Development of nitrogen protecting groups and synthesis and complexation of bridged bis-tetraazamacrocycles

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UMI
DEVELOPMENT OF NITROGEN PROTECTING GROUPS

AND

SYNTHESIS AND COMPLEXATION OF BRIDGED BIS-
TETRAAZAMACROCYCLES

BY

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M.S., University of New Hampshire, 1999

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THESIS

Submitted to the University of New Hampshire

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in

Chemistry

May, 2010
This thesis has been examined and approved.

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Dedication

For Erin and Rowan.
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Table of Contents

Dedication..........................................................................................................................iii
Acknowledgements............................................................................................................iv
Table of Contents................................................................................................................vi
Lists of Figures ....................................................................................................................ix
Lists of Schemes ..................................................................................................................xii
List of Tables.........................................................................................................................xvi
Abstract..................................................................................................................................xvii

Chapter I – Development of Nitrogen Protecting Groups

I) Introduction.......................................................................................................................1

II) Background.....................................................................................................................15

III) Results and Discussion................................................................................................36

A) Development of the N-allyl Protecting Group .............................................................37

   (1) Synthesis of di-NH cross-bridged cyclam 7 by way of deallylation.........................37

   (2) Synthesis of mono-benzyl cross-bridged cyclen 32 via deallylation.........................40

   (3) Synthesis of mono-benzyl cross-bridged cyclam 55..................................................48

B) Development of the N-PMB Protecting Group .............................................................51

   (1) Synthesis of di-NH cross-bridged cyclen 24 via the use of the N-PMB protecting group ..........................................................................................................................51

   (2) Synthesis of mono-benzyl cross-bridged cyclen 32 via the use of a N-PMB protecting group ..........................................................................................................................54

vi
(3) Progress toward the synthesis of di-NH cross-bridged cyclam 7 via the use of N-PMB protecting groups ..............................................56

C) Investigation of the N-Nap Protecting Group for Cross-bridged Tetraamines.................................................................58

(1) Progress toward the synthesis of mono-benzyl cross-bridged cyclen 33 via the use of an N-Nap protecting group .........................58

(2) Synthesis of di-Nap cross-bridged cyclen and cyclam (74 and 75) ....59

(3) Attempted synthesis of di-NH cross-bridged cyclam 7 via the use of an N-Nap protecting group ..................................................60

D) Synthetic Utility of the Mono-Benzyl Cross-bridged Cyclam 33 ..........61

(1) Synthesis of mono-benzyl mono-octyl cross-bridged cyclam 76 ......62

IV) Conclusions.................................................................................63

Chapter II – Synthesis and Complexation of Bridged Bis-tetraazamacrocycles

I) Introduction....................................................................................66

II) Background...................................................................................73

III) Results And Discussion.................................................................81

A) The Complexation of p-Xylyl-linked Adjacent-bridged Cyclam 102a with Copper(II).................................................................82

B) The Complexation of p-Xylyl-linked Cross-bridged Cyclen 94a and m-Xylyl-linked Cross-bridged Cyclen 94b with Copper(II)........85

(1) The complexation of p-xylyl-linked cross-bridged cyclen 94a with copper(II)...........................................................................85

(2) Discussion of the kinetic and electrochemical analyses of copper(II) complexes of 94a and 102a..............................................90

(3) The complexation of m-xylyl-linked cross-bridged cyclen 94b with copper(II).................................................................93
C) The Complexation of \( p \)-Xylyl-linked Cross-bridged Cyclen \( 94a \) and \( m \)-Xylyl-linked Cross-bridged Cyclen \( 94b \) with Zinc(II)...

(1) The complexation of \( p \)-xylyl-linked cross-bridged cyclen \( 94a \) with Zinc(II)...

(2) Progress toward the synthesis of a mononuclear Zn(II) • \( p \)-xylyl-linked cross-bridged cyclen \( 94a \) complex...

(3) The complexation of \( m \)-xylyl-linked cross-bridged cyclen \( 94b \) with Zinc(II)...

D) Synthesis of Crude Diamide \( p \)-Xylyl-linked Cross-bridged Cyclen 105...

E) Further Application of the N-Allyl Protecting Group – Synthesis of \( p \)-Xylyl-linked Cross-bridged Cyclen 31...

(1) Synthesis of N,N'-di-allyl \( p \)-xylyl-linked cross-bridged cyclen 107...

(2) Synthesis of \( p \)-xylyl-linked cross-bridged cyclen 31...

F) Progress toward the synthesis of the cross-bridged bis-cyclam 110...

G) Investigation of an aliphatic spacer group...

IV) Conclusions...

Chapter III – Experimental Section

I) General Methods...

II) Solvents...

III) Reagents...

IV) Syntheses...

Appendix

A) Spectral Index...

B) Compound Index...

List of References...
### List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Tetraazamacrocycles of interest to the Weisman/Wong research group: Cyclen 1, Cyclam 2 and Homocyclen 3</td>
<td>1</td>
</tr>
<tr>
<td>1.2</td>
<td>The adjacent- and cross-bridged tetraazamacrocycles 4 and 5, respectively</td>
<td>2</td>
</tr>
<tr>
<td>1.3</td>
<td>A conformation of 5b, where all four nitrogen lone pairs are convergent on a cavity and 5b complexed to a metal forming a diamond-lattice conformation that is stable and kinetically inert</td>
<td>3</td>
</tr>
<tr>
<td>1.4</td>
<td>Six of the nine configurations of metallo-cyclam complexes</td>
<td>3</td>
</tr>
<tr>
<td>1.5</td>
<td>A schematic of a radiopharmaceutical, which includes a bifunctional chelator (BFC) that is complexed to a radioactive metal ion and is also attached to a biologically-active targeting molecule</td>
<td>7</td>
</tr>
<tr>
<td>1.6</td>
<td>Ligands 6-10, the $^{64}$Cu complexes of these ligands have been tested for their in vivo stabilities</td>
<td>8</td>
</tr>
<tr>
<td>1.7</td>
<td>Ligands 11 and 12, the $^{64}$Cu complexes of these ligands have been tested for their in vivo stabilities</td>
<td>9</td>
</tr>
<tr>
<td>1.8</td>
<td>$^{64}$Cu complexes of ligands 13-17 have been tested for their in vivo stabilities</td>
<td>12</td>
</tr>
<tr>
<td>1.9</td>
<td>Compounds 27 and 28, with functional groups incorporated within the ligand framework</td>
<td>22</td>
</tr>
<tr>
<td>1.10</td>
<td>Compounds 29 and 30, where functional groups have been incorporated into the ligand framework for linking to biologically active target molecules of the radiopharmaceutical. “R” is the functional group that would be linked to the targeting molecule</td>
<td>22</td>
</tr>
<tr>
<td>1.11</td>
<td>The di-NH p-xylyl-linked cross-bridged cyclen, 31</td>
<td>23</td>
</tr>
<tr>
<td>1.12</td>
<td>(A): Hyperconjugative effects that may be responsible for the increased basicity of the nitrogen adjacent to the quaternary nitrogen, which has its lone pair placed within the cleft of the bisaminal for cyclam and cyclen bisaminals (cyclam is shown); (B): Empirical evidence of this increased basicity, demonstrated in an X-ray crystal structure of the mono-methylated cyclam bisaminal salt</td>
<td>44</td>
</tr>
</tbody>
</table>
Figure 1.13 An X-ray crystal structure of the BPh₄⁺ salt of protonated mono-allyl mono-benzyl cross-bridged cyclen 62..............................................................47

1.14 An X-ray crystal structure of the BPh₄⁺ salt of protonated mono-allyl mono-benzyl cross-bridged cyclam 55..............................................................51

2.1 The adjacent- and cross-bridged tetraazamacrocycles 4 and 5, respectively, and cross-bridged (79a and 80a) and adjacent-bridged (79b and 80b) bis-tetraazabicycles.............................................67

2.2 The bicyclam 81 (AMD3100), a stem cell mobilizer and anti-HIV agent.....68

2.3 Pendant-armed para- and meta-xylyl-linked cross-bridged systems (82-83) synthesized by Handel...............................................................70

2.4 Bis-cyclam complexes, with a variety of spacers, tested for their redox behavior.................................................................71

2.5 A bicyclen complex utilized for DNA binding..................................71

2.6 The meso and d/l stereoisomers of 90-91 and their time averaged symmetry..................................................74

2.7 The meso and d/l stereoisomers of 95a and its time-averaged symmetry.....75

2.8 Time-averaged symmetry (C₂ᵥ) of 94a...........................................75

2.9 Possible oligomer (96) and dialkyl (97) byproducts in the synthesis of 90 and 91..................................................76

2.10 The electronic spectrum of Cu₂ • 102a in 80% ethanol, where λ_max = 614 nm (Abs = 1.273). .................................................................82

2.11 (A) The electronic spectra and (B) the first order kinetics plot of the acid-decomplexation of (CuCl₂)₂(H₂O)₄ • 102a in 5M HCl at 50 °C. .........................83

2.12 The (A) cyclic voltammogram and (B) DVP of (CuCl₂)₂(H₂O)₄ • 102a (0.1 M NaOAc, pH=7). .................................................................84

2.13 The electronic spectrum of Cu₂ • 94a, in MeCN, where λ_max = 632 nm (Abs = 1.748). .................................................................86

2.14 Di-benzyl cross-bridged cyclen (22), which was complexed to Cu(II) by Weijun Niu.................................................................86
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.15</td>
<td>An X-ray crystal structure of ([\text{Cu(CH}_3\text{CN})(\text{ClO}_4)\text{]}_2 \cdot 94\text{a} (\text{CH}_3\text{OH})).</td>
<td>87</td>
</tr>
<tr>
<td>2.16</td>
<td>(A) The electronic spectra and (B) the first order kinetics plot of the acid-decomplexation of ([\text{Cu(CH}_3\text{CN})(\text{ClO}_4)\text{]}_2 \cdot 94\text{a} (\text{CH}_3\text{OH})) in 1M HCl at 30 °C.</td>
<td>88</td>
</tr>
<tr>
<td>2.17</td>
<td>The (A) cyclic voltammogram and (B) DVP of ([\text{Cu(CH}_3\text{CN})(\text{ClO}_4)\text{]}_2 \cdot 94\text{a} (\text{CH}_3\text{OH}) (0.1 \text{ M NaOAc, pH}=7)).</td>
<td>89</td>
</tr>
<tr>
<td>2.18</td>
<td>The structure of adjacent-bridged cyclam 103.</td>
<td>92</td>
</tr>
<tr>
<td>2.19</td>
<td>The electronic spectrum of Cu(<em>2 \cdot 94\text{b},) in MeOH, where (\lambda</em>{\text{max}} = 658 \text{ nm} (\text{Abs} = 0.895).)</td>
<td>94</td>
</tr>
<tr>
<td>2.20</td>
<td>A comparison of the (^1\text{H NMR spectra}) of the complexed ligand (Zn(_2 \cdot 94\text{a},) bottom), the free ligand in D(_2)O solvent (94\text{a}, middle), and the crude product from the attempted synthesis of the mononuclear Zn(II) complex (top).</td>
<td>96</td>
</tr>
<tr>
<td>2.21</td>
<td>The meso and d/l stereoisomers of 95\text{a} and the time averaged symmetry of 95\text{a} and 31.</td>
<td>100</td>
</tr>
<tr>
<td>2.22</td>
<td>The (^1\text{H NMR spectra comparing the starting material (91\text{a, upper spectrum}) with the allylated crude product (91\text{a, 108 and the mono-allylated intermediate, lower spectrum). The &quot;**&quot; is used to identify the (\beta)-methylene resonances in the product 108 (and possibly the mono-allylated intermediate).}</td>
<td>107</td>
</tr>
<tr>
<td>2.23</td>
<td>The bis-cyclam 81.</td>
<td>109</td>
</tr>
</tbody>
</table>
List of Schemes

<table>
<thead>
<tr>
<th>Scheme</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>The overall synthesis of di-NH cyclen, 24, and di-NH cyclam, 7</td>
<td>15</td>
</tr>
<tr>
<td>1.2</td>
<td>The regioselective process involved in the di-alkylation of 19</td>
<td>16</td>
</tr>
<tr>
<td>1.3</td>
<td>The general synthetic design for functionalizing cross-bridged</td>
<td></td>
</tr>
<tr>
<td></td>
<td>tetraazamacrocycles, such as 7, with two different pendant arms – employing</td>
<td></td>
</tr>
<tr>
<td></td>
<td>orthogonal protecting groups</td>
<td>19</td>
</tr>
<tr>
<td>1.4</td>
<td>The general synthetic design for functionalizing a bisaminal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>tetraazamacrocycle with two different pendant arms – relying on selective</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mono-functionalization of the cross-bridged di-NH intermediate</td>
<td>21</td>
</tr>
<tr>
<td>1.5</td>
<td>The synthetic utility of the mono-benzyl cross-bridged cyclen 32 and cyclam</td>
<td></td>
</tr>
<tr>
<td></td>
<td>33</td>
<td>25</td>
</tr>
<tr>
<td>1.6</td>
<td>Hill’s synthesis of the mono-methyl cross-bridged cyclen 45 and cyclam</td>
<td></td>
</tr>
<tr>
<td></td>
<td>46</td>
<td>26</td>
</tr>
<tr>
<td>1.7</td>
<td>Reed’s attempted synthesis of mono-benzyl cross-bridged cyclam 33</td>
<td>27</td>
</tr>
<tr>
<td>1.8</td>
<td>The synthetic design for the synthesis of the mono-t-butyl ester cross-bridged</td>
<td></td>
</tr>
<tr>
<td></td>
<td>cyclen 50</td>
<td>28</td>
</tr>
<tr>
<td>1.9</td>
<td>The two synthetic routes used in the attempted synthesis of the mono-t-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>butylcarboxymethyl mono-benzyl cyclam bisaminal salt 52</td>
<td>29</td>
</tr>
<tr>
<td>1.10</td>
<td>The proposed synthesis of the mono-t-butylcarboxymethyl cross-bridged</td>
<td></td>
</tr>
<tr>
<td></td>
<td>cyclam 53</td>
<td>29</td>
</tr>
<tr>
<td>1.11</td>
<td>The proposed synthesis of the mono-benzyl cross-bridged cyclam/cyclen</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(32/33) when employing an orthogonal protecting group</td>
<td>31</td>
</tr>
<tr>
<td>1.12</td>
<td>Bist’s synthesis of mono-allyl mono-benzyl cross-bridged cyclam, 55</td>
<td>31</td>
</tr>
<tr>
<td>1.13</td>
<td>Bist’s unsuccessful attempt at the deallylation of 55 using Pd(PPh₃)₄</td>
<td>32</td>
</tr>
<tr>
<td>1.14</td>
<td>Bist’s unsuccessful attempt at deallylation of 55, utilizing slightly basic pH</td>
<td></td>
</tr>
<tr>
<td></td>
<td>conditions</td>
<td>32</td>
</tr>
<tr>
<td>Scheme</td>
<td>Page</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>1.15</td>
<td>Bist’s unsuccessful attempt at deallylation of 55 using t-butoxide in DMSO</td>
<td>33</td>
</tr>
<tr>
<td>1.16</td>
<td>Widger’s unsuccessful attempt at deallylation using Pd(PPh₃)₄ and sodium p-toluene sulfinate</td>
<td>33</td>
</tr>
<tr>
<td>1.17</td>
<td>Deallylation conditions employed by David Martin</td>
<td>34</td>
</tr>
<tr>
<td>1.18</td>
<td>The model system and protecting groups that were chosen for investigation</td>
<td>36</td>
</tr>
<tr>
<td>1.19</td>
<td>Synthesis of di-allyl cross-bridged cyclam 60, developed by Fagan</td>
<td>37</td>
</tr>
<tr>
<td>1.20</td>
<td>Initial attempts at deallylation, employing microwave heating</td>
<td>38</td>
</tr>
<tr>
<td>1.21</td>
<td>The mechanism for the formation of the enamine, which then hydrolyzes in the presence of acid to complete the N-deallylation</td>
<td>39</td>
</tr>
<tr>
<td>1.22</td>
<td>Conditions developed for deallylation of diallyl cross-bridged cyclam 60</td>
<td>40</td>
</tr>
<tr>
<td>1.23</td>
<td>Synthesis of mono-allyl mono-benzyl cross-bridged cyclen 62</td>
<td>41</td>
</tr>
<tr>
<td>1.24</td>
<td>A small amount of deallylation of mono-allyl mono-benzyl bisaminal salt (54, 61) occurs, followed by reduction – thus yielding adjacent-bridged side-product (63, 64)</td>
<td>42</td>
</tr>
<tr>
<td>1.25</td>
<td>The mechanism for reductive ring opening of the di-alkylated cyclen/cyclam bisaminal salt to form the cross-bridged ligand</td>
<td>43</td>
</tr>
<tr>
<td>1.26</td>
<td>The mechanism for reductive ring opening of the mono-alkylated cyclen/cyclam bisaminal salt to form the adjacent-bridged ligand</td>
<td>43</td>
</tr>
<tr>
<td>1.27</td>
<td>Synthesis of mono-benzyl cross-bridged cyclen 32</td>
<td>48</td>
</tr>
<tr>
<td>1.28</td>
<td>Synthesis of mono-allyl mono-benzyl cross-bridged cyclam 55</td>
<td>49</td>
</tr>
<tr>
<td>1.29</td>
<td>Synthesis of mono-benzyl cross-bridged cyclam 33</td>
<td>50</td>
</tr>
<tr>
<td>1.30</td>
<td>Synthesis of di-PMB cross-bridged cyclen 66</td>
<td>52</td>
</tr>
<tr>
<td>1.31</td>
<td>Synthesis of di-NH cross-bridged cyclen 24 via cleavage of the N-PMB bond</td>
<td>53</td>
</tr>
<tr>
<td>1.32</td>
<td>The accepted mechanism for cleavage of the N-PMB bond in TFA</td>
<td>53</td>
</tr>
</tbody>
</table>
Scheme Page
1.33 Synthesis of mono-benzyl mono-PMB cross-bridged cyclen 68. ..................54
1.34 Synthesis of mono-benzyl cross-bridged cyclen 32. .............................55
1.35 The di-allyl (60) and di-benzyl (23) cross-bridged cyclams proved to be unreactive when exposed to the dealkylation conditions used to remove the PMB protecting group. .................................................................56
1.36 The attempted synthesis of the di-PMB cyclam bisaminal salt 69. ............56
1.37 Synthesis of mono-benzyl mono-Nap cross-bridged cyclen 71. .................59
1.38 Syntheses of di-Nap cross-bridged cyclen and cyclam (74 and 75). ..........60
1.39 Attempted synthesis of di-NH cross-bridged cyclam 7, via the oxidative cleavage of the N-Nap bond. .................................................................61
1.40 Synthesis of mono-benzyl mono-octyl cross-bridged cyclam 76, via reductive amination. .......................................................................................62
1.41 Summary of the protecting group work and the potential use of the mono-benzyl cross-bridged system. .................................................................63
1.42 Work done by Stigers to synthesize 78. .................................................63
1.43 The three alternate methods now available for the synthesis of cross-bridged ligands – with sensitivity to acid, base, or hydrogenolysis conditions.........64
1.44 Martin’s utilization of the N-allyl protecting group in synthesizing 57.........65
2.1 Synthesis of the di-methyl para- and meta-xylyl-linked cross-bridged bis-tetraazamacrocycles............................................................................73
2.2 Proposed side reactions – yielding 99 and 100. ......................................77
2.3 Synthesis of the adjacent-bridged bis-tetraazamacrocycles 101 and 102. .....78
2.4 Jolly’s Complexation of 94a/b with 2 Li(ClO₄)........................................79
2.5 Jolly’s Complexation of 94a/b with 2 Zn(CH₃CO₂) • 2H₂O......................79
2.6 Complexation of p-xylyl-linked adjacent-bridged cyclam 102a with Cu(II)...82
2.7 Complexation of p-xylyl-linked cross-bridged cyclen 94a with Cu(II)........86
<table>
<thead>
<tr>
<th>Scheme</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.8</td>
<td>Complexation of <em>m</em>-xylyl-linked cross-bridged cyclen 94b with Cu(II)......93</td>
</tr>
<tr>
<td>2.9</td>
<td>Complexation of <em>p</em>-xylyl-linked cross-bridged cyclen 94a with Zn(II)......94</td>
</tr>
<tr>
<td>2.10</td>
<td>The attempted complexation of <em>p</em>-xylyl-linked cross-bridged cyclen 94a with one equivalent of Zn(II).................................................................96</td>
</tr>
<tr>
<td>2.11</td>
<td>Complexation of <em>m</em>-xylyl-linked cross-bridged cyclen 94b with Zn(II).......97</td>
</tr>
<tr>
<td>2.12</td>
<td>Overall synthesis of diamide <em>p</em>-xylyl-linked cross-bridged cyclen 105.......98</td>
</tr>
<tr>
<td>2.13</td>
<td>Overall synthesis of <em>di</em>-allyl <em>p</em>-xylyl-linked cross-bridged cyclen 107........101</td>
</tr>
<tr>
<td>2.14</td>
<td>Synthesis of di-NH <em>p</em>-xylyl-linked cross-bridged cyclen 31......................101</td>
</tr>
<tr>
<td>2.15</td>
<td>The proposed synthesis of di-NH <em>p</em>-xylyl-linked cross-bridged cyclam 110.................................................................104</td>
</tr>
<tr>
<td>2.16</td>
<td>The proposed synthesis of the <em>di</em>-allyl <em>p</em>-xylyl-linked cyclam bisaminal salt 108.................................................................105</td>
</tr>
<tr>
<td>2.17</td>
<td>Synthesis of 108, with the mono-allylated intermediate included.................106</td>
</tr>
<tr>
<td>2.18</td>
<td>The proposed synthesis of 111. ..................................................................110</td>
</tr>
</tbody>
</table>
## List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>The half-lives of Cu(II) complexes of a 8, 11, 16 and 17</td>
<td>13</td>
</tr>
<tr>
<td>2.1</td>
<td>The half-lives of Cu(II) complexes of a variety of bridged and non-bridged tetraazamacrocycles</td>
<td>91</td>
</tr>
<tr>
<td>2.2</td>
<td>Electrochemical analysis of Cu(II) complexes of a variety of bridged and non-bridged tetraazamacrocycles (0.1 M NaOAc, pH=7)</td>
<td>92</td>
</tr>
<tr>
<td>2.3</td>
<td>Chromatographic conditions applied toward the purification of 31</td>
<td>103</td>
</tr>
<tr>
<td>2.4</td>
<td>The reaction solvents and times for the attempted di-allylation of 91a</td>
<td>106</td>
</tr>
<tr>
<td>3.1</td>
<td>The method, incorporating a ramp in power, used for the NaBH₄ MW-assisted reduction of 66</td>
<td>133</td>
</tr>
<tr>
<td>3.2</td>
<td>The method, incorporating a ramp in power, used for the NaBH₄ MW-assisted reduction of 68</td>
<td>136</td>
</tr>
<tr>
<td>3.3</td>
<td>The method, incorporating a ramp in power, used for the NaBH₄ MW-assisted reduction of 105</td>
<td>150</td>
</tr>
</tbody>
</table>
Abstract

Development of Nitrogen Protecting Groups

And

Synthesis and Complexation of Bridged Bis-tetraazamacrocycles

By

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University of New Hampshire, May, 2010

The development of nitrogen-protecting groups that are orthogonal to the N-benzyl group for use in cross-bridged tetraazamacrocycles and the synthesis and complexation of bridged bis-tetraazamacrocycles are presented. The allyl, p-methoxybenzyl (PMB), and 2-naphthylmethyl (Nap) nitrogen-protecting groups were investigated for use on cross-bridged tetraazamacrocycle systems and conditions for deprotection were found for the N-allyl and N-PMB protecting groups. The conditions applied for the cleavage of the N-allyl and N-PMB groups and the systems that these conditions were employed on are illustrated below. This research has demonstrated that the N-allyl and N-PMB protecting groups are applicable to a variety of systems. These protecting groups have the potential to be utilized in the synthesis of novel cross-bridged bis-tetraazamacrocycles where the N-benzyl protecting group may not be applicable. These cross-bridged systems can be further functionalized, in particular the mono-benzyl cross-bridged cyclam system 33, for a
variety of biomedical purposes including positron emission tomography (PET) imaging.

The N-allyl protecting group was also used to synthesize a cross-bridged bis-cyclen 31 that can now be further functionalized for a variety of biomedical purposes. Copper (II) and zinc (II) complexes of 94a and 94b and the diamide cross-bridged bis-tetraazamacrocycle 105 were also synthesized. These bis-tetraazamacrocycle systems may find use as potential anti-HIV agents or for stem-cell mobilization.
CHAPTER I

DEVELOPMENT OF NITROGEN PROTECTING GROUPS

I. Introduction

Polyazamacrocycles, particularly tetraazamacrocycles, are of continuing interest due to their ability to selectively form stable complexes with a variety of metal cations. Macrocyclic systems often exhibit unique properties that can give rise to complexes which are more kinetically inert and thermodynamically stable than their linear, non-cyclic analogs – this increase in stability is called the “macrocyclic effect”.1 With this increase in stability the complexes are able to retain their integrity under a variety of conditions.

Lindoy describes macrocyclic molecules as “compounds that contain at least three donor atoms where the macrocyclic ring consists of a minimum of nine atoms.”2 The tetraazamacrocycles that have been the focus of our research are based on the well-known compounds 1,4,7,10-tetraazacyclododecane (cyclen, 1), 1,4,8,11-tetraazacyclotetradecane (cyclam, 2), and 1,4,7,10-tetraazacyclotridecane (homocyclen, 3) shown in Figure 1.1. These compounds have four donor atoms and 12, 14, and 13 atom ring sizes, respectively.

Figure 1.1 Tetraazamacrocycles of interest to the Weisman/Wong research group: Cyclen 1, Cyclam 2 and Homocyclen 3.
The work performed by Gary Weisman’s research group, in collaboration with Edward Wong’s research group at the University of New Hampshire, has recently focused on the variations of the tetraazabicyclic units 4 and 5, shown below in Figure 1.2. Compounds 4a and 4b, where an ethylene bridge connects the two adjacent nitrogens in the macrocycle, have received attention because of their increased “structural rigidity” and metal ion selectivity over their non-bridged analogues.

Compounds 5a and 5b, in which the two nitrogens located across from each other in the macrocycle are joined by an ethylene bridge, are called “cross-bridged” macrocyclic tetraamines and were first synthesized in the Weisman research lab (5a, where R, R' = Me was first synthesized by Micheloni, et al.). Cross-bridged macrocycles, such as 5a and 5b, are flexible ligands that are able to adopt conformations where all four nitrogen lone pairs are convergent on a cleft or cavity for complexation to metal ions, as represented below in Figure 1.3 (using 5b). These cross-bridged compounds are proven to have an increased degree of basicity. Ligand 5b is able to take on a diamond-lattice conformation when complexed to a metal ion, giving the complex an added degree of stability and inertness (demonstrated below on the right of Figure 1.3). This increased
rigidity and stability is characteristic of the cross-bridged cyclam metal complexes and is of great interest to the Weisman and Wong research groups, as well as to others because of potential biological, redox, and selective metal complexation applications. These cross-bridged ligands form exclusively cis-folded isomers when complexed to metals, as demonstrated below in Figure 1.3.

![Figure 1.3](image)

**Figure 1.3** A conformation of 5b, where all four nitrogen lone pairs are convergent on a cavity and 5b complexed to a metal forming a diamond-lattice conformation that is stable and kinetically inert.

The six configurations of metallo-cyclam complexes are shown below in Figure 1.4. Cross-bridged cyclam is known to form the cis-V coordination exclusively while the adjacent-bridged cyclam is known to prefer the trans coordination of transition metal ions.

![Figure 1.4](image)

**Figure 1.4** Six of the nine possible configurations of metallo-cyclam complexes.
Unlike the cyclam analog, these bridged complexes have the ability to remain in one preferred configuration in solution when complexed to a metal and this can be of great advantage when utilizing them for particular purposes. If one configuration is preferred over others, this can be used to help predict, and hopefully guide, the biological properties of their metal complexes.

Members of the Weisman and Wong research groups have prepared and characterized a variety of cross-bridged copper(II) complexes of 5a and 5b.\textsuperscript{10, 12, 16, 25-29} This research has demonstrated that these ligands are capable of forming stable, inert complexes with copper(II) and has led toward the investigation of these ligands in \textsuperscript{64}Cu-based positron emission tomography (PET) imaging. Molecular imaging can be defined as “the visualization of \textit{in vivo} biological processes at the molecular or cellular level using specific imaging probes”\textsuperscript{30} and it may be used for investigating the effectiveness of drugs, and for the early detection, characterization, and real-time monitoring of disease.\textsuperscript{30-32} Currently, PET is one of the most sensitive molecular imaging techniques – it has the necessary sensitivity to visualize most interactions between physiological targets and ligands such as between neurotransmitters and brain receptors.\textsuperscript{30} Since its inception, molecular probes for PET imaging have been developed and employed for diagnostic clinical studies, studies aimed toward the understanding of human biochemical processes in neurobiology, and in pre-clinical studies where mostly nonhuman primates and rodents are used.\textsuperscript{30} Most recently, PET imaging has assisted in drug development by giving researchers greater insight into the understanding of drug action in the body, helping to establish drug dosage regimens for central nervous system drugs and to assist in developing drug treatment strategies.\textsuperscript{30}
In PET imaging, a pharmacologically-active molecule containing a positron-emitting radionuclide is delivered to the tissue or organ of interest and as this radionuclide decays it ejects a positron from its nucleus. This positron then travels a short distance before being annihilated with an electron, whereupon it releases two gamma rays 180° apart from each other that are captured by the detectors of the PET scanner – this process results in images of the radiotracer’s location within the organism. There are several factors that weigh in choosing which radionuclide to use when designing a radiopharmaceutical. These factors include: the radionuclide’s half-life must allow time for sufficient uptake and decay to provide the appropriate contrast and quality images; the radionuclide emission energies have to be appropriate for proper detection by the equipment; and the availability of the radionuclide. Transition metals (including $^{94m}$Tc, $^{66}$Ga, $^{68}$Ga, $^{86}$Y, $^{90}$Y, $^{45}$Ti, and $^{60/61/62/64}$Cu) have gained interest in recent years as candidate radionuclides due to their increased production and availability. In fact, a great deal of research has been dedicated to copper radionuclides since they offer a wide range of half-lives and positron energies. Also, the coordination chemistry of copper is widely studied and well understood and it has been shown to react with a wide variety of chelator systems that can be linked to biologically-active compounds such as antibodies, proteins, peptides, and other small molecules.

Since the cross-bridged ligands had been demonstrated to form stable, inert complexes with copper(II), a collaboration was initiated with Carolyn Anderson (at the Washington University School of Medicine) to investigate the cross-bridged ligands for use in PET imaging with the $^{64}$Cu radionuclide. Current research is focused on the $^{64}$Cu metal ion since it has potential as both a radionuclide for PET imaging and targeted
radiotherapy due to its half-life ($t_{1/2} = 12.7$ h), decay characteristics ($\beta^+ = 19\%$ and $\beta^- = 40\%$), and the fact that it can be produced on a large scale with high specific activity in a biomedical cyclotron.\textsuperscript{29} When choosing a ligand for use with $^{64}$Cu in PET imaging it is essential that the resulting complex enables high uptake of the copper radionuclide into the targeted tissue or organ and that there is as little non-selective binding or incorporation into other organs and tissues as possible.\textsuperscript{33} Therefore it is crucial that the radio-copper complex remains stable \textit{in vivo}, so that there is no loss of the radionuclide in transit in the body. When measuring the stability of the radio-copper complex \textit{in vivo}, it has been found that the kinetic inertness of the copper complex toward decomplexation is even more important than its thermodynamic stability.\textsuperscript{34} Also, since the reduction of copper(II) to copper(I) may result in a loss of the metal \textit{in vivo}, the resistance of the radio-copper complex toward Cu(II)/Cu(I) reduction and reversibility under biological conditions is an important feature.\textsuperscript{33} It is also important that the radio-copper complex can be formed easily – and so rapid complexation kinetics are desired. Along with these three requirements, the ligand in the radio-copper complex must have an available functional group that enables covalent linking to targeted peptides, proteins, and other biologically-relevant molecules. This ligand would then act as a bifunctional chelator (BFC, shown below in Figure 1.5) – a BFC is a metal complexing ligand which has a functional group incorporated into its structure that can be used for attachment to a biologically-active targeting molecule (e.g., proteins, peptides, etc).
In collaboration with Carolyn Anderson’s research group, the Weisman and Wong research groups have studied the use of various cross-bridged ligands as BFCs. One prominent tetraazamacrocyclic cycle that has been bound to peptides for use in PET imaging is 1,4,8,11-tetraazacyclotetradecane-1,4,8,11-tetraacetic acid (TETA, 6), shown below in Figure 1.6. It has been found that $^{64}\text{Cu}$ was dissociated from the TETA-D-Phe$^1$-octreotide (TETA-OC) bioconjugate in rat liver, *in vivo*, and bound to superoxide dismutase (SOD). Since cross-bridged ligands synthesized in the Weisman and Wong research groups have been shown to form kinetically inert complexes with copper(II), studies were begun with a variety of these cross-bridged ligands to determine their *in vivo* stability. It has been shown that the stability of $^{64}\text{Cu}$–ligand complexes can be used to gauge the stability of the respective BFCs for these complexes – and, with that in mind, the $^{64}\text{Cu}$ complexes of the cross-bridged ligands shown below in Figure 1.6, compounds 7-10, were examined.
Copper(II) complexes of the bridged compounds 7-10 were found to be stable in serum for up to 24 hours. The positively-charged $^{64}\text{Cu}$(II) complexes of ligands, 7, 9, and 10, were found to exhibit rapid uptake into the non-target liver and kidneys, but had slow clearance. In comparison, the charge-neutral $^{64}\text{Cu}$-CB-TE2A 8 complex was found to clear rapidly from all tissues, it showed a greater resistance to transchelation than $^{64}\text{Cu}$-TETA in rat liver metabolism studies, and it had the most rapid clearance through blood, liver, and kidneys in normal rats. It has been shown in the literature that neutral $^{64}\text{Cu}$(II)-azamacrocycles demonstrate better clearance through the body. And so, the fact that the $^{64}\text{Cu}$-CB-TE2A complex had the greatest in vivo clearance of the compounds studied may be attributed to the neutral charge of the complex, due to the presence of the two carboxymethyl pendant arms.

These initial promising results led to the further study of the $^{64}\text{Cu}$-cross-bridged ligand complex for possible use as a PET imaging agent. The UNH research groups, in
collaboration with the WUSM group, then studied the \textit{in vivo} properties of the $^{64}\text{Cu}$-complexed cross-bridged analog to CB-TE2A, called CB-DO2A (11, Figure 1.7), to gain further understanding of the effects of the cross-bridge on the $^{64}\text{Cu}$-tetraazamacrocycle's \textit{in vivo} properties.\textsuperscript{27} It was found that the $^{64}\text{Cu}$-CB-DO2A complex had more favorable \textit{in vivo} clearance and decreased transchelation (to proteins such as SOD) than a non-bridged analog, DOTA (12, Figure 1.7) – which has also been used as a chelator for PET imaging.\textsuperscript{37} These results give further evidence that the addition of the ethylene cross-bridge to the tetraazamacroyclic ligand, and the resulting increased kinetic inertness that this bridge imparts on the $^{64}\text{Cu}$ complex, yields $^{64}\text{Cu}$-labelled complexes that have better \textit{in vivo} characteristics than their non-bridged analogs.

With this initial research having been done on the $^{64}\text{Cu}$–tetraazamacrocycle complexes, the next step was to test the \textit{in vivo} properties of CB-TE2A when it was utilized as a BFC.\textsuperscript{38} The targeting molecule that was used in the BFC for this study was the somatostatin analog octapeptide Tyr$^3$-octreotate (Y3-TATE). The $^{64}\text{Cu}$-CB-TE2A-Y3-TATE radiopharmaceutical was tested and compared to the TETA analog \textit{in vivo} with tumor-bearing rats and these experiments yielded promising results for $^{64}\text{Cu}$-CB-TE2A-Y3-TATE. The results were that the CB-TE2A-based radiopharmaceutical demonstrated
better liver and blood clearance, which gives higher tumor-to-tissue ratios, and it was found to have increased tumor detection sensitivity over the TETA analog. However, it was also determined that $^{64}$Cu-TETA-Y3-TATE demonstrated greater clearance from the kidneys and it is suspected that this difference in uptake may be due to the difference in charge between the two BFCs – the $^{64}$Cu-TETA-Y3-TATE has a (-)1 charge while the $^{64}$Cu-CB-TE2A-Y3-TATE has a (+)1 charge. Based upon available research, it is believed that if the compound were neutral, or even negative in charge, it would clear the kidneys more readily. The above results have focused the efforts of the Weisman and Wong research groups toward the goal of altering the charge of the cross-bridged ligand, and therefore the resulting BFC.

In a further effort to reach a better understanding of $^{64}$Cu-cross-bridged ligand complexes and their in vivo stabilities, the kinetic inertness and electrochemical behavior of both $^{64}$Cu-CB-TE2A and $^{64}$Cu-CB-DO2A were studied and compared to their non-bridged analogs TETA and DOTA, respectively. As stated above, both kinetic inertness and resistance toward Cu$^{II}$/Cu$^{I}$ reduction and reversibility are desired characteristics for $^{64}$Cu-radiolabelled complexes. These characteristics were studied further using acid decomplexation, and both differential-pulse (DPV) and cyclic (CV) voltammetry. As stated above, it has been found that the kinetic inertness of the copper complex toward decomplexation is more important than its thermodynamic stability when considering in vivo stability and that the resistance of the radio-copper complex toward Cu(II)/Cu(I) reduction and reversibility under biological conditions is another important feature since the reduction of copper(II) to copper(I) may result in a loss of the metal in vivo. A greater understanding of the kinetic inertness and Cu$^{II}$/Cu$^{I}$ reduction
characteristics of the copper(II) cross-bridged complexes will give greater insight into their in vivo stability.

It was found that $^{64}$Cu-CB-TE2A was the most resistant to acid decomplexation, which can be correlated with the kinetic inertness of the metal complex. $^{64}$Cu-CB-TE2A also maintained the longest-lived Cu(I) complex – meaning that CB-TE2A is most able to accommodate the dissimilar coordination preferences of both Cu(II) and Cu(I). These results demonstrate that ethylene cross-bridge incorporated into the larger, cyclam tetraazamacrocycle, in addition to the two carboxymethyl pendant arms, yield a copper complex that is more stable toward acid-assisted and reductive decomplexation. The fact that the CB-TE2A was shown to be kinetically inert and stable toward reductive decomplexation, two characteristics that have been shown to demonstrate in vivo stability, $^{33,34}$ compares well with what has been found in the in vivo experiments with this copper(II) complexed ligand.$^{29}$

Other cross-bridged tetraazamacrocycles have also been synthesized and investigated – these ligands, compounds 13-17, are shown below in Figure 1.8.$^{28,40}$ The cross-bridged monoamides, 13-15, were synthesized as model compounds for the peptide-conjugated CB-TE2A.$^{28}$ The same acid decomplexation conditions that were used for the $^{64}$Cu-CB-TE2A complex were employed to determine the kinetic inertness of the $^{64}$Cu complexes of these $^{64}$Cu-cross-bridged monoamide complexes. Unfortunately, the amide arms were hydrolyzed under these harsh conditions and so it was not possible to directly measure the kinetic stabilities of these complexes.
Figure 1.8 $^{64}$Cu complexes of ligands 13-17 have been tested for their in vivo stabilities.\textsuperscript{12, 25}

However, in vivo studies that were performed on the $^{64}$Cu complexes of ligands 13-15 demonstrated that these complexes, like the $^{64}$Cu-CB-TE2A complex, have satisfactory clearance from blood, liver, and bone marrow. These results strongly suggest that $^{64}$Cu complexes of ligands 13-15 share the same improved in vivo stability as the “parent” $^{64}$Cu-CB-TE2A complex, which is greater than that of the $^{64}$Cu-TETA complex.

In addition to the above research to gain deeper insight into the basic backbone of the cross-bridged ligands used, further research was done to explore the influence of the pendant arm length on the observed improved in vivo properties, kinetic inertness, and redox stability of these cross-bridged complexes. Toward that end, new cross-bridged cyclam and cyclen ligands with longer N-carboxyethyl pendant arms (16, 17), and their respective copper(II) complexes, were synthesized and studied.\textsuperscript{25} Lengthening the N-carboxymethyl pendant arm by one methylene group yielded mixed results. Shown below in Table 1.1, the results of the acid decomplexation experiments demonstrated that Cu-CB-TE2A was about as inert as the copper(II) complex of its analog with lengthened N-carboxymethyl pendant arms, 16, and both of these complexes were more stable than
the analogous cross-bridged cyclen complexes – with Cu-17 being more stable than Cu-CB-DO2A.

<table>
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<tr>
<th>Complex</th>
<th>5 M HCl, 90 °C</th>
<th>Half-life</th>
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<tr>
<td>Cu-CB-TE2A</td>
<td>154(6) h</td>
<td></td>
</tr>
<tr>
<td>Cu-CB-DO2A</td>
<td>&lt; 3 min</td>
<td></td>
</tr>
<tr>
<td>Cu-CB-cyclam</td>
<td>11.8(2) min</td>
<td></td>
</tr>
<tr>
<td>Cu-CB-DO2LA</td>
<td>&lt; 3 min</td>
<td></td>
</tr>
<tr>
<td>Cu-CB-TE2LA</td>
<td>~100 h</td>
<td></td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Complex</th>
<th>1 M HCl, 30 °C</th>
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<tbody>
<tr>
<td>Cu-CB-DO2A</td>
<td>4.0(1) h</td>
</tr>
<tr>
<td>Cu-CB-DO2LA</td>
<td>37.2(5) min</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Complex</th>
<th>12 M HCl, 90 °C</th>
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<tbody>
<tr>
<td>Cu-CB-TE2A</td>
<td>1.6(2) h</td>
</tr>
<tr>
<td>Cu-CB-TE2LA</td>
<td>39(5) min</td>
</tr>
</tbody>
</table>

Table 1.1 The half-lives of Cu(II) complexes of 8, 11, 16 and 17.²⁵

In the electrochemical studies this research has found that the cross-bridged cyclam copper(II) complexes of 16 and CB-TE2A gave quasi-reversible electrochemistry, while the cyclen complexes were both irreversible. A reversible reduction is a sign that the ligand is capable of adjusting coordination in a relatively stable manner around Cu(I) and this would imply that the metal is retained within the cleft of the ligand and the complex remains relatively stable on the electrochemical timescale. Unfortunately, it was found that the cross-bridged cyclam complex with lengthened N-carboxyethyl pendent arms, Cu-16, was significantly less resistant toward reduction, making Cu-16 more likely to be susceptible to physiological reductants. Once Cu(II) has been reduced to Cu(I), the resulting complex should not be as kinetically inert, and the copper(I) may dissociate more easily. Consistent with this, the in vivo biodistribution studies demonstrated that the longer-armed ⁶⁴Cu-labelled analogs do not clear the body as well as their carboxymethyl-armed homologs. These results suggest
that the higher Cu(II) reduction potential of the lengthened N-carboxyethyl pendant-armed analogs may make them less optimal candidates for PET imaging agents and that the lower reduction potential of the Cu-CB-TE2A complex may be essential to its superior in vivo performance.

Thus far, CB-TE2A has proven to be the more promising candidate for PET imaging, but further modifications can still be made to improve this ligand. This is a major focus of research that is presently taking place in the Weisman and Wong research labs. Currently, students in the Weisman research group are performing research that includes modifications to the tetraazamacrocycle backbone and the addition of pendant arms beyond the carboxymethyl group. This research is also focused on modifying the basic CB-TE2A ligand such that when complexed to copper(II) a charge neutral BFC can be made, which may improve the resulting radiopharmaceutical’s clearance through the kidneys. Further modifications may also be made to the attached pendant arms, lengthening them to tune the lipophilicity of the ligand – thus modifying the biodistribution of the BFC.

As is demonstrated with the results from the above research, subtle changes to the ligand may have more dramatic effects on the biodistribution, clearance, and other important in vivo features of the BFC. Consequently, the goal to make these changes to the basic CB-TE2A structure has necessitated the development of auxiliary protecting groups that enable greater versatility when designing synthetic pathways and target molecules. The progress that the Weisman group has made in designing the cross-bridged cyclam species and, in particular, the work that has been done to add differentiated pendant arms will be discussed further in the Background section below.
II. Background

The “di-NH” cross-bridged cyclam 7 (Scheme 1.1) is the basic starting material that the Weisman and Wong research groups use to make the most successful of the PET imaging agents that have been discussed thus far. The overall synthesis of 7, as well as the cyclen analog 24, carried out by Mark Rogers and Dan Hill, was first published in 1996 by Weisman et al., and is shown below in Scheme 1.1.¹⁰,⁴¹

In this synthesis, the macrocycle is first condensed with glyoxal to form a cis-fused tetracyclic bisaminal (18, 19), followed by two consecutive regioselective alkylations to yield a bis-quaternary ammonium dibromide salt (20, 21). The final step involves a reductive cleavage through iminium ion intermediates to produce a double ring-expanded N,N'-dibenzyl bicyclic cross-bridged system (22, 23).

The Weisman group has studied the regioselectivity of the above alkylations. While in solution, the bisaminal (18, 19) undergoes a series of four nitrogen inversions
and two ring inversions to yield its enantiomer (this process is called enantiomerization), shown to the left of Scheme 1.2 for 19. The first alkylation to form 25 occurs on a more sterically-accessible exo nitrogen lone pair, which is located on the convex face of the bisaminal. Following this first alkylation the structure is locked, due to the inability of the quaternary nitrogen to invert, and the process of enantiomerization no longer occurs. The second alkylation, generating 26, regioselectively occurs on the other sterically accessible exo nitrogen lone pair located on the convex face of the tetracyclic species across from the quaternary nitrogen. Scheme 1.2 shows this sequence for 19. The process is analogous for 18.

![Scheme 1.2](image)

**Scheme 1.2**  The regioselective process involved in the di-alkylation of 19.

The synthetic route illustrated in Scheme 1.1 has worked well for the Weisman research group for some time, but there has long been interest in finding another protecting group to use in place of, or in addition to, the benzyl group. In recent years the goal of finding a new protecting group has become more of a necessity due to the presence of particular functional groups present either in synthetic intermediates or in current target ligands. In Scheme 1.1, the bisaminal has to be alkylated on both of the nitrogens that are across from each other in the ring in order for the reductive ring opening to yield the cross-bridged ligand. If the bisaminal is only mono-alkylated then the ethylene bridge will remain between the two nitrogens that are adjacent to each other in the macrocycle. This adjacent-bridged mono-benzyl tetraazamacrocycle was first
synthesized in the Weisman lab by Christine Elia (see 4b, Figure 2, where $R = \text{benzyl}$). The mechanism of this reductive ring opening, for both the cross- and adjacent-bridged ligands, will be discussed below in greater depth (see Schemes 1.25-1.26).

As mentioned in the introduction, the Weisman and Wong research groups have made modifications to the cross-bridged cyclam- and cyclen-based ligands in an effort to further fine-tune and optimize the \textit{in vivo} properties of the resulting radiopharmaceuticals they are incorporated into. There are two ways to modify the cross-bridged ligands for further optimization – either by attaching different pendant arms to the nitrogens in the cross-bridged macrocycle or by modifying the macrocycle’s frame itself. The greatest limitation to the modifications that can be made to the tetraazamacrocycle is that whatever new functional groups are incorporated into the ligand must be amenable to the current basic synthetic pathway of dialkylation, followed by reductive ring opening, then deprotection and further functionalization. Any necessary modifications to this basic synthetic strategy (shown in Scheme 1.1) should be reasonably efficient in design – that is to say, yields should be moderate to high and only a minimum number of steps are preferred.

When considering options for adding pendant arms to the cross-bridged ligand, these arms can be added either before or after the reductive ring opening. If a pendant arm is added before the reduction, then this pendant arm must be able to survive the rigors of the reduction (and the corresponding workup) as well as the conditions for debenzylation (if one benzyl is employed) – i.e., the second and third steps shown above in Scheme 1.1. To further complicate this issue, alkylation of the bisaminal depends upon precipitation of the mono- or di-alkylated bisaminal salt from solution as it is
produced. This precipitation of the product drives the reaction toward completion, and without it a mixture of products (most likely consisting of starting material, and mono- and di-alkylated product along with the unreacted alkylating agent) is often obtained.

There are two basic synthetic sequences that can be utilized to generate a ligand that has two different pendant arms. Both pathways necessitate the use of protecting group(s). The first method employs two orthogonal protecting groups (i.e., one protecting group can be removed without removing the other), shown below in Scheme 1.3. In this general synthetic strategy the bisaminal is first mono-alkylated, then alkylated with a different alkylating agent to yield a bisaminal with two separate pendant arms, both of which can survive the conditions used for reductive ring opening (shown in Steps 1-3). One pendant arm is then selectively removed, the mono-alkylated cross-bridge is then functionalized with another pendant arm that could not have survived the conditions used for reductive ring opening and/or the initial deprotection (Step 4-5) and this second mono-alkylated cross-bridge (the product from Step 6) is then further functionalized with another pendant arm that may have complicated the initial synthetic steps (Steps 1-6). The final product would be a cross-bridged ligand that has two different pendant arms, with unique functional groups.
Scheme 1.3 The general synthetic design for functionalizing cross-bridged tetraazamacrocycles, such as 7, with two different pendant arms — employing orthogonal protecting groups.

When employing the synthetic sequence shown in Scheme 1.3 there are several complications that may arise. First, the mono-alkylated bisaminal must not over-alkylate. This is normally achieved by using a solvent that the mono-alkylated bisaminal salt is insoluble in, thus precipitating it from solution and preventing further alkylation. This precipitation of product also serves to drive the alkylation, which may be an equilibrium process, toward product. In addition to this, when the second protecting group is added in Step 2, this dialkylated product must also precipitate from solution. The precipitation of the di-protected bisaminal in Step 2 not only helps to drive the reaction toward product but it also serves to inhibit any de-alkylation and re-alkylation of the product. If any de-alkylation occurs and PG1 is removed in this process, then under the reaction conditions employed in Step 2 it is more likely that any re-alkylation will lead to a di-PG2 armed product rather than the desired mono-PG1 mono-PG2 product. Finally, the protecting
groups employed should have the same basic characteristics of most protecting groups – they must have high-yielding reactions when applied to the molecule as well as when they are clipped – that is to say, it should be efficiently added and removed.

Scheme 1.4, below, shows the synthetic pathway that is employed if two different pendant arms R and R' are added to the basic cross-bridged di-NH ligand (e.g., 7). If the pendant arms were added after the reductive ring opening and de-protection then they would not be limited to functional groups that must survive Steps 1-3. However, this pendant arm R must be one that, upon addition to the cross-bridged species in Step 4, promotes precipitation or through some other manner renders the mono-alkylated species less reactive. If the reaction is not driven toward completion in this manner, then the other secondary nitrogen available in the di-NH cross-bridged macrocycle may also be alkylated. If further alkylation occurs, then this process may yield a mixture of mono- and di-alkylated cross-bridged species, in addition to any unreacted di-NH cross-bridge (i.e. a low-yielding mixture could be obtained). If Step 4 efficiently synthesizes mono-alkylated cross-bridge, then the next step would be to add the second pendant arm to obtain the final product. In other words, this route depends upon the viability of Step 4.
As stated above, there is also the possibility of placing a functional group within the actual backbone of the cross-bridged macrocycle itself — represented by the Y in both Schemes 1.3 and 1.4. If Y is a functional group that is more reactive than methylene (which is more likely than not), then the conditions used for alkylation, reduction, and the protection/deprotection steps must also be compatible with this new functional group. This functional group within the framework of the ligand could be used to link the ligand to the biologically-active targeting molecule of the radiopharmaceutical (see Figure 1.5, above). If this linker is a part of the ligand framework, then it allows for greater freedom in choosing what pendant arms may be attached. Most importantly it would allow both pendant arms to be free for coordination to the copper(II) metal — thus leading to a more kinetically inert metal complex.

Members of the Weisman group have worked on incorporating functional groups within the ligand framework that are capable of linking to the biologically-active targeting molecule of the radiopharmaceutical. Examples of this type of cyclam-based...
ligand that can be used for PET imaging in the literature are shown below in Figure 1.9.45-48

![Figure 1.9 Compounds 27 and 28, with functional groups incorporated within the ligand framework.](image)

The target molecules that Weisman group members have worked on are the dibenzo-annelated cyclam 29 and the β-functionalized 30, both analogs of CB-TE2A – which David Martin (29) and Antoinette Odendaal (30) have made progress on.49,50 These target compounds are shown below in Figure 1.10.

![Figure 1.10 Compounds 29 and 30, where functional groups have been incorporated into the ligand framework for linking to biologically active target molecules of the radiopharmaceutical. “R” is the functional group that would be linked to the targeting molecule.](image)

The dibenzo-annelated cyclam analog of CB-TE2A, 29, is an example of a system where the conditions used for alkylation, reduction, and the protection/
deprotection steps were not compatible with the new functional group present in the ligand. There are two benzylic sites within the ligand framework of 29, labeled with an asterisk "*" in Figure 1.10. If the general synthetic strategy that is shown above in Scheme 1.1 were employed to make 29 then the presence of these benzylic sites (i.e., those labeled with *) would make the debenzylation step a concern since it is expected that there would be little or no differentiation between the benzylic sites within the ring and those that are present in the benzylic pendant arms that would be applied as protecting groups. The dibenzo-annelated system, 29, is a good example of how changing the structure of the cross-bridged ligand framework necessitated the development of an alternative to benzyl protecting group. Following critical work by Jeff Condon and Yijie Peng, David Martin made much progress in further developing the synthesis of 29, but his synthetic method did not involve the steps that are used in Scheme 1.1 – a different reducing agent and other newly-developed methods were utilized. The synthetic scheme used to synthesize the di-NH precursor of 29 will be further discussed below in the Conclusion of this chapter (see section IV, Scheme 1.44).

Another application for a protecting group whose removal will not cleave N-benzylic sites is in the synthesis of the di-NH p-xylyl-linked cross-bridged cyclen, 31 – shown below in Figure 1.11. This compound is of great interest in other areas of this author’s research, and will be discussed further in Chapter 2 of this dissertation.

![Figure 1.11 The di-NH p-xylyl-linked cross-bridged cyclen, 31.](image)
Work has also been done to try to follow the basic design laid out in Scheme 1.3 – where mono-alkylation of the bisaminal was performed, then a second alkylation (the protecting group), followed by a reductive ring opening, and finally removal of the protecting group. This method leaves open a secondary nitrogen on the cross-bridged ligand that can then be functionalized with a variety of groups (beyond the carboxymethyl) capable of linking to the biologically-active targeting molecule of the radiopharmaceutical. Scheme 1.5 illustrates the synthetic utility of this mono-benzyl cross-bridged cyclam/cyclen ligand (32/33). In Reaction (A), two moles of 32/33 could be linked using a di-acid chloride and, following reduction, this linked system (34) could be debenzylated to yield an aliphatically linked di-NH bis-tetraazamacrocycle (as opposed to the para- and meta-xylene linked systems that the Weisman group has synthesized in the past).\textsuperscript{53-55} In Reactions (B) and (C), the “shor-armed” and “long-armed” phosphonate esters (35 and 36, respectively) could be further modified and explored for use as PET imaging agents. Weisman group member Dannon Stigers has made great progress on these phosphonate-armed cross-bridged systems in his doctoral research.\textsuperscript{56} The phosphonate-armed cross-bridged ligands would be capable of balancing the charge of the bifunctional chelator – thus yielding a neutral BFC.
Reaction (A)
1.)
1/2 eq Cl\ conseguência de nenhum
2.) Reduction (LiAlH₄)

Reaction (B)
1.) (CH₂O)ₙ THF
2.) P(O(But)₃)

Reaction (C)
O

Reaction (D)

Reaction (E)

Scheme 1.5 The synthetic utility of mono-benzyl cross-bridged cyclen 32 and cyclam 33.

Reaction (D) alkylates the secondary nitrogen on 32/33 via reductive alkylation. If a long alkyl chain is attached (e.g., R = (CH₂)₈), as with 37, then this will affect the lipophilicity of the BFC – possibly leading to an improvement in the resulting BFC’s in vivo biodistribution. And finally, as shown in Reaction (E), the addition of a t-butoxycarbonyl (BOC) protecting group, to form 38a, would yield a compound that can be debenzylated to give a mono-BOC-protected cross-bridged ligand 38b that can be further
functionalized and subsequently deprotected under acidic conditions. These few examples demonstrate the possible utility of the mono-benzyl cross-bridged ligand.

The methodology used to prepare a mono-alkyl cross-bridged cyclam via a process analogous to what was shown above in the first four steps of Scheme 1.3 was first developed by Dan Hill.\textsuperscript{41} As shown below in Scheme 1.6, Hill synthesized mono-methyl cross-bridged cyclam 45 and cyclen 46 by first mono-benzylating the bisaminal macrocycle (18, 19), then regioselectively methylating the product (39, 40) with methyl iodide.

\begin{align*}
\text{Scheme 1.6 Hill's synthesis of the mono-methyl cross-bridged cyclen 45 and cyclam 46.}^\text{41}
\end{align*}

The penultimate step employed standard reductive ring opening conditions, using NaBH\textsubscript{4}, to yield di-alkylated cross-bridged intermediates (43, 44). Finally, debenzylation of 43 and 44 by way of hydrogenolysis afforded the mono-methylated cross-bridged tetraazamacrocycles (45, 46).
Following Hill’s work, David Reed attempted to prepare the mono-benzylated cross-bridged cyclam 33 by direct monobenzylation of the di-NH cross-bridged cyclam 7. It was hoped that, once 7 was mono-benzylated, the resulting product (33) would be protonated (by the HBr that results from the alkylation) and therefore be less likely to undergo further $S_{N}2$ chemistry with any remaining benzyl bromide in solution. Unfortunately, as seen below in Scheme 1.7, this did not occur and a mixture of unreacted starting material (7), mono-benzylated (33), and di-benzylated (23) products were obtained.

![Scheme 1.7 Reed's attempted synthesis of mono-benzyl cross-bridged cyclam 33.](image)

Scheme 1.7 Reed’s attempted synthesis of mono-benzyl cross-bridged cyclam 33.$^{57}$

Then, in a further effort to have the mono-benzylated product (33) precipitate from solution, Reed altered the reaction conditions to use toluene (a less polar solvent) and ran the reaction at room temperature. In this case, however, Reed obtained a white solid that was found to be 23, the di-benzylated product.

Since the $t$-butylcarboxymethyl pendant arm is of great interest to our research, a year later Jeff Condon began investigating conditions to that would append this group on one of the nitrogens, with a benzyl group on the other non-adjacent nitrogen.$^{51}$ Condon developed the synthesis, shown below in Scheme 1.8, on cyclen. Cyclen, currently the less expensive analog of cyclam, is commonly used to work out the conditions that will be used in our synthetic methods. Unfortunately, Condon ran into problems with the hydrogenolysis and was unable to fully develop the final step to synthesize 50.
Fortunately, Peng was able to determine the proper hydrogenolysis conditions and synthesized 50, and Peng then went on to work on the addition of other pendant arms. Unfortunately, problems were encountered when work was repeated on the cyclam analog.52

Scheme 1.8 The synthetic design for the synthesis of the mono-\(t\)-butyl ester cross-bridged cyclen 50.51

Peng applied two routes to synthesize the mono-\(t\)-butylcarboxymethyl mono-benzyl cyclam bisaminal salt 52. In Route 1 the goal was to alkylate the bisaminal with the \(t\)-butyl bromoacetone first and then benzylate (A in Scheme 1.9, below). While in Route 2 the bisaminal was benzylated first and then the plan was to alkylate with \(t\)-butyl bromoacetone (B in Scheme 1.9, below).41,52 Unfortunately, both routes yielded mixtures of products and were abandoned due to these difficulties.
The next logical step was the synthesis of the mono-\(\tau\)-butylcarboxymethyl cross-bridged cyclam, 53, by alkylating the cross-bridged di-NH cyclam – this synthesis is shown below in Scheme 1.10.

Peng attempted to synthesize the mono-\(\tau\)-butylcarboxymethyl cross-bridged cyclam using conditions typically used in the group to alkylate our cross-bridged ligands and, not unexpectedly, met with a statistical alkylation. The crude product was 45-50% mono-alkylated product (with a mixture of di-alkylated product and unreacted di-NH) and a
39% yield was obtained after purification.\textsuperscript{52} While Peng was able to isolate 53 and perform further research with it, the yield of the reaction is not ideal.

The difficulties that have been encountered in synthesizing a mono-\textit{t}-butylcarboxymethyl cross-bridged cyclam led to even greater interest in the development of another protecting group that can be used as an alternative to, or is complementary to, the N-benzyl group. In searching for a protecting group, there are certain features that are essential in prospective candidates. Not only must the protecting group be efficiently added by alkylation and removed from the ligand, but other conditions must also be met. First, the deprotection conditions employed must not react with any other functional groups on the ligand and, second, the protecting group must survive the rigorous reaction conditions (i.e., the reductive ring opening) used to make the cross-bridged tetraazamacrocycles. Especially important for the synthesis of a mono-alkylated cross-bridged cyclam is that any chemistry involved with this new protecting group must not interfere with the N-benzyl protecting group. That is to say, any additional chemistry used to add or remove the protecting group must not cleave the N-benzyl bond. The general synthesis of the mono-benzyl cross-bridged cyclam (and cyclen) is shown below in Scheme 1.11.
There are many N-protecting groups to choose from in the literature, but one that has been investigated previously by two Weisman group members is the N-allyl group. The reason that the N-allyl protecting group has been chosen is that there are several available routes for removal established in the literature—many of which should leave the N-benzyl group intact. Shanta Bist was the first to investigate the versatile N-allyl protecting group and, toward this end, synthesized the mono-allyl mono-benzyl cross-bridged cyclam—shown below in Scheme 1.12.

The synthesis of 40, first developed by Hill (see Scheme 1.6), is followed by a regioselective allylation in MeCN for 11 days—to yield 54. This salt, 54, is then
reduced using the reductive ring opening conditions previously developed in the Weisman group.\textsuperscript{8} [Note: These conditions for the reductive ring opening of 54 were later found to lead to unwanted side-products – for further information see part A, section 3 in the Results and Discussion section of this chapter] With 55 in hand, Bist then went on to attempt the deallylation of 55. Her first attempt at deallylation was using tetrakis(triphenylphosphine)palladium(0) and morpholine,\textsuperscript{60} shown below in Scheme 1.13. Unfortunately, this did not lead to the deallylation of 55.

![Scheme 1.13](image)

Scheme 1.13 Bist’s unsuccessful attempt at the deallylation of 55 using Pd(PPh$_3)_4$.

The next unsuccessful attempt at deallylation was using hydroxide to promote deallylation from protonated cross-bridged mono-allyl mono-benzyl cyclam, 55. This reaction (along with the proposed electron pushing demonstrating the deallylation) is shown below in Scheme 1.14.

![Scheme 1.14](image)

Scheme 1.14 Bist’s unsuccessful attempt at deallylation of 55, utilizing slightly basic pH conditions.

Bist’s final attempt at deallylation involved the use of $t$-butoxide in DMSO to promote a 1,3-hydrogen shift – yielding an enamine.\textsuperscript{60} This enamine would then be hydrolyzed upon treatment with acid, followed by a basic workup – to give the mono-benzyl cross-bridged cyclam, 33. The overall reaction is shown below in Scheme 1.15.

![Scheme 1.15](image)

Scheme 1.15 Bist’s final attempt at deallylation of 55, utilizing $t$-butoxide in DMSO to promote a 1,3-hydrogen shift – yielding an enamine, which is then hydrolyzed upon treatment with acid, followed by a basic workup to give the mono-benzyl cross-bridged cyclam, 33.
Unfortunately, Bist had only limited success with this method. After running with a “large excess” of t-butoxide at reflux for 12 days only a small amount of what was believed to be the enamine was observed in the $^1$H NMR spectrum. It was determined that most of what was in the crude product was unreacted starting material.

Unfortunately, this reaction was not reproducible when run a second time, and Bist did not have time to further investigate these conditions.

Scheme 1.15 Bist’s unsuccessful attempt at deallylation of 55 using t-butoxide in DMSO.

Peter Widger later attempted a deallylation using tetrakis(triphenylphosphine)-palladium(0) [Pd(PPh$_3$)$_4$], employing reaction conditions that were similar to those used by Bist. However, in this case sodium p-toluenesulfinate was employed as an allyl scavenger rather than morpholine, which had been used by Bist. This reaction is shown below in Scheme 1.16. Unfortunately, some debenzylation also seemed to have occurred under these reaction conditions, and so this method was abandoned.

Scheme 1.16 Widger’s unsuccessful attempt at deallylation using Pd(PPh$_3$)$_4$ and sodium p-toluene sulfinate.

Some years later, David Martin attempted palladium-catalyzed N-deallylation of 56, utilizing a different allyl-scavenger and set of phosphine ligands than those employed
by Bist and Widger\textsuperscript{49} The reaction conditions, shown below in Scheme 1.17, involved the use of 2-mercaptobenzoic acid and tris(dibenzylideneacetone)dipalladium(0) (Pd\textsubscript{2}(dba)\textsubscript{3}) in the presence of 1,4-bis-(diphenylphosphino)butane (dpbb), refluxed in THF for two hours. Martin’s analysis of the crude material by \textsuperscript{1}H NMR demonstrated that a significant degree of deallylation did occur. Unfortunately, this crude reaction mixture was found to contain not just di-NH, but also mono-N-allyl and di-N-allyl species (estimated at 4.7/1.4/1 ratio, respectively) – thus demonstrating that the reaction was incomplete under these conditions. However, these results were promising and these reaction conditions can be revisited and possibly optimized if the need should arise in the future.

Scheme 1.17 Deallylation conditions employed by David Martin\textsuperscript{49}

Further work can still be done with the N-allyl protecting group, due to the fact that there are numerous methods available to remove it that have not yet been attempted\textsuperscript{58, 59} Also, applying microwave irradiation to heat a reaction mixture, rather than conventional heating, has been shown to improve reaction yields and efficiencies\textsuperscript{62-65} Therefore, this relatively new technology can be utilized to re-visit previously explored reaction conditions to see if these reactions may still be viable methods for protection/deprotection. Beyond applying new technology, there are also several other protecting groups available in the literature that have demonstrated themselves to be
orthogonal in relation to the N-benzyl group. These candidates will be discussed further in the Results and Discussion section below.
III. Results and Discussion

Several examples of nitrogen-protecting groups that have been added and removed in the presence of an N-benzyl group can be found in the literature \(^5\) – but any candidate must also meet the qualifications discussed above. That is, the prospective candidate must survive the conditions used to make the cross-bridged tetraazamacrocycle and any new reaction conditions that are employed to add or remove this new protecting group must not react with any functional groups already present on the cross-bridged tetraazamacrocycle. In searching the literature, \(^58,66\) the candidates that were chosen for investigation were the aforementioned N-allyl group \(^59,60,67-72\) as well as the N-para-methoxybenzyl (PMB) \(^73-77\) and N-2-naphthylmethyl (Nap) \(^78-84\) groups – shown below in Scheme 1.18. In each case the strategy was to synthesize the mono-protected mono-benzylated cross-bridged cyclen as a model system and subject this compound to the selective deprotection conditions in order to test the efficiency of the reaction conditions.

![Scheme 1.18](image).

Scheme 1.18 The model system and protecting groups that were chosen for investigation.
A. Development of the N-allyl Protecting Group

(1) Synthesis of di-NH cross-bridged cyclam 7 by way of deallylation

As summarized above in the Background section of this chapter, several methods of deallylation have already been investigated in the Weisman research group. In light of the fact that the application of microwave-assisted heating can sometimes yield better results in reactions where conventional heating had been utilized, some previously investigated methods of deallylation were first re-visited. Di-allyl cross-bridged cyclam (60, shown below in Scheme 1.19) was used as a model compound for testing the conditions used for deallylation.

The synthesis of di-allyl cross-bridged cyclam 60 was carried out by employing the method developed by Maureen Fagan (Scheme 1.19). The reductive ring opening was performed conventionally via Fagan's method. Microwave-assisted heating (78 °C, 20 min, open-vessel) was also employed to perform this reductive ring opening of 59. This small-scale experiment yielded cross-bridged product 60 of equal purity to what had been found when conventional heating was employed and, in the future, microwave-assisted heating can be employed to perform this reductive ring opening.

Microwave-assisted chemical reactions, while not yet fully understood, have demonstrated great potential for performing chemistry more efficiently than conventionally heated reactions. The deallylation reaction conditions that were first
revisited were what could be considered the “simplest” methods run by Bist as a part of her research. As mentioned in the Background, it was hoped that the ligand would be at the appropriate pH for the allylated nitrogen to be protonated and that hydroxide present in the solution would deallylate this nitrogen. First the ligand was placed into a Discover microwave 10 mL reaction tube with water, sealed, and heated at 150 °C for 30 minutes. In the first attempt the pH was pre-adjusted, using a solution of HCl, to 9.5 and in the second attempt the pH was not adjusted at all (pH≈12) – shown below in Scheme 1.20. It was hoped that these conditions would facilitate deallylation, but in both cases no reaction occurred and starting material was recovered. Since these reaction conditions did not lead to any deallylation at all, the reaction conditions that employed t-butoxide in DMSO were then investigated.

![Reaction Conditions]

1.) Reaction Conditions
MW, 150 °C, 30 min.
closed vessel, H₂O

2.) aq KOH/PhCH₃

**Reaction Conditions:**
A: pH = 9.5 (adjusted by adding HCl)
B: pH ≈ 12 (after adding the ligand to H₂O, no acid added)

**Scheme 1.20** Initial attempts at deallylation, employing microwave heating.

Methods for de-allylation of both N-allyl and O-allyl groups using t-butoxide in DMSO are found in the literature. The mechanism, illustrated below in Scheme 1.21, involves t-butoxide deprotonating DMSO to form the dimsyl anion, which then goes on to facilitate the 1,3-hydrogen shift that results in the formation of the enamine.
Scheme 1.21 The mechanism for the formation of the enamine, which then hydrolyzes in the presence of acid to complete the N-deallylation.

Fortunately, the reaction conditions of t-butoxide in DMSO employing microwave irradiation were found to deallylate 60. The optimal conditions were determined to be heating 60 (0.36 mM in DMSO) for 45 minutes at 100 °C in an open-vessel (under nitrogen) with 1.5 molar equivalents of t-butoxide (1.17 mM in DMSO) per allyl group – as presented below in Scheme 1.22. The pH of the crude reaction mixture was then lowered to pH≈0 by adding HCl – thus hydrolyzing the enamine to yield the crude di-NH cross-bridged cyclam HCl salt. The free ligand 7 is then extracted from a basic solution (pH≈14) using toluene. Based upon what has been found in the literature the DMSO reaction solvent, often difficult to remove, remained in the aqueous layer when performing the toluene extraction (if residual DMSO still remained after concentrating the sample, then it could be removed by performing another extraction with
toluene from basic water). SAFETY WARNING: The heating of DMSO with t-butoxide (2.09 mM in DMSO) under pressure led to an explosion and should be avoided. Therefore, open-vessel microwave conditions must be employed. There is some literature precedent of explosions occurring with DMSO when heated under pressure over 220 °C and when DMSO is reacted with compounds such as sodium hydride to form the dimethyl anion.

With these conditions worked out for the model system, 60, the next step was to perform the deallylation in the presence of an N-benzyl group to determine if the N-benzyl group is stable to the reaction conditions.

(2) Synthesis of mono-benzyl cross-bridged cyclen 32 via deallylation

Once the conditions for deallylation had been developed for di-allyl cross-bridged cyclam 60, they were applied to the mono-allyl mono-benzyl cross-bridged cyclen system. The synthesis of mono-allyl mono-benzyl cross-bridged cyclen is based on the synthesis of the cyclam analogue, first developed by Bist. The synthesis, shown below in Scheme 1.23, involves the benzylation of cyclen bisaminal 18 using the procedure developed by Hill followed by regioselective allylation of 39 using excess allylbromide to yield 61. Unfortunately, difficulties were met when performing the reductive ring
opening employing the conditions commonly used—excess sodium borohydride in 95% ethanol.\textsuperscript{12,14}

![Scheme 1.23: Synthesis of mono-allyl mono-benzyl cross-bridged cyclen 62.](image)

Use of NaBH\textsubscript{4} resulted in a small amount of deallylation (3-10\%) of 61, which occurred prior to the reduction of the bisaminal. This deallylation is caused by the presence of hydroxide or ethoxide present in the solution. In the 95\% ethanol reaction solvent some NaBH\textsubscript{4} will react with the water present, as well as ethanol, to yield NaOH and NaOEt.\textsuperscript{94} This NaOH and NaOEt will then act as nucleophiles reacting further with the N-allylated bisaminal salt to cleave off the allyl group from a quaternary nitrogen. Once this allyl group is cleaved, the mono-benzylated cyclen bisaminal salt would then reductively ring open to the mono-benzyl adjacent-bridged macrocycle—as shown below in Scheme 1.24 for 61.
Scheme 1.24 A small amount of deallylation of mono-allyl mono-benzyl bisaminal salt (54, 61) occurs, followed by reduction – thus yielding adjacent-bridged side-product (63, 64).

It is known that these mono-alkylated salts reduce to form the adjacent-bridged tetraazabicycles.\textsuperscript{43, 44} The mechanism for the reductive ring opening of the di-alkylated cyclen and cyclam bisaminal to form the cross-bridged ligand is shown below in Scheme 1.25. This mechanism proceeds via the formation of an iminium ion that is reduced by NaBH\textsubscript{4} – this reduction occurs twice in the molecule to afford the cross-bridged tetraazabicycle.
Scheme 1.25 The mechanism for reductive ring opening of the di-alkylated cyclen/cyclam bisaminal salt to form the cross-bridged ligand.

A possible mechanism for the formation of the adjacent-bridged tetraazabicycle begins with protonation of the nitrogen adjacent to the quaternary nitrogen in the ring as shown below in Scheme 1.26. This nitrogen is believed to be the most basic of the nitrogens within the ring system.

Scheme 1.26 The mechanism for reductive ring opening of the mono-alkylated cyclen/cyclam bisaminal salt to form the adjacent-bridged ligand.

The rationale for the increased basicity of the nitrogen adjacent to the quaternary nitrogen is based upon hyperconjugative stabilization as shown below in Figure 1.12A. This
rationale is supported by the literature since an X-ray crystal structure of the protonated mono-methylated cyclam bisaminal salt, shown below in Figure 1.12B, demonstrated that this nitrogen is protonated. Following the protonation of this nitrogen the mechanism is similar to the reductive ring opening of the cross-bridged analog. The process of the iminium ion forming followed by its reduction occurs twice after the initial protonation to yield an adjacent-bridged product – as shown above in Scheme 1.26.

**Figure 1.12 (A):** Hyperconjugative effects that may be responsible for the increased basicity of the nitrogen adjacent to the quaternary nitrogen, which has its lone pair placed within the cleft of the bisaminal for cyclam and cyclen bisaminals (cyclam is shown); (B): Empirical evidence of this increased basicity, demonstrated in an X-ray crystal structure of the mono-methylated cyclam bisaminal salt.\(^{22}\)

Alternative reducing agents were investigated by this author and David Martin (who was having similar problems with a di-allylated bisaminal precursor of 56) for use in the reductive ring-opening step. Sodium triacetoxyborohydride (NaBH(OAc)\(_3\)) and sodium cyanoborohydride (NaBH\(_3\)(CN)) were investigated since they are more mild reducing agents that are less reactive with water and reasonable alternatives to NaBH\(_4\). Both NaBH(OAc)\(_3\) and NaBH\(_3\)(CN) are less basic than NaBH\(_4\), and therefore less likely to cause the formation of \(\text{\textsuperscript{1}}\text{OH}\) (as well as \(\text{\textsuperscript{1}}\text{OEt}\) or \(\text{\textsuperscript{1}}\text{OMe}\), when in EtOH or MeOH, respectively) in solution. Martin’s research lead to the development of NaBH\(_3\)(CN) for use on a dibenzo-annelated system.\(^{49}\) It was also found that NaBH\(_3\)(CN) in MeCN was a viable alternative to NaBH\(_4\) in 95% EtOH when performing the reductive ring opening to
yield 62. An aprotic non-polar solvent, MeCN, was chosen over 95% EtOH since running the reaction in this solvent eliminates the potential for the side reactions that yielded the NaOH and NaOEt – which were responsible for the deallylation of the macrocycle that subsequently lead to the unwanted adjacent-bridged side products. These reaction conditions (NaBH$_3$(CN) in MeCN) were not only found to be applicable for the reduction of 61 but also for 31 and 54 – as described later in this dissertation. The conditions used to reduce 61, shown above in Scheme 1.23, have also been adapted for use in the microwave reactor (MW-assisted reaction conditions: 0.066 mM 61 and 2.7 mM NaBH$_3$(CN) in MeCN; closed vessel, 110 °C for 30 min). The conditions, which yield comparable results, for running this reduction in the microwave involve performing the reaction in a closed vessel at 110 °C for 30 minutes. Unfortunately, when run in an open-vessel in the microwave the deallylated product was slightly less pure – with small peaks present in the baseline of the $^1$H NMR spectrum. This small difference in purity of product, and the fact that it is preferred not to run this reaction in a closed vessel (due to the possible evolution of HCN) lead to the use of conventional heating when performing this reductive ring opening.

It was also found that sodium triacetoxyborohydride (NaBH(OAc)$_3$) could be a viable alternative for NaBH$_4$ as well as NaBH$_3$(CN). The reduction did not proceed toward product when employing abs. EtOH and 95% EtOH with analogous molar equivalents of starting ligand (0.025 mM 61) and reducing agent (1.0 mM NaBH(OAc)$_3$) – which are commonly used when utilizing NaBH$_4$ (see the experimental section, where NaBH$_4$ is used in 95% EtOH). The reductions were run at room temperature for 17 h (in both abs. EtOH and 95% EtOH) and 3 weeks (in 95% EtOH) and in each case mostly
unreacted starting material was recovered (note: this “unreacted starting material” has the bisaminal carbons cleaved out to produce mono-allyl mono-benzyl cyclen during the basic workup – as seen in the $^1$H and $^{13}$C NMR spectra that are obtained from crude product). Also, no 62 was observed in the crude products obtained after workup under these reaction conditions. Therefore, when considering the success that was recently found when employing NaBH$_3$(CN), MeCN was explored as an alternative solvent. Conditions that gave the best results when using MeCN were those run in an open vessel (under N$_2$) in the microwave at 82 °C for 90 min – with a 0.023 millimolar concentration in terms of starting material 61 and a 0.94 millimolar concentration of NaBH(OAc)$_3$ in MeCN. These reaction concentrations are more dilute compared to the reaction concentrations that employ NaBH$_3$(CN) (0.035 mM 61, and 1.38 mM NaBH$_3$(CN)) which will slow the reaction. These conditions yielded product in \( \approx 90\% \) purity (69% crude yield), with some of the impurities determined to be unreacted starting material (again, as was the case with the reactions employing NaBH(OAc)$_3$ above, this “unreacted starting material” was mono-allyl mono-benzyl cyclen – as determined by $^1$H and $^{13}$C NMR analysis). The fact that product is yielded in \( \approx 90\% \) purity (the low yield could be improved when reaction conditions are modified) shows that NaBH(OAc)$_3$ is a viable alternative to NaBH$_3$(CN) and that it should be further investigated. A variety of possible experiments could be run to optimize this reduction, including: running the reaction longer in the microwave; applying conventional heating for 1-3 days; or running the reaction at higher concentrations (the molar concentrations of the starting material and NaBH(OAc)$_3$ were diluted due to the fact that NaBH(OAc)$_3$ has a higher molar mass than NaBH$_4$ and NaBH$_3$(CN) and, if the same number of molar equivalents of
NaBH(OAc)$_3$ toward the di-allyl salt are used, the reaction mixture becomes more like a thick slurry. Further, sodium triacetoxyborohydride would be a preferred alternative to NaBH$_3$(CN) due to its toxicity and the fact that the production of HCN is a concern when dealing with NaBH$_3$(CN).

During the process of purifying 62, an X-ray grade crystal of its tetraphenyl borate salt was obtained which allowed its structural determination – shown below in Figure 1.13. Note that one of the bridging nitrogens (N2) is protonated, implying that the intramolecular H-bonding pattern in the solid state is most favorable for protonation at the bridgehead. It is interesting to note that N1, N3, and N4 all have their lone pairs convergent into the cleft of the macrocycle – implying that these nitrogens are helping to stabilize the proton that is bonded to N2.

![Image](image_url)

**Figure 1.13** An X-ray crystal structure of the BPh$_4^-$ salt of protonated mono-allyl mono-benzyl cross-bridged cyclen 62.

With mono-allyl mono-benzyl cross-bridged cyclen 62 synthesized, the deallylation conditions that were developed for di-allyl cross-bridged cyclam 60 were
applied to 62. For 1.5 eq. \( t \)-butoxide in DMSO, it was found that the reaction was complete after 25 min of heating (via microwave irradiation) at 100 °C in an open vessel, under nitrogen (Scheme 1.27). Purification by Kugelrohr distillation gave 32 in 77% yield. There was no evidence of debenzylation in the \(^1\)H and \(^{13}\)C NMR spectra. It should be noted that one reported literature method of cleaving an N-benzyl group utilizes potassium \( t \)-butoxide and oxygen in DMSO (at room temperature for 20 minutes) to cleave the N-benzyl bond on a variety of systems.\(^{95}\) So, it is fortunate that no debenzylation was observed under the reaction conditions used in Scheme 1.27, since no special precautions were taken to remove ambient oxygen from the DMSO used (the reaction was run under nitrogen).

![Scheme 1.27 Synthesis of mono-benzyl cross-bridged cyclen 32.](image)

(3) **Synthesis of mono-benzyl cross-bridged cyclam 55**

Two former Weisman group members had developed all but the final deprotection step of the synthesis of mono-benzyl cross-bridged cyclam 55 (Scheme 1.28). Hill was the first to synthesize the mono-benzyl cyclam bisaminal salt 40,\(^{41}\) but he recorded a 62% yield.\(^{44}\) This yield was increased to 94% by this author in the course of repeating this synthesis. The percent yield was improved by not only filtering the crude reaction mixture of 40 as originally instructed by Hill,\(^{41}\) but by also working to recover product from the resulting filter cake. More product was obtained from the filtrate by removing the solvent under reduced pressure and then washing the concentrated filtrate (now a
white solid) with toluene then filtering. The resulting washed filter cake was then combined with the original filter cake (after confirming its purity via $^1$H NMR analysis) to improve the percent yield. Apparently nearly a third of the product was being lost to the filtrate during the original workup. The regioselective allylation of 40 was first performed by Bist as was the reductive ring opening of 54 using NaBH$_4$.$^60$

\[ \text{Scheme 1.28} \quad \text{Synthesis of mono-allyl mono-benzyl cross-bridged cyclam 55.} \]

Unfortunately, difficulties similar to what were seen in the synthesis of 62 (see Part A Section 2 above) were encountered when employing NaBH$_4$ to reduce 54. The same process of deallylation followed by reduction to form the adjacent-bridged mono-benzyl tetraazamacrocycle (in this case 64, shown above in Scheme 1.24) was found to have occurred when reducing 54 with NaBH$_4$. The percentage of adjacent-bridged side-product was less than was seen in the case with the cyclen-based synthesis (ranging from 4-6%, with a higher percentage of side-product in the larger scale reactions). In fact, a singlet from adjacent-bridged side-product (attributed to benzyl protons, found at $\approx$3.5 ppm) can be seen in the $^1$H NMR spectrum of 55 in Bist’s thesis, but it was present to such a small extent that it was misinterpreted as being part of a nearby multiplet and the
presence of 64 was obscured by noise in the accompanying $^{13}$C NMR spectrum. However, when running this reduction on a larger scale, the amount of side-product is increased and not as easily dismissed. Fortunately, NaBH$_3$(CN) proved to work just as well in performing this reductive ring opening, giving product in a 94% yield after workup.

The same reaction conditions that were used for the deallylation of 62, 1.5 eq. t-butoxide in DMSO for 25 min at 100 °C (in an open-vessel), were applied to the cyclam-based system 55 (Scheme 1.29). The product was obtained in 90% yield after purification by Kugelrohr distillation. No debenzylation was observed.

![Scheme 1.29](image)

Scheme 1.29 Synthesis of mono-benzyl cross-bridged cyclam 33.

Fortunately, these deallylation conditions were found to work on all three N-allyl cross-bridged systems (55, 60, and 62) and they were also found to have utility with a bis-tetraazabicycle system (31, vide infra). Further, the use of this protecting group was also applicable on the dibenzo-annelated cyclam system 56, as demonstrated by David Martin.

During the process purifying 55, an X-ray grade crystal was obtained of its tetraphenylborate (BPh$_4^-$) salt (Figure 1.14). In this case, its structure revealed that the nitrogen (N61) attached to the benzyl group is protonated, implying that the intramolecular H-bonding pattern in the solid state is most favorable for protonation at this site. It is also interesting to note that the nitrogens N60 and N62 have their lone pairs
pointed into the macrocycle’s cleft, while N63 has its lone pair pointed outside of the cleft. This implies that N60 and N62 are helping to stabilize the proton that is bonded to N61, while N63 does not interact with this proton at all. While work was being done on the development of an N-allyl protecting group, efforts were also put into developing the N-PMB and N-Nap protecting groups.

![Figure 1.14](image)

**Figure 1.14** An X-ray crystal structure of the BPh₄⁺ salt of protonated mono-allyl mono-benzyl cross-bridged cyclam 55.

### B. Development of the N-PMB Protecting Group

1. **Synthesis of di-NH cross-bridged cyclen 24 via the use of the N-PMB protecting group**

   There are two methods known to cleave the N-PMB bond without cleavage of nearby N-benzyl bonds. These include oxidative removal using 1,2-dichloro-4,5-dicyanoquinone (DDQ) and its removal from nitrogen in triazoles, imidazoles, indoles, and pyrazoles using trifluoroacetic acid (TFA). However, before these conditions
were applied to our systems a model compound was synthesized, di-PMB cross-bridged cyclen 66 (Scheme 1.30).

![Scheme 1.30 Synthesis of di-PMB cross-bridged cyclen 66.](image)

The dialkylation of 18 proceeds in 3 days, similar to the analogous di-benzylolation reaction, to afford product in an 89% yield. The microwave-assisted reductive ring opening was then performed using NaBH₄, to obtain product with a 98% yield [Note: when running a reduction in the microwave using NaBH₄ in 95% EtOH, there are potential hazards associated with foaming of the reaction mixture – see the appropriate endnote]⁹⁷

With model compound 66 in hand, cleavage of the N-PMB group was investigated. The procedure (shown below in Scheme 1.31) is based upon the conditions used to remove PMB from N-PMB pyrrole.⁷⁵,⁷⁶ The reaction is run neat in TFA, heated for 18 hours at 60 °C, and anisole was added as a scavenger for the p-methoxybenzyl carbocation. Conditions utilizing microwave-assisted heating were also developed. It was found that this same reaction could be run at 85 °C for 10 minutes (closed vessel, due to the boiling point of TFA, 72 °C) to afford product in comparable yields.
Scheme 1.31 Synthesis of di-NH cross-bridged cyclen 24 via cleavage of the N-PMB bond.

The accepted mechanism for this deprotection, proceeding via the formation of a para-methoxybenzyl carbocation, is shown below in Scheme 1.32.\(^6\) As demonstrated below, the para-methoxy group stabilizes the carbocation intermediate. Without an electron-donating substituent to stabilize the benzylic carbocation, these conditions are far less likely to cleave an N-benzyl bond – proven experimentally as shown below in Schemes 1.34 and 1.35.

Scheme 1.32 The accepted mechanism for cleavage of the N-PMB bond in TFA.
The anisole is employed as a scavenger for the carbocation, shown in the mechanism in Scheme 1.32, and its use did result in a cleaner crude product.

(2) Synthesis of mono-benzyl cross-bridged cyclen 32 via the use of a N-PMB protecting group

The above conditions were then applied to mono-benzyl mono-PMB cross-bridged cyclen system 68 in an attempt to synthesize mono-benzyl cross-bridged cyclen 32 and determine if the N-benzyl group would be cleaved under these reaction conditions. The synthesis, similar to analogous mono-benzyl mono-alkylated cross-bridged cyclen procedures, is shown below in Scheme 1.33. The regioselective alkylation was carried out for 6 days in MeCN to afford mono-benzyl mono-PMB cyclen bisaminal salt 67 in 92% yield. Reductive ring opening of 67 was then performed using NaBH₄ via microwave-assisted heating at 78 °C for 10 minutes give 68 in a 79% yield after extractive workup. [Note: when running a reduction in the microwave using NaBH₄ in 95% EtOH, there are potential hazards associated with foaming of the reaction mixture – see the appropriate endnote]⁹⁷

Scheme 1.33 Synthesis of mono-benzyl mono-PMB cross-bridged cyclen 68.
The method used to cleave the N-PMB bond in the di-PMB system 66 was then applied to 68 (Scheme 1.34). Product was obtained in 72% yield (≈97% pure) after Kugelrohr distillation. No debenzylation was observed in the \(^1\)H and \(^{13}\)C NMR spectra – thus demonstrating that the N-PMB group is a viable orthogonal protecting group to the N-benzyl system.

![Scheme 1.34 Synthesis of mono-benzyl cross-bridged cyclen 32.](image)

In order to test if these reaction conditions would also remove the N-allyl group, di-allyl cross-bridged cyclam 60 was also put through the same reaction conditions with no reaction – represented below in Scheme 1.35. This result is important since it demonstrates that the N-PMB protecting group is orthogonal to both the N-benzyl group and the N-allyl group. Also, the reaction conditions used for this deprotection were applied to the dibenzyl cross-bridged cyclam 23, and again only starting material was recovered. This experiment confirms that the N-benzyl bond would not be cleaved from cross-bridged cyclam either, thus this method of deprotection could also be applied to cyclam-based systems. Therefore, the PMB group could be added and removed from molecules having both N-benzyl and N-allyl groups present – making it an important addition to the synthetic tools available for the production of azamacrocycles that contain a variety of functional groups.
1.) xs Trifluoroacetic acid, Anisole, 60 °C, 18 h  
2.) aq. HCl/PhCH₃  
3.) aq KOH/PhCH₃

No Reaction

R = allyl (60) or benzyl (23)

Scheme 1.35 The di-allyl (60) and di-benzyl (23) cross-bridged cyclams proved to be unreactive when exposed to the dealkylation conditions used to remove the PMB protecting group.

(3) Progress toward the synthesis of di-NH cross-bridged cyclam 7 via the use of N-PMB protecting groups

An attempt was also made to synthesize di-NH cross-bridged cyclam 7 via the use of N-PMB protecting groups. Unfortunately, this work did not move beyond the first step. Following the same synthetic method as was applied for the cyclen-based system (Scheme 1.30, vide supra), the first step in the synthesis was to di-alkylate the cyclam bisaminal 19 to give di-PMB cyclam bisaminal salt 69 (Scheme 1.36).

Scheme 1.36 The attempted synthesis of the di-PMB cyclam bisaminal salt 69.

The reaction mixture was stirred at room temperature for 11 days and a milky white precipitate was observed. This precipitate was separated from the reaction solvent via centrifugation and washed with Et₂O before removing residual solvent under reduced pressure. Once isolated, the white precipitate was analyzed via ¹H NMR spectroscopy in D₂O. Surprisingly, the spectrum for the precipitate matched with the spectrum of cyclam bisaminal 19 (which is deuterated when in D₂O) and no evidence of alkylation (mono- or di-alkylation) was observed. The ¹H NMR spectrum of the supernatant appeared to be unreacted para-methoxybenzyl bromide and 19, as well as other impurities that included
another set of *para*-methoxybenzyl aromatic multiplets. Later the puzzling results of this reaction were made clear when Kaitlyn Dugan and Gary Weisman worked toward the synthesis of a mono-benzyl mono-PMB cyclam bisaminal salt. A white precipitate was obtained as a crude product and this precipitate was analyzed in D$_2$O, as had been done when working toward the synthesis of 69. Similar to what had been seen when analyzing the precipitate that was found as a result of the reaction shown in Scheme 1.36, Dugan and Weisman found the unreacted starting ligand 40 in the $^1$H and $^{13}$C NMR spectra. It was then determined that the product, a mono-benzyl mono-PMB cyclam bisaminal salt, had in fact been synthesized, but that the product was hydrolyzed shortly after being dissolved in the D$_2$O. This lead to $^1$H and $^{13}$C NMR spectra that contained “recovered” mono-benzyl cyclam bisaminal salt 40 and what is believed to be *para*-methoxybenzyl alcohol. Therefore this work can be revisited and further investigated since the precipitate from the reaction shown in Scheme 1.36 was most likely 69 and another solvent such as deuterated DMSO (which worked for Dugan and Weisman when analyzing the mono-benzyl mono-PMB cyclam bisaminal salt product) could be used to analyze this precipitate. However, once 69 has been made, the conditions used to perform the reductive ring opening would probably have to be modified so that 95% EtOH is not used as the reaction solvent -- since the PMB group may be cleaved in this solvent as well. Perhaps NaBH$_3$CN or NaBH(OAc)$_3$ in MeCN could be explored as an alternative. But, since the cyclen analog, 66, worked just as well in testing the viability of this protecting group and synthesis of di-NH cross-bridged cyclam 7 has already been well developed, this research direction was not further pursued.
Alongside this work, and the work that was being done on the N-allyl protecting group system, efforts were put into the development of a potential N-Nap protecting group. The results for this protecting group did not meet with such success, as described below. However, the synthesis of the N-Nap cross-bridged tetraazamacrocycles was straightforward and successful.

C. Investigation of the N-Nap Protecting Group for Cross-bridged Tetraamines

(1) Progress toward the synthesis of mono-benzyl cross-bridged cyclen 33 via the use of an N-Nap protecting group

The N-Nap group was the third candidate investigated for use as a protecting group. These Nap-armed cross-bridged tetraazamacrocycle target molecules are also interesting for other applications. Tetraazamacrocycles in the literature have had pendant arms incorporating large aromatic systems, such as 1- or 2-naphthylmethylene, appended to them for possible fluorescence applications and the di-Nap and mono-benzyl mono-Nap cross-bridged tetraazamacrocycles may have some applications in this area as well.

There are a few methods (both oxidative and reductive) known to cleave the N-Nap bond selectively over the N-benzyl bond. In order to test the selectivity of these methods in cleaving the N-Nap bond over the N-benzyl bond in cross-bridge tetraamines, mono-benzyl mono-Nap cross-bridged cyclen 71 was synthesized. The strategy for synthesis of 71 is shown below in Scheme 1.37. Mono-benzyl cyclen bisaminal salt 39 was regioselectively alkylated using excess 2-(bromomethyl)naphthalene in MeCN, and crude 70 was washed with Et₂O, though excess alkylating agent was not fully removed in this process. The reductive ring opening was then performed with excess NaBH₄ in 95%
EtOH stirred for 6 days – affording 71 in 94% yield after extraction. If there is renewed interest in this compound in the future, the microwave-assisted conditions that have been developed could also be applied in making this reductive ring opening more efficient. The conditions for cleaving the N-Nap bond will first be tested on a di-Nap cross-bridged species before testing these conditions on the mixed armed species 71, and so these di-Nap cross-bridged species were also synthesized using similar conditions as were used in Scheme 1.37.

![Scheme 1.37 Synthesis of mono-benzyl mono-Nap cross-bridged cyclen 71.](image)

(2) Synthesis of di-Nap cross-bridged cyclen and cyclam (74 and 75)

While work was being done to synthesize the mixed armed cross-bridge (71) for use in testing deprotection conditions in the presence of an N-benzyl group, work was also directed toward the synthesis of the di-Nap cross-bridged cyclen/cyclam systems. The synthesis of the di-Nap cross-bridged cyclen 74 and the di-Nap cross-bridged cyclam 75 are shown below in Scheme 1.38. The syntheses went smoothly for the di-alkylated salts of both the cyclen and cyclam analogs (72 and 73). The only problem with the purification of 72 was that residual alkylating agent was difficult to remove when washing with MeCN. However, this impurity did not prove to be a problem when
performing the reductive ring opening of 72, the next step in the synthesis. The reductive ring openings of these systems, yielding 74 and 75, were successful, giving reasonable yields after extraction. Microwave-assisted conditions were also found to be successful for ring-expansion of these N-Nap systems.

Scheme 1.38 Syntheses of di-Nap cross-bridged cyclen and cyclam (74 and 75).

(3) Attempted synthesis of di-NH cross-bridged cyclam 7 via the use of an N-Nap protecting group

With 74 and 75 in hand, cleavage of the N-Nap was investigated. The first method attempted was the oxidative cleavage of the N-Nap bond using DDQ based upon literature methods. As shown below in Scheme 1.39, 75 was stirred with DDQ for 3.5 hours in a 4:1 mixture of CH₂Cl₂/MeOH – following what have been described as “typical” reaction conditions in the literature. Unfortunately, no reaction was observed and only starting material was obtained.
Scheme 1.39 Attempted synthesis of di-NH cross-bridged cyclam 7, via the oxidative cleavage of the N-Nap bond.

This reaction was only run once and further work can still be done in this area. Either these conditions could be more fully developed (e.g., run longer or with heat, exploring different solvent systems, etc.) or other methods of cleavage could be used. There are selective reductive methods of deprotection in the literature (using H₂, Pd/C) as well as other agents that can be explored for oxidative cleavage (such as cerium(IV) ammonium nitrate (CAN)). However, since the conditions to cleave both the N-allyl and N-PMB bonds had proven successful, work toward developing the N-Nap deprotection was not pursued further.

D. Synthetic Utility of the Mono-Benzyl Cross-bridged Cyclam 33

As discussed above in the Background, the secondary nitrogen in the mono-benzyl cross-bridged cyclam can be substituted with a variety of potential pendant arms (see above in Scheme 1.5). These pendant arms can be used to modify the ligand for a variety of purposes. In Reaction D in Scheme 1.5, a long alkyl chain is added to the mono-benzyl cross-bridged cyclam to increase to the ligand’s lipophilicity. Increasing the lipophilicity of the ligand will alter its biological properties and biodistribution, possibly in beneficial ways. Toward this end, the secondary nitrogen was alkylated with a long hydrocarbon chain via reductive alkylation.
(1) **Synthesis of mono-benzyl mono-octyl cross-bridged cyclam 76**

A common method of alkylating a secondary nitrogen is to perform a reductive alkylation using a mild reducing agent such as NaBH$_3$(CN)$\textsuperscript{101,102}$ or NaBH(OAc)$_3$$\textsuperscript{103}$. The reaction using the former (Scheme 1.40) was straightforward, yielding 79% crude material (>90% purity) after acid wash and extraction from base using toluene. The <10% impurities are unknown, but the impurities in the $^1$H and $^{13}$C NMR spectra of the crude product 76 do not match with unreacted starting material 33. Chromatography was investigated as a method for purification of this material. Thin-layer chromatography experiments were performed using CH$_2$Cl$_2$:MeOH:(28% aq) NH$_3$ (10:2:0.25; 10:3:0.25; 15:2:0.25; on basic alumina) but, as is often the case with amines, the crude material streaked and ran together at reasonable $R_f$ values. Other conditions for chromatography could be investigated or recrystallization could be attempted (possibly via an HBr salt, etc.) as methods to purify this material further. Once purified this compound may give insight into what effects altering the lipophilicity of the cross-bridged cyclam ligands could have on their properties as PET imaging agents (e.g., how its biodistribution my be affected) and 76 could also be complexed to copper(II), to study its properties.

![Scheme 1.40 Synthesis of mono-benzyl mono-octyl cross-bridged cyclam 76, via reductive amination.](image)
IV. Conclusions

The work described in this chapter has led to the development of two protecting groups that can be used to aid in the synthesis of a variety of new, unsymmetrically functionalized bridged tetraazamacrocycles. Mono-benzyl cross-bridged cyclen 32 or cyclam 33 can now be synthesized employing two different protecting groups, N-PMB or N-allyl, and the resulting secondary nitrogen can then be used to perform further chemistry making a variety of mixed-arm cross-bridged species as demonstrated below in Scheme 1.41. The mono-benzyl mono-armed species could then be hydrogenolyzed to remove the N-benzyl group, leaving open the resulting NH for further functionalization if desired. Hill, Peng, and Bist have already utilized this type of elaboration to a mix-armed species.\(^{41,52,60}\)

![Scheme 1.41](image)

**Scheme 1.41** Summary of the protecting group work and the potential use of the mono-benzyl cross-bridged system.

Dannon Stigers has specifically utilized 33 (made by this author via the use of an N-allyl protecting group) to synthesize a mono-phosphonic acid pendant-armed cross-bridged cyclam 78 – shown below in Scheme 1.42.\(^{56}\)

![Scheme 1.42](image)

**Scheme 1.42** Work done by Stigers to synthesize 78.\(^{56}\)
As illustrated below in Scheme 1.43, there are now three potential protecting
groups that can be added and removed from tetraazamacrocyclic systems – one of these
groups can be removed using reductive methods, another using basic conditions, and the
third using acidic conditions. This affords greater synthetic versatility when modifying
our systems by allowing researchers to add a variety of functional groups that may have
sensitivity to acid, basic, or reductive reaction conditions to the frame of the
tetraazamacrocycle.

![Scheme 1.43](image)

Scheme 1.43 The three alternate methods now available for the synthesis of cross-bridged ligands – with
sensitivity to acid, base, or hydrogenolysis conditions.

Indeed, the deallylation conditions have already found utility in David Martin’s synthesis
of di-NH cross-bridged dibenzo-annelated cyclam 57 – shown below in Scheme 1.44.49
The N-allyl protecting group has also been applied successfully to the p-xylyl-linked
cross-bridged cyclen system – discussed in Chapter II.
While the conditions for removal of the N-Nap moiety were not found, more work can be done toward this goal if the need for this protecting group should arise. As stated above, further research could include revisiting the conditions utilizing DDQ (see Scheme 1.39) or investigating reductive methods for removal. These Nap pendant-armed systems may also be of interest for applications in fluorescence research.

It is this author’s hope that these protecting groups, and the methods developed to remove them, will continue to find use in the Weisman and Wong research groups and by others. Though the N-allyl and N-PMB protecting groups have proved successful with several cross-bridged systems, the work done here does not end the search for other protecting groups that can be used on cross-bridged tetraazamacrocycles. Novel protecting groups, and novel removal methods of pre-existing protecting groups, are constantly being developed – as is evidenced by the large number of protecting group related research found in the literature as well as the continued publication of the ever-larger monographs on the topic.
CHAPTER II

SYNTHESIS AND COMPLEXATION OF BRIDGED BIS-TETRAAZAMACROCYCLES

I. Introduction

Linked tetraazamacrocycles have added chemical utility over their non-linked analogs in that they are capable of binding to two separate metal ions simultaneously. These bis-tetraazamacrocycles, with their ability to place two separate metal ions near each other, can create complexes with unique electronic, catalytic, and redox properties. The resulting bimetallic complexes can also serve as models for the charge transfer, electron transport, and allosteric behavior found in many metal-containing biochemical systems.\textsuperscript{104-106} Bis-tetraazamacrocycles are also of interest for use as potent anti-HIV-1 and HIV-2 agents.\textsuperscript{107-113} Depending on the choice of linker, the type of pendant arm attached, and the size of the macrocycle, the chemical properties of the bis-tetraazamacrocycle can be fine-tuned for particular applications.\textsuperscript{114-122}

The bis-tetraazamacrocycles of interest in this author’s research are based on compounds 4 and 5 (shown again in Figure 2.1), discussed in Chapter I. In the pursuit of more stable and selective bis-tetraazamacrocycles, this research incorporates the use of cross-bridged and adjacent-bridged tetraazamacrocycles 4 and 5. Also, the linkers or “spacers” that are incorporated into these bis-tetraazamacrocycles are either \textit{para-} or \textit{meta}-xylyl units, thus creating compounds of the general design shown in Figure 2.1.
These cross-bridged (79a and 80a) and adjacent-bridged (79b and 80b) type bis-tetraazabicycles will be discussed in greater detail below.

![Chemical Structures](image)

Where:

\[4a \text{ and } 5a: n = 0\]
\[4b \text{ and } 5b: n = 1\]
\[R, R' = H, \text{Me, benzyl, etc}\]

**Figure 2.1** The adjacent- and cross-bridged tetraazamacrocycles 4 and 5, respectively, and cross-bridged (79a and 80a) and adjacent-bridged (79b and 80b) bis-tetraazabicycles.

Compounds similar to 79a/b and 80a/b have been investigated in recent years for use in a variety of applications.\(^{105, 107, 114}\) They have received a great deal of attention in the literature for their biological applications, including their aforementioned anti-HIV-1 and HIV-2 properties. Below in Figure 2.2 is the bicyclam AMD3100 (81, formerly JM3100), which has generated attention for its possible applications – mostly due to its potent activity against the virus.\(^{119, 123}\) Unfortunately, the compound was found to have significant cardiac side effects and has been withdrawn for consideration as an anti-HIV agent.\(^{124}\) However, the compound continues to spark interest in other applications.
Recently it was investigated for use as a ligand in a possible PET imaging agent\textsuperscript{125} and in late 2008 the FDA approved \textit{81} (under the name MOZOBIL and, most recently, Plerixafor – owned by Genzyme Inc.) for use as a stem cell mobilizer.\textsuperscript{126,127}

![Figure 2.2](image)

\textbf{Figure 2.2} The bicyclam \textit{81} (AMD3100), a stem cell mobilizer and anti-HIV agent.\textsuperscript{119,123}

The ability of \textit{81} to act as an anti-HIV agent and a stem cell mobilizer is based on its ability to interact with the CXCR4 chemokine receptor on a cell. Much research has been directed toward understanding this interaction between bis-polyazamacrocycles and this receptor so as to produce optimal activity with minimal physical side effects.

Bridger, De Clercq, and others have studied the structural characteristics that are required for these bis-tetraazamacrocycles to be active against the virus.\textsuperscript{111,112,119,123,128-136}

Further information on this topic can be found in this author’s original Master’s thesis\textsuperscript{55} as well as in research published on bicyclam complexes by Sadler and Liang.\textsuperscript{106,126,137-139}

Initial work by Bridger and coworkers also investigated the activities of a series of metal complexes of AMD3100.\textsuperscript{123,124} The following order of activity was found: \textit{81}-Zn\textsuperscript{II} > AMD3100 > \textit{81}-Ni\textsuperscript{II} > \textit{81}-Cu\textsuperscript{II} >> \textit{81}-Co\textsuperscript{III} >> \textit{81}-Pd\textsuperscript{II}. It should also be noted that not only does the Zn(II)\textsubscript{2} complex of AMD3100 exhibit greater anti-HIV activity than the free ligand, but it also has lower toxicity. On the other end of the spectrum, the Pd(II)\textsubscript{2} complex inactivates the drug’s activity.\textsuperscript{140} Research by Sadler \textit{et al.} has further elucidated the relationship between the metal complexes of AMD3100 and the CXCR4 receptor, with a focus on the complexation of Zn(II).\textsuperscript{137-140} Sadler’s investigations into how the metallomacrocycles interact with the CXCR4 receptor may aid in tailoring bis-
tetraazamacrocycles that have activity similar to that of AMD3100, but lack the adverse physical side effects. And, in recent years, there has been interest from other researchers in the field to develop bridged bis-tetraazamacrocycles, and their metal complexes, for this purpose.

Since this author’s Master’s research was completed it should be noted that some of these compounds have been published in the literature by other researchers. The adjacent-bridged para- and meta-xylyl-linked cyclen (101a, and 101b, respectively, shown below in Scheme 2.3) were the first to be studied (called a “side-bridged” system by the author). Other authors have also found interest in both the Zn(II) and Cu(II) complexes of the para-xylyl-linked adjacent-bridged cyclam-based ligand 102a for use as an anti-HIV agent – and these complexes were found to have notable anti-HIV activity. Most recently, a Cu(II) complex of the di-methyl para-xylyl-linked cross-bridged cyclam-based ligand (95a, in Scheme 2.1 below) has also been found to have significant anti-HIV activity in in vitro testing. While these results are promising and demonstrate the potential of these systems for use as anti-HIV agents it is still not certain how well these ligands would be able to retain the copper and zinc cations in vivo. The loss of these metals from the ligand due to transmetallation, yielding the free ligand, within the body may lead to health problems due to similar cardiac issues as were found for 81 in clinical trials. Therefore, these bis-tetraazamacrocycles will have to be further modified (i.e., through the addition of pendant arms that could aid in securing the metal in place) and studied before they could be seriously considered for such applications. This fact also necessitates the development of an alternate protecting group to the N-benzyl group (and the currently used N-methyl group) for use on these bis-
tetraazamacrocycles. Handel has also synthesized and studied pendent-armed \textit{para}- and \textit{meta}-xylyl-linked cross-bridged cyclen-based systems – shown below in Figure 2.3.\textsuperscript{109} These systems are the cyclen- and cyclam-based bridged systems that are currently found in the literature.

![Figure 2.3](image)

\textbf{Figure 2.3} Pendant-armed \textit{para}- and \textit{meta}-xylyl-linked cross-bridged systems (82-83) synthesized by Handel.\textsuperscript{109}

There has also been research investigating the redox behavior of metal complexes of bis-cyclams\textsuperscript{114} and bis-cyclens.\textsuperscript{142,143} For example, Ciampolini and coworkers tested the redox behavior of [Ni(II)$_2$(bis-cyclam)](ClO$_4$)$_2$ complexes with a variety of spacers, as shown in Figure 2.4.\textsuperscript{144} In these experiments, the \(\Delta E_{\frac{1}{2}}\) of the stepwise oxidation of the [Ni(II)$_2$(bis-cyclam)](ClO$_4$)$_2$ complexes varied according to the type of spacer joining the two cyclam rings. They discovered that the potential difference was the largest with the shortest spacer system, and that the \(\Delta E_{\frac{1}{2}}\) decreased with the increase in separation between the rings. Research such as this indicates that the cyclam and cyclen ring systems may serve as convenient and versatile building blocks in the production of multi-electron redox systems. This research also demonstrates the importance of the type of the spacer used when designing the bis-tetraazamacrocycles. The length and rigidity of the linker will play a significant role in determining how far apart the metals that are bound to the bis-tetraazamacrocycles will be and this will help in determining the redox and biomimetic properties of the resulting ligand-metal complexes.
Eiichi Kimura and coworkers have carried out significant research into the interaction of bis-cyclen metal complexes is with biologically-relevant compounds. Complex 88, shown below in Figure 2.5, has the ability to interact with a variety of such compounds including DNA in aqueous solution at physiological pH. Their research has also revealed that the meta-xylyl analog of 88 shows similar promise for a variety of related biological applications.

Biomimetics is another area where bis-tetraazamacrocycles have a great potential for use. Dinuclear metal complexes have been investigated for such biomimetic purposes and bis-tetraazamacrocycles have a great deal of potential. One enzyme of interest in the literature is superoxide dismutase (SOD). SOD catalyzes the dismutation (the process of simultaneous oxidation and reduction) of the superoxide anion (O$_2^-$) and plays an important role in the protection of cells from oxidative damage. The active site of the Cu-Zn SOD enzyme has a bimetallic center with one
Cu(II) and one Zn(II) ion bridged by an imidazolate moiety. Several researchers have designed and investigated systems where the Zn(II) and Cu(II) metal complexes are placed in the appropriate distance from each other, bridged by imidazolate moieties, for possible activity as SOD biomimetic agents.\textsuperscript{161-168} Bis-tetraazamacrocycles have a possible application in this area of research, due to the fact that the linked macrocycles have the ability to place two metals (e.g., Cu(II) and Zn(II)) in close proximity and these complexes could be further modified as an SOD-mimic.

The substantial amount of literature documenting the synthesis of bis-tetraazamacrocycles\textsuperscript{55, 169, 170} to date involves modifying these ligands so that they can better interact with the CXCR4 receptor for use as anti-HIV agents,\textsuperscript{171-178} and as stem cell mobilizers.\textsuperscript{106, 119, 127, 179-185} These compounds also have a great deal of potential for use in bimetallic chemistry.\textsuperscript{105, 173, 174, 176-178, 186-188} A key advantage to the use of bis-tetraazamacrocycles is their versatility – they are easily functionalized and their structures can be altered in subtle ways leading to considerable changes in the stability of their resulting metal complexes. The addition of an ethylene bridge to each macrocycle of these bis-tetraazamacrocycles, to add structural rigidity to the resulting complexes, is one modification that has been made and deserves further investigation. Below, in the Background section, is a summary of the work that has been done in this group on bridged bis-tetraazamacrocycles.
II. Background

Our synthetic design of the cross-bridged bis-tetraazabicycles is based on previous research involving the synthesis of mono-benzyl mono-methyl analogs. This original research, carried out by Mark Rogers and Dan Hill, was further developed by Christine Elia to synthesize the adjacent-bridged mono-benzylated cyclam. Weisman et al. have adapted these methods to develop the synthesis of linked macrocyclic systems. The basic synthetic design developed is shown below in Scheme 2.1.

Anthony Harris began work on the synthesis of the cross-bridged bis-tetraazabicycles by synthesizing 91a and as well as crude 93a and 91b. This author continued that work by synthesizing and fully characterizing 94a, 95a, 95b, and 102a (see Scheme 2.3 for 102a). This author also synthesized 101a and 102b, but did not complete their characterization.
(see Scheme 2.3 for 101a and 102b). Sam Jolly continued the project and was able to fully characterize 94b.$^{54}$

As discussed in Chapter I, the regioselectivity of the alkylation of 18 and 19 has been studied thoroughly by the Weisman group. This process is also shown in Scheme 1.2 and discussed in the Background of Chapter I. In terms of stereochemistry, there are three possible isomeric products for 90/91 and 92/93 – each has possible meso and the d/l configurations. Figure 2.6 shows the time-averaged symmetry of 90/91 (meso) and 90/91 (d/l). The meso form of 90/91 has a $\sigma$ plane; while the d/l enantiomers of 90/91, have $C_2$ symmetry. An X-ray grade crystal of 91a was obtained and analyzed in this author’s M.S. research (Figure 1.20, page 31 of the M.S. thesis).$^{55}$ The crystal was the meso isomer (misreported as the d/l isomer in the actual M.S. thesis), implying that the mother liquor was enriched with the d/l isomer and that the meso isomer crystallized first under the conditions used (i.e., slow evaporation of D$_2$O). This same symmetry is carried on into the methylation products (i.e., 92 and 93) as well as the reductive ring opened bridged compounds 95a and 95b (which are conformational diastereomers) – shown below in Figure 2.7 for 95a.

![Figure 2.6](image)

*Figure 2.6* The meso and d/l stereoisomers of 90-91 and their time averaged symmetry.

74
While it can be said with relative certainty that both the respective meso and d/l diastereomers are produced, the $^1$H and $^{13}$C NMR analysis of 90a and 92a gave no evidence of their existence. It is believed that this is simply due to the fact that the NMR resonances associated with the diastereomers happen to be isochronous, and not that a single diastereomer is being formed.

The cross-bridges of the cyclen-based bis-tetraazamacrocycles, 94a and 94b, are of particular interest due to the fact that a single stereoisomer is formed upon reduction of 92a/92b. As shown below in Figure 2.8 for 94a, the time-averaged symmetry ($C_{2v}$) inherent in the molecule rules out the possibility of a diastereomeric mixture.
The fact that the compound exists as one single isomer is fortunate, since there is no need to worry about further purification of diastereomers in order to use it in metal complexation and other, possibly biological, applications.

The synthetic route for the cross-bridged bis-tetraazabicycles, shown above in Scheme 2.1, is analogous to that for the non-linked systems. The macrocycle (1 or 2) is first condensed with glyoxal to form a cis-fused tetracyclic bisaminal (18/19), which is then alkylated with one half an equivalent of \(\alpha,\alpha'-\text{dibromo-p-xylene, } 89a\) (or \(\alpha,\alpha'-\text{dibromo-m-xylene, } 89b\)). Upon washing with MeCN, a diastereomeric mixture of the dibromide salt (90/91) is >95% pure at that point and gives yields ranging from 83% - 94% yield. It is believed that over-alkylation, i.e., the formation of an oligomer (96) or a dialkyl salt (97), is circumvented (or greatly reduced) due to the fact that when 90/91 is produced it precipitates from solution, making it essentially inert toward further substitution. Possible oligomer or dialkyl salt side-products are shown below in Figure 2.9.

![Figure 2.9 Possible oligomer (96) and dialkyl (97) byproducts in the synthesis of 90 and 91.](image)

The penultimate step, shown above in Scheme 2.1, is the synthesis of 92/93. The reaction mixture, consisting of 90/91 with an excess of Mel in MeCN, is stirred for 24 hours in a sealed flask. This process affords product, as a mixture of diastereomers, in 65-
90% crude yield. This wide range in yield is due to the difference in solubility between 92 and 93. The more soluble the salt is in MeCN, the greater the likelihood that the iodide (or bromide) will act as a nucleophile to de-alkylate the bis-tetraazamacrocycle (shown below in Scheme 2.2) – thus regenerating 18/19 and a mono-alkylated tetraazamacrocycle, 98.

\[
\begin{align*}
\text{90/91} & \quad \text{2Br}^- \\
\text{98} & \quad \text{I}^- \\
\text{99} & \quad \text{2I}^- \\
\text{100} & \quad \text{Mel}
\end{align*}
\]

Scheme 2.2 Proposed side reactions – yielding 99 and 100.

Compound 18/19 is then di-methylated by excess Mel in solution. The mono-alkylated product 98 also goes on to be alkylated by excess Mel in solution, then de-alkylated, re-alkylated, etc. Compounds 98 and 100 are not seen in either the supernatant or the washed precipitate due to their solubility in MeCN. It is believed that 98 and 100 would also react in a similar way as 90/91 in solution to yield more 92/93. Also, while Br\(^-\) is the stronger nucleophile in MeCN, the abundance of I\(^-\) in the solution means that both are probable nucleophiles. Evidence of this process taking place is demonstrated in the fact
that the major component observed in the $^1$H and $^{13}$C NMR spectra of the concentrated supernatant matches the spectra of previously synthesized 99.

The final step in the synthesis (shown above in Scheme 2.1) involves the reductive ring opening of 92/93. This reaction has been performed using excess NaBH$_4$ at room temperature for 2 days in 95% EtOH. The process gives moderate yields ranging from 52-79% after extraction. These crude products are >95% pure before purification. Chromatography was needed to further purify compounds 95a and 95b, while compounds 94a and 94b were pure enough to be fully characterized without the need of further purification.

The synthesis of the adjacent-bridged bis-tetraazamacrocycles, shown below in Scheme 2.3, is similar to that of the cross-bridged analog. The dibromide salt (90/91) was reduced using excess NaBH$_4$ at room temperature for 2 days in 95% EtOH. The crude material is reasonably pure (>90%). Unfortunately, 102a and 102b were not fully characterized, but preliminary TLC experiments reveal that chromatography should work to purify these compounds. Fortunately, 101a was readily recrystallized using hot MeCN in 64% yield facilitating its full characterization.55

Scheme 2.3 Synthesis of the adjacent-bridged bis-tetraazamacrocycles 101 and 102.55
Upon completion of this author’s M.S. degree, Sam Jolly continued work on the bis-tetraazamacrocycle research with some additional development in ligand synthesis as well as complexation studies.\textsuperscript{54} As mentioned above, Jolly was able to complete the characterization of 94b. The main focus of Jolly’s research was on the synthesis and complexation of the \textit{para}- and \textit{meta}-xylyl-linked 94a and 94b with lithium and zinc(II).

Below, in Scheme 2.4, are the conditions that Jolly used to synthesize 94a/b. He analyzed the product by \textsuperscript{1}H NMR spectroscopy and confirmed that he had the desired complex by comparing his results with work done by Hill on the non-linked, mono-benzyl mono-methyl cross-bridged cyclen analog.\textsuperscript{41}

\begin{equation}
\begin{align*}
\text{94a/b} & \xrightarrow{2 \text{ eq Li(ClO}_4\text{)}} \text{CD}_3\text{CN} \quad 2 \text{ Li(ClO}_4\text{)} \cdot 94\text{a/b} \\
\text{Scheme 2.4 Jolly’s Complexation of 94a/b with 2 Li(ClO}_4\text{).}\textsuperscript{54}
\end{align*}
\end{equation}

Jolly also complexed 94a and 94b with zinc(II) acetate, as shown below in Scheme 2.5.\textsuperscript{54} Taking steps to avoid water in the reaction mixture, he was able to synthesize the target compounds, \textit{Zn}_294\text{a/b}, and confirm their presence using \textsuperscript{1}H and \textsuperscript{13}C NMR as well as IR spectroscopy.

\begin{equation}
\begin{align*}
\text{94a/b} & \xrightarrow{2 \text{ eq Zn(CH}_3\text{CO}_2\text{)}_2 \cdot 2\text{H}_2\text{O}} \text{anhydrous MeOH, reflux, N}_2 \quad 2 \text{ Zn(CH}_3\text{CO}_2\text{)}_2 \cdot 2\text{H}_2\text{O} \cdot 94\text{a/b} \\
\text{Scheme 2.5 Jolly’s Complexation of 94a/b with 2 Zn(CH}_3\text{CO}_2\text{)} \cdot 2\text{H}_2\text{O}.}\textsuperscript{54}
\end{align*}
\end{equation}
This brief synopsis of where the Weisman and Wong groups’ research relating to the synthesis and complexation of bis-tetraazamacrocycles stood upon this author’s return to graduate school reveals that there was a clear opportunity to begin complexing these ligands with Cu(II) and further investigate their complexations with Zn(II). Also, there is obvious utility for the N-allyl protecting group developed in the work covered in Chapter I with these xylyl-linked bis-tetraazamacrocycles. While the N-methyl group itself can be utilized as a protecting group,\textsuperscript{58} conditions for its deprotection are not selective enough for use with these compounds.
IIII. Results and Discussion

There is a great deal of work that can be done with bis-tetraazamacrocycles—whether it be varying the linker, the pendant arms, or the nature of macrocycles that are incorporated into the system. Upon this author's return to the Ph.D. program at UNH, this chemistry was picked up from where Jolly had left off and metal complexation of the systems was first explored. If these systems are to be used in biological systems one day, then work toward a greater understanding how these ligands complex to biologically relevant metals such as copper(II) and zinc(II), metals that have been well-studied when complexed to their non-linked analogs in the Weisman and Wong research groups, was clearly the next step. Also, though much work had been done to develop the synthesis of the cross-bridged bis-tetraazamacrocycles, most of the systems had been synthesized with methyl groups in place in order to have the reductive ring opening of the system generate a cross-bridged system. Therefore there was interest in the development of a protecting group to be used in the place of the methyl group, one that could be removed to allow the synthesis of a "di-NH" cross-bridged bis-tetraazamacrocycle. Once methyl groups have been replaced with hydrogens in systems such as 94 and 95, these bis-tetraazamacrocycles can then be functionalized with a variety of pendant arms or these cross-bridged bis-tetraazamacrocycles themselves (e.g., 31, Figure 1.11) can be investigated for their possible anti-HIV properties and whatever other biomedical applications they might have.
A. **The Complexation of p-Xylyl-linked Adjacent-bridged Cyclam 102a with Copper(II)**

The synthesis for the starting material, 102a, shown above in Scheme 2.3, was performed following the method developed in this author’s Master’s research.\(^5\) Once 102a was in hand, the di-Cu(II) complex was synthesized following the reaction conditions shown below in Scheme 2.6.\(^1\) The resulting Cu(II) complex was analyzed by UV-vis spectroscopy and found to have octahedral coordination about Cu(II) in solution (in 80% ethanol). The electronic spectrum of Cu\(_2\) • 102a is shown below in Figure 2.10.

\[
\text{Scheme 2.6 Complexation of p-xylyl-linked adjacent-bridged cyclam 102a with Cu(II).}
\]

![Scheme 2.6](image)

**Figure 2.10** The electronic spectrum of Cu\(_2\) • 102a in 80% ethanol, where \(\lambda_{\text{max}} = 614\) nm (Abs = 1.273).

Professor Ed Wong performed acid dissociation experiments on (CuCl\(_2\))(H\(_2\)O)\(_4\) • 102a in 5M HCl at 50 °C under pseudo-first order conditions. The results, shown below in Figure 2.11, demonstrated that the half-life for decomplexation under these conditions was 1.6(1) hours.
Figure 2.11 (A) The electronic spectra and (B) The first-order non-linear fit (monitored at 585 nm) of the experimental absorbance/time data for the acid-decomplexation of (CuCl₂)₂(H₂O)₄ • 102a in 5M HCl at 50 °C.

Professor Wong also performed electrochemical analyses of (CuCl₂)₂(H₂O)₄ • 102a. The resulting cyclic voltammogram of (CuCl₂)₂(H₂O)₄ • 102a yielded an irreversible reduction at -0.71 V as illustrated below in Figure 2.12A. Also, the differential pulse
voltammogram, obtained by Wong, resulted in reduction of \((\text{CuCl}_2)_2(\text{H}_2\text{O})_4 \cdot 102a\) at -0.67V – shown below in Figure 2.12B.

Figure 2.12 The (A) cyclic voltammogram and (B) DVP of \((\text{CuCl}_2)_2(\text{H}_2\text{O})_4 \cdot 102a\) (0.1 M NaOAc, pH=7).

As mentioned above, other researchers have also demonstrated interest in these compounds for their anti-HIV properties. In 2007 a copper(II) complex of 102a was published by Archibald and coworkers; synthesized in a similar manner.\(^{112}\) It was found that the complex of Cu\(_2\) \cdot 102a had better anti-HIV activity than the copper(II) complex of the bis-cyclam analog 81 (AMD3100) as well as better activity toward the virus over the free ligand 102a. However, the Cu\(_2\) \cdot 102a complex was found to have lower anti-
HIV activity than the Zinc(II) complex of the same ligand – also made by the authors.\textsuperscript{113} Archibald, Sadler, and Hubin have continued work in these adjacent- and cross-bridged ligands for their potential anti-HIV activity – most recently publishing the synthesis and analysis of the copper(II) complex of cross-bridged analog, 95a.\textsuperscript{111}

B. The Complexation of \textit{p}-Xylyl-linked Cross-bridged Cyclen 94a and \textit{m}-Xylyl-linked Cross-bridged Cyclen 94b with Copper(II)

(1) The complexation of \textit{p}-xylyl-linked cross-bridged cyclen 94a with copper(II)

The synthesis for the starting material, 94a, shown above in Scheme 2.1, was performed following the method developed by this author.\textsuperscript{55} Once 94a was in hand, the Cu(II) complex was synthesized following similar reaction conditions that are typically applied to make a Cu(II) complex of the cross-bridged di-benzyl cyclam analogs – shown below in Scheme 2.7.\textsuperscript{12} After purification by crystallization, the product was obtained in 65\% yield as dark-blue crystals. The resulting Cu(II) complex was analyzed by UV-vis spectroscopy and found to have octahedral coordination in solution (in MeCN). The electronic spectrum of 94a is shown below in Figure 2.13. Its molar absorptivity was determined to be 280 M$^{-1}$ cm$^{-1}$. This is nearly twice the molar absorptivity of the analogous di-benzyl cross-bridged cyclen (22, shown below in Figure 2.14) copper(II) complex synthesized by Weijun Niu ($\lambda_{\text{max}}(\epsilon) = 647$ nm (162 M$^{-1}$ cm$^{-1}$), in MeCN) which is attributed, in part, to the fact that there are two times the copper(II)-complexed ligands per molecule in Cu$_2$ • 94a.\textsuperscript{190}
Scheme 2.7  Complexation of p-xylyl-linked cross-bridged cyclen 94a with Cu(II).

Figure 2.13  The electronic spectrum of Cu$_2$ • 94a, in MeCN, where $\lambda_{max} = 632$ nm (Abs = 1.748).

Figure 2.14  Di-benzyl cross-bridged cyclen 22, which was complexed to Cu(II) by Weijun Niu.

Recrystallization of Cu$_2$ • 94a from MeCN and benzene/Et$_2$O (1:1, diffusion chamber), gave an X-ray grade crystal. The crystal structure was initially determined by Arnold L. Rheingold (University of California - San Diego, La Jolla, CA) and James A. Golen (University of Massachusetts Dartmouth, North Dartmouth, MA). Jonathan M. White (University of Melbourne, Parkville, Australia) and Gary Weisman carried out some further refinements. The structure, shown below in Figure 2.15, illustrates that the ligand is in a square-pyramidal geometry around the copper(II) in the solid state – where N103 (Cu36-N103 bond distance = 2.134 Å) is the apical nitrogen and the remaining nitrogens in the ring (along with a nitrogen in the coordinated MeCN) are the basal
nitrogens (Cu-N bond distances ranging between 1.949-2.079 Å) in the pyramid. The Addison and Reedjick τ-parameter, for describing five-coordinate geometries along the Berry rearrangement between a trigonal bipyramid and a square pyramid, can be used to demonstrate that the geometry is in fact square pyramidal. The equation yielded a value of \( \tau = 0.052 \) [where \( \tau = (\beta - \alpha)/60 \), with \( \tau = 1 \) for trigonal bipyramidal and \( \tau = 0 \) for square pyramidal; the values for \( \beta \) and \( \alpha \) are taken from the two largest N-Cu-N angles in the complex]. This \( \tau \) value sets the complex well within the region where it would be considered to have square pyramidal geometry. It is also interesting to compare the similarity in the intramolecular distance between the two copper(II) cations for \( \text{Cu}_2 \cdot 94a \), which is 11.59 Å, to that of an analogous \( \text{para-xylyl-linked non-bridged cyclen copper(II) perchlorate complex synthesized by Handel et al.} \) where the distance is 11.54 Å.

Figure 2.15 An X-ray crystal structure of \([\text{Cu(CH}_3\text{CN})(\text{ClO}_4)_2]_2 \cdot 94a \text{ (CH}_3\text{OH})\).

Acid dissociation experiments on \([\text{Cu(CH}_3\text{CN})(\text{ClO}_4)_2]_2 \cdot 94a \) (CH\(_3\)OH) in 1M HCl at 30 °C under pseudo-first order conditions were also performed by Professor
Wong. The results, shown below in Figure 2.16, revealed that the half-life for decomplexation in these conditions were 18.8(2) min.

![Electronic Spectra for Cu$_2$ • 94a](image1)

(A) The electronic spectra and (B) the first order kinetics plot of the acid-decomplexation of [Cu(CH$_3$CN)(ClO$_4$)$_2$ • 94a (CH$_3$OH) in 1M HCl at 30 °C.

![First-order Non-linear Fit of Experimental Data for Cu$_2$ • 94a](image2)
Electrochemical analyses of \([\text{Cu(CH}_3\text{CN)}(\text{ClO}_4)_2](\text{CH}_3\text{OH}) \cdot 94\text{a}\) was also performed by Wong. The resulting cyclic voltammogram of \([\text{Cu(CH}_3\text{CN)}(\text{ClO}_4)_2](\text{CH}_3\text{OH}) \cdot 94\text{a}\) yielded an irreversible reduction at -0.69 V – as shown below in Figure 2.17A. Wong also obtained a differential pulse voltammogram that gave reduction of \([\text{Cu(CH}_3\text{CN)}(\text{ClO}_4)_2] \cdot 94\text{a} (\text{CH}_3\text{OH})\) at -0.62V as shown below in Figure 2.17B.

![Cyclic Voltammogram](image)

**Figure 2.17** The (A) cyclic voltammogram and (B) DVP of \([\text{Cu(CH}_3\text{CN)}(\text{ClO}_4)_2] \cdot 94\text{a} (\text{CH}_3\text{OH})\) (0.1 M \(\text{NaOAc}, \text{pH}=7\)).
(2) Discussion of the kinetic and electrochemical analyses of copper(II) complexes of 94a and 102a

The kinetics and electrochemical results from Professor Wong's work can be compared to what has been found in the literature for similar bridged and non-bridged tetraazamacrocycles. Aqueous acid-assisted decomplexation of Cu(II) complexes of tetraazamacrocycles is an indicator of the kinetic inertness of these complexes.26 Listed below in Table 2.1 are the results of acid decomplexation studies of Cu(II) complexes of analogous tetraazamacrocycles. The adjacent-bridged cyclam complex Cu₂ • 102a was found to have a longer $t_{\frac{1}{2}}$ (1.6(1) hours, in 5M HCl at 50 °C) than the di-methyl cross-bridged cyclen analog (Cu₂ • 94a) when Cu₂ • 102a is exposed to less acidic conditions at lower temperatures (18.8(2) minutes, in 1M HCl at 30 °C). As seen below in Table 2.1, the Cu(II) complexes of cross-bridged cyclen are not as kinetically stable as their bridged and non-bridged cyclam analogs. This decrease in kinetic inertness is attributed mainly to the fact that the cyclen ring is smaller, and thus the Cu(II) metal does not fit as snugly into its cleft as it does for the cyclam analog. However, as would be expected, the Cu-CB-DO2A (Cu • 12) complex is more stable than Cu₂ • 94a due to the two carboxymethyl pendant arms that coordinate to the Cu(II) and help to hold the metal in the cleft. The adjacent-bridged Cu₂ • 102a complex is not as stable as Cu-TETA — its half-life is only half that of Cu-TETA (1.6 h for Cu₂ • 102a compared to 3.2 h for Cu-TETA). Adjacent-bridged cyclam (103, shown below in Figure 2.18) has been found to be about as stable as its non-bridged analog (i.e., cyclam) in the literature.192 Fabbrizzi and co-workers found that the half-life of Cu • AB cyclam 103 was only slightly more kinetically inert than Cu • cyclam — $t_{\frac{1}{2}} = 23.5$ (±2.1 days) compared to $t_{\frac{1}{2}} = 22.0$ (±2.0 days) for cyclam in 1M HClO₄ at 60 °C. Therefore, unlike the ethylene bridge that joins non-adjacent
nitrogens (i.e., the "cross-bridge"), the ethylene bridge that joins the adjacent nitrogens (i.e., the "adjacent-bridge") does not add any significant kinetic inertness to resulting metal complexes with copper(II). The adjacent-bridge does however lend selectivity to the cyclam frame (as well as cyclen) for particular metals over others and for trans-coordination to metals (see Figure 1.4, page 4, for coordination geometry nomenclature). In fact, it was demonstrated that both adjacent-bridged cyclam macrocycles of 102a coordinate in a trans-II fashion when complexed to Zn(II).

<table>
<thead>
<tr>
<th>Complex</th>
<th>Half-life</th>
</tr>
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<tbody>
<tr>
<td>Cu-CB-TE2A</td>
<td>154(6) h</td>
</tr>
<tr>
<td>Cu-CB-DO2A</td>
<td>&lt; 3 min</td>
</tr>
<tr>
<td>Cu-TETA</td>
<td>4.5(5) min</td>
</tr>
<tr>
<td>Cu-DOTA</td>
<td>&lt; 1 min</td>
</tr>
<tr>
<td>Cu-CB-cyclam</td>
<td>11.8(2) min</td>
</tr>
<tr>
<td>Cu-CB-DO2LA</td>
<td>&lt; 3 min</td>
</tr>
<tr>
<td>Cu-CB-TE2LA</td>
<td>~100 hr</td>
</tr>
<tr>
<td>Cu-CB-TE2LA</td>
<td>154(6) h</td>
</tr>
<tr>
<td>Cu-CB-cyclam</td>
<td>11.8(2) min</td>
</tr>
<tr>
<td>Cu-CB-DO2LA</td>
<td>&lt; 3 min</td>
</tr>
<tr>
<td>Cu-CB-TE2LA</td>
<td>~100 hr</td>
</tr>
</tbody>
</table>

Table 2.1 The Half-lives of Cu(II) complexes of a variety of bridged and non-bridged tetraazamacrocycles.25, 26
The electrochemical results for several cross-bridged and non-bridged cyclen and cyclam copper(II) complexes are listed below in Table 2.2, along with the results obtained for Cu₂ • 94a and Cu₂ • 102a. The cross-bridged cyclam compounds have been found to have quasi-reversible reductions, and a Cu(I) complex of cross-bridged dibenzyl cyclam has been characterized in the literature, thus demonstrating that some cross-bridged cyclam macrocycles have the ability to adjust coordination to both copper(II) and copper(I). This is not the case for the cross-bridged cyclen Cu-CB-DO2LA, and the non-bridged Cu-DOTA. Both complexes were found to have irreversible reductions, unlike cross-bridged analogs of cyclam. The irreversible reductions found for both Cu₂ • 94a and Cu₂ • 102a imply that neither is able to adapt to the coordination requirements of copper (I) and stabilize that oxidation state of copper.

<table>
<thead>
<tr>
<th>Complex</th>
<th>Peak Potential, E_{Reduction} (V)</th>
<th>DVP Results, E_{Reduction} (V)</th>
<th>Reversibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu-CB-TE2A</td>
<td>-1.08</td>
<td>-1.06</td>
<td>quasi-reversible</td>
</tr>
<tr>
<td>Cu-CB-TE2LA</td>
<td>-0.68</td>
<td>-0.68</td>
<td>quasi-reversible</td>
</tr>
<tr>
<td>Cu-DOTA</td>
<td>-0.92</td>
<td>-0.85</td>
<td>irreversible</td>
</tr>
<tr>
<td>Cu-CB-DO2LA</td>
<td>-0.78</td>
<td>-0.67</td>
<td>irreversible</td>
</tr>
<tr>
<td>Cu-CB-cyclam</td>
<td>-0.68</td>
<td>--</td>
<td>quasi-reversible</td>
</tr>
<tr>
<td>Cu₂-94a</td>
<td>-0.69</td>
<td>-0.62</td>
<td>irreversible</td>
</tr>
<tr>
<td>Cu₂-102a</td>
<td>-0.71</td>
<td>-0.67</td>
<td>irreversible</td>
</tr>
</tbody>
</table>

Table 2.2 Electrochemical analysis of Cu(II) complexes of a variety of bridged and non-bridged tetraazamacrocycles (0.1 M NaOAc, pH=7).
(3) The complexation of m-xylyl-linked cross-bridged cyclen 94b with copper(II)

The synthesis of the starting material, 94b, shown above in Scheme 2.1, was performed following the method developed in this author's Master's research. After synthesizing 94b, the Cu(II) complex was then synthesized following similar reaction conditions as were used to make the Cu(II) complex of 94a (Scheme 2.8). After purification by crystallization (MeOH/Et2O, diffusion chamber), dark-blue crystals were obtained in 97% crude yield. The resulting Cu(II) complex was analyzed via UV-vis spectroscopy and found to have octahedral coordination in solution (in MeOH). The electronic spectrum of Cu2 • 94b is shown below in Figure 2.19. Its molar absorptivity (in MeOH) was determined to be 202 M⁻¹ cm⁻¹. Attempts were made to purify the compound by utilizing the same conditions that were used with the p-xylyl-linked system, but Cu2 • 94b did not crystallize as well. It should be noted that this sample also did not pass chemical analysis due to the fact that the %H is off by more than 0.4% of the calculated value – however the %C and %N were within the expected range.

Scheme 2.8 Complexation of m-xylyl-linked cross-bridged cyclen 94b with Cu(II).
Figure 2.19 The electronic spectrum of Cu₂ • 94b, in MeOH, where λₘₐₓ = 658 nm (Abs = 0.895).

C. The Complexation of p-Xylyl-linked Cross-bridged Cyclen 94a and m-Xylyl-linked Cross-bridged Cyclen 94b with Zinc(II)

(1) The complexation of p-xylyl-linked cross-bridged cyclen 94a with Zinc(II)

The complexation of 94a with Zn(II) was performed following similar reaction conditions that were applied to make Zn(II) complexes of the cross-bridged cyclam analogs as well as those used by Jolly in his research shown below in Scheme 2.9. The ¹H and ¹³C NMR spectra matched those obtained by Jolly in his research. A 94% crude yield is reported since recrystallization experiments did not yield purified product. The most promising system, using a water and acetone diffusion chamber did not consistently yield purified crystals. This could be due to the fact that Zn(II) may be readily displaced from the metal-ligand complex in time in water, forming Zn(OH)₂ and the protonated ligand.

Scheme 2.9 Complexation of p-xylyl-linked cross-bridged cyclen 94a with Zn(II).
(2) Progress toward the synthesis of a mononuclear Zn(II) \(\cdot\) \(p\)-xylyl-linked cross-bridged cyclen 94a complex

An effort was made to synthesize a mononuclear Zn(II) complex of cross-bridged bis-cyclen 94a for the purposes of placing a Cu(II) into the other linked macrocycle and yielding a bimetallic system with two different metals coordinated within the linked system. Zinc(II) was chosen for synthesis of the mononuclear complex since \(^1\)H NMR spectroscopy could be used to analyze the results—compared to UV-vis analysis for the Cu(II) mononuclear complex, which may yield spectra that are more difficult to interpret. The reaction conditions were based loosely on work that had been done by Handel in which the adjacent-bridged analog (101a) was complexed to one equivalent Cu(II) per bis-macrocycle\(^{141}\) where Handel had used 0.9 eq of Cu(ClO)_4 \(\cdot\) H_2O per mole 101a in “frozen ethanol” (i.e., temp = 0 °C) and the mono-nuclear product precipitated from solution before further complexation could occur. The conditions used, as shown below in Scheme 2.10, involved utilizing 0.9 equivalents of anhydrous ZnCl_2 per bis-macrocycle (94a), in MeOH for 24 hours at room temperature. While our systems need to be refluxed in order to promote complexation, it was hoped that if the reaction were run at room temperature this would be a sufficient temperature to promote complexation, but a low-enough temperature that the mono-nuclear complex would precipitate from solution. These reaction conditions are diluted to \(\approx\) 1/3 the concentration of the reaction conditions used for the dinuclear complexation that yielded Cu\(_2\) \(\cdot\) 94a and Cu\(_2\) \(\cdot\) 102a—in order to promote precipitation of the mono-nuclear product (Zn \(\cdot\) 94a). Unfortunately, the product did not precipitate from solution as hoped and the crude product was a mixture of non-complexed starting material, complexed ligand, and another species—which is most likely the mono-nuclear product Zn \(\cdot\) 94a (Figure 2.20).
Scheme 2.10 The attempted complexation of p-xylyl-linked cross-bridged cyclen 94a with one equivalent of Zn(II).

Figure 2.20 A comparison of the $^1$H NMR spectra of the complexed ligand (Zn$_2$ • 94a, bottom), the free ligand in D$_2$O solvent (94a, middle), and the crude product from the attempted synthesis of the mononuclear Zn(II) complex (top).

Further work can be done toward the goal of synthesizing a hetero-dinuclear complex of 94a. Such work could include altering the reaction conditions to make further attempts at the synthesis of the mononuclear Zn(II) • 94a (i.e., change concentrations, reaction solvents, etc.) or attempts could be made to synthesize the mononuclear Cu(II) • 94a complex prior to adding the Zn(II) metal to the bis-tetraazamacrocycle. In either case, after the mono-nuclear • 94a complex has been
synthesized the next step would be to complex the non-coordinated macrocycle to the appropriate metal to make the mono-Cu(II) mono-Zn(II) • 94a complex and to investigate this system for its properties. A mono-Cu(II) mono-Zn(II) • 94a system could then be investigated and possibly studied as an SOD biomimic.

(3) The complexation of m-xylyl-linked cross-bridged cyclen 94b with Zinc(II) 

m-Xylyl-linked cross-bridged cyclen 94b was complexed with Zn(II) following the same basic procedure as described above for the p-xylyl-linked analog, shown below in Scheme 2.11. The 1H and 13C NMR spectra were consistent with Jolly’s results.54 A 98% crude yield is reported since, as was seen with the p-xylyl-linked analog, recrystallization experiments did not yield purified product. Water and acetone were again the most promising diffusion chamber solvents used – but purified material was not obtained.

D. Synthesis of Crude Diamide p-Xylyl-linked Cross-bridged Cyclen 105

The synthesis of an amide pendent armed cross-bridged bis-cyclen is of interest since the amide pendant arm could potentially coordinate to the metal that is complexed to the cross-bridged cyclen – thus potentially adding stability and kinetic inertness to the overall ligand-metal complex. This amide pendant arm could also be hydrolyzed, using methods previously developed in the Weisman research group,56 to a carboxymethyl
pendant arm that would also be capable of coordinating to available sites on the metal that is seated within the ligand cleft. The synthesis of diamide cross-bridged bis-cyclen 105, shown below in Scheme 2.12, is analogous to the synthesis of 94a (above in Scheme 2.1).

![Scheme 2.12 Overall synthesis of diamide p-xylyl-linked cross-bridged cyclen 105.](image)

A diastereomeric mixture of 90a was regioselectively alkylated with excess 2-bromoacetamide, to afford 104 in 82% crude yield. The impurities at that point were mainly unreacted 2-bromoacetamide, which was difficult to wash away. The resulting salt, 104, was then reductively ring opened to 105 using NaBH₄ in 95% EtOH under microwave-assisted heating at 78°C for 10 minutes (open-vessel). [Note: when running a reduction in the microwave using NaBH₄ in 95% EtOH, there are potential hazards associated with foaming of the reaction mixture – see the appropriate endnote] A quantitative crude yield was obtained but this material, which was >90% pure, was not purified further. Some experiments exploring potential recrystallization solvents found it to be only slightly soluble in hot acetonitrile or insoluble in toluene or dichloromethane both at room temperature and with heating. Further work can be done to purify this compound, possibly using chromatography. After purification, 105 can be complexed to
Cu(II) and Zn(II) and compared with the non-linked analogs that have been studied in the group. Also, as stated above, hydrolysis of the amide bond would yield the carboxymethyl pendant-armed bis-cyclen – another ligand worthy of complexation to both Cu(II) and Zn(II) and further study. The added binding of the pendant arm to the metal bound to the macrocycle should help to stabilize the metal-ligand complex.

E. Further Application of the N-Allyl Protecting Group – Synthesis of p-Xylyl-linked Cross-bridged Cyclen 31

After having developed the N-allyl protecting group for use with the non-linked cross-bridged systems (discussed in Chapter I), it was applied as a protecting group for the bis-cross-bridged systems. A parent cross-bridged bis-tetraazamacrocyclic system could then be tested for its potential anti-HIV activity, for its biomimetic properties, or a variety of other uses where the non-bridged systems have found utility. These cross-bridged bis-tetraazamacrocycles could also be further functionalized at the NH groups with a variety of pendant arms that could modify and optimize the linked system for these same potential applications. The advantage of the cross-bridged bis-cyclen 31 over its dimethyl p-xylyl-linked cross-bridged bis-cyclam analog (i.e., 95a) is that 31 has a greater degree of symmetry ($C_2v$) and is therefore synthesized as a single isomer – unlike 94a, which is produced as a mixture of 3 stereoisomers. These isomers, and their time-averaged symmetry, are illustrated below in Figure 2.21. When synthesizing the cross-bridged bis-cyclam compounds, unlike the bis-cyclen systems, the product is a meso and d/l diastereomeric mixture. If these bis-tetraazamacrocycles, and their resulting metal complexes, are to be used for biomedical applications, then the synthesis of a compound
that is one single stereoisomer eliminates the need to purify the active stereoisomer from the less active, inert, or even possibly harmful stereoisomers.

![Diagram of stereoisomers](image.png)

**Figure 2.21** The meso and d/l stereoisomers of 95a and the time averaged symmetry of 95a and 31.

(1) **Synthesis of N,N'-di-allyl p-xylyl-linked cross-bridged cyclen 107**

Synthesis of the di-allyl p-xylyl-linked cross-bridged cyclen was based upon that of the di-methyl analog (94a, shown above in Scheme 2.1). The overall synthesis, shown below in Scheme 2.13, began with the regioselective di-allylation of 90a in MeCN with excess allyl bromide. The crude di-allyl salt product, which was >95% pure, was then reductively ring opened using the same conditions that were applied to the mono-allyl mono-benzyl systems (Chapter I). Sodium cyanoborohydride was employed to avoid the potential adjacent-bridged side-products that were discussed in Chapter I. Fortunately,
the process was straightforward and the material was purified by simple extraction. Once 107 was in hand, deallylation was attempted employing the conditions applied for the non-linked analogs.

\[ \text{Scheme 2.13 Overall synthesis of di-allyl } p\text{-xylyl-linked cross-bridged cyclen 107.} \]

(2) Synthesis of \( p\text{-xylyl-linked cross-bridged cyclen 31} \)

With the di-allyl \( p\text{-xylyl-linked cross-bridged cyclen} \) in hand, the deprotection conditions developed for the non-linked systems (discussed in Chapter I) were applied. The reaction conditions are shown below in Scheme 2.14. A longer reaction time was necessary for full deallylation to occur than had been the case for the mono-allyl mono-benzyl analogs (55 and 62), but the deallylation was complete after 45 minutes – the same reaction time that was necessary for di-allyl cross-bridged cyclam 60.

Unfortunately, the product was not as easily purified as the non-linked systems.

\[ \text{Scheme 2.14 Synthesis of di-NH } p\text{-xylyl-linked cross-bridged cyclen 31.} \]
The purification of the non-linked analogs (55 and 62) was accomplished by performing an extraction followed by Kugelrohr distillation, but the larger molecular weight of 31 made distillation more difficult. The product was distilled (with a 61% recovery), but the conditions for Kugelrohr distillation were rather harsh since the air bath temperature had to be raised to 240 °C (0.1 mmHg). While the crude product was purified, at these high temperatures some impurities were also distilled over with the pure material – yielding a still impure distillate. An HBr salt was also made of the crude material, and this salt was then recrystallized in EtOH/water (≈3.75:1), but this process did not fully remove the color (the crude product lightened from brown to yellow) and some impurities still remained as determined as shown in the \(^1\)H and \(^{13}\)C NMR spectra.

Since chromatography had worked to purify several of the bis-tetraazabicycles in this author’s M.S. research, these methods were also investigated for this compound.\(^{55,195}\) Several chromatographic conditions were investigated via TLC, listed below in Table 2.3. Unfortunately, the components of the crude mixture streaked under each set of conditions (unless the mixture did not move from the baseline) and no separation was observed. A simple basic alumina plug was also employed, but the brown-colored impurity filtered through along with the product and no separation was achieved. The ligand was also reacted with \(\text{NH}_4\text{BF}_4\) to make the HBF\(_4\) salt and recrystallizations were attempted in MeCN – but these conditions did not yield purified product. An effort was made to purify the compound somewhat before distillation by making the HBr salt of the ligand first, recrystallizing the HBr salt and then recovering the purified free ligand and distilling it. The intention was to purify the ligand to some degree prior to distillation,
thus removing some of the impurities that come over with the distillate and reducing the amount of residue that remains in the distillation pot, which possibly impedes distillation.

<table>
<thead>
<tr>
<th>Mobile Phase Solvents</th>
<th>Solvent Ratios</th>
<th>Stationary Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHCl₃-MeOH</td>
<td>10-1</td>
<td>basic alumina</td>
</tr>
<tr>
<td>CHCl₃-MeOH-NH₃ (28% aq.)</td>
<td>10-3-0.25</td>
<td>basic alumina</td>
</tr>
<tr>
<td>MeOH-HOAc-H₂O</td>
<td>4-1-1</td>
<td>silica</td>
</tr>
<tr>
<td>EtOAc-MeCN-H₂O</td>
<td>7-1-1</td>
<td>silica</td>
</tr>
<tr>
<td>nBuOH-HOAc-H₂O</td>
<td>4-1-1</td>
<td>alumina</td>
</tr>
<tr>
<td>nBuOH-HOAc-H₂O</td>
<td>4-1-3</td>
<td>alumina</td>
</tr>
<tr>
<td>nBuOH-HOAc-H₂O</td>
<td>4-1-5</td>
<td>alumina</td>
</tr>
<tr>
<td>nBuOH-HOAc-H₂O</td>
<td>8-1-1</td>
<td>alumina</td>
</tr>
<tr>
<td>nBuOH-HOAc-H₂O</td>
<td>6-1-1</td>
<td>alumina</td>
</tr>
<tr>
<td>nBuOH-HOAc-H₂O</td>
<td>6-0.5-0.5</td>
<td>alumina</td>
</tr>
<tr>
<td>nBuOH-HOAc-H₂O</td>
<td>5-0.5-0.5</td>
<td>alumina</td>
</tr>
<tr>
<td>CHCl₃-MeOH-NH₃ (28% aq.)</td>
<td>4-4-2</td>
<td>silica</td>
</tr>
<tr>
<td>CHCl₃-MeOH-NEt₃</td>
<td>4-4-2</td>
<td>silica</td>
</tr>
<tr>
<td>CHCl₃-MeOH</td>
<td>10-1</td>
<td>propylamine functionalized silica</td>
</tr>
<tr>
<td>CHCl₃-MeOH</td>
<td>10-3</td>
<td>propylamine functionalized silica</td>
</tr>
</tbody>
</table>

Table 2.3 Chromatographic conditions applied toward the purification of 31.

While this did remove much of the colored impurities and cleaned up the product before distillation, the material was still not fully purified after distillation. An attempt was also made to sublime the material. The material was heated up to 190 °C (at 0.1 mm Hg) in a silicone oil bath. While sublimation did not occur, some product distilled onto the sublimation cold finger under these conditions, and this material was pure.

Unfortunately, this resulted in only a 7% overall yield of product. When the material was distilled in the sublimation apparatus at higher temperatures (240 °C), impurities distilled over along with the product.

There are other distillation methods used to separate high boiling compounds for purification. A molecular still could be employed as well as a thin-film evaporator – a
type of molecular still.\textsuperscript{196} Like a Kugelrohr distillation apparatus, a thin-film evaporator separates volatile materials from non-volatile materials, but this apparatus does so by heating the material for only a brief amount of time and with little distance for the vapor to travel before it is re-condensed. With less distance for the material to travel, lower temperatures are required for the distillation to occur. Since the material was purified at 190 °C in the sublimation apparatus, this may be a viable method of purification.

\textbf{F. Progress toward the synthesis of the cross-bridged bis-cyclam 110}

Once the deallylation conditions had been worked out on the bis-cyclen system an effort was made to apply these reaction conditions toward the synthesis of a di-NH cross-bridged bis-cyclam. The general design of the synthesis, laid out below in Scheme 2.15, includes the same basic reaction conditions as were used for the bis-cyclen system.

\textbf{Scheme 2.15} The proposed synthesis of $p$-xylyl-linked cross-bridged cyclam 110.

Unfortunately, though most cyclen- and cyclam-based systems are comparable in many ways, these two systems do not always behave in the same manner under the same reaction conditions and difficulties were met in the very first step. The general reaction conditions for the first step, to synthesize the di-allyl $p$-xylyl-linked cyclam bisaminal salt 108, are shown below in Scheme 2.16.
This step is an equilibrium that is driven forward by the precipitation or crystallization of the product from solution. If the product does not precipitate, or it has some degree of solubility in the chosen reaction solvent, then the reaction may not proceed completely to product (i.e., it will reach an equilibrium) and some percentage of starting material as well as possible intermediates may still remain. With these limitations, several other solvents and reaction times were utilized in order to promote precipitation to occur and hence increase the reaction yield.

The reaction solvents, and reaction times, are listed below in Table 2.4.

Acetonitrile was first used since this is the same solvent that was employed with the cyclen analog (i.e., 106). When this was seen to yield very little allylated material, dichloromethane was tried, in the hopes that the product would be more likely to precipitate from this less polar solvent. Unfortunately, this reaction solvent did not yield better results since less allylated product was seen in the $^1$H NMR spectrum. With these disappointing results, diethyl ether was added (MeCN / Et$_2$O, 80:20) to help facilitate precipitation and the amount of MeCN was halved (relative to the dibromide salt) – neither of these solvent systems yielded a substantial amount of product even after 21 days.
It is difficult to determine how far the reaction progressed since the $^1$H NMR spectrum of the mono-allylated intermediate (where one side of the bis-cyclam salt is allylated and the other is not, shown below in Scheme 2.17) will look very similar to the $^1$H NMR spectrum of both the non-allylated starting material (i.e., 91a) and the product (i.e., 108).

Therefore, it is not as simple as observing the disappearance of the starting material in the $^1$H NMR spectrum, since the intermediate will have a very similar spectrum to both the product 108 and the starting material 91a. Also, the presence of diastereomers (explained in the Introduction, see Figure 2.6) further complicates the $^1$H and $^{13}$C NMR analysis. Shown in Figure 2.21 is the $^1$H NMR spectrum of the crude product that employed MeCN / Et$_2$O (80:20) solvent mixture (lower spectrum) compared to the starting material (91a, upper spectrum). Note that in the lower spectrum the "*" is used to identify the $\beta$-methylene resonances in the product (108) (and possibly the mono-allylated intermediate). Also, the allyl resonances of the product (and possibly the mono-allylated intermediate) are evident in the 5.8-6.2 ppm region. Figure 2.22 demonstrates that the reaction is progressing using the MeCN / Et$_2$O (80:20) solvent system, but these
conditions are still not appropriate and further work needs to be done to find the right reaction conditions.

![NMR Spectra](image)

**Figure 2.22** The $^1$H NMR spectra comparing the starting material (91a, upper spectrum) with the allylated crude product (91a, 108 and the mono-allylated intermediate, lower spectrum). The "*" is used to identify the $\beta$-methylene resonances in the product 108 (and possibly the mono-allylated intermediate).

Unfortunately, the proper reaction conditions to synthesize 108 were not found, and therefore other conditions would need to be explored or an alternative approach found to synthesize di-allyl $p$-xylyl-linked cross-bridged cyclam 109. Other solvents can be investigated that may enable product to precipitate from solution, such as toluene or THF. Another alternative is to change the alkylating agent. David Martin utilized allyl tosylate when synthesizing the analogous dibenzo-annelated bisaminal salt precursor to 56. Martin even ran the reaction neat with a large excess of allyl tosylate. Allyl bromide was used first, and was the preferred alkylating agent, since it has a lower boiling point than allyl tosylate (and is thus easier to remove when the reaction is
complete) and allyl tosylate needs to be made prior to use and has a limited shelf-life compared to allyl bromide. Therefore, further actions that can be taken to synthesize 108 is to try the allylation reaction using allyl tosylate – with reaction conditions based upon Martin’s. Once 108 has been made, the reductive ring opening can be performed, as well as the deallylation that follows, both steps should proceed as smoothly as was seen for the bis-cyclen analog (107) and the non-linked systems (55 and 62). Also, since the N-PMB protecting group has been developed for cross-bridged cyclen systems (see Chapter I, in Section B of the Results and Discussion section), a di-PMB p-xylyl-linked cross-bridged cyclam could be synthesized as a route toward the di-NH p-xylyl-linked cross-bridged cyclam. It has been shown that the TFA deprotection conditions will not remove an N-benzyl group, and so there should be no cleavage of the N-xylyl bonds. If PMB were investigated as a protecting group for this system, then similar problems to what were found by Weisman and Dugan may be encountered with this system.98 The PMB group may be cleaved in solvents such as H2O, MeOH or EtOH. Since 95% EtOH is the solvent used for the reductive ring-opening step when employing NaBH4, this may mean that other solvents or reducing agents may be necessary. Perhaps NaBH3CN or NaBH(OAc)3 in MeCN could be explored as an alternative.

The di-NH p-xylyl-linked cross-bridged cyclen system 31, as well as the cyclam analog (110, once it has been synthesized), and their Cu(II) and Zn(II) metal complexes could all have potential use as anti-HIV agents and could also be used for stem cell mobilization just as the bis-cyclam 81 system (shown below in Figure 2.23) has been utilized. It could be that these bridged systems will not suffer the same fate as 81, and there will be no cardiac problems associated to their use. Also, with the secondary
nitrogen available on these bridged systems, 31 and 110 (once it is synthesized) could be further functionalized with pendant arms that could be used to aid in complexing (and stabilizing) metals that are bound to the bridged macrocycles. As was seen with the non-linked di-NH systems (7, 24), once the NH group has been deprotected and is available to perform further chemistry, a number of options are then open for modifying these ligands for a variety of uses.

\[
\text{NH} \quad \text{N} \quad \text{—} \quad \text{N} \quad \text{HN} \\
\text{NH} \quad \text{HN} \quad \text{N} \quad \text{HN} \\
\text{HN} \quad \text{HN} \quad \text{HN} \quad \text{HN} \\
\text{L}^+ \quad \text{J} \\
\text{81} \cdot 8 \text{HCl} \quad \text{J}^\text{J} \\
\]

**Figure 2.23** The bis-cyclam 81.

G. **Investigation of an aliphatic spacer group**

An exploratory effort was made to synthesize a cross-bridged bis-cyclen system incorporating an aliphatic linker, with 62 as the starting material. In the approach, shown below in Scheme 2.18, two equivalents of 62 were to be linked to one another via homo-metathesis using Grubb's 2\textsuperscript{nd} generation catalyst.\textsuperscript{197-199} Unfortunately the results were inconclusive. The crude product did not match what was expected – mainly there were no distinct signals in the \textsuperscript{1}H and \textsuperscript{13}C NMR spectra that could be attributed to a carbon-carbon double bond being present on the molecule. Neither the starting material 62 nor what would be expected for the desired product 111 were found in the spectra of the crude product. A possible further complication is that similar conditions may also be applied for the de-allylation of N-allyl functional groups – as has been demonstrated in the literature.\textsuperscript{59} Although it should be noted that the mono-benzyl cross-bridged cyclen 32 (the product of the deallylation of 62) was not observed in the crude \textsuperscript{1}H and \textsuperscript{13}C NMR
spectra of the product. Unfortunately, it is difficult to assign a particular structure to the crude $^1$H and $^{13}$C NMR spectra. With these disappointing results, no further attempts were made to synthesize 111 via this method. If research to synthesize an aliphatic spacer were re-visited, perhaps the same reaction conditions used in Scheme 2.18 could be employed at higher concentrations. But, since the first attempt gave no clear indication that any product was formed, it may be better to simply try another strategy.

The reaction conditions utilized in Reaction A in Scheme 1.5 (i.e., linking with a di-acid chloride followed by reduction via LiAlH$_4$ to yield 34) are a possible alternative to this method that could be explored.

Scheme 2.18 The proposed synthesis of 111.
IV. Conclusions

Progress was made in both the complexation of the bis-tetraazamacrocycle systems that were first synthesized in the Weisman research lab as well as in the synthesis of new cross-bridged bis-cyclen systems. Copper(II) complexes of both the *meta*-- and *para*-xylyl-linked cross-bridged cyclen (94a and 94b) and *para*-xylyl-linked adjacent-bridged cyclam 102a were synthesized and analyzed and the zinc(II) complexes of the *meta*-- and *para*-xylyl-linked cross-bridged cyclen ligands (94a and 94b) were also prepared. The copper(II) *para*-xylyl-linked cross-bridged cyclen system (Cu₂ • 94a) and the copper(II) *para*-xylyl-linked adjacent-bridged bis-cyclam system (Cu₂ • 102a) were found to be less kinetically inert than a variety of copper(II) cross-bridged cyclam tetraazamacrocycle complexes (see Table 2.1). Complexes Cu₂ • 94a and Cu₂ • 102a demonstrated irreversible reductions when analyzed using cyclic voltammetry. The fact that these copper(II) complexes are not as kinetically inert (demonstrated by their shorter half-lives in the same acidic conditions, as illustrated in Table 2.1) and are more likely to lose copper upon 1-electron reduction from Cu(II) to Cu(I) as compared to their cross-bridged cyclam analogs makes them less useful for biomedical purposes. As stated in the introduction of Chapter I, it has been found that the kinetic inertness of the copper complex toward decomplexation is even more important than the thermodynamic stability when measuring the stability of the radio-copper complex *in vivo* and the reduction of copper(II) to copper(I) *in vivo* may result in a loss of the metal, therefore the resistance of the radio-copper complex toward Cu(II)/Cu(I) reduction and reversibility under biological conditions is an important feature. These results demonstrate the need to replace the N-methyl group with a protecting group that can be removed to yield 31.
With an N-H functional group in place of an N-Me group, the cross-bridged bis-tetraazamacrocycle 31 can be more easily modified and adapted for particular uses. Work toward the synthesis of a mono-zinc(II) complex of the cross-bridged bis-cyclen 94a did not progress as hoped, but if a mono-Zn(II) complex of 94a is made then work toward a mono-Zn(II) mono-Cu(II) complex of 94a could be attempted and this dinuclear complex could be investigated as an SOD biomimic.

The N-allyl protecting group developed in Chapter I was successfully applied to the di-allyl para-xylyl-linked cross-bridged cyclen system (i.e., 107). While further work should be done to develop a more efficient process for its purification, 31 can now be complexed with copper(II) and zinc(II) and the properties of these complexes can be studied. Since a variety of linked and non-linked tetraazamacrocycles have been able to bind to the CXCR4 receptor, 31 may also be useful as an anti-HIV agent or a stem-cell mobilizer. And, unlike the di-methyl bis-cyclam analog 95a, 31 exists as only one stereoisomer so there are no concerns about the presence of diastereomers which may be less active or potentially harmful to the body. Further work will be necessary to synthesize di-NH cross-bridged bis-cyclam 110. Once this compound has been made it can be determined if this ligand has improved anti-HIV properties over the copper(II) complex of its di-methyl analog (Cu₂ • 95a) tested by Hubin.¹¹¹

The diamide para-xylyl-linked cross-bridged cyclen 105 has also been synthesized, but further work needs to be done to purify this compound and complete its characterization. This compound, as well as its di-carboxymethyl-armed derivative, can also be studied further for its copper(II) and zinc(II) complexation characteristics. It
could be that the resulting complexes will be more kinetically stable and therefore have greater promise for biological applications.

While progress has been made with the complexation and synthesis of several new bis-tetraazamacrocycles, there is still much more that can be done. These systems have great promise for a variety of potential biological applications, many of which are based upon their ability to bind with the CXCR4 receptor, but these systems can also be used for other possible biomimetic purposes, in PET imaging, and as MRI contrasting agents. As stated in Chapter I, tetraazamacrocycles can be modified for a variety of purposes by making adjustments to the macrocycle backbone itself as well as by affixing a variety of pendant arms. Bis-tetraazamacrocycles can be modified in the same way, but can be further altered when changing, or functionalizing, the spacer group between macrocycles. Therefore, considering the great deal of versatility of the basic bridged bis-tetraazabicycles, there is a great deal of potential research yet to be done with these compounds.
CHAPTER III

EXPERIMENTAL SECTION

I. General Methods

Melting Points (mp) were recorded on a Thomas Hoover capillary melting point apparatus and are uncorrected.

Infrared Spectra (IR) were run on a Nicolet MX-1 FT-IR spectrometer with EZ OMNIC 6.0a software. Absorptions are reported in wavenumbers (cm$^{-1}$).

$^1$H NMR Spectra ($^1$H NMR) were acquired on Varian NMR spectrometer systems operating at 500 MHz with VNMR 6.1c software. Chemical shift ($\delta$) values are reported in parts per million (ppm) relative to Me$_4$Si (TMS) unless otherwise noted. Coupling constants ($J$ values) are reported in Hertz (Hz).

$^{13}$C NMR Spectra ($^{13}$C NMR) were acquired on a Varian NMR spectrometer system operating at 125.7 MHz with VNMR 6.1c software. Chemical shift ($\delta$) values are reported in parts per million (ppm) relative to Me$_4$Si (TMS) unless otherwise noted.

Electrospray Ionization Mass Spectra (ESIMS) was obtained on a Thermoquest LCQ mass spectrometer at the University of New Hampshire.

High Resolution Mass Spectra (HRMS) Compounds were analyzed by the Department of Chemistry and Biology at the University of Notre Dame using a JEOL AX505HA high resolution mass spectrometer.

X-Ray Crystallography (X-Ray) was performed by Arnold L. Rheingold (compounds 55 and 62) and James A. Golen at the University of California, San Diego, La Jolla,
California, USA. with some further refinements for compound 31 performed by Jonathan M. White and Gary Weisman at the University of Melbourne, Parkville, Australia,

Microwave Synthesis The CEM Discover® research scale manual microwave synthesizer using Synergy v.1.21 software was utilized for microwave-assisted syntheses.

Centrifugation: was performed on an International Equipment Company International Clinical Centrifuge Model CL.

Elemental Analysis (CHN) was performed by Atlantic Labs Inc., Georgia, USA.

pH Measurements were performed on a Hanna Instruments HI 221 microprocessor-based pH/mV/°C bench meter.

Ultra-Violet Visible Spectra (UV-Vis) were taken on a Varian Cary 5E UV-Vis-NIR spectrophotometer. Absorbance maxima are reported in nanometers (nm).
II. Solvents

*Absolute Ethanol* (EtOH, ACS/USP grade) was obtained from Pharmco Products Inc. and was used without further purification.

*Acetonitrile* (CH$_3$CN) was obtained from EMD Chemicals Inc. and stored in an Innovative Technology Inc. Pure-Solv Solvent Purification System. Prior to use, the solvent was passed through the system’s silica column under low pressure to remove trace impurities.

*Benzene* (C$_6$H$_6$, ACS grade) was obtained from EMD Chemicals Inc. and was used without further purification.

*Chloroform* (CHCl$_3$, HPLC grade) was obtained from EMD Chemicals Inc. and was used without further purification.

*Diethyl Ether* (Et$_2$O) was obtained from EMD Chemicals Inc. and stored in an Innovative Technology Inc. Pure-Solv Solvent Purification System. Prior to use, the solvent was passed through the system’s silica column under low pressure to remove trace impurities.

*Dimethyl Sulfoxide* (DMSO, ACS grade) was obtained from EMD Chemicals Inc. and was stored over 3Å molecular sieves.

*Deuterated NMR Solvents* were obtained from Cambridge Isotope Laboratories and both DMSO-d$_6$ and CDCl$_3$ were stored over 3Å molecular sieves.

*Ethanol* (95% EtOH, ACS grade) was obtained from EMD Chemicals Inc. and was used without further purification.

*Methanol* (MeOH, Reagent/ACS/USP/NF grade) was obtained from Pharmco Products Inc. and stored in an Innovative Technology Inc. Pure-Solv Solvent Purification System.
Prior to use, the solvent was passed through the system’s silica column under low pressure to remove trace impurities.

_Tetrahydrofuran_ (THF) was obtained from EMD Chemicals Inc. and stored in an Innovative Technology Inc. Pure-Solv Solvent Purification System. Prior to use, the solvent was passed through the system’s silica column under low pressure to remove trace impurities.

_Toluene_ (PhCH₃, ACS grade) was obtained from EMD Chemicals Inc. and stored in an Innovative Technology Inc. Pure-Solv Solvent Purification System. Prior to use, the solvent was passed through the system’s silica column under low pressure to remove trace impurities.
III. **Reagents**

Allyl Bromide was obtained from Aldrich Chemical Company.

Alumina: Aluminum oxide powder (activated, basic, Brockmann 1, 150 mesh) "suitable for chromatography" was obtained from Aldrich Chemical Co.

Ammonium hydroxide (28%, NH₃ in water) was obtained from Fisher Chemical Co.

Anisole was obtained from Aldrich Chemical Co.

Benzyl Bromide was obtained from Alfa Aesar.

2-(Bromomethyl)naphthalene was obtained from Aldrich Chemical Co.

Diatomaceous Earth (Celite® 545) was obtained from Aldrich Chemical Company.

1,3-Dibromopropane was obtained from Aldrich Chemical Co.

α,α'-Dibromo-m-xylene was obtained from Aldrich Chemical Co.

α,α'-Dibromo-p-xylene was obtained from Aldrich Chemical Co.

Ethylenediamine was obtained from Aldrich Chemical Company.

Glyoxal (40% aq) was obtained from Aldrich Chemical Company.

Hydrochloric Acid (12 M HCl) was obtained from Fisher Chemical Company and was used without further purification.

Hydrobromic Acid (47-49% in water) was obtained from Aldrich Chemical Company.

p-Methoxybenzylbromide (PMB-Br) was obtained from Aldrich Chemical Company.

Methyl iodide (Mel) was obtained from Aldrich Chemical Co.

Potassium Hydroxide (KOH) was obtained from EMD Chemicals Incorporated.

Potassium carbonate (K₂CO₃) was obtained from Fisher Chemical Co.

Potassium tert-Butoxide (KO'Bu) was obtained from Aldrich Chemical Company.
Silica Gel (230-400 mesh ASTM) for column chromatography was obtained by EM Science.

Sodium Borohydride \((\text{NaBH}_4)\) was obtained from Aldrich Chemical Company.

Sodium Cyanoborohydride \((\text{NaBH}_3\text{CN})\) was obtained from Aldrich Chemical Company.

Sodium Triacetoxyborohydride \((\text{NaBH(OAc)}_3)\) was obtained from Aldrich Chemical Company.

Sodium Hydroxide \((\text{NaOH})\) was obtained from EM Science Company.

Sodium Sulfate \((\text{Na}_2\text{SO}_4)\) was obtained from Fisher Chemical Company.

Sodium Tetraphenylborate \((\text{NaBPh}_4)\) was obtained from J.T. Baker Chemical Company.

1,4,7,10-Tetraazacyclododecane \((\text{cyclen})\) was obtained from Strem Chemical Co.

1,4,8,11-Tetraazacyclotetradecane \((\text{cyclam})\) was obtained from Strem Chemical Co.

Trifluoroacetic Acid \((\text{TFA, 99\%})\) was obtained from Alfa Aesar.
IV. Syntheses

Note: All routine solvent evaporations were carried out on a standard rotary evaporator using aspirator pressure unless otherwise noted. All reactions were carried out under N₂ atmosphere unless otherwise noted.

1,4,8,11-Tetraazabicyclo[6.6.2]hexadecane (7). 4,11-Diallyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane 60 (0.0510 g, 0.1665 mmol) was massed into a 5 mL round-bottomed reaction flask, and to this oil 0.46 mL of dry DMSO (stored over 4Å sieves under N₂) was added via syringe followed by potassium t-butoxide (0.0603 g, 0.5374 mmol) (NOTE: All reagents were handled under N₂ in a dry bag). The reaction mixture was stirred under N₂ before inserting the reaction flask into a CEM Discover microwave reactor, making sure that there were no deposits of potassium t-butoxide adhered to the inner wall of the reaction flask. Once in the microwave reactor, the reaction flask was fitted with an air condenser with a nitrogen inlet adapter attached and the open vessel reaction was run under microwave irradiation at 100 °C for 45 minutes. A brown pungent-smelling mixture remained (the sample was capped at all times when handled outside of the hood). 6M HCl was added dropwise to the crude mixture and the resulting solution was concentrated to an oil under reduced pressure. [Note: At this point, while the crude reaction mixture is still acidic, a wash could be performed using the appropriate volume of toluene]. This oil was then dissolved in H₂O (8 mL) and KOH pellets were added to make the solution strongly basic (pH 14). The basic solution was extracted with toluene (5 x 8 mL) and the combined extracts were dried over Na₂SO₄. Solvent was removed under reduced pressure to yield a crude brown oil (0.0280 g, 74.5 %). The crude brown oil may be further purified via Kugelrohr distillation (100 °C air
bath temperature, 0.06 mmHg) to yield a clear oil. The $^1$H NMR spectrum was consistent with the literature spectra of pure 7.\textsuperscript{10,12}

**Note:** If DMSO is present in the crude sample after toluene extraction, it can be removed by re-dissolving the sample in water, making the solution strongly basic (pH $\approx$14), and extracting the sample again with toluene as above. This procedure can be repeated until all of the DMSO is removed. Also, if the deallylation reaction is not complete, the crude product can be reacted again with an excess of $t$-butoxide in dry DMSO to complete the deallylation.

**1,4,7,10-Tetraazabicyclo[5.5.2]tetradecane (24).** 4,10-Bis-(4-methoxybenzyl)-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane \( \mathbf{66} \) (0.2055 g, 0.4685 mmol) in a 10 mL round-bottomed flask was treated with anisole (93 $\mu$L, 0.85 mmol) followed by trifluoroacetic acid (2.60 mL). The reaction mixture was stirred, under N\(_2\), at 60 °C for 18 h. Upon cooling, 75 mL Et\(_2\)O was added to the resulting light-orange colored reaction mixture (to aid in the removal of TFA) and it was concentrated to a thick yellow oil. H\(_2\)O (20 mL) was added dropwise to the crude mixture, followed by 10 mL 6M HCl, and the resulting solution was extracted with toluene (5 x 20 mL). The aqueous phase was made strongly basic (pH 14) by slow addition of KOH pellets, with cooling, and this basic solution was then extracted with toluene (5 x 25 mL). The combined extracts were dried over Na\(_2\)SO\(_4\) and then the solvent was removed under reduced pressure to yield a yellow and white solid (0.0766 g, 82% crude yield). The crude product was Kugelrohr distilled (75 °C air bath temperature, 0.06 mmHg) to yield a white solid (0.0438 g, 47%): $^1$H NMR and
ON the basis of the spectra, the purity is estimated to be >97%.

(Note: This reaction can also be run via microwave heating, for 10 min at 85°C.)

4-[(4-(1,4,7,10-Tetraazabicyclo[5.5.2]tetradec-4-ylmethyl)-benzyl]-1,4,7,10-
tetraaza-bicyclo[5.5.2]tetradecane (31). 4-Allyl-10-[(4-allyl-1,4,7,10-
tetraazabicyclo[5.5.2]tetradec-4-ylmethyl)-benzyl]-1,4,7,10-tetraazabicyclo[5.5.2]-
tetradecane 107 (0.8191, 1.415 mmol) in a 50 mL round-bottomed reaction flask was
treated with 8 mL of dry DMSO (stored over 4Å sieves under N₂) followed by potassium
\textit{t}-butoxide (0.4895 g, 4.362 mmol) (NOTE: all reagents were handled under N₂ in a dry
bag). The reaction mixture was pre-stirred in an oil bath, at about 60 °C, under N₂ before
inserting the reaction flask into a CEM Discover microwave reactor – making sure that
there were no deposits of potassium \textit{t}-butoxide adhered to the inner wall of the reaction
flask. Once in the microwave reactor, the reaction flask was fitted with an air condenser
with a nitrogen inlet adapter attached and the open-vessel reaction was run under
microwave irradiation at 100 °C for 45 minutes (maximum power set to 100 W). A
brown pungent-smelling mixture remained (the sample was capped at all times when
handled outside of the hood). H₂O (10 mL) was added dropwise to the crude mixture,
followed by 6M HCl (50 mL) and the resulting solution was concentrated to an oil under
reduced pressure. This oil was then dissolved in H₂O (200 mL) and KOH pellets were
added to make the solution strongly basic (pH 14). The basic solution was extracted with
toluene (5 x 150 mL) and the combined extracts were dried over Na₂SO₄. Solvent was
removed under reduced pressure to yield a light brown solid (0.6195 g, 75 % crude).
This crude product was precipitated from a hot EtOH/water (≈3.75:1) solution as an HBr salt, which was then filtered to yield an off-white powder. The crude product was recovered from the HBr salt. The salt was dissolved in H₂O (10 mL), KOH pellets were added to make the solution strongly basic (pH 14), the basic solution was extracted with toluene (5 x 10 mL), and the combined extracts were dried over Na₂SO₄. Solvent was removed under reduced pressure and the crude light-yellow solid (0.1934 g) was distilled (190 °C, 0.1 mmHg) to yield a white solid (0.0513 g, 7 %): mp 100-103 °C; ¹H NMR (500 MHz, C₆D₆) δ 2.42-2.52 (m, 12H), 2.53-2.64 (m, 12H), 2.64-2.66 (app br s, 8H, ethylene cross-bridge), 2.70-2.83 (m, 8H), 3.47 (br s, 2H, NH), 3.58 (s, 4H, CH₂Ar), 7.46 (s, 4H, Ar); ¹³C{¹H} NMR (125.68 MHz, C₆D₆) δC 48.32, 52.03, 52.90, 53.62, 55.67, 62.06, 129.91, 138.67; IR 3223, 3035, 2910, 2839, 2794, 1671, 1449, 1369, 1316, 1162, 1134, 1111, 1037, 1019, 973, 930, 778 cm⁻¹; HRFABMS, m/z (M+H)⁺ exact mass calcd for C₂₈H₅₁N₈: 499.4237; Found: 499.4239 (error +0.3 mmu/+0.5 ppm).

Note: If DMSO is present in the crude sample after toluene extraction, it can be removed by re-dissolving the sample in water, making the solution strongly basic (pH ≈14), and extracting the sample again with toluene as above. This procedure can be repeated until all of the DMSO is removed. Also, if the deallylation reaction is not complete, the crude product can be reacted again with an excess of t-butoxide in dry DMSO to complete the deallylation.

This 7% yield was the first “cut” from the sublimation/distillation. When the temperature was increased to 240 °C for the second cut, the rest of the impurities came over and the 2nd cut was not as pure (cut 2 was 0.0829 g). This accounts for the lost mass, the rest of the material was remaining in the pot and lost to transfer of the samples.
4-Benzyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane (32). [via N-allyl protecting group] 4-Allyl-10-benzyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane 62 (0.6426 g, 1.967 mmol) was transferred into the 25 mL round-bottomed reaction flask, and to this oil 5.4 mL of dry DMSO (stored over 4Å sieves under N$_2$) was added via syringe followed by potassium t-butoxide (0.3298 g, 2.939 mmol) (NOTE: All reagents were handled under N$_2$ in a dry bag). This mixture was stirred under N$_2$ before inserting the reaction flask into a CEM Discover microwave reactor, making sure that there were no deposits of potassium t-butoxide adhered to the inner wall of the reaction flask. Once in the microwave reactor, the reaction flask was placed under an air condenser with a nitrogen inlet adapter attached and the open vessel reaction was run under microwave irradiation at 100 °C for 25 minutes. A brown pungent-smelling mixture remained (the sample was capped at all times when handled outside of the hood). H$_2$O (10 mL) was added dropwise to the crude mixture, followed by 20 mL 6M HCl, and the resulting solution was concentrated to an oil under reduced pressure. This oil was then dissolved in H$_2$O (70 mL) and KOH pellets were added to make the solution strongly basic (pH 14). The basic solution was extracted with toluene (5 x 80 mL) and the combined extracts were dried over Na$_2$SO$_4$. Solvent was removed under reduced pressure and the crude brown oil was Kugelrohr distilled (145 °C air bath temperature, 0.1 mmHg) to yield a clear, colorless oil (0.4394 g, 77.4 %): $^1$H NMR (500 MHz, C$_6$D$_6$) δ 2.43-2.79 (m, 20H), 3.21 (br s, 1H, NH), 3.58 (s, 2H, CH$_2$Ph), 7.09-7.13 (m, 1H, Ph), 7.20-7.24 (m, 2H, Ph), 7.34-7.40 (m, 2H, Ph); $^{13}$C {$^1$H} NMR (125.68 MHz, C$_6$D$_6$, C$_6$D$_6$ secondary ref. (center peak) set to 128.06) δ$_C$ 48.83, 52.56, 53.59, 54.03, 56.00, 61.84, 127.17, 128.43,
129.82, 140.06; IR 3226 (R\_2N-H), 3083, 3058, 3026, 2801, 1948, 1876, 1809, 1672, 1452, 1372, 1160, 1130, 1030, 977, 729, 700 cm\(^{-1}\); HRFABMS, m/z (M+H)\(^+\) exact mass calcd for C\(_{17}\)H\(_{29}\)N\(_4\): 289.2392; Found: 289.2393 (error +0.1 mmu/+0.4 ppm).

**Note:** If DMSO is present in the crude sample after toluene extraction, it can be removed by re-dissolving the sample in water, making the solution strongly basic (pH ≈14), and extracting the sample again with toluene as above. This procedure can be repeated until all of the DMSO is removed. Also, if the deallylation reaction is not complete, the crude product can be reacted again with an excess of \(\tau\)-butoxide in dry DMSO to complete the deallylation.

**4-Benzyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane (32).** [via N-PMB protecting group] 4-Benzyl-10-(4-methoxybenzyl)-1,4,7,10-tetraazabicyclo[5.5.2]-tetradecane 67 (0.2227 g, 0.5451 mmol) in a 5 mL round-bottomed flask was treated with anisole (54 \(\mu\)L, 0.49 mmol) followed by trifluoroacetic acid (1.47 mL). The reaction mixture was stirred, under N\(_2\), at 60 °C for 18 h. Upon cooling, 75 mL Et\(_2\)O was added to the resulting light-orange colored reaction mixture (to aid in the removal of TFA) and it was concentrated to a thick yellow oil. H\(_2\)O (10 mL) was added dropwise to the crude mixture, followed by 15 mL 6M HCl, and the resulting solution was extracted with toluene (5 x 15 mL). The aqueous phase then had H\(_2\)O (10 mL) added and was made strongly basic (pH 14) by slow addition of KOH pellets, with cooling. This basic solution was then extracted with toluene (5 x 25 mL) and the combined extracts were dried over Na\(_2\)SO\(_4\). The solvent was then removed under reduced pressure and the resulting yellow oil was Kugelrohr distilled (150 °C air bath temperature, 0.1 mmHg) to
yield a clear oil (0.1132 g, 72%): \(^1\text{H}\) NMR and \(^{13}\text{C}\{^1\text{H}\}\) NMR spectra were consistent with spectra for \(32\) reported in this dissertation. On the basis of the spectra, the purity is estimated to be >97%.

(Note: This reaction can also be run via microwave heating, for 10 min at 85° C.)

4-Benzyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (33). 4-Allyl-11-benzyl-1,4,8,11-tetraaza-bicyclo[6.6.2]hexadecane 55 (0.6367 g, 1.79 mmol) was transferred into the 25 mL round-bottomed reaction flask, and to this oil 5.0 mL of dry DMSO (stored over 4Å sieves under \(\text{N}_2\)) was added via syringe followed by potassium \(\gamma\)-butoxide (0.3096 g, 2.76 mmol) (Note: All reagents were handled under \(\text{N}_2\) in a dry bag). This mixture was stirred under \(\text{N}_2\) before inserting the reaction flask into a CEM Discover microwave reactor, making sure that there were no deposits of potassium \(\gamma\)-butoxide adhered to the inner wall of the reaction flask. Once in the microwave reactor, the reaction flask was placed under an air condenser with a nitrogen inlet adapter attached and the open vessel reaction was run under microwave irradiation at 100 °C for 25 minutes. A brown pungent-smelling mixture remained (the sample was capped at all times when handled outside of the hood). \(\text{H}_2\text{O}\) (10 mL) was added dropwise to the crude mixture, followed by 10 mL 6M HCl, and the resulting solution was concentrated to an oil under reduced pressure. This oil was then dissolved in \(\text{H}_2\text{O}\) (70 mL) and KOH pellets were added to make the solution strongly basic (pH 14). The basic solution was extracted with toluene (5 x 95 mL) and the combined extracts were dried over \(\text{Na}_2\text{SO}_4\). Solvent was removed under reduced pressure and the crude brown oil was Kugelrohr distilled (145 °C air bath temperature, 0.1 mmHg) to yield a clear, colorless oil (0.5094 g, 89.9
\[\text{\textsuperscript{1}H NMR (500 MHz, C}_6\text{D}_6\delta 1.26-1.56 (m, 4H, CH}_2\text{CH}_2\text{CH}_2\text{), 2.01-2.10 (m, 2H), 2.14-2.24 (m, 2H), 2.27 (ddd, 1H, } J = 14.0, 11.5, 2.8 \text{ Hz), 2.35 (tt, 1H, } J = 11.2, 2.9 \text{ Hz), 2.37-2.56 (m, 7H), 2.65 (ddd, 1H, } J = 14.3, 11.4, 3.3 \text{ Hz), 2.78 (td, 1H, } J = 11.7, 5.9 \text{ Hz), 2.87 (td, 1H, } J = 12.3, 4.3 \text{ Hz), 2.97 (ttdd, 1H, } J = 10.9, 5.8, 0.8 \text{ Hz), 3.14 (d, 1H, } J = 13.1 \text{ Hz, CHHFPh), 3.16-3.28 (m, 2H), 3.36-3.43 (m, 1H), 3.57 (d, 1H, } J = 13.1 \text{ Hz, CHHFPh), 4.46-4.54 (m, NH, 1H), 7.09-7.13 (m, 1H, Ph), 7.16-7.22 (m, 2H, Ph), 7.24-7.28 (m, 2H, Ph); \text{\textsuperscript{13}C{\textsuperscript{1}H}} \text{ NMR (125.68 MHz, C}_6\text{D}_6\delta C 26.08, 28.14, 49.14, 49.34, 49.37, 50.00, 54.81, 55.19, 57.14, 58.09, 59.17, 59.21, 59.82, 126.79, 128.12, 129.40, 140.51; IR 3238 (R}_2\text{N-H), 3084, 3061, 3026, 2799, 1944, 1872, 1804, 1494, 1454, 1360, 1138, 1113, 1013, 735, 699 cm}^{-1}; \text{ HRFABMS, m/z (M+H)}^+ \text{ exact mass calcd for C}_{19}\text{H}_{33}\text{N}_4: 317.2705; \text{ Found: 317.2693 (error -1.2 mmu/-3.9 ppm).}

\textbf{Note:} If DMSO is present in the crude sample after toluene extraction, it can be removed by re-dissolving the sample in water, making the solution strongly basic (pH \approx 14), and extracting the sample again with toluene as above. This procedure can be repeated until all of the DMSO is removed. Also, if the deallylation reaction is not complete, the crude product can be reacted again with an excess of \text{-}butoxide in dry DMSO to complete the deallylation.

\textbf{4-Allyl-11-benzyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (55).} \textit{cis-15-1-}

\textbf{Allyl-8-benzyl-4,11-diaza-1,8-diazoniatetracyclo[6.6.2.0.4\textsuperscript{16},0\textsuperscript{11},15]hexadecane dibromide 54} (3.0036 g, 5.8397 mmol) was added in one portion to 169 mL of acetonitrile, under N\textsubscript{2}. This mixture was stirred for several minutes and NaBH\textsubscript{3}CN (14.7258 g, 234.34 mmol) was then added in small portions over several minutes. The mixture was stirred at
reflux for 24 h (*caution HCN evolution!*). The solvent was then removed under reduced pressure (the rotary evaporator was vented to the hood) to yield a white solid, the solid was dissolved in 1:1 (vol:vol) MeOH:H₂O and 6M HCl was added dropwise to the reaction mixture (to pH 1). ( Decomposition of the excess NaBH₃CN, with evolution of HCN, may take an hour. The absence of bubbles forming in the solution indicates the process is complete.) The solvent was then removed under reduced pressure yielding a white paste (the rotary evaporator was vented to the hood). Methanol and water were then added in portions so as to dissolve all solids (typically requiring a 1:1 (vol:vol) MeOH:H₂O solution). The solution was made strongly basic (pH 14) by slow addition of KOH pellets, with cooling, and this basic solution was then extracted with toluene (5 x 180 mL). The combined extracts were dried over Na₂SO₄ and the solvent was removed under reduced pressure to yield clear, colorless product as an oil that required no further purification (1.9638 g, 94.3%): ¹H NMR (500 MHz, C₆D₆) δ 1.25-1.57 (m, 4H, CH₂CH₂CH₂), 2.16-2.25 (m, 2H), 2.31-2.59 (m, 11H), 2.68-2.79 (m, 3H), 2.95 (ddd, 1H, J = 12.9, 11.9, 4.0 Hz), 3.07 (d, 1H, J = 13.5 Hz, CH/HPh), 3.15 (ddt, 1H, J = 14.1, 4.8, 1.7 Hz), 3.22-3.35 (m, 2H), 3.72 (td, 1H, J = 11.6, 4.3 Hz), 3.75 (d, 1H, J = 13.4 Hz, CH/HPh), 4.05 (td, 1H, J = 11.7, 3.9 Hz), 5.01-5.05 (dm, 1H, J = 10.2 Hz, CH₂CH=CHH₂), 5.10-5.16 (dm, 1H, J = 17.2 Hz, CH₂CH=CHH₂), 5.87 (dddd, 1H, J = 17.2, 10.2, 7.7, 4.9 Hz, CH₂CH=CH₂), 7.09-7.13 (m, 1H), 7.18-7.23 (m, 2H, Ph), 7.33-7.37 (m, 2H, Ph); ¹³C {¹H} NMR (125.68 MHz, C₆D₆) δc 28.44, 28.78, 51.79, 51.99, 54.24, 54.85, 57.21, 57.24, 57.73, 57.79, 57.97, 58.01, 58.30, 60.33, 116.07, 126.92, 128.36, 129.25, 137.60, 141.27; IR 3064, 3026, 2910, 2794, 1943, 1867, 1835, 1805, 1743, 1679, 1642, 1602, 1493, 1453, 1363, 1243, 1125, 916, 733, 699 cm⁻¹; HRFABMS,
$m/z$ (M+H)$^+$ exact mass calcld for C$_{22}$H$_{37}$N$_4$: 357.3018; Found: 357.3001 (error -1.7 mmu/-4.8 ppm).

**(8bR,8cR)-rel-Decahydro-2a-allyl-6a-benzyl-4a,8a-diaza-2a,6a-diazone cyclonaphthylenium dibromide (61).** Allyl Bromide (11.00 g, 90.91 mmol) was added dropwise to a stirred mixture of (8bR,8cR)-rel-decahydro-2a-benzyl-4a,6a,8a-triaza-2a-azoniacyclonaphthylenene bromide 39 (3.32 g, 9.09 mmol) in MeCN (145 mL). The reaction mixture was stirred at room temperature for 4 days in a tightly stoppered flask, in the dark. The fine white precipitate was then isolated by suction filtration and washed with MeCN. Residual solvent was removed under reduced pressure to yield 4.11 g of a white solid (93.7 %). *(NOTE: The yield of this reaction can be increased by concentrating the filtrate, washing it with MeCN, and, if it is of comparable purity, combining it with the washed precipitate): mp 178-186 °C (dec); $^1$H NMR (500 MHz, D$_2$O, CH$_3$CN secondary ref. set to 2.05) δ 2.99-3.07 (m, 1H), 3.08-3.21 (m, 2H), 3.31-3.46 (m, 3H), 3.52-3.70 (m, 4H), 3.72-3.80 (dm, 1H, $J = 13.0$ Hz), 3.82-3.99 (m, 3H), 4.07 (ddd, 1H, $J = 12.1$, 10.5, 4.2 Hz), 4.20 (dd, 1H, $J = 12.6$, 5.8 Hz, $\text{CH}_2\text{CH}_2\text{CH}=\text{CH}$), 4.20-4.30 (m, 1H), 4.37 (dd, 1H, $J = 13.4$, 7.8 Hz, CH$\text{HCH}=\text{CH}_2$), 4.59 (d, 1H, $CH$, $J = 2.5$ Hz), 4.69 (d, 1H, CH, $J = 2.5$ Hz), 4.75 (d, downfield line partially overlapped with HDO, 1H, $J = 12.8$ Hz, CH$\text{HCH}=\text{CH}_2$), 4.92 (d, 1H, $J = 13.3$ Hz, CH$\text{HPh}$), 5.76-5.82 (dm, 1H, $J = 16.9$ Hz, CH$_2$CH=CH$\text{H}_2$), 5.79-5.83 (dm, 1H, $J = 10.0$ Hz, CH$_2$CH=CH$\text{H}_2$), 6.09 (dddd, 1H, $J = 16.9$, 10.2, 7.6, 6.9 Hz, CH$_2$CH=CH$_2$), 7.54-7.66 (m, 5H, Ph); $^{13}$C{$^1$H} NMR (125.68 MHz, D$_2$O, CH$_3$CN secondary ref. set to 1.70) δ$_C$ 43.25, 43.46, 46.77, 47.01, 55.75, 56.40, 60.83(C-9), 61.48, 61.75 (Benzyl), 61.84, 77.94,
(C-11), 126.82 (ipso-\(C_6H_3\)), 130.38, 130.40, 131.98, 133.14 (\(o-C_6H_5\)); IR (KBr) 3430, 3005, 1618, 1438, 1401, 1287, 954, 929, 709 cm\(^{-1}\); Anal Calcd for C\(_{20}\)H\(_{30}\)N\(_4\)Br\(_2\)-1.5H\(_2\)O: C, 46.80; H, 6.48; N, 10.91; Br, 31.13. Found: C, 46.60; H, 6.55; N, 10.74; Br, 30.77; HRFABMS, \(m/z\) (M)\(^+\) exact mass calcd for C\(_{20}\)H\(_{30}\)N\(_4\)Br: 357.3018; Found: 405.1665 (error +1.2 mmu/+2.8 ppm).

4-Allyl-10-benzyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane (62). (8bR,8cR)-rel-Decahydro-2a-allyl-6a-benzyl-4a,8a-diaza-2a,6a-diazenia-cyclopent[fg]-acenaphthylene dibromide 61 (3.0254 g, 6.2214 mmol) was added in one portion to 180 mL of acetonitrile, under N\(_2\). This mixture was stirred for several minutes and NaBH\(_3\)CN (15.6505 g, 249.53 mmol) was then added in small portions over several minutes. The mixture was stirred at reflux for 24 h (caution HCN evolution!). The solvent was then removed under reduced pressure (the rotary evaporator was vented to the hood) to yield a white solid, the solid was dissolved in 1:1 (vol:vol) MeOH:H\(_2\)O and 6M HCl was added dropwise to the reaction mixture (to pH 1). (Decomposition of the excess NaBH\(_3\)CN, with evolution of HCN, may take an hour. The absence of bubbles forming in the solution indicates the process is complete.) The solvent was then removed under reduced pressure yielding a white paste (the rotary evaporator was vented to the hood). Methanol and water were then added in portions so as to dissolve all solids (typically requiring a 1:1 (vol:vol) MeOH:H\(_2\)O solution). The solution was made strongly basic (pH 14) by slow addition of KOH pellets, with cooling, and this basic solution was then extracted with toluene (5 x 180 mL). The combined extracts were dried over Na\(_2\)SO\(_4\) and the solvent was removed under reduced pressure to yield clear,
colorless product as an oil that required no further purification (2.0134 g, 99.7%): $^1$H NMR (500 MHz, $\text{C}_6\text{D}_6$) $\delta$ 2.57-2.84 (m, 16H), 3.06-3.08 (dm, 2H, $J = 6.3$ Hz), 3.08-3.19 (m, 4H, AA'BB', ethylene cross-bridge), 3.59 (s, 2H, $CH_2$Ph), 5.00-5.02 (m, 0.5H, $CH_2$CH=CHH), 5.02-5.05 (m, 1H, $CH_2$CH=CHH), 5.06-5.08 (m, 0.5H, $CH_2$CH=CHH), 5.79-5.88 (m, 1H, $CH_2$CH=CH$_2$), 7.09-7.13 (m, 1H, Ph), 7.18-7.23 (m, 2H, Ph), 7.28-7.32 (m, 2H, Ph); $^{13}$C($^1$H) NMR (125.68 MHz, $\text{C}_6\text{D}_6$) $\delta$C 56.96, 57.88, 57.99, 58.35, 58.72, 59.94, 61.53, 115.99, 126.89, 128.34, 129.11, 137.18, 140.88; IR 3063, 3026, 2913, 2806, 1944, 1869, 1835, 1806, 1675, 1641, 1493, 1451, 1371, 1104, 1076, 1050, 1031, 994, 916, 729, 699 cm$^{-1}$; HRFABMS, $m/z$ (M+H)$^+$ exact mass calcd for C$_{20}$H$_{33}$N$_4$: 329.2705; Found: 329.2707 (error +0.1 mmu/+0.4 ppm).

$(8bR,8cR)$-rel-Decahydro-2a,6a-bis-(4-methoxybenzyl)-4a,8a-diaza-2a,6a-diazeniacyclopent[fg]acenaphthylene dibromide (65). $p$-Methoxybenzyl bromide (1.3276 g, 6.405 mmol, stabilized with 0.0398 g K$_2$CO$_3$) was added dropwise to a stirred solution of $(8bR,8cR)$-rel-decahydro-2a,4a,6a,8a-tetraazacyclopent[fg]acenaphthylene 18 (0.2057 g, 1.0588 mmol) in MeCN (8 mL). The reaction mixture was stirred, under N$_2$, at room temperature for 3 days. The white precipitate was then isolated by suction filtration and washed with Et$_2$O (6 x 10 mL). Residual solvent was removed under reduced pressure to yield 0.5609 g of product as a white solid (89%): mp 186-190 °C (dec); $^1$H NMR (500 MHz, D$_2$O, CH$_3$CN secondary ref. set to 2.05) $\delta$ 3.08-3.16 (m, 2H), 3.35-3.44 (m, 4H), 3.55-3.66 (m, 6H), 3.80-3.88 (m, 2H), 3.87 (s, 6H, OCH$_3$), 4.20-4.28 (m, 2H), 4.74 (d, 2H, $J = 13.3$ Hz, CHHPh), 4.74-4.75 (br s, 2H, CH), 4.91 (d, 2H, $J = 13.3$ Hz, CHHPh), 7.09-7.15 (XX' of AA'XX', 4H, Ar), 7.52-7.57 (AA' of AA'XX', 4H, Ar).
$^{13}$C{$^1$H} NMR (125.68 MHz, D$_2$O, CH$_3$CN secondary ref. set to 1.70) $\delta_C$ 43.65, 46.92, 55.42, 56.36, 61.55, 61.63, 78.27, 115.88, 119.22, 134.81, 161.90; IR (KBr) 3475, 3413, 2963, 2856, 2048, 1612, 1582, 1515, 1284, 1259, 1184, 1044, 1030, 947, 842 cm$^{-1}$; HRFABMS, $m/z$ (M)$^+$ exact mass calcd for C$_{26}$H$_{36}$BrN$_4$O$_2$: 515.2022; Found: 515.2029 (error +0.8 mmu/+1.5 ppm).

4,10-Bis-(4-methoxybenzyl)-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane (66).

(8bR,8cR)-rel-Decahydro-2a,6a-bis-(4-methoxy-benzyl)-4a,8a-diaza-2a,6a-diazonia cyclopent[fg]acenaphthlenium dibromide 65 (0.4814 g, 0.8072 mmol) was added in one portion to 21 mL of 95% EtOH in a 100 mL round-bottomed reaction flask, under N$_2$. This mixture was stirred for several minutes and NaBBU (1.2273 g, 31.60 mmol) was then added, with cooling, in small portions over several minutes. This mixture was stirred under N$_2$ for approximately 25 minutes before inserting the reaction flask into a CEM Discover microwave reactor. Once in the microwave reactor, the reaction flask was fitted with an air condenser topped by a water condenser with a nitrogen inlet adapter and the open vessel reaction was run under microwave irradiation at 78 °C for 10 minutes. [Note: For this experiment the CEM Synergy software method was written such that the microwave irradiation was ramped up in 5 stages, shown below in Table 3.1. See the appropriate reference for further important safety information.97] The reaction flask was removed from the microwave and 6M HCl was added dropwise to the reaction mixture (to pH 1), with cooling. The solvent was then removed under reduced pressure to yield a white solid, which was dissolved in water (40 mL). The solution was made strongly basic (pH 14) by slow addition of KOH pellets, with cooling, and this
basic solution was then extracted with toluene (5 x 30 mL). The combined extracts were
dried over Na₂SO₄ and the solvent was removed under reduced pressure to yield product
as a clear oil that required no further purification (0.3481 g, 98%): ¹H NMR (500 MHz,
C₆D₆) δ 2.65-2.86 (m, 16H), 3.23 (s, 4H, ethylene cross-bridge), 3.35 (s, 6H, CH₃O), 3.58
(s, 4H, CH₂Ar), 6.81-6.85 (XX' of AA'XX', 4H, Ar), 7.22-7.26 (AA' of AA'XX', 4H, Ar);
¹³C{¹H} NMR (125.68 MHz, C₆D₆) δC 54.73, 57.00, 58.02, 58.68, 61.22, 113.91, 130.36,
132.82, 159.14; IR 2995, 2913, 2830, 2061, 1881, 1671, 1611, 1511, 1455, 1368, 1300,
1246, 1173, 1102, 1035, 820 cm⁻¹; HRFABMS, m/z (M+H)+ exact mass calcd for
C₂₆H₃₉N₄O₂: 439.3073; Found: 439.3072 (error -0.1 mmu/-0.2 ppm).
(Note: This reduction was also run conventionally, at room temperature, for four days.)

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Table 3.1 The method, incorporating a ramp in power, used for the NaBH₄ MW-assisted reduction of 66.

(8bR,8cR)-rel-Decahydro-2a-benzyl-6a-(4-methoxybenzyl)-4a,8a-diaza-2a,6a-
diazoniacyclopent[fg]acenaphthlenium dibromide (67). p-Methoxybenzyl bromide
(9.2028 g, 45.77 mmol) was added dropwise to a stirred mixture of (8bR,8cR)-rel-
decahydro-2a-benzyl-4a,6a,8a-triaza-2a-azoniacyclopent[fg]acenaphthylene bromide 39
(2.75 g, 7.52 mmol) in MeCN (120 mL). The reaction mixture was stirred, under N₂, at
room temperature for 6 days. The white precipitate was then isolated by suction filtration
and washed with Et₂O (3 x 25 mL). Residual solvent was removed under reduced
pressure to yield 3.9216 g of product as a white solid (92 %): mp 179-182 °C (dec); ¹H
NMR (500 MHz, CD$_3$OD, central peak of secondary reference CHD$_2$OD set to 3.31 ppm) $\delta$ 3.22-3.30 (m, 2H), 3.35-3.41 (app dm, 2H, $J = 13.4$ Hz), 3.46-3.51 (dm, 1H, $J = 13.1$ Hz), 3.49-3.53 (dm, 1H, $J = 13.1$ Hz), 3.53-3.64 (m, 6H), 3.78-3.90 (m, 2H), 3.85 (s, 3H), 4.32-4.46 (m, 2H), 4.89 (br d, X of AX, 1H, $J = 13.3$ Hz, CHHAr), 4.95 (br d, X of AX, 1H, $J = 13.3$ Hz, CHHAr), 5.07 (d, A of AX, 1H, $J = 13.3$ Hz, CHHAr), 5.08-5.09 (br app s, 1H, CH), 5.12-5.13 (br app s, 1H, CH), 5.14 (d, A of AX, 1H, $J = 13.0$ Hz, CHHAr), 7.06-7.11 (XX' of AA'XX', 2H, p-MeOPh), 7.54-7.60 (m, 3H, Ar), 7.60-7.65 (AA' of AA'XX', 2H, p-MeOPh), 7.69-7.73 (m, 2H, Ar); $^{13}$C{$^1$H} NMR (125.68 MHz, CD$_3$OD, central peak of secondary ref. CD$_3$OD set to 49.00) $\delta_C$ 43.92, 43.95, 47.48, 47.51, 55.69, 55.81, 56.01, 61.86, 61.92, 61.99, 62.20, 78.85, 79.32, 116.11, 119.72, 128.21, 130.81, 132.30, 133.74, 135.22, 163.28; IR (KBr) 3435, 2960, 2855, 1611, 1518, 1404, 1256, 1192, 1044, 1022, 930, 839, 708 cm$^{-1}$; HRFABMS, m/z (M-Br)$^+$ exact mass calcd for C$_{25}$H$_{34}$BrN$_4$O: 485.1916; Found: 485.1896 (error -2.0 mmu/-4.0 ppm).

4-Benzy1-10-(4-methoxybenzyl)-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane (68). (8bR,8cR)-rel-Decahydro-2a-benzyl-6a-(4-methoxybenzyl)-4a,8a-diaza-2a,6a-diazoniacyclopent[f,g]acenaphthlenium dibromide 67 (0.3037 g, 0.5365 mmol) was added in one portion to 21 mL of 95% EtOH in a 100 mL round-bottomed reaction flask, under N$_2$. This mixture was stirred for several minutes and NaBH$_4$ (0.8121 g, 20.91 mmol) was then added, with cooling, in small portions over several minutes. This mixture was stirred under N$_2$ for approximately 25 min before inserting the reaction flask into a CEM Discover microwave reactor. The reaction flask was fitted with an air condenser topped by a water condenser with a nitrogen inlet adapter and the open-vessel
reaction was run under microwave irradiation at 78 °C for 10 minutes. [Note: For this experiment the CEM Synergy software method was written such that the microwave irradiation was ramped up in 5 stages, shown below in Table 3.2. See the appropriate reference for further important safety information.] The reaction flask was removed from the microwave reactor and 6M HCl was added dropwise to the reaction mixture (to pH 1), with cooling. The solvent was then removed under reduced pressure to yield a white solid, which was dissolved in water (35 mL). The solution was made strongly basic (pH 14) by slow addition of KOH pellets, with cooling, and this basic solution was then extracted with toluene (5 x 25 mL). The combined extracts were dried over Na₂SO₄ and the solvent was removed under reduced pressure to yield product as a clear oil that required no further purification (0.1735 g, 79%): ¹H NMR (500 MHz, C₆D₆) δ 2.63-2.72 (m, 8H), 2.72-2.85 (m, 8H), 3.14-3.25 (m, 4H, AA'BB', ethylene cross-bridge), 3.34 (s, 3H, OCH₃), 3.57 (s, 2H, CH₂Ar), 3.60 (s, 2H, CH₂Ar), 6.80-6.85 (XX' of AA'XX', 2H, p-MeOPh), 7.09-7.13 (m, 1H, Ph), 7.18-7.22 (m, 2H, Ph), 7.22-7.25 (AA' of AA'XX', 2H, p-MeOPh), 7.28-7.31 (m, 2H, Ph); ¹³C⁻¹H) NMR (125.68 MHz, C₆D₆) δC 54.68, 56.94, 57.86, 57.93, 58.62, 58.69, 61.17, 61.25, 113.85, 126.89, 128.35, 129.09, 130.28, 132.72, 140.82, 159.05; IR 3060, 3026, 2912, 2807, 2280, 2064, 1944, 1878, 1806, 1674, 1611, 1585, 1510, 1452, 1369, 1300, 1246, 1171, 1145, 1103, 1035, 996, 822, 729, 699 cm⁻¹; HRFABMS, m/z (M+H)⁺ exact mass calcd for C₂₅H₃₇N₄O: 409.2967; Found: 409.2965 (error -0.2 mmu/-0.6 ppm).

(Note: This reduction was also run conventionally, at room temperature, for four days.)
Table 3.2 The method, incorporating a ramp in power, used for the NaBH₄ MW-assisted reduction of 68.

(8bR,8cR)-rel-Decahydro-2a-benzyl-6a-(naphthalen-2-ylmethyl)-4a,8a-diaza-2a,6a-diazoniacyclopent[fg]acenaphthylenium dibromide (70). 2-(Bromomethyl)naphthalene (10.04 g, 45.41 mmol) was added in portions to a stirred solution of (8bR,8cR)-rel-decahydro-2a-benzyl-4a,6a,8a-triaza-2a-azoniacyclopent[fg]-acenaphthylene bromide 39 (2.76 g, 7.55 mmol) in MeCN (120 mL). The reaction mixture was stirred, under N₂, at room temperature for 6 days. The white precipitate was then isolated by suction filtration and washed with Et₂O (3 x 25 mL) [Note: several washes may be required to completely remove unreacted 2-(bromomethyl)naphthalene]. Residual solvent was removed under reduced pressure to yield 4.05 g of product as a white solid (92%): mp 281-284 °C (dec); ¹H NMR (500 MHz, CD₃OD, central peak of secondary reference CHD₂OD set to 3.31 ppm) δ 3.27-3.31 (m, 1H, downfield region partially overlapped with CHD₂OD solvent peak), 3.32-3.36 (m, 1H), 3.36-3.45 (m, 2H), 3.46-3.52 (dm, 1H, J = 13.14 Hz), 3.53-3.68 (m, 6H), 3.72 (td, 1H, J = 13.2, 1.9 Hz), 3.80-3.90 (m, 2H), 4.41-4.48 (m, 1H), 4.55 (td, 1H, J = 10.7, 4.2 Hz), 4.99 (d, 1H, J = 13.0 Hz, CH/Ph), 5.14 (d, 1H, J = 13.1 Hz, CHHAr), 5.17 (d, 1H, J = 13.1 Hz, CHHAr), 5.22 (d, 1H, J = 2.5 Hz, CH), 5.27 (d, 1H, J = 2.5 Hz, CH), 5.33 (d, 1H, J = 13.2 Hz, CHHNap), 7.51-7.64 (m, 5H, Ph), 7.68-7.75 (m, 3H, Nap), 7.91-7.96 (m, 1H, Nap), 7.99-8.04 (m, 2H, Nap), 8.28-8.31 (m, 1H, Nap); ¹³C{¹H} NMR (125.68 MHz, CD₃OD, secondary reference CD₃OD set to 49.00 ppm) δC 43.86, 43.94, 47.48, 47.50, 55.59,
55.87, 61.83, 62.03, 62.16, 62.22, 79.12, 79.22, 125.32, 128.08, 128.23, 128.81, 129.04, 129.24, 129.54, 130.60, 130.70, 132.16, 133.61, 134.41 (2 carbons), 135.35; IR (KBr) 3414, 3009, 2954, 2913, 2843, 1629, 1600, 1456, 1403, 1288, 1199, 1041, 937, 826, 760, 705, 479 cm⁻¹; HRFABMS, m/z (M-Br)⁺ exact mass calcd for C₂₈H₃₄BrN₄: 505.1967; Found: 505.1941 (error -2.6 mmu/-5.2 ppm).

4-Benzyl-10-(naphthalen-2-ylmethyl)1,4,7,10-tetraazabicyclo[5.5.2]tetradecane (71). (8bR,8cR)-rel-Decahydro-2a-benzyl-6a-(naphthalen-2-ylmethyl)-4a,8a-diaza-2a,6a-diazoniacycopent[f,g]acenaphthylene dibromide 70 (0.1003 g, 0.01717 mmol) was added in one portion to 6 mL of 95% EtOH in a 50 mL round-bottomed reaction flask, under N₂. This mixture was stirred for several minutes and NaBH₄ (0.2593 g, 6.6761 mmol) was then added, with cooling, in small portions over several minutes. The mixture was stirred under N₂ for 4 days. Then 3M HCl was added dropwise to the reaction mixture (to pH 1), with cooling. The solvent was then removed under reduced pressure yielding a white solid, which was then dissolved in water (8 mL). The solution was made strongly basic (pH 14) by slow addition of KOH pellets, with cooling, and this basic solution was extracted with toluene (5 x 10 mL). The combined extracts were dried over Na₂SO₄ and the solvent was removed under reduced pressure to yield product as a clear oil (0.0691 g, 94%): ¹H NMR (500 MHz, C₆D₆) δ 2.60-2.88 (m, 16H), 3.15-3.27 (m, 4H, AA'BB', ethylene cross-bridge), 3.59 (s, 2H, CH₂Ph), 3.72 (s, 2H, CH₂Nap), 7.09-7.14 (m, 1H, Ph), 7.18-7.24 (m, 2H, Ph), 7.24-7.32 (m, 4H, Ar), 7.51-7.56 (m, 1H, Nap), 7.62-7.72 (m, 4H, Nap); ¹³C{¹H} NMR (125.68 MHz, C₆D₆) δc 57.01, 57.88, 57.91, 58.75, 58.81, 61.31, 61.74, 125.65, 126.14, 126.96, 127.46, 127.84,
(8bR,8cR)-rel-Decahydro-2a,6a-bis(naphthalen-2-ylmethyl)-4a,8a-diaza-2a,6a-diazeniacyclopent[f/g]acenaphthylene dibromide (72). 2-Bromomethyl-naphthalene (3.4543 g, 15.623 mmol) was added to a stirred solution of (8bR,8cR)-rel-decahydro-2a,4a,6a,8a-tetraazacyclopent[f/g]acenaphthylene 18 (0.5057 g, 2.603 mmol) in MeCN (19.4 mL). The reaction mixture was stirred, under N₂, at room temperature for 3 days. The white precipitate was then isolated by suction filtration and washed with MeCN (6 x 5 mL). Residual solvent was removed under reduced pressure to yield 1.4838 g of product as a white solid (90%): mp 185-189 °C (dec); ¹H NMR (500 MHz, CD₃OD, secondary ref. central peak of CD₃OD set to 3.31) δ 3.33-3.40 (m, 2H), 3.39-3.45 (dm, 2H, J = 12.9 Hz), 3.49-3.54 (dm, 2H, J = 13.0 Hz), 3.56-3.66 (m, 4H), 3.71 (td, 2H, J = 13.1, 1.7 Hz), 3.83 (td, 2H, J = 12.5, 2.7 Hz), 4.55 (td, 2H, J = 10.4, 3.2 Hz), 5.17 (d, 2H, J = 13.1 Hz, CHNap), 5.35 (d, 2H, J = 13.2 Hz, CHNap), 5.41 (s, 2H, CH), 7.53-7.59 (m, 4H, Ar), 7.66 (dd, 2H, J = 8.4, 1.8 Hz, Ar), 7.82-7.86 (m, 2H, Ar), 7.90-7.96 (m, 4H, Ar), 8.21-8.23 (m, 2H, Ar), ¹³C {¹H} NMR (125.68 MHz, CD₃OD, secondary ref. central peak of CD₃OD set to 49.00) δC 44.05, 47.59, 55.96, 62.23, 62.33, 79.26, 125.21, 128.31, 128.82, 129.12, 129.15, 129.52, 130.67, 134.45, 134.49, 135.48; IR (KBr) 3333, 3277, 3055, 3015, 2840, 2128, 1626, 1599, 1337, 1287, 1196, 1040, 934,
815, 759, 475 cm$^{-1}$; HRFABMS, m/z (M-Br)$^+$ exact mass calcd for C$_{32}$H$_{36}$BrN$_4$: 555.2123; Found: 555.2108 (error -1.6 mmu/-2.8 ppm).

cis-Decahydro-3a,8a-bis(naphthalen-2-ylmethyl)-5a,10a-diaza-3a,8a-
diazoniapryene dibromide (73). 2-Bromomethyl-naphthalene (3.0526 g, 13.807 mmol) was added to a stirred solution of cis-decahydro-1H,6H-3a,5a,8a,10a-tetraaza-pyrene 19 (0.5116 g, 2.3011 mmol) in MeCN (19 mL). The reaction mixture was stirred, under N$_2$, at room temperature for 3 days. The white precipitate was then isolated by suction filtration and washed with MeCN (5 x 5 mL). Residual solvent was removed under reduced pressure to yield 1.1316 g of product as a white solid (74 %): mp 134-137 °C (dec); $^1$H NMR (500 MHz, CD$_3$OD, secondary ref. central peak of CHD$_2$OD set to 3.31) $\delta$ 1.80-1.89 (dm, 2H, $J = 14.9$ Hz), 2.17-2.32 (m, 2H), 3.10-3.20 (m, 6H), 3.25-3.33 (m, 2H), 3.45 (dd, 2H, $J = 13.3$, 3.0 Hz), 3.71-3.80 (tm, 2H, $J = 12.4$ Hz), 4.08 (td, 2H, $J = 13.0$, 2.8 Hz), 4.51 (td, 2H, $J = 12.9$, 3.7 Hz), 5.19 (d, 2H, $J = 12.9$ Hz, CH$H$Nap), 5.61 (d, 2H, $J = 12.9$ Hz, CH$H$Nap), 5.75 (s, 2H, $CH$), 7.52-7.58 (m, 4H, Nap), 7.60-7.65 (dm, 2H, $J = 8.5$ Hz, Nap), 7.77-7.82 (m, 2H, Nap), 7.83-7.87 (dm, 2H, $J = 8.4$ Hz, Nap), 7.93-7.98 (m, 2H, Nap), 8.18-8.22 (m, 2H, $Ar$); $^{13}$C{$^1$H} NMR (125.68 MHz, CD$_3$OD, secondary ref. central peak of CD$_3$OD set to 49.00) $\delta$C 19.78, 46.91, 48.18, 52.41, 61.55, 63.04, 78.38, 124.05, 128.18, 128.80, 129.10, 129.52, 130.00, 130.26, 134.32, 135.20, 135.38; IR (KBr) 3396, 3048, 2999, 2952, 2904, 2853, 1628, 1599, 1449, 1437, 1355, 1133, 865, 821, 756, 481 cm$^{-1}$; HRFABMS, m/z (M-Br)$^+$ exact mass calcd for C$_{34}$H$_{40}$BrN$_4$: 583.2436; Found: 583.2415 (error -2.2 mmu/-3.7 ppm).
4,10-Bis-(naphthalen-2-ylmethyl)-1,4,7,10-tetraaza-bicyclo[5.5.2]tetradecane (74). (8bR,8cR)-rel-Decahydro-2a,6a-bis-(naphthalen-2-ylmethyl)-4a,8a-diaza-2a,6a-diazeniacyclopent[f,g]acenaphthlenium dibromide 72 (0.0755 g, 0.119 mmol) was stirred in 4.8 mL of 95% EtOH in a 25 mL round-bottomed reaction flask, under N$_2$. NaBH$_4$ (0.1841 g, 4.740 mmol) was then added to the mixture, with cooling, in small portions over several minutes. This mixture was stirred under N$_2$ for approximately 0.5 h before inserting the reaction flask into a CEM Discover microwave reactor. Once in the microwave reactor, the reaction flask was fitted with an air condenser topped by a water condenser with a nitrogen inlet adapter attached and the open vessel reaction was run under microwave irradiation at 78 °C for 10 minutes. [Note: The maximum irradiation power was adjusted until the reaction mixture reached 78 °C. When running a reduction in the microwave using NaBH$_4$ in 95% EtOH, there are potential hazards associated with foaming of the reaction mixture – see the appropriate endnote] The reaction flask was removed from the microwave and 3M HCl was added dropwise to the reaction mixture (to pH 1), with cooling. The solvent was then removed under reduced pressure to yield a white solid, which was dissolved in water (6 mL). The solution was made strongly basic (pH 14) by slow addition of KOH pellets, with cooling, and this basic solution was then extracted with toluene (5 x 6 mL). The combined extracts were dried over Na$_2$SO$_4$ and the solvent was removed under reduced pressure to yield product as a white solid that required no further purification (0.0432 g, 76 %): mp 116-119 °C; $^1$H NMR (500 MHz, C$_6$D$_6$) δ 2.60-2.90 (m, 16H), 3.25 (s, 4H, ethylene cross-bridge), 3.72 (s, 4H, C$_2$/Nap), 7.24-7.33 (m, 4H, Nap), 7.55 (dd, 2H, $J$ = 8.4, 1.6 Hz, Nap), 7.63-7.73 (m, 8H, Nap); $^{13}$C{$^1$H} NMR (125.68 MHz, C$_6$D$_6$) δC 57.03, 57.91, 58.80, 61.71, 125.66, 126.15,
127.46, 127.84, 128.03, 128.16, 133.26, 133.96, 138.49; IR (KBr) 3048, 2969, 2927, 2904, 2812, 1668, 1597, 1504, 1445, 1361, 1330, 1102, 1033, 821, 757 cm⁻¹; HRFABMS, m/z (M+H)⁺ exact mass calcd for C₃₂H₃₉N₄: 479.3175; Found: 479.3155 (error -2.0 mmu/-4.1 ppm).

4,11-Bis-(naphthalen-2-ylmethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (75). cis-Decahydro-3a,8a-bis(naphthalen-2-ylmethyl)-5a,10a-Diaza-3a,8a-diaza-pyrene dibromide 73 (0.5055 g, 0.7617 mmol) was added in one portion to 30.3 mL of 95% EtOH in a 100 mL round-bottomed reaction flask, under N₂. This mixture was stirred for several minutes and NaBH₄ (1.1159 g, 28.731 mmol) was then added, with cooling, in small portions over several minutes. This mixture was stirred under N₂ for approximately 0.5 h before inserting the reaction flask into a CEM Discover microwave reactor. Once in the microwave reactor, the reaction flask was fitted with an air condenser topped by a water condenser with a nitrogen inlet adapter attached and the open vessel reaction was run under microwave irradiation at 78 °C for 10 minutes. [Note: The maximum irradiation power was adjusted until the reaction mixture reached 78 °C. When running a reduction in the microwave using NaBH₄ in 95% EtOH, there are potential hazards associated with foaming of the reaction mixture – see the appropriate endnote]⁹⁷ The reaction flask was removed from the microwave and 3M HCl was added dropwise to the reaction mixture (to pH 1), with cooling. The solvent was then removed under reduced pressure to yield a white solid, which was dissolved in water (20 mL). The solution was made strongly basic (pH 14) by slow addition of KOH pellets, with cooling, and this basic solution was then extracted with chloroform (5 x 40 mL). The
combined extracts were dried over Na₂SO₄ and the solvent was removed under reduced pressure to yield product as an off-white solid (0.3662 g, 95 %): mp 130-134 °C; ¹H NMR (500 MHz, C₆D₆) δ 1.32-1.45 (m, 2H), 1.51-1.63 (m, 2H), 2.19-2.26 (dm, 2H, J = 12.7), 2.30-2.37 (dm, 2H, J = 13.0), 2.43-2.58 (m, 8H), 2.59-2.68 (XX' of AA'XX', 2H, ethylene cross-bridge), 2.99 (td, 2H, J = 12.8, 3.6 Hz), 3.25 (d, 2H, J = 13.4 Hz, CHH Nap), 3.38-3.48 (AA' of AA'XX', 2H, ethylene cross-bridge), 3.89 (d, 2H, J = 13.3 Hz, CHH Nap), 4.10 (td, 2H, J = 11.8, 3.8 Hz), 7.24-7.33 (m, 4H, Ar), 7.57-7.62 (m, 2H, Ar), 7.65-7.73 (m, 8H, Ar); ¹³C {¹H} NMR (125.68 MHz, C₆D₆) δC 28.75, 52.26, 55.04, 57.24, 57.98, 58.03, 60.63, 125.68, 126.17, 127.68, 127.92, 128.07, 128.13, 128.30, 133.31, 134.03, 138.88; IR (KBr) 3052, 2915, 2798, 1673, 1631, 1599, 1506, 1457, 1447, 1363, 1245, 1118, 858, 821, 475 cm⁻¹; HRFABMS, m/z (M+H)⁺ exact mass calcd for C₃₄H₄₃N₄: 507.3488; Found: 507.3501 (error +1.3 mmu/+2.6 ppm). [Note: The NMR spectra indicated that the sample was contaminated (<5%) with toluene and silicone grease]

4-Benzyl-11-octyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (76). Acetic acid (30.5 µL, 0.534 mmol) was added dropwise over 5 minutes to a stirred mixture of octanal (0.4095 g, 3.194 mmol), 4-benzyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane 33 (0.0968 g, 0.306 mmol), sodium cyanoborohydride (0.0593 g, 0.944 mmol) and 1.25 mL MeCN in a two-neck round-bottomed flask. The reaction mixture was stirred under N₂ for two hours before adding a second portion of acetic acid (30.5 µL, 0.534 mmol) dropwise over 2 minutes. The reaction mixture was then stirred under N₂ for an additional hour before it was concentrated under reduced pressure to a white solid. This solid was dissolved in
1:1 (vol:vol) MeOH: H₂O and 6M HCl was added dropwise, to pH 1 (*caution HCN evolution!*). The methanol was then removed under reduced pressure (the rotary evaporator was vented to the hood) and H₂O (10 mL) and 6M HCl (to pH 1) were added to this solution before washing with toluene (5 x 20 mL). Additional H₂O (5 mL) was added to the aqueous solution before making it strongly basic (pH 14) by slow addition of KOH pellets, with cooling, and this basic solution was then extracted with toluene (5 x 25 mL). The combined extracts were dried over Na₂SO₄ and the solvent was removed under reduced pressure to yield crude product as a clear, colorless oil (0.1041 g, 79% crude):

¹H NMR (500 MHz, C₆D₆) δ 0.91 (t, 3H, J = 6.95 Hz, CH₃CH₇H₁₄), 1.22-1.58 (m, 18H), 2.16 (ddd, 1H, J = 12.5, 8.3, 4.7 Hz), 2.20 (ddd, 1H, J = 13.5, 3.7, 2.2 Hz), 2.27 (ddd, 1H, J = 12.9, 4.6, 3.5 Hz), 2.33-2.57 (m, 9H + impurity peaks), 2.57-2.61 (m, 1H), 2.76-2.88 (m, 2H), 2.97 (td, 1H, J = 12.5, 4.0 Hz), 3.08 (d, 1H, J = 13.4 Hz, CH₃Ph), 3.30-3.39 (m, 2H, ethylene cross-bridge), 3.72 (td, 1H, J = 11.8, 4.0 Hz), 3.77 (d, 1H, J = 13.4 Hz, CH₃Ph), 4.10 (td, 1H, J = 11.8, 3.9 Hz), 7.09-7.14 (m, 1H), 7.18-7.24 (m, 2H, Ph), 7.33-7.38 (m, 2H, Ph); ¹³C{¹H} NMR (125.68 MHz, C₆D₆) δC 14.34, 23.01, 28.09, 28.76, 28.83, 28.94, 29.77, 29.98, 32.23, 51.76, 51.97, 54.88, 55.28, 55.56, 57.20, 57.35, 57.56, 58.01, 58.52, 59.58, 60.30, 126.87, 128.32, 129.22, 141.22; IR 3061, 3026, 2922, 2852, 2793, 1942, 1868, 1803, 1722, 1678, 1598, 1494, 1455, 1363, 1324, 1244, 1159, 1125, 999, 734, 698 cm⁻¹; HRFABMS, m/z (M+H)+ exact mass calcd for C₂₇H₄₈N₄: 429.3957; Found: 429.3952 (error +1.3 mmu/0.6 ppm). [Note: the ¹H NMR spectrum indicated that the sample was 90% pure. Thin layer chromatography experiments were performed on the crude product utilizing the following conditions: CH₂Cl₂:MeOH:(28% aq) NH₃]
but the crude material streaked and ran together at reasonable \( R_f \) values.]

\[
\text{(CuCl}_2\text{)}_2(\text{H}_2\text{O})_4 \cdot 5\cdot [4-(1,5,8,12-\text{Tetraazabicyclo}[10.2.2]\text{hexadec-5-ylmethyl})\text{phenyl}]\text{methyl}]-1,5,8,12-\text{tetraazabicyclo}[10.2.2]\text{hexadecane (Cu}_2 \cdot 102\text{a). A}
\]

solution of 0.0666 g (0.3907 mmol) CuCl\(_2\) \cdot 2 H\(_2\)O in methanol (3 mL) was added dropwise to a stirred solution of 0.1071 g (0.1930 mmol) 5-[4-(1,5,8,12-tetraazabicyclo-10.2.2]hexadec-5-ylmethyl)phenyl]methyl]1,5,8,12-tetraazabicyclo[10.2.2]hexadecane 102a in methanol (6 mL). The mixture was refluxed under N\(_2\) for 2 hours. The resulting dark-blue solution was cooled to room temperature and the methanol was removed under reduced pressure to yield a light-blue solid (0.1708 g). This crude solid was then dissolved in 9.5 mL of 80% aq ethanol and any undissolved material was removed via centrifugation. The supernatant was subjected to slow diffusion from benzene/Et\(_2\)O (1:1) solution yielding dark blue crystals. IR (KBr) 3423, 3118, 2927, 2869, 1630 cm\(^{-1}\); UV-vis: (80% ethanol) \( \lambda_{\text{max}} \) 614 nm (\( e = 661 \text{ M}^{-1} \text{ cm}^{-1} \)); Anal Calcd for 2(CuCl\(_2\))\( \cdot C_{32}H_{58}N_8 \cdot 4H_2O: \) C, 42.90; H, 7.43; Cl, 15.83; N, 12.51; Found: C, 43.12; H, 7.43; N, 12.57; Cl, 16.25.

(Note: Compound has been published and fully characterized in the literature.\(^{45}\))

\[
[\text{Cu(CH}_3\text{CN})(\text{ClO}_4)_2]_2(\text{CH}_3\text{OH}) \cdot 4\cdot \text{Methyl-10-[4-(10-methyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradec-4-ylmethyl)-benzyl]-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane (Cu}_2 \cdot 94\text{a). A solution of 0.1566 g (0.4226 mmol) Cu(\text{ClO}_4)_2 \cdot 6 H\text{O in methanol (3 mL) was added dropwise to a stirred solution of 0.1094 g (0.2078}
\]
mmol) 4-methyl-10-[4-(10-methyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradec-4-ylmethyl)-
benzyl]-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane 94a in methanol (3 mL). The mixture
was refluxed under N₂ for 2 hours. The resulting dark-blue solution was cooled and the
methanol was removed under reduced pressure to yield a light-blue solid. This crude
solid was then mixed with 12 mL acetonitrile and any undissolved material was removed
via centrifugation. The supernatant was subjected to slow diffusion from a benzene/ether
(1:1) solution to yield dark-blue crystals (0.1499 g, 63.6% yield). IR (KBr) 2938, 2881,
1484, 1094, 751, 623 cm⁻¹; Anal Calcd for [Cu(CH₃CN)(ClO₄)]₂(C₂H₅OH) • C₃oH₅₄N₈:
C, 36.06; H, 5.53; Cl, 12.16; N, 12.01. Found: C, 36.01; H, 5.61; Cl, 12.01; N, 11.60.
UV-vis: (MeCN) λ_max, 632 nm (ε = 280 M⁻¹ cm⁻¹).

[Cu(NO₃)₂][H₂O]₄ • 4-Methyl-10-[3-(10-methyl-1,4,7,10-tetraazabicyclo-
[5.5.2]tetradec-4-ylmethyl)-benzyl]-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane (Cu₂ •
94b). A solution of 0.1005 g (0.423 mmol) Cu(NO₃)₂ • 3 H₂O in methanol (3 mL) was
added dropwise to a stirring solution of 0.1079 g (0.2050 mmol) 4-Methyl-10-[3-(10-
methyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradec-4-ylmethyl)-benzyl]-1,4,7,10-
tetraazabicyclo[5.5.2]tetradecane 94b in methanol (7 mL). The reaction mixture was
refluxed under N₂ for 2.5 hours. After transfer into test tubes, diethyl ether diffusion into
the dark-blue solutions yielded dark-blue crystals (0.1799 g, 97.3% yield). IR (KBr)
3442, 3269, 2938, 2879, 1647, 1473, 1399, 1094, 623 cm⁻¹; UV-vis: (MeOH) λ_max, 658
nm (ε = 202 M⁻¹ cm⁻¹); Anal Calcd for 2(Cu(NO₃)₂) • C₃oH₅₄N₈ • 4H₂O: C, 36.99; H,
6.42; N, 17.26; Found: C, 36.66; H, 5.84; N, 17.34 [Note: This sample does not pass

145
chemical analysis due to the fact that the %H is off by more than 0.4% of the calculated value.

\[
(ZnCl_2)_2 \cdot 4\text{-Methyl-10-[4-(10-methyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradec-4-ylmethyl)-benzyl]-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane (Zn}_2 \cdot 94a) \]

Anhydrous ZnCl\(_2\) (0.1072 g, 0.7865 mmol) was added in one portion to a stirred suspension of 0.2075 g (0.3941 mmol) 4-methyl-10-[4-(10-methyl-1,4,7,10-tetraazabicyclo[5.5.2]-tetradec-4-ylmethyl)-benzyl]-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane 94a in MeCN (25 mL). This mixture was refluxed under N\(_2\) for 24 h. The solvent was removed under reduced pressure to yield an off-white solid. This crude solid was then mixed with 10 mL H\(_2\)O and any undissolved material was separated via centrifugation and washed with 2 mL H\(_2\)O. This wash was combined with the supernatant and concentrated under reduced pressure to yield a white solid (0.2923 g, 94% crude yield). \(^1\)H NMR (500 MHz, D\(_2\)O, CH\(_3\)CN secondary ref. set to 2.05) \(\delta\) 2.68 (s, 6H, CH\(_3\)), 2.81 (ddd, 4H, \(J = 15.9, 12.2, 5.4\) Hz), 2.90-3.03 (m, 16H), 3.08-3.24 (m, 16H), 3.61 (dd, 4H, \(J = 15.5, 6.5\) Hz), 4.14 (s, 4H, CH\(_2\)Ph), 7.52 (s, 4H, Ph); \(^13\)C\(\{^1\)H\} NMR (125.68 MHz, D\(_2\)O, CH\(_3\)CN secondary ref. set to 1.70) \(\delta_c\) 48.25, 51.48, 55.88 (degenerate resonances), 55.92, 59.56, 61.04, 131.83, 135.37; IR (KBr) 3448, 2925, 2875, 1655, 1459, 1400, 1360, 1102, 1073, 1037, 1001 cm\(^{-1}\).

\[
(ZnCl_2)_2 \cdot 4\text{-Methyl-10-[3-(10-methyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradec-4-ylmethyl)-benzyl]-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane (Zn}_2 \cdot 94b) \]

Anhydrous ZnCl\(_2\) (0.1099 g, 0.8063 mmol) was added in one portion to a stirred
suspension of 0.2148 g (0.4080 mmol) 4-Methyl-10-[3-(10-methyl-1,4,7,10-tetra-
azabicyclo[5.5.2]tetradec-4-ylmethyl)-benzyl]-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane
94b in MeCN (25 mL). The reaction mixture was refluxed under N\textsubscript{2} for 24 h. The
solvent was removed under reduced pressure to yield a light brown solid. This crude
solid was then dissolved in 10 mL H\textsubscript{2}O and insoluble material was separated via
centrifugation then washed with 2 mL H\textsubscript{2}O. This wash was combined with the
supernatant and concentrated at reduced pressure to yield a light brown solid (0.3159 g,
98% crude yield). \textsuperscript{1}H NMR (500 MHz, D\textsubscript{2}O, CH\textsubscript{3}CN secondary ref. set to 2.05) \(\delta\) 2.68
(s, 6H, CH\textsubscript{3}), 2.80 (ddd, 4H, \(J=15.0, 12.3, 5.4 \text{ Hz}\)), 2.89-3.04 (m, 16H), 3.05-3.25 (m,
16H), 3.61 (dd, 4H, \(J=15.4 6.5 \text{ Hz}\)), 4.16 (s, 4H, CH\textsubscript{2}Ph), 7.49-7.56 (m, 4H, Ph);
\textsuperscript{13}C\{\textsuperscript{1}H\} NMR (125.68 MHz, D\textsubscript{2}O, CH\textsubscript{3}CN secondary ref. set to 1.70) \(\delta\)C 48.29, 51.49,
55.79, 55.87, 55.90, 59.82, 61.02, 129.64, 131.52, 133.91, 135.79.

\((8bR,8'bS,8cS,8'cR)-rel-6a,6'a-[1,4-Phenylenebis(methylene)]bis[decahydro-
2a-acetamido-4a,8a-diaza-2a,6a-diazeniacyclopent[fg]acenaphthylene] tetrabromide
[\approx 1:1 meso/d/l diastereomeric mixture] (104). 2-Bromoacetamide (7.1211 g, 0.0516
mol) was added in portions to a stirred mixture of \((8bR,8'bS,8cS,8'cR)-rel-2a,2'a-[1,4-
phenylenebis(methylene)]bis[decahydro-4a,6a,8a-triaza-2a-azoniacyclopent[fg]-
acenaphthylene] dibromide (\approx 1:1 meso/d/l diastereomeric mixture) 90a (2.1074 g, 3.23
mmol) in MeCN (50 mL). The reaction mixture was stirred, under N\textsubscript{2}, at room
temperature for 4 days. The off-white precipitate was then isolated by suction filtration
and washed with MeCN (3 x 40 mL). Several more washes with MeCN served to reduce
the amount of unreacted 2-bromoacetamide in the crude product, but a small amount still
remained. Residual solvent was removed under reduced pressure to yield 2.4617 g of crude product as a white solid (82 %): The product was >95% pure after washing. It was taken on without further purification. This product was analyzed to be a ≈1:1 mixture of meso and d/l diastereomers, as demonstrated by several doubled peaks in the $^{13}$C NMR spectrum: crude mp 198-204 °C (dec); $^1$H NMR (500 MHz, D$_2$O, CH$_3$CN secondary ref. set to 2.05, all signals meso and d/l) δ 3.07-3.18 (m, 4H), 3.18-3.27 (m, 2H), 3.36-3.50 (m, 6H), 3.55-3.77 (m, 8H), 3.88-3.97 (m, 2H), 4.06-4.33 (m, 10H), 4.53 (d, 2H, J = 15.9 Hz, CHHCONH$_2$), 4.68 (d, 2H, J = 15.9 Hz, CHH CONH$_2$), 4.71-4.75 (m, 2H, CH), 4.82 (d, 2H, J = 13.3 Hz, CHHPH), 4.85-4.90 (m, 2H, CH), 5.02 (d, 2H, J = 13.3 Hz, CHHPH), 7.77 (s, 4H, Ph); $^{13}$C{$^1$H} NMR (125.68 MHz, D$_2$O, CH$_3$CN secondary ref. set to 1.70 ) δC 43.61, 43.64, 46.88, 47.80, 55.65, 55.71, 57.55, 58.80, 60.91, 61.83, 61.89, 64.94, 78.52, 78.60, 79.67, 130.10, 134.50, 134.52, 165.55; IR (KBr) 3409, 3150, 2964, 2852, 1693, 1621, 1437, 1403, 1288, 1195, 1042 cm$^{-1}$; HRFABMS, m/z (M)$^+$ exact mass calcd for C$_{32}$H$_{52}$Br$_3$N$_{10}$O$_2$: 845.1825; Found: 845.1803 (error −2.2 mmu/+2.6 ppm).

2-{(10-[4 -(10-Carboxamylmethyl)-1,4,7,10-tetraaza-bicyclo[5.5.2]tetradec-4- ylmethyl]-benzyl)-1,4,7,10-tetraaza-bicyclo[5.5.2]tetradec-4-yl]-acetamide (105). A (≈1:1) meso/d/l diastereomeric mixture of (8bR,8'bS,8cS,8'cR)-rel-6a,6'a-[1,4-Phenylenebis(methylene)]bis[decahydro-2a-acetamido-4a,8a-diaza-2a,6a-diazonia-cyclopent[f/g]acenaphthylene] tetrabromide 104 (1.0474 g, 1.13 mmol) was added in one portion to 22.25 mL of 95% EtOH in a 100 mL round-bottomed reaction flask, under N$_2$. This mixture was stirred for several minutes and NaBH$_4$ (1.1408 g, 29.37 mmol) was then added, with cooling, in small portions over several minutes. This mixture was
stirred under N₂ for approximately 25 before inserting the reaction flask into a CEM
Discover microwave reactor. Once in the microwave reactor, the reaction flask was fitted
with an air condenser topped by a water condenser with a nitrogen inlet adapter and the
open vessel reaction was run under microwave irradiation at 78 °C for 10 minutes. [Note:
For this experiment the CEM Synergy software method was written such that the
microwave irradiation is ramped up in 5 stages, shown below in Table 3.3. See the
appropriate reference for further important safety information.97] The reaction flask was
removed from the microwave and 6M HCl was added dropwise to the reaction mixture
(to pH 1), with cooling. The solvent was then removed under reduced pressure to yield a
white solid, which was dissolved in water (35 mL). The solution was made strongly
basic (pH 14) by slow addition of KOH pellets, with cooling, and this basic solution was
then extracted with CHCl₃ (5 x 30 mL). The combined extracts were dried over Na₂SO₄
and the solvent was removed under reduced pressure to yield crude product as a light
yellow solid (0.7904 g, >100%): mp 64-68 °C; ¹H NMR (500 MHz, C₆D₆) δ 2.52-2.63
(m, 8H), 2.65-3.00 (m, 30H), 3.05 (ddd, 4H, J = 14.7, 4.9, 3.7 Hz), 3.31 (s, 4H,
CH₂CONH₂), 3.78 (s, 4H, CH₂Ph), 5.50 (br s, 2H, CONH), 7.20 (s, 4H, Ph), 8.98 (br s,
2H, CONH₂); ¹³C {¹H} NMR (125.68 MHz, C₆D₆) δC 51.46, 57.49, 57.64, 57.66, 58.60,
59.53, 60.01, 128.43, 138.18, 177.27; IR 3424, 3178, 2918, 2815, 1674, 1452, 1373,
1140, 1106, 1033, 996, 751 cm⁻¹; HRFABMS, m/z (M+H)⁺ exact mass calcd for
C₃₂H₅₇N₁₀O₂: 613.4666; Found: 613.4643 (error –2.3 mmu/–3.8 ppm).
Table 3.3 The method, incorporating a ramp in power, used for the NaBH₄ MW-assisted reduction of 105.

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(8bR,8'bS,8cS,8'cR)-rel-6a,6'a-[1,4-Phenylenebis(methylene)]bis[decahydro-2a-allyl-4a,8a-diaza-2a,6a-diazoniacyclopen[fg]acenaphthylene] tetrabromide

[≈1:1 meso/d/l diastereomeric mixture] (106). Allyl bromide (14.94 g, 123.5 mmol) was added dropwise to a stirred mixture of (8bR,8'bS,8cS,8'cR)-rel-2a,2'a-[1,4-phenylenebis(methylene)]bis[decahydro-4a,6a,8a-triaza-2a-azoniacyclopen[fg]-acenaphthylene] dibromide (≈1:1 meso/d/l diastereomeric mixture) 90a (4.03 g, 6.18 mmol) in MeCN (97 mL). The reaction mixture was stirred at room temperature for 14 days in a tightly stoppered flask, in the dark. The solvent and excess allyl bromide was then removed under reduced pressure (the rotary evaporator was vented to the hood, and an additional cold trap was used to collect the allyl bromide). The resulting solid was then transferred to 4 test tubes (25 mL MeCN was used to facilitate this transfer) and washed with MeCN (3 x 25 mL). The solid was centrifuged from the wash solutions and residual solvent was removed under vacuum to give 5.03 g of a fine white solid (91 %). The product was >95% pure after washing. It was taken on without further purification. This product was analyzed to be a ≈1:1 mixture of meso and d/l diastereomers, evident in the ¹H NMR by doubling of the benzyl peaks as well as several doubled peaks in the ¹³C NMR: crude mp 197-218 °C (dec); ¹H NMR (500 MHz, D₂O, CH₃CN secondary ref. set to 2.05, all signals meso and d/l) δ 3.07-3.13 (m, 4H), 3.20-3.28 (m, 2H), 3.34-3.40 (dm,
$2H, J = 13.9 \text{ Hz}$), $3.41-3.47 \text{ (dm, 4H, } J = 7.1 \text{ Hz})$, $3.55-3.74 \text{ (m, 8H)}$, $3.74-3.80 \text{ (dm, 2H, } J = 12.9 \text{ Hz})$, $3.84-4.00 \text{ (m, 6H), 4.07 (td, 2H, } J = 11.3, 4.1 \text{ Hz})$, $4.23 \text{ (dd, 2H, } J = 13.4, 6.6 \text{ Hz, } CHHCH=CH_2)$, $4.28 \text{ (td, 2H, } J = 11.2, 4.4 \text{ Hz})$, $4.41 \text{ (dd, 2H, } J = 13.4, 7.9 \text{ Hz, } CHHCH=CH_2)$, $4.69-4.72 \text{ (m, 2H, } CH)$, $4.71-4.75 \text{ (m, 2H, } CH)$, $4.81 \text{ (d, 1H, } J = 13.4 \text{ Hz, } CHHPh)$, $4.82 \text{ (d, 1H, } J = 13.4 \text{ Hz, } CHHPh)$, $5.01 \text{ (d, 1H, } J = 13.4 \text{ Hz, } CHHPh)$, $5.02 \text{ (d, 1H, } J = 13.4 \text{ Hz, } CHHPh)$, $5.78-5.84 \text{ (dm, 2H, } J = 16.9 \text{ Hz, } CH_2CH=CHH_2)$, $5.80-5.84 \text{ (dm, 2H, } J = 10.2 \text{ Hz, } CH_2CH=CHH_2)$, $6.05-6.15 \text{ (m, 2H, } CH_2CH=CH_2)$, $7.77 \text{ (s, 4H, } Ph)$; $\text{¹C}^{1H} \text{ NMR (125.68 MHz, D}_2\text{O, CH}_3\text{CN secondary ref. set to 1.70} \delta_C 43.31, 43.51, 46.85, 46.87, 47.14, 55.86, 55.93, 56.52, 60.84, 61.00, 61.75, 61.79, 61.98, 78.13, 78.14, 78.52, 78.61, 124.33, 130.12, 130.49, 130.51, 134.50, 134.51; IR (KBr) 3412, 3004, 1636, 1440, 1336, 1289, 1199, 1154, 1105, 1043, 958, cm$^{-1}$; HRFABMS, $m/z$ (M-Br)$^+$ exact mass calcd for $C_{34}H_{34}N_8Br_3$: 811.2022; Found: 811.2042 (error +2.0 mmu/+2.5 ppm).

4-Allyl-10-[4-(10-allyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradec-4-ylmethyl)-benzyl]-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane (107). A ($≈1:1$) meso/d/l diastereomeric mixture of (8b$R$,8$b'S$,8c$S$,8'c$R$)-rel-6a,6'a-[1,4-phenylenebis(methylene)]-bis[decahydro-4a,8a-diaza-2a,6a-diaziacyclopent[f,g]acenaphthylene tetrabromide 106 (2.2653 g, 2.5326 mmol) was added in one portion to 146 mL of acetonitrile, under N$_2$, in an efficient hood. This mixture was stirred for several minutes and NaBH$_3$(CN) (12.7909 g, 203.55 mmol) was then added in small portions over several minutes. The mixture was stirred at reflux for 24 h. The solvent was then removed under reduced pressure (the rotary evaporator was vented to the hood) to yield a white solid. These white solids were
completely dissolved by the addition of 95% EtOH (50 mL) followed by 150 mL 6M HCl (caution HCN evolution!), initially added dropwise, to the reaction mixture (to pH 1). (Decomposition of the excess NaBH₃(CN), with evolution of HCN, may take an hour. The absence of bubbles forming in the solution indicates the process is complete.) The solvent was then removed under reduced pressure yielding a white paste (the rotary evaporator was vented to the hood). Methanol and water were then added in portions so as to dissolve all solids (typically requiring a 1.5:1 (vol:vol) H₂O:MeOH solution). The solution was made strongly basic (pH 14) by slow addition of KOH pellets, with cooling, and the MeOH was then removed by rotary evaporation. After the MeOH had been removed, the crude material formed a more distinct separate layer from the aqueous layer. The resultant basic aqueous solution was then extracted with toluene. The combined extracts were dried over Na₂SO₄ and the solvent was removed under reduced pressure to yield white solid product (0.8967 g, 61%): mp 54-57°C; ¹H NMR (500 MHz, C₆D₆) δ 2.57-2.63 (m, 4H), 2.65-2.85 (m, 28H), 3.05-3.10 (dm, 4H, J = 6.3 Hz), 3.09-3.21 (m, 8H, AA′BB′, ethylene cross-bridge), 3.62 (s, 4H, CH₂Ph), 5.00-5.04 (dm, 2H), 5.06 (dq, 2H, J = 17.2, 1.5 Hz), 5.84 (ddt, 2H, J = 17.1, 10.1, 6.3 Hz), 7.32 (s, 4H, Ph); ¹³C{¹H} NMR (125.68 MHz, C₆D₆) δC 56.92, 57.88, 57.98, 58.31, 58.73, 59.94, 61.45, 115.97, 129.04, 137.18, 139.29; IR 3073, 2914, 2805, 2415, 1675, 1640, 1509, 1450, 1419, 1372, 1104, 1033, 9934, 916, 750 cm⁻¹; HRFABMS, m/z (M+H)⁺ exact mass calcd for C₃₄H₅₉N₈: 579.4863; Found: 579.4846 (error -1.7 mmu/-3.0 ppm).
Appendix A:

Spectral Index
secondary sol. cont. peak of CDCl₃ at 7.26
secondary vs. central peaks of CHD set to 3:1

73

CDCl₃/OD

8.0 7.0 6.0 5.0 4.0 3.0 2.0 ppm
The first-order non-linear fit (monitored at 585 nm) of the experimental absorbance/time data for the acid decomplexation of Cu$_2$•102a.
The first-order non-linear fit (monitored at 693 nm) of the experimental absorbance/time data for the acid decomplexation of Cu₂ • 94a

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104, meso and d,l diastereomeric mixture

CH3CN secondary ref. set to 2.06

3.79 ppm
104. meso and d/l diatereomeric mixture

CH₃CN secondary ref. set to 1.70
% Transmittance

Wavenumbers (cm⁻¹)

Diagram showing a graph with a curve indicating a spectral analysis of a compound. The compound is labeled as 104, meso and d/l diatereomeric mixture with KBr as the supporting material. The graph includes molecular structures with labels such as H₂N and O, indicating functional groups like amine and carboxylic acid.
106, meso and dl diatereomeric mixture

D2O

CH3CN secondary ref. set to 1.70

ppm
Appendix B:

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</table>
LIST OF REFERENCES


57. Reed, D. P. Synthesis of Vicinal Bisimidines and Bisaminals for the Preparation of Tetraazamacrocycles and the Synthesis of Cross-bridged Cyclam Derivatives and


94. See the appropriate material safety data sheet for Sodium Borohydride.
97. Foaming of the reaction mixture up the condenser can be an issue and the best way to prevent this is to limit the microwave reactor’s irradiation power. The risk of foaming is greater in the beginning of the run and if the reaction flask is more than ½ full, but it is always an issue during such NaBH₄ reductions. For that reason, the reaction should be monitored at all times. If foaming occurs during the run, then the best solution is to lower the power setting to zero and wait for it to subside. If the foaming is allowed to get out of control, then the material that floods the column will cool the reaction mixture down after falling back down from the condenser. The microwave software will respond to this lowering of the reaction mixture’s temperature by increasing the irradiation power – to increase the reaction mixture’s temperature – and this, in turn, will lead to an increase in foaming. This cyclic process of the refluxed material cooling the reaction mixture and the reaction mixture being irradiated at higher power will cause more foaming and, if left unmonitored, eventually result in foam flowing into the tubing attached to the top of the condenser. In.
98. Information taken from conversations with Gary R. Weisman about the research performed by Kaitlyn E Dugan.


144. Ciampolini, M.; Fabbri, L.; Perotti, A.; Poggi, A.; Seghi, B.; Zanobini, F., Dinickel and dicopper complexes with N,N-linked bis(cyclam) ligands. An ideal system


