2\) is a decrease of the emission intensity at 575 nm (Figure 163). Since there was no observable increase at 470 nm, the different responses produced by probe 3.8 towards the \(d^8\) ions (Ni\(^{2+}\) and Pd\(^{2+}\)) are due to the increase in ionic radius.
Figure 163. Pd(OAc)$_2$ fluorescence titration with probe 3.8.

$\lambda_{ex}$ 408 nm) Titration carried out in 100% DMSO. $[3.8] = 1.56 \times 10^{-5}$ M, $[Pd(OAc)_2] = 3.11 \times 10^{-4}$ M (equivalences used: 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0, 4.0, and 5.0).

The addition of Pb$^{2+}$ ions resulted in a slight increase in the emission intensity of probe 3.8 along with a 6 nm blue shift from 569 to 563 nm (Figure 164). From a Benesi-Hildebrand plot a $K_{11}$ binding constant was calculated to be $1.7 \times 10^4$ M$^{-1}$. 


313
**Figure 164.** Pb(OAc)$_2$ fluorescence titration with probe 3.8.

(λ$_{ex}$ 408 nm) Titration carried out in 100% DMSO. [3.8] = 1.56 × 10$^{-5}$ M [Pb(OAc)$_2$] = 3.11 × 10$^{-4}$ M (equivalences used: 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0, 4.0, and 5.0).

The emission spectrum of probe 3.8 underwent a slight decrease along with a hypsochromic shift and the increase in emission intensity at 490 nm upon the addition of Mg$^{2+}$ ions (Figure 165). The significant spectroscopic response observed from probe 3.8 with the addition of Mg$^{2+}$ ions indicates that the second nitrogen atom in the pyrimidine ring is influencing the metal ion coordination (cooperative binding). From a Benesi-Hildebrand plot a $K_{11}$ binding constant was calculated to be 1.6 × 10$^4$ M$^{-1}$ (HypSpec log $K_{11}$ = 5.1).
Figure 165. Mg(OAc)$_2$ isotherm and fluorescence titration with probe 3.8.

(λ$_{ex}$ 408 nm) Titration carried out in 100% DMSO. [3.8] = 1.56 × 10$^{-5}$ M [Mg(OAc)$_2$] = 3.11 × 10$^{-4}$ M (equivalences used: 0.0-2.0 by increments of 0.1, 2.5, 3.0, 4.0, and 5.0).

The addition of Ca(OAc)$_2$ resulted in the inhibition of the probe 3.8 ESIPT

(Figure 166). The coordination of the Ca$^{2+}$ ion induced a significant increase in the
emission intensity along with a 94 nm blue shift, from 576 to 482 nm. A pseudo isoemissive point is observed at 629 nm. From a Benesi-Hildebrand plot a $K_{11}$ binding constant was calculated to be $1.8 \times 10^4 \text{ M}^{-1}$ (HypSpec log $K_{11} = 4.4$).

**Figure 166.** Ca(OAc)$_2$ isotherm and fluorescence titration with probe 3.8.

($\lambda_{ex}$ 408 nm) Titration carried out in 100% DMSO. [3.8] = $1.56 \times 10^{-5}$ M [Ca(OAc)$_2$] = $3.11 \times 10^{-4}$ M (equivalences used: 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.5, 3.0, 4.0, and 5.0).
Unique to probe 3.8, the addition of the Hg$^{2+}$ ion induced an increase in the emission intensity of the initial maximum emission (Figure 167). The increase in emission intensity was accompanied by a 28 nm blue shift. From the isotherm provided in Figure 3.106 the plateau and decrease in emission intensity after two equivalents indicates that the probe 3.8 coordination of the Hg$^{2+}$ ion occurs in a 1:2 (L:M) ratio [3.8(Hg)$_2$]. From HypSpec a log $K_{11}$ binding constant was calculated to be 6.5.
Figure 167. Hg(OAc)$_2$ isotherm and fluorescence titration with probe 3.8.

$\lambda_{ex}$ 408 nm) Titration carried out in 100% DMSO. [3.8] = 1.56 × 10$^{-5}$ M [Hg(OAc)$_2$] = 3.11 × 10$^{-4}$ M (equivalences used: 0.0-2.0 by increments of 0.1, 2.5, 3.0, 4.0, and 5.0).

The addition of Zn(OAc)$_2$ to probe 3.8 (Figure 168) induced a similar response as observed with probes 3.6, 3.7, and 2.16. The ESIPT inhibition as a result of the
coordination of the Zn\(^{2+}\) ion resulted in a 61 nm blue shift and an increase in the emission intensity. From a Benesi-Hildebrand plot, a \(K_{11}\) binding constant was calculated to be \(2.3 \times 10^4\) M\(^{-1}\).

Figure 168. Zn(OAc)\(_2\) isotherm and fluorescence titration with probe 3.8.

\(\lambda_{ex} 408\) nm Titration carried out in 100% DMSO. [3.8] = 1.56 \times 10^{-5} M [Zn(OAc)\(_2\)] = 3.11 \times 10^{-4} M\) (equivalences used: 0.0-2.0 by increments of 0.1, 2.5, 3.0, 4.0, and 5.0).
Upon the addition of the Cd$^{2+}$ ions, the ESIPT of probe 3.8 was inhibited Figure 169. As in the case with Ca$^{2+}$ ions, the spectroscopic response observed from the larger Cd$^{2+}$ ion (compared to the Zn$^{2+}$ ion) indicates that the second nitrogen atom of the pyrimidine ring influences the coordination of the metal ions. From a Benesi-Hildebrand plot a $K_{11}$ binding constant was calculated to be $6.8 \times 10^4$ M$^{-1}$ (HypSpec log $K_{11} = 5.7$). HypSpec also provided another binding constant that fit the experimental data log $K_{21} = 9.7$ (Cd(3.8)$_2$).
Figure 169. Cd(OAc)$_2$ isotherm and fluorescence titration with probe 3.8. 

(λ$_{ex}$ 408 nm) Titration carried out in 100% DMSO. [3.8] = 1.56 × 10$^{-5}$ M [Cd(OAc)$_2$] = 3.11 × 10$^{-4}$ M (equivalences used: 0.0-2.0 by increments of 0.1, 2.5, 3.0, 4.0, and 5.0).

To summarize all of the spectroscopic responses observed from the probes in the presence of various metal ions, the changes in emission intensity at 514 nm are listed in
Table 23. A challenge that arises when detecting a specific $d^{10}$ metal ion ($\text{Zn}^{2+}$, $\text{Cd}^{2+}$, and $\text{Hg}^{2+}$ ions) is false positives from other $d^{10}$ metal ions as well as $\text{Mg}^{2+}$ and $\text{Ca}^{2+}$ ions (section 1.1.6). Therefore, with each probe an * is placed next to the $d^{10}$ metal ion that induced the largest change. The negative values indicate that the metal ion produced a decrease in emission intensity of the probe.
Table 23

*The Δ EM at 514 nm by probes 2.15, 2.16, and 3.6-3.8 with metal acetate salts*

<table>
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<tr>
<th>Probes</th>
<th>2.15</th>
<th>2.16</th>
<th>3.6</th>
<th>3.7</th>
<th>3.8</th>
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<td>Na⁺</td>
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<td>-0.048</td>
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<td>Ag⁺</td>
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<td>-0.024</td>
<td>-0.063</td>
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<td>Mn²⁺</td>
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<td>-0.049</td>
<td>-0.006</td>
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<td>0.059</td>
<td>0.003</td>
<td>-0.016</td>
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</tr>
<tr>
<td>Fe²⁺</td>
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<td>-0.028</td>
<td>-0.007</td>
<td>-0.038</td>
<td>0.000</td>
</tr>
<tr>
<td>Pd²⁺</td>
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<td>-0.305</td>
<td>-0.106</td>
<td>-0.368</td>
<td>-0.037</td>
</tr>
<tr>
<td>Co²⁺</td>
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<td>-0.437</td>
<td>-0.105</td>
<td>-0.324</td>
<td>-0.034</td>
</tr>
<tr>
<td>Ni²⁺</td>
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<td>0.110</td>
<td>-0.105</td>
<td>-0.296</td>
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<tr>
<td>Cu²⁺</td>
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<td>-0.367</td>
<td>-0.038</td>
</tr>
<tr>
<td>Mg²⁺</td>
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<td>-0.056</td>
<td>-0.049</td>
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<td>Ca²⁺</td>
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<tr>
<td>Hg²⁺</td>
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<td>0.026</td>
<td>0.095</td>
<td>0.226</td>
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<tr>
<td>Cd²⁺</td>
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<td>0.215</td>
<td>0.961*</td>
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<tr>
<td>Zn²⁺</td>
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<td>0.219*</td>
<td>0.883*</td>
<td>0.566*</td>
<td>0.567</td>
</tr>
</tbody>
</table>

Note: Numerical values are from the two equivalence screenings shown in Figures 132 - 136. The change in emission intensity at 514 nm is calculated from the following equation: (emission intensity at 514 nm with metal ion - emission intensity at 514 nm without metal ion).

The data presented in this section and in section 3.2.7 both indicate that all of the probes produce the largest fluorescence emission intensity increase in the presence of
Zn$^{2+}$ ions regardless of the metal salt used, with the exception of probe 3.8 in the presence of Cd(OAc)$_2$. While the presence of the acetate ion increases the spectroscopic response displayed by each probe towards Zn$^{2+}$ ions, it also results in an emission signal change induced by one of the other four metal ions (Mg$^{2+}$, Ca$^{2+}$, Hg$^{2+}$, and Cd$^{2+}$).

3.2.10 Detection Limits

With the binding ratios determined and the spectroscopic responses recorded, the limit of detection was investigated. First the Zn$^{2+}$ ion detection limit was calculated using the probe 3.8 in DMSO (Figure 170). The following parameters were used in the detection limit calculation three times the standard deviation with n = 20, df= 18, and at a CI= 95% ($t_{\text{value (95%)}} = 2.086$). The probe 3.8 Zn(OAc)$_2$ detection limit was calculated at $1.1 \times 10^{-7}$ mol•dm$^{-3}$, roughly 7.4 ppb Zn$^{2+}$ ions.
Next, probe 3.8 was used to investigate the Cd$^{2+}$ ion detection limit. Initially the Cd(OAc)$_2$ salt was used, but the results were inconsistent and illogical (negative intercepts). This is attributed to the acetate ions not fully deprotonating the enaminone. Therefore an equivalence of TBAOAc was added to the probe 3.8 solution and was
allowed to stir to ensure deprotonation. CdCl$_2$ was then titrated into the deprotonated probe 3.8 solution (Figure 171). The following parameters were used in the detection limit calculation three times the standard deviation with $n = 20$, $df = 18$, and at a CI= 95% ($t_{value (95\%)} = 2.086$). The probe 3.8 Cd$^{2+}$ ions detection limit was calculated to be $1.1 \times 10^{-7}$ mol•dm$^{-3}$, roughly 12.5 ppb Cd$^{2+}$. This value falls within the accepted WHO limit of 4-40 ppb Cd$^{2+}$ ions.$^{42}$
Figure 171. Probe 3.8 Cd$^{2+}$ ions detection limit.

L.o.D. = $1.1 \times 10^{-7}$ mol dm$^{-3}$ (≈ 12.5 ppb of Cd$^{2+}$)
3.3 Summary

The probes 2.15, 2.16, and 3.6-3.8 were synthesized (46 to 82 % yield range, section 3.4) as a novel small library of coumarin-enaminones for the screening and detection of metal ions. Both solid state and solution based techniques were investigated. The crystal structures of the [Zn(2.15)₂] complex (Figure 68) showed that probe 2.15 coordinates the Zn²⁺ ions in a T₄ geometry through the enaminone binding site, which is in agreement with zinc azomethine complexes. The IR spectra (Figure 70 and 71) of the [Zn(2.15)₂] complex supports the crystal structure by the disappearance of the enamine N-H at 3239 cm⁻¹ and the new C-O (1213 cm⁻¹) and Zn-O (557 and 534 cm⁻¹) stretches. The crystal structure of the [Zn(3.8)₂] complex is shown in Figure 69; however, during the crystal growing process the probe 3.8 underwent hydrolysis.

The [Zn(2.15)₂] and [Zn(3.8)₂] complex were investigated using ¹H and ¹³C NMR spectroscopy (Figures 87-92). The analysis of the NMR spectra for both complexes showed that the coordination of the Zn²⁺ ions resulted in the formation of a single isomer as well as downfield shifts of the C(9) and C(10) ¹³C NMR signals.

A Zn(OAc)₂ ¹H NMR titration was investigated with probes 2.16 (Figure 93) and 3.8 (Figure 94). Both NMR titrations show that during the Zn²⁺ ions coordination the first step is the deprotonation of the enamine N-H by free acetate ions in solution (Schemes 66-68). The disappearance of the N-H signal was accompanied by the appearance of the imine C-H signal.

The optical spectroscopic (colorimetric and fluorescence) response towards metal ions was investigated with all five probes (sections 3.2.7-3.2.9). The effect on the
spectroscopic response due to changing solvent system and the excitation wavelength was investigated. It was observed that the response is independent of the excitation wavelength (section 3.2.3). This is attributed to the ESIPT process will predominately emit from the S₁ keto-enamine state, regardless of the excitation wavelength: imine-enol ($\lambda_{ex}$ 339 nm) or the keto-enamine tautomer ($\lambda_{ex}$ 408 nm). Only small changes were observed with the use of a different solvent.

The results from sections 3.2.8 and 3.2.9 show that the acetate ion is necessary to observe large spectroscopic responses. The responses can be seen from metal acetates (sections 3.2.8 and 3.2.9) or from a metal chloride with TBAOAc (Figure 80).

In section 1.1.6 it was discussed that the detection of $d^{10}$ metal ions often suffer from false positives from Mg$^{2+}$, Ca$^{2+}$, and from each other, therefore full titrations were carried out with all five probes with all five metal (Mg$^{2+}$, Ca$^{2+}$, Zn$^{2+}$, Cd$^{2+}$, and Hg$^{2+}$) ions. From the data shown in this chapter, the five probes can be grouped in two groups with one outlier based on their spectroscopic responses: probes (2.15 and 3.7), (2.16 and 3.6), and probe 3.8 as the outlier. The probes 2.15 and 3.7 show smaller responses towards the various metal ions than the other three probes, with the largest response seen with Zn$^{2+}$ ions. The small responses is attributed to probe 2.15 contains no nitrogen atoms in the aromatic ring connected to the enamine; while probe 3.7 does have a nitrogen atom in the pyridine, it is in a poor position (meta). Both probes 2.16 and 3.6 have a nitrogen atom in the pyridine ring like compound 3.7, but showed a larger spectroscopic response to the metal ions. The Zn$^{2+}$ and Cd$^{2+}$ ions gave rise to large responses from both probes, with the former inducing the largest signal change. The increased response from these
two probes is attributed to the nitrogen atom in the pyridine ring is in a good position ortho (3.6) and para (2.16). Finally the outlier probe 3.8 contains a nitrogen atom in both ortho positions. This resulted in a large response from the majority of the metal ions screened; however, probe 3.8 is labeled as an outlier since the largest fluorescence emission intensity increase arose from the addition of Cd$^{2+}$ ions and not Zn$^{2+}$ ions. Detection limits for the Zn$^{2+}$ and Cd$^{2+}$ ions were investigated with probe 3.8 and were calculated to be 7.4 and 12.5 ppb respectively.

Each screening and titration presented in this chapter is the result of a single run. For a more accurate and detailed investigation of the optical spectroscopic response of each probe duplicate experiments are needed. A collaborator is currently investigating the multiple experiments and is applying LDA analysis to interpret the data.

3.4 Experimental Procedure

Following the literature procedures,64,66 the commercially available reagents 2,4,6-trichlorophenol (2.17) and malonic acid (1.11) were refluxed for three hours in phosphorus (V) oxychloride under anhydrous conditions. The reaction was then allowed to cool to room temperature and was quenched with cold deionized water then neutralized with saturated sodium bicarbonate solution. The resulting solid bis-(2,4,6-trichlorophenyl)malonate (1.14) was collected by vacuum filtration and recrystallized from ethyl acetate in a 90 % yield, (Scheme 48). The product was confirmed by the singlet that represented the four aromatic protons at $\delta$ (ppm) 7.53 and the singlet 3.85 ($s, 2H, CH_2$); $^{13}$C NMR $\delta$ (ppm): 168.8, 149.0, 128.6, 123.8, 123.5 (DMSO-$d_6$).
1.14 (9.26 g, 20.0 mmol) and 3-diethylaminophenol (1.13) (3.30 g, 20.0 mmol), and anhydrous toluene (50 mL) were refluxed for three hours. The reaction was allowed to cool to room temperature. The precipitate, 7-(diethylamino)-4-hydroxycoumarin (1.2) was collected by vacuum filtration and washed with toluene (3.50 g, 75 % yield) (Scheme 48). ¹H NMR (300 K, DMSO-d₆, 400 MHz): δ (ppm) 11.91 (s, 1H, OH), 7.55 (d, 1H, J = 9.0 Hz, ArH), 6.65 (dd, 1H, J = 9.0 and 2.2 Hz, ArH), 6.45 (d, 1H, J = 2.2 Hz, ArH), 5.25 (s, 1H, CH), 3.42 (dq, 4H, J = 14.0 and 7.0 Hz, CH₂), 1.11 (t, 6H, J = 7.0, CH₃); ¹³C NMR (300 K, DMSO-d₆, 100 MHz): δ (ppm) 166.9, 163.2, 156.6, 151.3, 124.6, 108.6, 103.9, 98.9, 86.6, 44.4, 12.8.

1.2 (233.3 mg, 1.0 mmol), and aniline (91.1 μL, 0.9997 mmol), and triethyl orthoformate (250 μL, 1.5 mmol) were refluxed in 2-propanol (5 mL) for two hours (Scheme 2.17). The reaction was allowed to cool to room temperature and the resulting solid (2.15) was collected by vacuum filtration then washed with 2-propanol, which gave a yield of 268 mg (0.98 mmol, 80 % yield); ¹H NMR (300 K, CDCl₃, 400 MHz): δ (ppm) 13.65 (d, 1H, J = 12.5 Hz, NH), 8.82 (d, 1H, J = 13.3 Hz, CH enimine), 7.87 (d, 1H, J = 9.0 Hz, CHcoumarin), 7.45 (t, 2H, J = 7.9 Hz, CHaromatic), 7.30 (dt, 3H, J = 15.9 and 7.5 Hz, CHaromatic), 6.59 (dd, 1H, J = 9.0 and 2.4 Hz, CHcoumarin), 6.38 (d, 1H, J = 2.3 Hz, CHcoumarin), 3.45 (q, 4H, J = 7.1 Hz, CH₂), 1.15 (t, 6H, J = 7.1 Hz, CH₃); ¹³C NMR (300 K, CDCl₃, 100 MHz): δ (ppm) 181.0, 164.5, 157.2, 153.5, 152.9, 138.2, 130.8, 127.3, 126.6, 118.0, 108.8, 108.4, 98.1, 97.2, 44.9, 12.9; ESI-MS m/z [M+H]⁺ = 337.0; IR (ATR solid): (cm⁻¹) 3239 (w) vNH, 3059 (w) vC=O(enamine), 2970 (w) vCH, 1716 (s) vCO (δ
lactone), 1571 νCO (ketone); HRMS observed for C_{20}H_{20}N_{2}O_{3} = 336.1484; Calculated for C_{19}H_{19}N_{3}O_{3} = 336.1474. Crystals were grown from slow evaporation in DMSO.

**1.2** (466.5 mg, 2.0 mmol), and 4-aminopyridine (188.1 mg, 2.0 mmol), and triethyl orthoformate (500 μL, 3.0 mmol) were refluxed in 2-propanol (10 mL) overnight (Scheme 2.17). The reaction was allowed to cool to room temperature and the resulting solid (2.16) was collected by vacuum filtration then washed with 2-propanol, which gave a yield of 553.7 mg (1.64 mmol, 82 % yield); ^1H NMR (300 K, DMSO-d$_6$, 400 MHz): δ (ppm) 13.25 (d, J = 12.8 Hz, 1H), 11.46 (d, J = 13.9 Hz, 1H), 8.88 (t, J = 11.3 Hz, 1H), 8.57 (d, J = 5.5 Hz, 2H), 7.74 (t, J = 7.7 Hz, 1H), 7.62 (t, J = 7.7 Hz, 2H), 6.69 (d, J = 7.2 Hz, 1H), 6.43 (d, J = 17.9 Hz, 1H), 3.45 (dd, J = 13.7, 6.7 Hz, 4H), 1.14 (t, J = 6.9 Hz, 6H); ^13C NMR (300 K, CDCl$_3$, 100 MHz): δ (ppm) 181.1, 163.9, 157.3, 153.4, 152.2, 151.6, 145.0, 127.7, 111.8, 108.7, 108.6, 100.1, 97.2, 44.9, 12.5; ESI-MS; m/z for [M+H]$^+$ = 338.2; IR (ATR solid); (cm$^{-1}$) 3059 (w) νC=C(enamine), 2970 (w) νCH, 1716 (s) νCO (δ lactone), 1571 νCO (ketone) cm$^{-1}$; HRMS observed for C$_{19}$H$_{19}$N$_3$O$_3$ = 337.1430; Calculated for C$_{19}$H$_{19}$N$_3$O$_3$ = 337.1426. Crystals were grown from slow evaporation in DMSO.

**1.2** (233.2 mg, 1.00 mmol) was dissolved in 10 mL IPA and was allowed to stir. TEOF (250 μL, 1.50 mmol) and 2-aminopyridine (94.2 mg, 1.00 mmol) were added to the solution. The reaction was allowed to reflux for three hours during which time a bright yellow solid (3.6) precipitated out of solution. The reaction was allowed to cool to slightly above room temperature and the solid was isolated by vacuum filtration (224 mg, 0.66 mmol, 66% yield); ^1H NMR (400 MHz, CDCl$_3$) δ (ppm) 13.57 (d, J = 12.2 Hz),
11.71 (d, J = 14.0 Hz), 9.60 (d, J = 13.6 Hz), 9.51 (d, J = 12.5 Hz), 8.48 – 8.39 (m), 7.95 (d, J = 9.0 Hz), 7.85 (d, J = 9.0 Hz), 7.74 (ddd, J = 9.4, 6.9, 1.8 Hz), 7.20 – 7.11 (m), 7.07 (dd, J = 24.0, 8.1 Hz), 6.56 (dd, J = 9.0, 2.4 Hz), 6.36 (d, J = 2.4 Hz), 3.43 (q, J = 7.1 Hz), 1.23 (t, J = 7.1 Hz); 13C NMR (100 MHz, CDCl3) δ (ppm) 182.2, 181.3, 177.6, 165.9, 164.2, 157.3, 156.9, 153.1, 153.0, 152.8, 151.2, 149.2, 138.8, 128.3, 127.5, 121.2, 113.0, 112.6, 108.7, 108.5, 108.4, 99.3, 97.2, 97.1, 44.9, 12.5; IR (ATR solid); 3060 (w) νNH, 2974 (w) νCH, 1714 (s) νCO (delta lactone), 1562 νCO (ketone) cm⁻¹; HRMS: [M]**: Calc for C19H19N3O3 = 337.1426; found for C19H19N3O3 = 337.1431 and [M-CH3]** Calc for C18H16N3O3 = 323.1192; found for C17H15N4O3 = 323.1197. Crystals were grown from slow evaporation in benzene followed by slow evaporation in CHCl3.

1.2 (233.4 mg, 1.00 mmol) was dissolved in 10 mL IPA and was allowed to stir. TEOF (250 μL, 1.50 mmol) and 3-aminopyridine (94.1 mg, 1.00 mmol) were added to the solution. The reaction was allowed to reflux for three hours during which time a bright yellow solid (3.7) precipitated out of solution. The reaction was allowed to cool to slightly above room temperature and the solid was isolated by vacuum filtration (155.6 mg, 0.46 mmol, 46% yield); 1H NMR (400 MHz, CDCl3) δ (ppm) 13.71 (d, J = 12.6 Hz, 1H), 11.64 (d, J = 13.0 Hz, 1H), 8.92 (d, J = 14.0 Hz, 1H), 8.77 (d, J = 12.9 Hz, 1H), 8.64 (dd, J = 8.0, 2.5 Hz, 1H), 8.51 (d, J = 4.1 Hz, 1H), 7.92 (d, J = 9.0 Hz, 1H), 7.84 (d, J = 9.0 Hz, 1H), 7.67 (t, J = 11.3 Hz, 1H), 7.39 (dd, J = 8.2, 4.7 Hz, 1H), 6.57 (dd, J = 9.0, 2.2 Hz, 1H), 6.35 (d, J = 2.1 Hz, 1H), 3.43 (q, J = 7.0 Hz, 5H), 1.23 (t, J = 7.1 Hz, 7H); 13C NMR (100 MHz, CDCl3) δ (ppm) 181.1, 164.1, 157.3, 157.0, 153.5, 153.2, 152.0, 147.6, 147.5, 140.7, 140.6, 135.0, 128.2, 127.5, 124.6, 124.3, 124.3, 108.7, 108.6, 108.6,
108.5, 99.8, 99.3, 97.2, 77.3, 77.0, 76.7, 44.9, 12.5. IR (ATR solid); 3066 (w) v$_{\text{NH}}$, 2966 (w) v$_{\text{CH}}$, 1711 (s) v$_{\text{CO}}$ (delta lactone), 1608 v$_{\text{CO}}$ (ketone) cm$^{-1}$; HRMS: [M]$^{+}$: Calc for C$_{19}$H$_{19}$N$_3$O$_3$ = 337.1426; found for C$_{19}$H$_{19}$N$_3$O$_3$ = 337.1430 and [M-CH$_3$]$^{+}$ Calc for C$_{18}$H$_{16}$N$_3$O$_3$ = 323.1192; found for C$_{17}$H$_{15}$N$_4$O$_3$ = 323.1191. Crystals were grown from slow evaporation in EtOH.

1.2 (466.5 mg, 2.00 mmol) was dissolved in 20 mL IPA and was allowed to stir. TEOF (500 μL, 3.00 mmol) and 2-aminopyrimidine (190.2 mg, 2.00 mmol) were added to the solution. The reaction was allowed to reflux for four hours during which time a dark orange solid (3.8) precipitated out of solution. The reaction was allowed to cool to slightly above room temperature and the solid was isolated by vacuum filtration (364.4 mg, 1.02 mmol, 51% yield); m.p. 225-227°C. 1H NMR (400 MHz, CDCl$_3$): δ (ppm) 13.20 (d, 1H, J = 12.2 Hz, NH), 11.60 (d, 1H, J = 12.8 Hz, NH), 9.62 (d, 1H, J = 13.6 Hz, CH$_{\text{enamine}}$), 9.47 (d, 1H, J = 12.7 Hz, CH$_{\text{enamine}}$), 8.64 (t, 2H, J = 5.7 Hz, CH$_{\text{aromatic}}$), 7.92 (dd, 1H, J = 24.5, 9.0 Hz, CH$_{\text{coumarin}}$), 7.12 (t, 1H, J = 4.8 Hz, CH$_{\text{aromatic}}$), 6.57 (dd, 1H, J = 9.0, 2.1 Hz, CH$_{\text{coumarin}}$), 6.35 (d, 1H, J = 2.1 Hz, CH$_{\text{coumarin}}$), 3.44 (q, 4H, J = 7.0 Hz, CH$_2$), 1.24 (t, 6H, J = 7.1 Hz, CH$_3$). 13C NMR (300 K, CDCl$_3$, 100 MHz): δ (ppm) 202.8, 181.0, 164.0, 158.7, 158.7, 157.3, 156.7, 153.3, 152.8, 128.5, 128.0, 117.9, 117.9, 108.8, 108.5, 100.8, 97.2, 97.1, 44.9, 12.5. IR (ATR solid); 3062 (w) v$_{\text{NH}}$, 2968 (w) v$_{\text{CH}}$, 1713 (s) v$_{\text{CO}}$ (δ lactone), 1601 and 1551 v$_{\text{CO}}$ (ketone) cm$^{-1}$; HRMS: [M]$^{+}$: Calc for C$_{18}$H$_{18}$N$_4$O$_3$ = 338.1379; found for C$_{18}$H$_{18}$N$_4$O$_3$ = 337.1375 and [M-CH$_3$]$^{+}$ Calc for C$_{17}$H$_{15}$N$_4$O$_3$ = 323.1140; found for C$_{17}$H$_{15}$N$_4$O$_3$ = 323.1154. Crystals were grown from slow evaporation in DMSO.
Following the literature procedures, a methanol solution (10-15 mL) containing 2.15 (149.8 mg, 0.45 mmol) was stirred and brought to a boil. Zn(OAc)₂ (48.8 mg, 0.22 mmol) was added to the solution, which resulted in the cloudy yellow solution turning clear and yellow in color. Within 20 minutes a bright yellow solid [Zn(2.15)₂] formed and precipitated out of solution. The reaction was allowed to boil and stir for an additional three hours adding MeOH as needed. The reaction was allowed to cool to room temperature, and the yellow solid was removed by vacuum filtration. While under vacuum the solid was cleaned with fresh MeOH, yield 85.9 mg. m.p. 277-278°C. Crystals were grown from slow evaporation in CHCl₃ and toluene.

A methanol solution (10-15 mL) containing 3.8 (200 mg, 0.59 mmol) was stirred and brought to a boil. Zn(OAc)₂ (129.7 mg, 0.59 mmol) was added to the solution, which resulted in the cloudy orange solution turning clear and yellow in color. Within 20 minutes a bright yellow solid [Zn(3.8)₂] formed and precipitated out of solution. The reaction was allowed to boil and stir for an additional three hours adding MeOH as needed. The reaction was allowed to cool to room temperature and the yellow solid was removed by vacuum filtration. While under vacuum the solid was cleaned with fresh MeOH, yield 100.2 mg. m.p. 322-327°C. Crystals were grown from slow evaporation in DMSO.

Stock solutions for titrations: Probe 2.15 (2.1 mg, 6.24 × 10⁻⁶ mol) was dissolved in 20.059 mL DMSO to give a stock solution concentration of 0.31 mM. Probes 2.16, 3.6, and 3.7, (2.1 mg, 6.22 × 10⁻⁶ mol) was dissolved in 20 mL DMSO to give a stock solution concentration of 0.31 mM. Probe 3.8 (2.2 mg, 6.50 × 10⁻⁶ mol) was dissolved in 20.8911
mL DMSO to give a stock solution concentration of 0.31 mM. All metal chloride and acetate salt solutions were made in DMSO with a stock concentration of 0.31 mM.

For UV-Vis studies, 50 μL of the probe was added to a 4 mL quartz cuvette and diluted to a volume of 1 mL with fresh DMSO to give a final working concentration of 15.6 μM. Full titrations were carried out by adding 5 μL (0.1 equivalence) of the metal salt to the probe solution. The spectrum was recorded after each addition up to two equivalents. From two to five equivalences 25 μL aliquots (0.5 equivalence) of the metal salt was added. The absorbance spectra were recorded over the range of 290-560 nm.

For fluorescence studies 100 μL of the probe was added to a 4 mL quartz cuvette and diluted to a volume of 2 mL with fresh DMSO to give a final working concentration of 15.6 μM. Full titrations were carried out by adding 10 μL (0.1 equivalence) of the metal salt to the probe solution. The spectrum was recorded after each addition up to two equivalents. From two to five equivalences 50 μL aliquots (0.5 equivalence) of the metal salt was added. For λ_{ex} 408 nm the slit widths were kept at 0.3 mm and the emission spectrum was recorded over the range of 420-780 nm. For λ_{ex} 339 nm the slit widths were kept at 0.3 mm and the emission spectrum was recorded over the range of 345-665 nm.
CHAPTER IV – CONCLUSION

A small library of coumarin-enaminone molecular probes was designed for the detection of NC\(^-\), Zn\(^{2+}\), Cd\(^{2+}\), and Hg\(^{2+}\) ions through ESIPT inhibition and CHEF. From the common coumarin intermediate (compound 1.2) the probes 2.15, 2.16, and 3.6-3.8 were synthesized in good yields (46 to 82 % yield range). The crystal structures for all five probes were solved, all in the keto-enamine tautomer. The ESIPT process was confirmed through theoretical calculations shown in Chapter II.

In Chapter II, the probes 2.15 and 2.16 were used for the detection of NC\(^-\) ions. It was determined by molecular modeling, mass spectrometry, and both 1D and 2D NMR spectroscopic experiments that both probes are detecting cyanide via a Michael addition to the C(9) carbon. The nucleophilic attack from cyanide breaks the conjugation of both probes and fully inhibits the ESIPT process. The conversion from the free probes to their respective adducts produces a ratiometric response in both the absorption and emission spectra. Cyanide ions induced a 70 nm blue shift absorbance spectrum along with decolorization of the probe solution as well as a 219 nm blue shift in the emission spectrum. Each probe showed a 1:1 reaction ratio with NC\(^-\) ions. The sensitivity of both probes was determined with detection limits of ppb with probe 2.15 and ppb with probe 2.16.

Of the other anions screened the F\(^-\) and OAc\(^-\) ions produced a significant spectroscopic response, which was attributed to the deprotonation of the enamine N-H. The deprotonation of the enamine was the key factor in the spectroscopic responses observed in Chapter III.
The five probes (2.15, 2.16, and 3.6-3.8) were screened against various metal chlorides. The largest emission response was seen upon the addition of the Zn$^{2+}$ ions. When comparing the responses observed from the metal chlorides and acetates it is noticed that the spectroscopic signal changes are smaller with the metal chlorides. This is attributed to the fact that the chloride ion is not strong enough to deprotonate the enamine N-H and therefore the coordination of a metal chloride does not significantly perturb the ESIPT process.

For the metal acetate salts, probes 2.15, 2.16, 3.6, and 3.7 gave the largest response in the presence of Zn$^{2+}$ ions, while probe 3.8 produced the largest response with Cd$^{2+}$ ions. The results shown in Chapter II along with Zn(OAc)$_2$ $^1$H NMR titrations, it was determined that the first step in metal ion coordination at the enaminone binding site required the deprotonation of the enamine N-H by the acetate ions. Probe 3.8 has a detection limit of 7.4 ppb for Zn$^{2+}$ ions and 12.5 ppb for Cd$^{2+}$ ions.

The results from Chapters II and III have created a framework whereby coumarin-enaminone probes can be synthesized and easily derivatized in three steps with high yields. This design approach can be used to improve on selectivity, sensitivity, and the ability to work in aqueous solutions.
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345


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