Part I Synthesis, functionalization and metal complexation of polyamine macrocycles Part II Synthesis and dynamic NMR studies of bicyclic ureas

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PART I: SYNTHESIS, FUNCTIONALIZATION AND METAL COMPLEXATION OF POLYAMINE MACROCYCLES

PART II. SYNTHESIS AND DYNAMIC NMR STUDIES OF BICYCLIC UREAS

By

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B.S. University of Southern Maine, 1990

DISSERTATION

Volume I

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Doctor of Philosophy in Chemistry

September, 1995
This dissertation has been examined and approved.

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DEDICATION

This Dissertation is dedicated to my wife Darlene, whom I love with all my heart.
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ABSTRACT

I. SYNTHESIS, FUNCTIONALIZATION AND METAL COMPLEXATION OF POLYAMINE MACROCYCLES

II. SYNTHESIS AND DYNAMIC NMR STUDIES OF BICYCLIC UREAS

by

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University of New Hampshire, September, 1995

Part I: The syntheses and characterizations of novel bicyclic tetraamines (shown below) have been accomplished.

\[
\begin{align*}
X = (CH_2)_m & \quad m,n = 0 \\
Y = (CH_2)_n & \quad m,n = 1 \\
R = H, CH_3, or CH_2Ph
\end{align*}
\]

The complexation of bicyclic tetraamines (R = CH_3) with Li^+ and Na^+ have been studied by NMR spectroscopy. These tetraamines are strong bases and selective Li^+ binders. The parent "cross-bridged cyclam" (R = H; X = Y = CH_2) was functionalized with a variety of

xxx
ligating groups containing heteroatoms.

\[ R = \]

\[ \text{ligands} \]

Several of the ligands were investigated as possible radiopharmaceutical \(^{99m}\text{Tc}\) imaging agent precursors.

Syntheses of non-adjacent selectively functionalized tetraazacycloalkanes have also been developed.

\[ \text{ligands} \]

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Part II: Synthesis and dynamic NMR studies of intramolecular transamidation of bicyclic urea 199 have been carried out.

![Chemical Structure](image)

Bicyclic urea 199 exhibited dynamic exchange broadening indicative of an enantiomerization process in D₂O at pD > 8. Below pD 8, bicyclic urea converts to guanidinium salt 216.

![Chemical Structure](image)

Semiempirical methods (AM1, PM3) were used to study possible intermediates of the transamidation reaction.
PART I: SYNTHESIS, FUNCTIONALIZATION AND METAL COMPLEXATION
OF POLYAMINE MACROCYCLES

Volume 1

Part 1
Chapter I

A Brief Introduction to Macrocyclic Chemistry

I. Introduction

To date, host/guest chemistry has been extensively reviewed,¹ and several books on the subject have been written.² Two major contributors to this field are Lehn³ and Cram⁴ who established the fundamental terminology and concepts in this field. The field of "Host/Guest Chemistry" is more frequently being referred to as "Supramolecular Chemistry" meaning "beyond the molecule" as described by Lehn.⁵ In this chapter the fundamentals of host/guest chemistry will be introduced and briefly reviewed.

Pharmaceutical applications are among the more prevalent uses for macrocyclic hosts. This is due to the fact that some macrocyclic compounds are able to transport ions across a lipophilic membrane. Macrocyclic hosts can encapsulate a metal ion and reveal only a lipophilic exterior to the environment. The first antibiotic capable of this action was Valinomycin, which was isolated in
and later synthesized in 1963. 

II. Background

Host/guest chemistry is the study of receptor molecules (hosts) having convergent binding sites which include or complex with other chemical species (guests) having divergent binding sites. The intermolecular forces between the host and guest are electrostatic in nature. These forces can be classified as hydrogen bonding, metal ion to ligand interactions, \( \pi \)-acid to \( \pi \)-base interactions, and van der Waals attractive forces.

A. General Types of Hosts

Coronands are defined as multidentate macrocyclic ligands containing heteroatoms such as nitrogen, oxygen, sulfur or phosphorus. Crown ether 1 and aza-crown 2 are examples of coronands.
Structural modifications, such as the incorporation of rigid groups, can often change the basicity and donor ability of the heteroatoms as in crown ether 3.5.

Podands are open chain compounds that can coordinate to metal ions much like coronands. For example tripodand 410, is structurally less rigid, and as a result generally forms weaker complexes with metal cations than coronands. The lack of preorganization exhibited by 4 makes it a weaker complexer of alkali metal cations when compared to macrocyclic ligands 1 - 3. This is an example of the "macrocyclic effect."
Podandocoronands including "lariat ethers"\textsuperscript{11} are coronands with attached coordinating arms. The incorporation of ligating arms can produce a three dimensional cavity suitable for a metal ion. Crown ethers with side arms containing donor atoms are referred to as "lariat ethers." An example of an N-functionalized lariat aza-crown ether containing two sidearms (5) is shown below.

The donor arms in 5 can complex with metal cations and therefore increase complex stability when compared to aza-crowns without coordinating arms. The cooperative ring-side arm interaction as described by Gatto et al.\textsuperscript{12} is responsible for the increased binding
strength of lariat ethers over crown ethers. Gokel and coworkers demonstrated that lariat ether complexes Na⁺ and K⁺ ions in a three dimensional fashion as indicated by the X-ray crystal structures. An example of a podandocoronand synthesized by Weitl and coworkers (6) is shown below.

Cryptands are formed by the bridging of monocyclic coronands to generate a bicyclic structure or "crypt." Conventional cryptands contain two bridgehead nitrogen atoms which are joined by three polyoxyethylene chains. The three dimensional cavity generated by this bicyclic system is well adapted for the encapsulation of metal ions. Cryptand 7 was synthesized by Lehn and coworkers and has been studied with respect to metal cation complexation.
B. Macrocyclic Polyamine Hosts

1. Historical

The first example of a saturated tetraaza macrocycle (cyclam, 8) was reported in 1937 by Van Alphen.\textsuperscript{16} The synthesis of this tetraaza macrocycle was improved by Stetter and Meyer\textsuperscript{17a} in 1961 and further improved in 1972 by Barefield.\textsuperscript{17b} Ongoing research in the area of macrocyclic polyamines ligands has resulted in the synthesis and study of a wide variety of polyamines. Some examples of macrocyclic polyamines containing 3-5 nitrogen atoms arranged within a saturated hydrocarbon ring system are shown below (8-12).
For many years following the first report of the synthesis of 8 little research on this compound was done, until the discovery of its coordination chemistry. When compared to their acyclic analogs, cyclam transition metal complexes are more stable and less susceptible towards dissociation. The inertness and unique physical properties of cyclam complexes of transition metal ions in general made them excellent candidates for further study. Co(II) and Ni(II) complexes of azamacrocycles were first reported by Curtis.\textsuperscript{18,19} The kinetics and mechanism of complexation of cyclam with Ni(II),\textsuperscript{20b,c} Co(II),\textsuperscript{21} Zn(II),\textsuperscript{22} Cu(II),\textsuperscript{23} Tc(V),\textsuperscript{24a,b} and Re(V)\textsuperscript{24c} have been studied in recent years. Recently, the cyclam complex with Ni(II) was reduced to the Ni(I) complex by pulse radiolysis and laser flash photolysis.\textsuperscript{20a} In this example, the reduction was carried out by solvated electrons, hydrogen atoms, and carbon dioxide radical anions and was shown to proceed by an inner-sphere mechanism. The coordination chemistry of macrocyclic polyamines has been extensively researched and reviewed.\textsuperscript{25}

A wide variety of macrocyclic polyamine ligands have been synthesized and studied with respect to their coordination ability. Some of these polyamine macrocycles were studied with respect to
their redox behavior$^{26}$ and basicity.$^{27}$

2. Factors Which Influence Complex Stability.

Varying the size of the macrocyclic ring in tetraamines changes metal cation complexation selectivity. In general, smaller ions complex more effectively with smaller macrocyclic ring sizes and larger ions complex most effectively with larger metal ions. This concept of size-match selectivity was developed to describe the nature of crown ether metal complexes.$^{3,4,5,25c,28}$ For instance, the complexation selectivities observed for crown ethers can be interpreted in terms of the ionic radius of the metal cation and the size of the macrocyclic cavity. For crown ethers, experimental evidence based on the determination of formation constants (in water) has shown K$^+$ fits best in 18-crown-6 and therefore forms the most stable complex when compared to Li$^+$, Na$^+$, Rb$^+$, and Cs$^+$. Polyamines, however, do not necessarily exhibit this simple size match relationship. The selectivity is more likely due to the flexibility of the macrocycle, and not the simple size-match relationship described above. Polyamine and crown ether macrocycles are flexible, and as a result, can form a number of
different complexing conformations each varying slightly in the size of the macrocyclic cavity. Hancock\textsuperscript{29} demonstrated that polyamine macrocycles can adopt a variety of conformations according to Molecular Mechanics (MM) calculations\textsuperscript{30} and thermodynamic studies.\textsuperscript{31}

For example, metal complexes of cyclam (8) can adopt at least three configurations. Each of these configurations has a different metal ion size preference (Figure 1.1).

Figure 1.1

![Diagram of cyclam configurations]

In the case of a metal complexes of cyclam, configuration c (trans I) is favored for larger ions whereas configuration a (trans III) and b (cis V) are favored for smaller metal ions. Configurations c and a are complexes in which ligand is trans coordinated to the metal and the ligand in configuration is cis coordinated.\textsuperscript{29} A distinguishing
feature that allows configuration c to more favorably complex larger metal ions is that the metal can rise out of the plane of the four nitrogens to more effectively adapt to the coordination geometry.

In addition to the conformational preferences mentioned above, the size of the chelate rings formed in a complex has a strong influence in determining complex stability. Typically, five-membered chelate rings are favored for larger metal ions and six-membered chelate rings are favored for smaller metal ions. This can be explained by comparison of the chelate ring conformation formed in a complex to the steric requirements of low-strain cycloalkanes. According to MM calculations, the ideal geometries for five and six-membered rings are those shown in Figure 1.2.

Figure 1.2

The six-membered case only allows for a metal-nitrogen bond distance of 1.6 Å if the minimum energy chair conformation is
retained. The five-membered case allows for a much longer metal-nitrogen bond distance (2.5 Å) for the minimum energy twist envelope conformation. Therefore, smaller metal ions form more stable complexes with six-membered chelate rings and larger metal ions form more stable complexes with five-membered rings.

3. Applications of Macrocyclic Polyamines

Several examples of crown ethers, aza-crowns and aza-macrocycles have been utilized in medical, chemical and pharmaceutical applications. For example, tetrabenzylcyclam 13 forms complexes with copper, gold, and silver which have been studied with respect to their antitumor activity.32

A commonly used method to treat tumors utilizes a ligand-radioisotope complex which is attached to an antibody. Cyclic polyamines form strong complexes with radioactive metal ions
which are kinetically inert and resistant to dissociation at physiological pH. These types of complexes have been described by several authors. The application of aza-crown macrocycle metal ion complexes as proton relaxation enhancement agents for Nuclear Magnetic Resonance Imaging (MRI) has received much attention. Several reports of metal complexes useful for this purpose have appeared in the literature. Macrocyclic compounds have long been used for the treatment of metal intoxication. Cations of metals such as arsenic, lead, mercury and nickel can be immobilized by complexation with a macrocyclic host. Derivatives of cyclam have successfully been used for Ni(II) detoxication by increasing the urinary excretion of Ni(II), presumably by complex formation. Several macrocyclic ligands have been tested with respect to their antimicrobial, anticonvulsive, antiarrhythmic, and antihypertensive activity. It has been suggested by Kimura that aza-crown macrocycles might be used for the treatment of kidney stones.

Extensive research in the area of polyamines has established many fundamental concepts concerning the complexation behavior, functionalization, and synthesis of tetraaza macrocycles. Some
macrocyclic complexes as described above have been shown to be of therapeutic and diagnostic value. The research described in this thesis has contributed to this area of study.
Chapter II

Bridged Tetraamine Ligands and Selectively Bridged Tetraamine Ligands

1. Introduction

Structurally-reinforced polyamine macrocycles and their complexes have been studied by a number of research groups. It is believed that the bridging of adjacent or non-adjacent nitrogens in a tetraamine macrocycle should change the proton-transfer and metal complexation characteristics significantly compared to non-bridged tetraamine macrocycles. This structural reinforcement will restrict conformational flexibility, which may result in increased complex stability.

We have developed a general synthetic strategy to cross-bridged tetraamines (Figure 2.1).
Several examples of compounds with functionalized non-adjacent nitrogens are described in detail in Chapter III. Some of the ligating groups contain ionizable moieties in which coordination might be controlled by altering the pH of the medium. There are many examples in the literature of macrocyclic tetraamine syntheses and functionalization but very few which deal with the ethylene cross bridging of non-adjacent nitrogens. A background of structurally reinforced polyamine ligands and their metal complexes will be discussed before the synthetic strategy utilized to make cross-bridged tetraamines is presented (Figure 2.1).

II. Background

A. Bridged Tetraamines
Structurally reinforced macrocycles 1 4 and 1 5, having adjacent nitrogens bridged with an ethanediyl (-CH₂CH₂-) linkage were first synthesized by Wainwright⁴², who reacted 1,2-dichloroethane with cyclam (8) in ethanol to give a mixture of 1 4 and 1 5 (Scheme 2.1).

**Scheme 2.1**

Macrocycles 1 4 and 1 5 were separable, however the separation procedure was lengthy and the yields were low. The bridging of adjacent nitrogens creates a steric barrier, according to molecular models. Therefore, these structurally reinforced macrocycles are forced to complex with metal cations in which the ligand is “trans” coordinated to the metal. For example, the Ni(II) complexes of 1 4 and 1 5 were prepared and exhibited square planar geometry.

An alternative procedure for the synthesis of 1 4 developed by Yamamoto⁴³ is shown in Scheme 2.2.
In this procedure, the reaction of cyclam (8) with aqueous glyoxal, by the method of Weisman,\textsuperscript{44} afforded tetracyclic glyoxal adduct 16. Reduction of 16 with diisobutylaluminum hydride (DIBAH) gave pure 14 in 96% yield. The second bridge was introduced by reaction of 14 with oxalyl chloride in dichloromethane (Scheme 2.3).

After purification by chromatography, the diamide 17 was reduced with DIBAH to give 15.

The bridging of adjacent nitrogens of cyclen was accomplished
by Wainwright\textsuperscript{25} using a method similar to that developed for the adjacent bridging of cyclam \textsuperscript{8,42} Reaction of cyclen (1\textsuperscript{11}) with 1,2-dichloroethane in the presence of potassium hydroxide gave \textsuperscript{18} as the major product (Scheme 2.4).

Scheme 2.4

The significant difference in volatility between \textsuperscript{18} and \textsuperscript{19} allowed separation by fractional sublimation. The Ni(II) complex of \textsuperscript{18} was prepared and the ligand was trans coordinated according to UV-Vis and \textsuperscript{13}C NMR spectra. The bridging of adjacent nitrogens causes the ligand to coordinate with Ni(II) in a trans fashion, whereas Ni(II) in the cyclen complex is cis coordinated.

Ciampolini and Micheloni\textsuperscript{46,47} reported the bridging of non-adjacent nitrogens in the 1,7-dimethyl-1,4,7,10-tetraaza-cyclododecane system (Figure 2.2). Their successful synthetic methodology enabled the synthesis of several bridged structures, differing in the nature of the bridging chain. A list of the types of

\textsuperscript{19}

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bridging units is shown in Figure 2.2.

Figure 2.2

\[
X = \begin{matrix}
\text{N} & \text{N} \\
\text{N} & \text{N} \\
\text{O} & \text{N} \\
\text{S} & \text{N} \end{matrix}
\]

The protonation and metal complexation abilities of these macrocycles were determined and evaluated in terms of the length of the bridging chain and the nature of any associated donor atoms. Most of these macrocycles are selective lithium binders, and a few of them are strong bases, as determined potentiometrically. These macrocycles are unique because conformational flexibility allows for the formation of a small "tridimensional" cavity, which makes the ligand well suited for complexation of a proton or metal cation. For example, 1,7-dimethylcyclen (20), readily reacted with succinyl chloride to give the diamide 21 in 64% yield (Scheme 2.5).
The reduction of the diamide 21 gave non-adjacent bridged macrocycle 22 in 69% yield. A recent report of the synthesis of 24 by the same authors has recently appeared in the literature. The synthetic approach utilized an acylation/reduction approach (Scheme 2.6) analogous to the approach used for the preparation of 22. Compound 24 was synthesized several years ago in our research group.
The X-ray crystal structure of the trihydrochloride salt of 24 was also reported.

B. The Synthesis of Cross-bridged Tetraamine Ligands by Reductive Ring Expansion of Tetracyclic Bis-Aminals.

N,N'-Dimethyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (25) was first reported by Rogers and Weisman in 1990. Reaction of cyclam (8) with aqueous glyoxal in acetonitrile gave 16 (Scheme 2.7) as previously reported.44

Scheme 2.7

Bisaminal 16 was subsequently regioselectively alkylated with 26.
excess methyl iodide to give bis-quaternary bis-aminal dimethiodide 2 6. The regioselectivity is a result of the conformation of 1 6, which has two readily accessible nitrogen lone pairs (Scheme 2.8). Compound 1 6 has a concave face and a convex face. The two nitrogens on the convex face are readily accessible to alkylation.

Scheme 2.8

The high temperature (100°C) ¹H-decoupled ¹³C NMR spectrum of bis-aminal 1 6 showed that the compound undergoes rapid enantiomerization (top of Scheme 2.8). The room temperature ¹³C NMR {¹H} spectrum of 1 6 exhibited six peaks (C₂ symmetry), two
pair of which broadened and coalesced with increasing temperature to give a total of four $^{13}$C NMR peaks at 100°C (time averaged $C_{2v}$ symmetry). The activation barrier for this process was measured\textsuperscript{44} $\Delta G^\ddagger = 15.36 \pm 0.2$ Kcal/mol (at $57.5 \pm 3$°C). The first alkylation (Scheme 2.8) freezes the conformational enantiomerization of 1,6 and thereby directs the second alkylation to the non-adjacent nitrogen (Scheme 2.9).

Scheme 2.9

The reduction of 2,6 with NaBH$_4$ in 95% EtOH gave cross-bridged tetraamine 2,5. It is believed that the mechanism of this reaction involves the intermediacy of iminium ion intermediates, which are reduced by borohydride to give tetraamine 2,5 (Scheme 2.10).
So far, experimental methods have been unable to provide information as to the detailed conformations of 25. However, it is evident that the central -CH₂CH₂- bridging unit cannot "tuck through" the center of the 14-membered ring on the NMR timescale. The ¹H NMR of 25 shows that all geminal pairs of hydrogens on the

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bridging unit are in slow exchange on the NMR timescale (360 MHz).

The first attempts at alkylation of bisaminal 16 with benzyl bromide were performed by Rogers. Benzyla
tion was accomplished by stirring 2 molar equivalents of PhCH₂Br with 16 in MeCN for 3-8 days. The white solid product which precipitated from the reaction mixture was a mixture of monoalkylated and dialkylated products as indicated by ¹H and ¹³C NMR. In one case the reaction mixture was heated, but this run gave decomposition products, complicating isolation of pure product. Pure dibenzylated 27 (>99%) was prepared when 16 was stirred in MeCN at room temperature with a large excess of benzyl bromide for 1 week (Scheme 2.11).
Bis-quaternary bisaminal dibromide salt 27 was reduced with NaBH₄ in 95% ethanol to give 28. The X-ray crystal structure of 28 shown in Figure 2.3 was determined by Jasinski and coworkers. The debenzylation of 28 was achieved by catalytic hydrogenolysis (1 atmosphere) with 10% Pd/C in glacial acetic acid. The crude product was worked up with aqueous base, extracted with benzene, and recrystallized from Et₂O at low temperature (-78 °C) to give pure 29. The successful synthesis of parent ethylene cross-bridged cyclam 29 established a reaction sequence which was subsequently
Figure 2.3
X-ray Crystal Structure of 28
utilized to prepare similar cross-bridged tetraamines (see Results and Discussion). IR and NMR spectra strongly suggest that the solution conformation of 2 9 is "diamond-lattice like." The NH chemical shift of 2 9 in the 1H NMR (C6D6) is shifted downfield (3.59 ppm) compared to typical secondary amines (0.5-3.0 ppm). This shift is clearly due to strong intramolecular hydrogen bonding which should move the NH resonance towards the lower field end of the 1H NMR spectrum. The IR spectrum of 2 9 in chloroform (0.13 M) exhibited an N-H stretching frequency at 3260 cm⁻¹ which is shifted somewhat towards longer wavelengths compared to typical secondary amines (3350-3310 cm⁻¹). Hydrogen bonding typically shifts the N-H stretching frequency of amines toward longer wavelengths. If 2 9 was not intramolecularly hydrogen bonded, a very dilute solution (<0.01 M) would show a shift in the N-H stretching frequency. A dilution study performed by Rogers⁵⁰ confirmed that 2 9 exhibited intramolecular hydrogen bonding since no observable shift in the N-H stretching frequency occurred upon dilution (0.05 M and 0.016 M) of the sample. It is worthy to note that 2 9 and related parent cross-bridged tetraamines are soluble in polar solvents (H₂O (pH<10), DMSO, CH₃CN) as well as non-polar
solvents (C₆H₆, pentane, CCl₄). The solubility in non-polar solvents is undoubtedly due to the fact that these compounds form strong intramolecular hydrogen bonds presenting a lipophilic exterior to the solvent. Compound 29 has subsequently been functionalized with ligating groups containing heteroatoms useful for coordination. These new functionalized derivatives are discussed in Chapter III.

Rogers⁵¹ first established that bisaminal 30 could be carried on through the alkylation/reduction sequence to give macrocycle 24 utilizing the same synthetic sequence used to make 25 (Scheme 2.12).

Scheme 2.12

\[
\begin{align*}
\text{Rogers} & \rightarrow \text{bisaminal} 30 \\
\text{alkylation/reduction} & \rightarrow \text{macrocycle 24}
\end{align*}
\]
C. Selectively Monofunctionalized Tetraamines

In comparison to their tetrafunctional analogs, monofunctionalized macrocyclic tetraamines will coordinate to metals, but in a less complicated fashion. Monofunctionalized cyclams (3 2) shown in Figure 2.4, have fewer possible modes of coordination since they contain only one coordinating arm. Some of the various approaches developed toward monofunctionalized macrocyclic tetraamines will be briefly discussed.

Figure 2.4

Parker and coworkers\(^5\) have synthesized tritosyl cyclam (3 3) which was monofunctionalized at the secondary amine and subsequently detosylated to give monofunctionalized cyclams (3 2). Fabbrizz\(^5\) and coworkers have synthesized one-armed macrocycle 3 4 starting with 3 3 and tosylaziridine.
Bernard\textsuperscript{54a} has prepared monofunctionalized tetraaza-macrocycles 3\textsuperscript{7} by protection of three of the nitrogens of 3\textsuperscript{5} as shown in Scheme 2.13.

Scheme 2.13

Tris(dimethylamino)borane reacts with tetraamines 3\textsuperscript{5} to form protected intermediates 3\textsuperscript{6}, which were N-alkylated and subsequently hydrolyzed to give monofunctionalized tetraamine macrocycles 3\textsuperscript{7}.

Yaouanc\textsuperscript{54b} and coworkers have successfully protected three of
the nitrogens of tetraazamacrocycles 3 5 as a chromium complex (3 8) as shown in Scheme 2.14.

Scheme 2.14

Treatment of tetraamine macrocycles 3 5 with chromium hexacarbonyl gave tridentate complexes 3 8, which were then N-alkylated and air oxidized in aq HCl. Workup with aqueous base gave monofunctionalized tetraamine macrocycles 3 7.

The synthesis and functionalization of 1,4,8-trimethyl-1,4,8,11-tetraazacyclotetradecane (3 9) has been reviewed by Kaden55 (Figure 2.5).
The study of azamacrocycles containing convergently arranged binding sites with respect to coordination of metal cations has been recently reviewed. The coordination chemistry of lithium ion, with emphasis on amine complexes and lithium selective ionophores has recently been reviewed.

General reviews covering the thermodynamic and kinetic data for macrocycle interaction with cations, anions, and neutral species have been compiled by Izatt and coworkers. These reviews contain extensive lists of macrocyclic compounds and tables which include macrocycle complexation parameters for transition metal ions, alkaline earth metal ions, anions, and neutral species. An earlier review also published by Izatt covers the thermodynamic and kinetic parameters of mainly...
polyazamacrocycles. This earlier review focuses on tetraaza-macrocycles and their transition metal, alkaline earth, and proton complexes.

Martell and coworkers have recently investigated the factors which affect the stability of macrocyclic and macrobicyclic complexes in solution. The major contributing factors are listed as follows: 1) size of the chelate ring, 2) basicity of the donor atom, 3) charge neutralization upon complex formation, 4) steric effects, and 5) preorganization of ligand.

Some of the first work on the coordination chemistry of cyclam (8) with Li⁺ was done by Truter and coworkers, who reacted

\[ \text{8} \]

8 with excess LiClO₄ in methanol to give a mononuclear LiClO₄•8 complex. Evaporation of the mother liquors of this reaction gave the bimetallic complex (LiClO₄)₂•8. More recently, the X-ray crystal
structure of tetraprotonated 8 was determined by Subramanian et al.\textsuperscript{62}

Herlinger\textsuperscript{63} has recently reported the preparation of the sodium and lithium thiocyanate complexes of 1,4,8,11-tetramethyl-1,4,8,11-tetraazacyclotetradecane (4 1).

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Ciampolini and coworkers\textsuperscript{46,47,65-71} have synthesized and studied several aza-cages. For example, the Li\textsuperscript{+} complexes of 4\textsuperscript{248} and 4\textsuperscript{370} were synthesized and characterized by X-ray crystallography.

![Chemical structures](image)

The second protonation constants of these macrocycles were determination potentiometrically while the first and third were respectively too high and too low to measure using this method.

Micheloni\textsuperscript{72} has studied azamacrocycles with more than six donor atoms (Figure 2.6).

Figure 2.6

![Chemical structure](image)

These polyazamacrocycles are polyprotic bases and can potentially
form polynuclear metal complexes. For example, polyaza-
macrocycles are strong bases in the first protonation step and are
weaker bases in the last protonation step. This decrease in basicity
can be explained by charge repulsion effects. It was shown that
when protonated, these polyazamacrocycles can form adducts with
polycharged anions.

Ab initio calculations on protonated 11 were performed in
order to compare the results to those obtained experimentally.

Dye and coworkers prepared the first alkalide of azacage 43.
This alkalide, Li+(43)Na-, was characterized by 23Na and 7Li NMR.
The alkalide is reasonably stable and did not thermally decompose
below 65°C.

Rogers showed that macrocyclic polyamine ligands 24 and
25 form complexes with lithium perchlorate as demonstrated by X-
ray crystallography, 1H NMR, and 1H-decoupled 13C NMR.

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The X-ray crystal structure of the lithium complex of 2 4 is shown in Figure 2.7. This complex exhibits distorted octahedral geometry at Li⁺ with perchlorate binding in a bidentate fashion. Each of the 9-membered rings in the bicyclic structure is in a [45] conformation.⁷⁴ The X-ray crystal structure of the lithium complex of 2 5 is shown in Figure 2.8. This complex is trigonal bipyramidal at Li⁺ with perchlorate binding in a monodentate fashion. Each of the 10-membered rings in the bicyclic structure is in a [2323] conformation⁷⁴ and the complex has C₂ symmetry.

Compounds 2 5 and 2 4 are strong bases as determined by Rogers.⁴⁹,⁵⁰a,c,d The X-ray crystal structures of the diprotonated and monoprotonated forms of ligands 2 5⁹ and 2 4¹⁰c,d respectively were determined by Jasinski and coworkers. The addition of NaBPh₄ to an aqueous solution of 2 4 resulted in the precipitation of the monoprotonated tetraphenylborate salt 4 4. An inspection of the X-ray crystal structure data of 4 4 (disordered) (Ortep diagram in Figure 2.9) reveals that the structure is inside-protonated at the methyl-substituted nitrogens. This is supported by the shorter C-N bond lengths to those nitrogens. Ligand 2 5 was titrated with trifluoroacetic acid (TFA) in CD₃CN. In the absence of acid,
Figure 2.7

- [45]/[45] ligand conformation
- distorted octahedral at Li⁺
- bidentate perchlorate

Figure 2.8

- [2323]/[2323] ligand conformation
- trigonal bipyramidal at Li⁺
- monodentate perchlorate
compound 2 5 exhibited 7 sharp lines in the $^{13}$C NMR spectrum. When TFA was added (0.5 equivalents), a separate set of 7 $^{13}$C NMR peaks was observed which indicated 2 5 and its monoprotonated complex were in slow dynamic exchange with respect to the NMR timescale. When more TFA was added (1.5 equivalents total) two sets of 7 $^{13}$C NMR peaks were observed, indicating that the diprotonated species was in slow dynamic exchange with the monoprotonated species. The X-ray crystal structure of 2 5$^\cdot$H$_2$2$^+$·bis-trifluoroacetate is shown in Figure 2.10. The structure has C$_2$ symmetry with each 10-membered ring in a slightly distorted [2323] conformation. Each N-H in this complex is within hydrogen bonding distance to two transannular nitrogens. Modeling of the dication with the AMBER force field in Macromodel gave a structure that was in good geometric agreement with the X-ray crystal structure.$^{50c,d}$ Rogers$^5$ has also performed a titration of ligand 2 4 with TFA. In this example the ligand and the monoprotonated species were in slow dynamic exchange.

The X-ray crystal structure of trihydrochloride dihydrate 4 5 was recently reported by Biancini$^{48}$ and coworkers, as mentioned previously.
They describe the structure of 45 as clamp-like, with the two N-CH₃ nitrogens protonated and a third proton shared between the two bridgehead nitrogens. An NMR titration of 24 (D₂O/DCI) indicated a monoprotonated species was present at pH 10 which exhibited time averaged Cᵥₐ symmetry, indicating a rapid exchange of a proton between the two methylated nitrogens. As more acid was added, the diprotonated species formed (pH 3.8) as indicated by the ¹H NMR spectrum. The ¹H NMR spectrum at pH 1 supports a distinct triprotonated species.

III. Results and Discussion

A. Synthesis of N,N'-Dimethyl Cross-bridged Tetraamines.

Tetraamines 8, 11, 46, and 47, used as starting materials for ligand synthesis, were either purchased or prepared in this laboratory.
Tetraamines 11, 46, and 47 were prepared by the Kellogg Cs₂CO₃ modification⁷⁵,⁷⁶ of the cyclization procedure of Richman and Atkins.⁷⁷ In this procedure, tosylamide 48, 51, or 54 was converted to the dicesium salt with 2 equivalents of Cs₂CO₃ and cyclized with 1 equivalent of the appropriate ditosylate (49, 52, or 49) in DMF at 100°C. The cyclized product (50, 53, or 55) was detosylated using the conc. H₂SO₄ method.⁷⁷,⁷⁸ Workup with base followed by extraction with CHCl₃ gave pure tetraamine 11, 46, or 47 (Scheme 2.15).
Tetraamine 47 and cyclen (11) were prepared on multigram scales by Fagan.\textsuperscript{79} Tetraamine 46 was prepared by the same method. Cyclam (8) was purchased from Strem Chemicals.

Weisman and Ho\textsuperscript{44} reported the synthesis of 30, 56, and 57 by the same route previously described to make bisaminal 16.
1. Synthesis of N,N'-Dimethyl-1,4,8,11-Tetraazabicyclo[6.6.2]-pentadecane (25).

The synthesis of 25 was previously discussed in the background section of this chapter. The reaction sequence was repeated several times and similar yields were obtained.

2. Synthesis of N,N'-Dimethyl-1,4,8,11-Tetraazabicyclo[6.5.2]-pentadecane (58).

Ho and Weisman first established that the reaction of 46 with 46.
aqueous glyoxal in MeCN gave two isomeric products, 5 6 and 5 9. Compound 5 6 was formed along with a side product 5 9, which was separable by chromatography (Scheme 2.16). The detailed structure of 5 9 was not established by Ho and Weisman, who reported a 1H NMR and obtained a 92% yield of the isomeric mixture (78% of 5 6, and 18% of 5 9).

Scheme 2.16

When the reaction was repeated on a larger scale a lower yield was obtained (50% of 5 6, and 5% of 5 9 after chromatography). The major component, 5 6, exhibited severe dynamic exchange broadening (intermediate) in both the 1H and 13C NMR spectrum at room temperature. Low temperature 13C NMR experiments performed by Weisman4 with 5 6 showed that the dynamic process, which is an enantiomerization, can be frozen out below -55 °C (see experimental section-preparation of 5 6). The minor component 5 9, exhibits two
distinct resonances in the $^1H$ NMR for the upfield methylene. The $^{13}C$ NMR of 5 9 spectrum exhibits five upfield signals and one downfield signal (one methine). The upfield methylene in the $^1H$ NMR ($\text{CH}_2\text{CH}_2\text{CH}_2$) exhibits two distinct multiplets, each integrating to one hydrogen. Trans fused product 6 1 can be ruled out since the upfield methylene in the $^1H$ NMR would exhibit a single multiplet due to symmetry ($C_2$).

Trans fused product 6 0 can also be ruled out since the downfield methine region in the $^{13}C$ NMR would exhibit two different resonances ($C_2$). In the case of 5 9 the enantiomerization is very likely in fast exchange with respect to the NMR timescale. At room temperature bisaminal 1 6 and its enantiomer are in slow dynamic exchange with respect to the $^{13}C$ NMR timescale (90.56 MHz) as discussed earlier. However, $^{13}C$ NMR spectra (20.25 MHz) indicate that 3 0 and its enantiomer are in fast dynamic exchange with
respect to the NMR timescale. To date, the characterization of compound 5 9 is incomplete (13C and 1H NMR only).

The regioselective methylation of 5 6 gave bis-quaternary salt 6 2 in 87-92% yield. NaBH₄ reduction of 6 2 gave cross-bridged macrocycle 5 8 in 96% yield (Scheme 2.17).

Scheme 2.17

![Scheme 2.17]

Compound 6 2 was characterized by IR, elemental analysis, 1H, and 13C NMR. Compound 5 8 was characterized by IR, high resolution mass spectrometry (HRMS), 1H, and 13C NMR.

3. Synthesis of N,N'-Dimethyl-1,4,7,10-tetraazabicyclo[5.5.2]-tetradecane (2 4).

The Rogers⁵⁰ synthesis of 2 4 was repeated several times with significant improvements in the yields (Scheme 2.18).
4. Synthesis of N,N'-Dimethyl Cross-bridged Isocyclam

The reaction of aqueous glyoxal with isocyclam (47) gave glyoxal adduct 57 in 50% yield after chromatography (Scheme 2.19). Compound 57 had been synthesized previously and reported as an oil.⁴⁴ Upon scaleup of this procedure, 57 was isolated as a low melting solid.
Scheme 2.19

A low temperature $^{13}$C NMR spectrum of 57 (-72 °C) indicated no observable dynamic exchange broadening; only slight changes in $^{13}$C NMR chemical shift values were noted. In addition, no separate set of peaks corresponding to a minor conformer were noted at this temperature. This experiment showed that 57 is very biased toward a single conformation. Inversion of all four nitrogens of a single enantiomer of 57 would give a diastereomeric conformation.

Scheme 2.20 shows the four possible isomers of 57 and describes their relationship to one another.
Fagan methylated isocyclam bisaminal 57 to give a mixture of products (63 and 64) which were not separated. The reduction of this mixture gave two polyamines 65 and 66 (in ratio 9:1 in one run; less selective in another) which could not be distinguished from one another (Scheme 2.21).
Scheme 2.21

The two products are identified as having either two ten membered rings (6\textsubscript{5}) or having a nine and an eleven membered ring (6\textsubscript{6}) in the macrobicyclic structure. The lack of selective methylation complicated the preparation of a single isomer (6\textsubscript{3} or 6\textsubscript{4}), and attempts to purify this mixture by recrystallization were unsuccessful. Compound 5\textsubscript{7} was characterized by IR, elemental analysis, $^1\text{H}$, and $^{13}\text{C}$ NMR.

B. Synthesis of Parent (N-H) Cross-bridged Ligands

1. Synthesis of 1,4,8,11-Tetraazabicyclo[6.6.2]hexadecane (2\textsubscript{9}).
The scaleup synthesis of the sequence first established by Rogers\textsuperscript{50a} (Scheme 2.22) resulted in yield improvements for every step. The yield of 27 was significantly improved (~100\%) by increasing the reaction time from one to three weeks.

Scheme 2.22

The precipitated product was isolated by filtration and no further purification was necessary. The NaBH\textsubscript{4} reduction of 27 was repeated on a large scale (20 g of 27) to yield dibenzyl cross-bridged cyclam (28, 89\%) which was >99\% pure according to \textsuperscript{1}H and \textsuperscript{13}C NMR. Toluene was substituted for benzene in the extraction step. The removal of the benzyl groups was accomplished by catalytic
hydrogenolysis (atmospheric pressure H₂; 24 hours) using 10% Pd/C in glacial acetic acid. The hydrogenolysis was stopped after the theoretical amount of H₂ (97% of theoretical) was taken up. After workup with aqueous base and extraction with benzene, the crude product 29 (oil) was crystallized using the Rogers method to give 85% yield of pure 29. The low temperature recrystallization is not essential since the crude oil product is >99% pure by NMR. Compound 27 was characterized by IR, elemental analysis, 1H, and 13C NMR.

2. Synthesis of 1,4,8,11-Tetraazabicyclo[6.5.2]pentadecane (67).

Macrocyclic tetraamine 67 was synthesized by the same basic procedure used to synthesize 29 (Scheme 2.23).
Scheme 2.23

The alkylation and reduction steps go cleanly in 99% and 66% yield respectively. However, the debenzylation step leading to 6,7 did not go cleanly and attempts to recrystallize the crude solid product from Et₂O (-78 °C) failed. The hydrogenolysis was stopped after the theoretical amount of H₂ (104% of theoretical) was taken up. So, after workup with aq KOH and toluene extraction, the crude product (~85-90% pure, 82% crude yield) was dissolved in absolute ethanol and converted to the hydrochloride salt by addition of a few drops of 12M HCl. The precipitated hydrochloride salt was isolated by
filtration and washed with cold ethanol. It is not known at this point whether this solid is a mono-, di-, or trihydrochloride salt. This salt was dissolved in aq KOH and the free amine was extracted into benzene. Concentration of the extracts gave an oil which was kugelrohr distilled. This material solidified upon standing and was shown to be pure 6 7 (47% yield). Compound 6 8 was characterized by IR, elemental analysis, \(^1\)H, and \(^{13}\)C NMR. Compounds 6 9 and 6 7 were characterized by IR, high resolution mass spectrometry (HRMS), \(^1\)H, and \(^{13}\)C NMR.

3. Synthesis of 1,4,7,10-Tetraazabicyclo[5.5.2]tetradecane (7 2).

The synthesis of 7 2 proceeded as described in Scheme 2.24 following the Rogers\(^a\) procedure. Rogers first established that 3 0 could be carried on through the alkylation/reduction sequence in much the same way as 1 6. His findings were based on \(^1\)H and \(^{13}\)C NMR spectra. A single attempt by Rogers to prepare 7 2 by hydrogenolysis of 7 1 gave a complex mixture of products.
The alkylation/reduction sequence was repeated in order to fully characterize 70 and 71. The yields for these two steps were significantly improved during scaleup. Several attempts at hydrogenolysis of 71 using 10% Pd/C in glacial acetic acid gave (atmospheric H₂; 24 hours) the desired product 72 but in only ~85% purity. The best yield of pure 72 was obtained by hydrogenolysis (atmospheric pressure) for 40 minutes using this procedure (2.3 equivalents of H₂ taken up). After workup with aq KOH and benzene extraction, the crude solid product was kugelrohr distilled. During the distillation, the collection bulb was changed such that two
portions were collected (see experimental section). The first fraction was a solid which was sublimed to give pure 7 2 in 55% yield. 1H NMR of the second fraction (liquid) showed that it consisted of a mixture of the desired product and some partially debenzylated material. An attempt to obtain more product by hydrogenolysis of the second fraction and the distillation residue was unsuccessful.

The 13C NMR of the crude reaction mixture indicated an impurity with shifts consistent with monoacetylated cyclen 7 3 (peak at 164.1 ppm present).

![Chemical structure of 7 3](image)

The 1H NMR of the crude product exhibited a sharp singlet at 1.74 ppm, likewise consistent with an acetyl group. Alternatively, the crude solid product could be purified by conversion to the hydrochloride salt by the same method used to purify 6 7. Mixing a solution of 7 2 in ethanol with a few drops of 12M HCl resulted in the precipitation of the trihydrochloride salt (CHN analysis). This
salt was recrystallized from acetonitrile containing a small amount of water. Subsequent workup with aqueous base, extraction with benzene, and removal of solvent gave solid product which was sublimed. The yield of pure product for this alternative procedure was 50%. Compound 70 was characterized by IR, elemental analysis, $^{1}H$, and $^{13}C$ NMR. Compounds 71 and 72 were characterized by IR, high resolution mass spectrometry (HRMS), $^{1}H$, and $^{13}C$ NMR.


Fully characterized 57 did not dibenzylate cleanly using the Rogers$^{50a}$ procedure.

Scheme 2.25

![Scheme 2.25](image-url)
There are several possible products of the reaction which are shown in Scheme 2.25. Stirring 5 7 in acetonitrile with an excess of bromide resulted in the crystallization of a solid product which was isolated by filtration. The $^1$H and $^{13}$C NMR of this solid indicated a complex mixture, possibly monoalkylated (7 4, 7 5, 7 6, 7 7) and dialkylated (7 8, 7 9) products as suggested in Scheme 2.25. The reaction mixture was filtered after 5 days at room temperature because darkening of the mixture was evident which could very well be attributed to the formation of decomposition products. This lack of selective benzylation was not unexpected given the results observed by Fagan\textsuperscript{79} for methylation of 5 7.

C. Synthesis of N-Methyl Non-adjacent Cross-bridged Tetraamines.

Significant progress was made towards the synthesis of N-methyl cross-bridged tetraamines (Figure 2.11).
To date, no attempts have been made to functionalize secondary amine portion with a group containing heteroatoms. These cross-bridged monofunctionalized tetraamines might best be described as "one-arm bandits" (Figure 2.12) since the pendant arm can assist in coordination to a metal cation.

Figure 2.12

\[ \text{"One-arm bandits"} \]

The initial attempts by Rogers to synthesize 27 by alkylation of bisaminal 16 with benzyl bromide in MeCN at room temperature showed that the reaction was slow, and heating of the mixture only resulted in the formation of decomposition products as discussed earlier (Scheme 2.26).
In one example, small amounts of $\textit{80}$ were detected by $^1\text{H}$ and $^{13}\text{C}$ NMR. This result suggested that it might be possible to prepare $\textit{80}$ by proper choice of reaction time and solvent.

1. Synthesis of $\text{N}$-Methyl-$1,4,8,11$-Tetraazabicyclo[6.6.2]-hexadecane ($\textit{81}$).

Clean monoalkylation of bisaminal $\textit{16}$ was achieved by adding 1 equivalent of benzyl bromide to a stirred solution of $\textit{16}$ in toluene. The quaternary salt $\textit{80}$, crystallized due to its limited solubility in toluene. After one week, the product was collected by filtration in 66% yield; it needed no further purification (Scheme 2.27).
Compound 80 analyzed as a monohydrate. The remaining non-
adjacent nitrogen was then be methylated by the same procedure
used for the methylation of bisaminal 16 as discussed previously in
this Chapter. Compound 82 (conceivably be a mixed salt) was
characterized by IR and NMR spectroscopy. It was then reduced with
excess NaBH$_4$ in 95% EtOH for 1 week, although a similar yield was
obtained in a separate run when the reaction was run for 2 days. The
two step yield from 80 to 83 ranged from 64 %to 68%.

Hydrogenolysis of 83 was carried out using 10% Pd/C (H$_2$, 1
atmosphere) in glacial acetic acid. The hydrogenolysis was stopped after 21 hours (103% of the theoretical uptake of H₂). The workup with aq base followed by extraction with toluene gave pure 81. The ¹H NMR of 81 exhibited a singlet corresponding to the methyl group at 2.01 ppm. The ¹H NMR chemical shift of the NH proton (4.75 ppm) of 81 is shifted considerably downfield (Figure 2.13A), compared to acyclic secondary amines, suggesting strong intramolecular hydrogen bonding. A D₂O exchange experiment confirmed the assignment of the downfield multiplet at 4.75 ppm as the NH proton as shown in Figure 2.13B. Compound 80 was characterized by IR, elemental analysis, ¹H, and ¹³C NMR. Compounds 83 and 81 were characterized by IR, high resolution mass spectrometry (HRMS), ¹H, and ¹³C NMR.
2. Synthesis of N-Methyl-1,4,7,10-Tetraazabicyclo[5.5.2]-
tetradecane (84).

The same sequence utilized to prepare 81 was applied to
bisaminal 30 (Scheme 2.28).

Scheme 2.28

The monobenzylation of 30 proceeded in 84% yield. Subsequent
methylation of 85 gave a mixed bis-quaternary bisaminal dihalide
salt (86) which was pure according to $^1$H and $^{13}$C NMR. The reduction
of 86 with excess NaBH$_4$ successfully produced 87. The workup
with aqueous base followed by benzene extraction gave 87, which
was >98% pure according to $^1$H and $^{13}$C NMR spectra. The two step
yield (70%) from 8 5 to 8 7 is reported since the mixed salt (8 6) was not completely characterized (IR, 1H, and 13C NMR only). Compound 8 5 was characterized by IR, CHN analysis, 1H, and 13C NMR. Compound 8 7 was characterized by IR, high resolution mass spectrometry (HRMS), 1H, and 13C NMR. The hydrogenolysis (atmospheric pressure) of 8 7 was carried out using 10% Pd/C in glacial acetic acid. The workup with base followed by toluene extraction gave impure 8 4. The product was about 75% pure (50% crude yield) after debenzylation and was not successfully purified by any of the following methods: 1) distillation of the crude product, 2) recrystallization of the hydrochloride salt of 8 4. To date the characterization of 8 4 is incomplete (IR, 1H and 13C NMR).

Alternative hydrogenolysis conditions (10% Pd/C, EtOH, 10% aq HCl) were attempted without success. A similar problem with the hydrogenolysis was encountered in the debenzylation step for the preparation of cross-bridged cyclen (7 2), but was eventually overcome. Perhaps fine tuning of the reaction conditions could provide cleaner 8 4. Choosing other solvents as well as catalysts might help solve this problem.
D. Complexation (Li+ and Na+) and Protonation Competition

Experiments with Cross-Bridged Tetraamine Ligands

In this section the measurement of the relative basicities and the relative Li+ and Na+ complexation abilities of three cross-bridged tetraamines (2, 5, 8, and 25) are reported.

Conformational flexibility allows the four nitrogen lone pairs to converge on a cleft or cavity when protonated or complexed with a metal cation as shown in Figure 2.14.
The variation in chelate ring sizes of 24, 58, and 25 might be expected to lead to observable variations in metal ion selectivity and basicity. In most of these competition experiments the ligands and their complexes or conjugate acids were in slow exchange and the concentrations of all species were determined using either \(^{1}H\) or \(^{13}C\) \{\(^{1}H\}\) NMR. Each component had a set of resonances corresponding to it, thus allowing the proportions to be determined by integration of specific \(^{13}C\) and \(^{1}H\) resonances. A competition equilibrium constant (\(K_{rel}\)) can be calculated since the concentrations of all the species can be measured in an appropriately designed competition.
experiment. Multiple integrations were run and enabled multiple calculations of $K_{rel}$. Error analyses were performed in order to obtain confidence intervals for the calculated equilibrium constants. In all cases a dramatic change in the appearance of $^1$H and $^{13}$C NMR spectra indicated conformational biasing as a result of metal ion complexation or protonation.


The chemical shifts of the tetraamine ligands 2 5, 2 4, and 5 8 and their NaBPh$_4$ and LiClO$_4$ complexes and CF$_3$COOH salts in CD$_3$CN were determined experimentally by $^1$H and $^{13}$C {1H} NMR (Table 2.1A, 2.1B, and 2.1C; $^{13}$C chemical shifts only). Tables 2.1A and 2.1B were produced from the results obtained by Rogers.$^{50a}$
Table 2.1A: $^{13}$C NMR Chemical Shifts of 25 and the Na+, Li+ and H+ Complexes (CD$_3$CN).

<table>
<thead>
<tr>
<th>Host (δ)</th>
<th>LiClO$_4$ complex (δ)(0.10 M)</th>
<th>NaBPh$_4$ complex (δ) (0.07 M)</th>
<th>Monoprotonated (CF$_3$CO$_2$H) (δ) (0.16 M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 (0.10 M)</td>
<td>28.70 (C$_6$,13)</td>
<td>25.58 (C$_6$,13)</td>
<td>25.02 (C$_6$,13)</td>
</tr>
<tr>
<td>43.02 (CH$_3$)</td>
<td>46.52 (CH$_3$)</td>
<td>43.74 (CH$_3$)</td>
<td></td>
</tr>
<tr>
<td>52.29</td>
<td>51.75</td>
<td>50.79</td>
<td>52.47</td>
</tr>
<tr>
<td>56.81</td>
<td>52.20</td>
<td>51.27</td>
<td>53.01</td>
</tr>
<tr>
<td>56.96</td>
<td>58.89</td>
<td>59.07</td>
<td>54.22</td>
</tr>
<tr>
<td>57.77</td>
<td>58.94</td>
<td>59.14</td>
<td>58.23</td>
</tr>
<tr>
<td>61.67</td>
<td>59.64</td>
<td>59.21</td>
<td>58.65</td>
</tr>
</tbody>
</table>

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Table 2.1B: $^{13}$C NMR Chemical Shifts for 24 and the Na$,^+$, Li$,^+$ and H$^+$ Complexes (CD$_3$CN).

<table>
<thead>
<tr>
<th>Host (δ)</th>
<th>LiClO$_4$ complex (δ) (0.17 M)</th>
<th>NaBPh$_4$ complex (δ) (0.20 M)</th>
<th>Monoprotonated (CF$_3$CO$_2$H) (δ) (0.17 M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>44.51 (CH$_3$)</td>
<td>46.95 (CH$_3$)</td>
<td>46.13* (br, CH$_3$)</td>
<td>44.12 (CH$_3$)</td>
</tr>
<tr>
<td>57.25 (C$_{13,14}$)</td>
<td>50.79 (C$_{13,14}$)</td>
<td>57.54*</td>
<td>48.97 (C$_{13,14}$)</td>
</tr>
<tr>
<td>57.60</td>
<td>57.45</td>
<td>61.60* (br)</td>
<td>49.83</td>
</tr>
<tr>
<td>60.59</td>
<td>61.62</td>
<td></td>
<td>53.89</td>
</tr>
</tbody>
</table>

*These chemical shifts actually correspond to dynamically averaged peaks of complexed and uncomplexed ligand since the ligand was incompletely complexed even in the presence of excess NaBPh$_4$. 

![Diagram of 24](image-url)
Table 2.1C: $^{13}$C NMR Chemical Shifts for 58 and the Na+, Li+ and H+ Complexes (CD$_3$CN).

<table>
<thead>
<tr>
<th>Host (δ)</th>
<th>LiClO$_4$ complex (δ) (0.11 M)</th>
<th>NaBPh$_4$ complex (δ) (0.17 M)</th>
<th>Monoprotonated (CF$_3$CO$_2$H) (δ) (0.25 M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>58 (0.11 M)</td>
<td>28.01 (C$_6$)</td>
<td>24.77 (C$_6$)</td>
<td>25.49 (C$_6$)</td>
</tr>
<tr>
<td></td>
<td>43.62 (CH$_3$)</td>
<td>45.43 (CH$_3$)</td>
<td>45.54 (CH$_3$)</td>
</tr>
<tr>
<td></td>
<td>44.19 (CH$_3$)</td>
<td>47.10 (CH$_3$)</td>
<td>46.89 (CH$_3$)</td>
</tr>
<tr>
<td></td>
<td>54.15</td>
<td>51.30</td>
<td>49.53</td>
</tr>
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<td>50.35</td>
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<td>54.86</td>
<td>56.69</td>
<td>51.89</td>
</tr>
<tr>
<td></td>
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<td>57.85</td>
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<td></td>
<td>56.79</td>
<td>58.73</td>
<td>58.30</td>
</tr>
<tr>
<td></td>
<td>58.06</td>
<td>59.32</td>
<td>59.54</td>
</tr>
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<td></td>
<td>58.25</td>
<td>59.56</td>
<td>59.73</td>
</tr>
<tr>
<td></td>
<td>60.54</td>
<td>60.46</td>
<td>61.33</td>
</tr>
<tr>
<td></td>
<td>61.16</td>
<td></td>
<td>61.83</td>
</tr>
</tbody>
</table>

* degenerate resonances

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The complexation reactions were performed using CD$_3$CN as the solvent (CD$_3$CN as ref. set at 1.93 ppm in the $^1$H NMR, CD$_3$CN as ref. set at 1.30 ppm in the $^{13}$C NMR). In the examples where $^{13}$C NMR peaks were integrated, no account was made for differences in $T_1$ values. Typically it is advisable to wait five $T_1$'s between pulses when using FT-NMR. To reduce error associated with differences in NOE corresponding carbons in the complex and uncomplexed ligand were integrated when possible.

a. Determination of $K_{r1}$ Where All Ligand and Complex Concentrations can be Measured.

In a competition where two ligands compete for a single guest metal cation, the following equilibrium expressions may be written, where $[L_1]$ and $[L_2]$ are the concentrations of the two free ligands at equilibrium and $[G^+]$ is the guest concentration at equilibrium. $G^+$ represents the metal cation or proton and $X^-$ represents the counterion. Equations 1 and 2 are separate equilibrium expressions describing the complexations of ligands 1 and 2 and $K_{s1}$ and $K_{s2}$ are associated nominal stability constants. Ion pairing effects are
ignored in this treatment since they have been shown to be
unimportant in CD$_3$CN in related cases.$^8$

\[
\begin{align*}
G^+ + L_1 & \quad \leftrightarrow \quad GL_1^+ \quad \text{(Equation 1)} \\
G^+ + L_2 & \quad \leftrightarrow \quad GL_2^+ \quad \text{(Equation 2)}
\end{align*}
\]

\[
K_{s1} = \frac{[GL_1^+]}{[L_1][G^+]} \quad \text{(Equation 3)} \quad K_{s2} = \frac{[GL_2^+]}{[L_2][G^+]} \quad \text{(Equation 4)}
\]

\[
K_{rel} = K_{s1} / K_{s2} = \frac{[GL_1^+][L_2]}{[GL_2^+][L_1]} \quad \text{(Equation 5)}
\]

The relative equilibrium constant $K_{rel}$ is the ratio of $K_{s1}$ (Equation 3) and $K_{s2}$ (Equation 4) as shown in Equation 5. The competitions were performed with the molar ratio of the three components ($G\times L_{1T}:L_{2T}$) being 1:1:1, where $L_{1T}$ and $L_{2T}$ are defined as follows: $L_{1T} = [L_1] + [GL_1^+]$, and $L_{2T} = [L_2] + [GL_2^+]$. $L_{1T}$ and $L_{2T}$ also correspond to the concentrations of ligands $L_1$ and $L_2$ in the absence of guest. The relative equilibrium constant $K_{rel}$ was used to calculate the free energy of competition for the reaction $\Delta G_{rel}^o$.

\[
\Delta G_{rel}^o = -RT\ln K_{rel} \quad \text{(Equation 6)}
\]
In most examples repetitive integrations were performed allowing several calculations of $K_{rel}$. Statistical analyses were performed on these results allowing the calculation of standard deviations, which were used to calculate confidence intervals (90%). The above analysis depends on having negligible free guest at equilibrium.

b. Determination of $K_{rel}$ When the Relative Concentrations of Only One Ligand and its Complex Can Be Measured.

In a competition between two ligands for a guest where only the concentrations of one ligand and its complex are measurable due to overlapping $^{13}$C or $^1$H NMR resonances, the $K_{rel}$ was obtained. For a 1:1:1 molar mixture of $L_1$, $L_2$, and $G$ the total concentration of free ligands at equilibrium ($[L_1]+[L_2]$) is equal to the concentration of complexes at equilibrium ($[L_1G^+]+[L_2G^+]$). It is also true that $[GL_1^+] = [L_2]$ and $[GL_2^+] = [L_1]$. The substitution shown below simplifies the equation used to calculate the competition equilibrium constant ($K_{rel}$) to Equation 7.
Since \([L_2] = [GL_1^+]\) and \([GL_2^+] = [L_1]\) then,

\[
K_{rel} = K_{s1} / K_{s2} = \frac{[GL_1^+]^2}{[L_1]^2} \text{ or } \frac{[GL_2^+]^2}{[L_2]^2} \quad \text{(Equation 7)}
\]

Equation 7 was used to determine \(K_{rel}\) if the relative concentration of only one ligand and its complex were measured. Identifiable \(^1\)H and \(^{13}\)C resonances of a single ligand \(L_1\) and the complex \(L_1G^+\) were integrated to provide the fraction of each component. Repetitive integrations were performed enabling multiple calculations of \(K_{rel}\). Again, statistical analyses allowed the calculation of standard deviations and confidence intervals for \(K_{rel}\).

c. Determination of Relative Equilibrium Constant in a Li\(^+\)/Na\(^+\) Competition.

In a competition where two ions compete for a single host the following equilibrium expressions may be written, where \([LiL^+]\) and \([NaL^+]\) are the concentrations of two complexes at equilibrium and
[Li+] and [Na+] are the concentrations of uncomplexed guest ions at equilibrium. Again, ion pairing effects are not accounted for.

Equations 8 and 9 are separate equilibrium expressions describing the complexations of each ion. The relative equilibrium constant \( K_{\text{rel}} \) is the ratio of \( K_{\text{Li+}} \) (Equation 10) and \( K_{\text{Na+}} \) (Equation 11) as shown in Equation 12.

\[
\begin{align*}
\text{L} + \text{Li}^+ & \rightleftharpoons \text{LiL}^+ \quad \text{(Equation 8)} \\
\text{L} + \text{Na}^+ & \rightleftharpoons \text{NaL}^+ \quad \text{(Equation 9)} \\
K_{\text{Li+}} & = \frac{[\text{LiL}^+]}{[\text{L}][\text{Li}^+]} \quad \text{(Equation 10)} \\
K_{\text{Na+}} & = \frac{[\text{NaL}^+]}{[\text{L}][\text{Na}^+]} \quad \text{(Equation 11)} \\
K_{\text{rel}} & = \frac{K_{\text{Li+}}}{K_{\text{Na+}}} = \frac{[\text{LiL}^+][\text{Na}^+]}{[\text{Li}^+][\text{NaL}^+]} \quad \text{(Equation 12)}
\end{align*}
\]

For a 1:1:1 molar mixture of L₁, sodium salt, and lithium salt, assuming no uncomplexed ligand is present, \([\text{LiL}^+] = [\text{Na}^+]\) and \([\text{Li}^+] = [\text{NaL}^+]\). The calculation of the relative equilibrium constant of Li⁺ relative to Na⁺ \( (K_{\text{rel}}) \) is shown in equation 13.
Since $[\text{Na}^+] = [\text{LiL}^+]$ and $[\text{NaL}^+] = [\text{Li}^+]$ then,

$$K_{\text{rel}} = \frac{[\text{LiL}^+]^2}{[\text{NaL}^+]^2} \quad (\text{Equation 13})$$

2. Determination of $pK_a$ Relative to that of DBU.

The $pK_a$ values of tetraamines 2 4, 5 8, and 2 5 relative to that of diazabicyclo[5.4.0]undec-7-ene (8 8, DBU) in CD$_3$CN were determined by monitoring $^{13}$C NMR chemical shift changes associated with DBU.

![Diagram of DBU](image)

Several research groups have studied the acidity/basicity of various compounds in acetonitrile. Anslyn and coworkers have recently studied the $pK_a$ of several ketones in acetonitrile. The $pK_a$ of the conjugate acid of 8 8 was determined in CD$_3$CN by Leffek (23.9) and more recently by Schwesinger (24.3).

In CD$_3$CN, the protonated and unprotonated forms of 8 are in fast dynamic exchange with respect to the $^{13}$C NMR timescale (90.56...
MHz), resulting in one set of $^{13}$C NMR resonances. Thus, in the presence of 1/2 equivalent of trifluoroacetic acid (TFA) the chemical shift values observed for 8 8 actually represent the concentration weighted average chemical shift values for protonated and unprotonated 8 8. Variation of $^{13}$C chemical shifts can therefore be used to measure the degree of protonation of 8 8. The pK$_a$ (of 2 4, 5 8, or 2 5) can be determined by mixing a 1:1:1 molar mixture of 8 8:TFA:tetraamine 2 4, 5 8, or 2 5 and measuring the $^{13}$C NMR chemical shift changes associated with 8 8. In our example, a tetraamine (2 4, 5 8, or 2 5) and 8 8 compete for the proton of trifluoroacetic acid (TFA). Equation 14-16 shows how the pK$_a$ of the tetraamine was calculated.
\[ \delta_A = \text{Chemical shift of unprotonated DBU} \]
\[ \delta_B = \text{Chemical shift of protonated DBU} \]
\[ \delta_C = \text{Chemical shift of DBU in competition} \]

\[ \frac{|\delta_C - \delta_A|}{|\delta_B - \delta_A|} \times 100 = \% \text{ Protonated DBU} \quad \text{(Equation 14)} \]

\[ -\log \left( \frac{(\% \text{ Protonated DBU})^2}{(100-\% \text{ Protonated DBU})^2} \right) = pK_{eq} \quad \text{(Equation 15)} \]

\[ pK_a + pK_{eq} = pK_a' \quad \text{(Equation 16)} \]
\[ pK_a = pK_a \text{ of DBU} \]
\[ pK_a' = \text{Calculated } pK_a \text{ of ligand} \]

The chemical shift values for the fully protonated and unprotonated 88 were determined experimentally beforehand. The tetraamine was then added to a solution of monoprotonated 88 in CD$_3$CN and the $^{13}$C NMR shift values of 88 were measured. There are nine different $^{13}$C resonances of 88 from which seven were selected since they exhibited large $\Delta$\delta's. Equation 16 was used to calculate the $pK_a$ of the tetraamine relative to that of 88.

3. Results of Ligand/Ligand Competitions with 2 4, 5 8, and 2 5.

a. 5 8 vs. 2 5 Competition for LiClO$_4$ in CD$_3$CN.
A competition between ligands 5 8 and 2 5 for Li+ (LiClO₄) was performed and quantified by ¹H NMR. For this competition ¹H NMR resonances corresponding to the free ligands and their complexes were clearly resolved. This allowed the integration of representative resonances from which the relative equilibrium constant could be determined. A ¹H NMR spectrum of a 1:1 molar mixture of free 5 8 and 2 5 in which the peaks corresponding to the ligands are labelled is shown in Figure 2.15A. The peak at 3.37 ppm (ddd) corresponds to ligand 5 8 and integrates to one proton. The peak at 3.73 ppm (td) corresponds to ligand 2 5 and integrates to two protons. The ¹H NMR spectrum of the 1:1 mixture of 5 8 and 2 5 after the addition of one equivalent of LiClO₄ is shown in Figure 2.15B. The peak at 1.83 ppm (dd) corresponds to one proton of the lithium complex of ligand 5 8. The peak at 1.73 ppm (dm) corresponds to two protons of the lithium complex of ligand 2 5. These clearly resolved
25 vs. 58 Competition for LiClO₄ in CD₃CN

Figure 2.15A
1:1 2 5:5 8
(No LiClO₄)

Figure 2.15B
1:1:1 2 5:5 8:LiClO₄

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\( ^1H \) NMR resonances corresponding to all four species were integrated. The total concentration of a single amine component (either 5 8 or 2 5) was 0.09 M. The signal to noise ratio for this example was 80:1. Multiple integrations of these resonances were performed and the results are shown in Table 2.2. The competition equilibrium constant (2 5 relative to 5 8) derived from this data is 

\[ K_{rel} = 1.22 \pm 0.04 \]

The free energy of competition (2 5 relative to 5 8) was calculated \( \Delta G^\circ = -0.12 \pm 0.004 \) kcal/mol at 25\(^\circ\) C. It is clear that 2 5 is a slightly better complexer of Li\(^+\) than 5 8.

b) 2 4 vs. 5 8 Competition for LiClO\(_4\) in CD\(_3\)CN.

Method of detection: \(^{13}\)C \{\(^1\)H\} NMR

Signal:Noise = 50:1

Concentration of a single amine component: 0.27 M

Peak Integrated (uncomplexed 5 8): 27.92 ppm (C\(_6\))
Peak Integrated (complexed 5 8): 24.80 ppm (C₆)

Multiple integrations of these resonances were performed and the results are shown in Table 2.3. The competition equilibrium constant (5 8 relative to 2 4) is $K_{r e l} = 138 \pm 11$. The free energy of competition was calculated (5 8 relative to 2 4) $\Delta G^\circ = -2.92 \pm 0.23$ kcal/mol at 25° C. It is clear that 5 8 is a better complexer of Li+ than 2 4.

c) 2 5 vs. 2 4 Competition for LiClO₄ in CD₃CN.

Method of detection: ¹H NMR

Signal:Noise = 60:1

Total concentration of a single amine component: 0.21 M in both experiments.

Peak Integrated (uncomplexed 2 5): 3.73 ppm (td, 2H)

Peak Integrated (complexed 2 5): 1.73 ppm (dm, 2H)
For this competition two separate NMR experiments were run with the same sample. Each result was analyzed separately to determine the reproducibility of the technique. The results of these competitions are shown in Tables 2.4 and 2.5. The calculated competitive equilibrium constant (2 5 relative to 2 4) in each case was determined: experiment #1 $K_{rel} = 168 \pm 8$, experiment #2 $K_{rel} = 186 \pm 18$. The free energy of competition was calculated in each case: experiment #1 $\Delta G^{\circ}_{298 K} = -3.03 \pm 0.14$ kcal/mol, experiment #2 $\Delta G^{\circ}_{298 K} = -3.09 \pm 0.27$ kcal/mol. These results are within experimental error, demonstrating that this technique (integration of corresponding $^1$H NMR peaks) gives reproducible results. It is clear that 2 5 is a better complexer of Li$^+$ than 2 4.
Table 2.2  

<table>
<thead>
<tr>
<th>Integration #</th>
<th>5 8</th>
<th>2 5*</th>
<th>5 8* LiClO₄</th>
<th>2 5* LiClO₄*</th>
<th>( K_{\text{rel}} ) (25 relative to 58)</th>
<th>( \frac{1}{N} \sum (X_i - \bar{X})^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.3420</td>
<td>2.5235</td>
<td>3.4761</td>
<td>4.3739</td>
<td>1.1678</td>
<td>3.091e-3</td>
</tr>
<tr>
<td>2</td>
<td>2.2660</td>
<td>2.3921</td>
<td>3.4519</td>
<td>4.4251</td>
<td>1.2144</td>
<td>8.10e-5</td>
</tr>
<tr>
<td>3</td>
<td>2.2788</td>
<td>2.3987</td>
<td>3.3626</td>
<td>4.3921</td>
<td>1.2409</td>
<td>3.052e-4</td>
</tr>
<tr>
<td>4</td>
<td>2.2788</td>
<td>2.3578</td>
<td>3.3274</td>
<td>4.3946</td>
<td>1.2765</td>
<td>2.817e-3</td>
</tr>
<tr>
<td>5</td>
<td>2.3170</td>
<td>2.3705</td>
<td>3.4640</td>
<td>4.3899</td>
<td>1.2387</td>
<td>2.338e-4</td>
</tr>
<tr>
<td>6</td>
<td>2.2431</td>
<td>2.3646</td>
<td>3.3626</td>
<td>4.3876</td>
<td>1.2378</td>
<td>2.067e-4</td>
</tr>
<tr>
<td>7</td>
<td>2.2894</td>
<td>2.3266</td>
<td>3.3553</td>
<td>4.3951</td>
<td>1.2890</td>
<td>4.297e-3</td>
</tr>
<tr>
<td>8</td>
<td>2.1694</td>
<td>2.3232</td>
<td>3.3680</td>
<td>4.3876</td>
<td>1.2165</td>
<td>4.778e-5</td>
</tr>
<tr>
<td>9</td>
<td>2.1098</td>
<td>2.4100</td>
<td>3.3587</td>
<td>4.3945</td>
<td>1.1454</td>
<td>6.082e-3</td>
</tr>
<tr>
<td>10</td>
<td>2.2963</td>
<td>2.3945</td>
<td>3.4908</td>
<td>4.4037</td>
<td>1.2098</td>
<td>1.855e-4</td>
</tr>
<tr>
<td>11</td>
<td>2.2660</td>
<td>2.3936</td>
<td>3.3842</td>
<td>4.3829</td>
<td>1.2261</td>
<td>7.108e-6</td>
</tr>
<tr>
<td>12</td>
<td>2.2850</td>
<td>2.4513</td>
<td>3.3626</td>
<td>4.3940</td>
<td>1.2181</td>
<td>2.834e-5</td>
</tr>
</tbody>
</table>

*Integral values shown have been divided by 2 for the determination of \( K_{\text{rel}} \).

\( S = \frac{\sqrt{\sum (X_i - \bar{X})^2}}{N-1} \)

\( \bar{X} = 1.22 \)
\( S = 0.04 \)

\( N = 12 \)

\( t = 1.80 \)

\( K_{\text{rel}} = 1.22 \pm 0.04 \)

(25 relative to 58)

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### Table 2.3

**b) 58 vs. 24 Competition for \( \text{LiClO}_4 \) in CD3CN.**

<table>
<thead>
<tr>
<th>Integration #</th>
<th>58</th>
<th>58•Li+</th>
<th>( K_{\text{rel}} ) (58 relative to 24)</th>
<th>( (X_i - \bar{X})^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2158</td>
<td>2.7637</td>
<td>164.01</td>
<td>650.25</td>
</tr>
<tr>
<td>2</td>
<td>0.2823</td>
<td>2.8079</td>
<td>98.93</td>
<td>1566.58</td>
</tr>
<tr>
<td>3</td>
<td>0.2119</td>
<td>2.6709</td>
<td>158.87</td>
<td>414.53</td>
</tr>
<tr>
<td>4</td>
<td>0.2317</td>
<td>2.7925</td>
<td>145.26</td>
<td>45.56</td>
</tr>
<tr>
<td>5</td>
<td>0.2236</td>
<td>2.6850</td>
<td>144.19</td>
<td>32.26</td>
</tr>
<tr>
<td>6</td>
<td>0.2254</td>
<td>2.7638</td>
<td>150.35</td>
<td>140.19</td>
</tr>
<tr>
<td>7</td>
<td>0.2333</td>
<td>2.6729</td>
<td>131.26</td>
<td>52.56</td>
</tr>
<tr>
<td>8</td>
<td>0.2333</td>
<td>2.6510</td>
<td>129.12</td>
<td>88.17</td>
</tr>
<tr>
<td>9</td>
<td>0.2338</td>
<td>2.6462</td>
<td>128.65</td>
<td>97.22</td>
</tr>
<tr>
<td>10</td>
<td>0.2273</td>
<td>2.6355</td>
<td>134.44</td>
<td>16.56</td>
</tr>
</tbody>
</table>

---

\( S = \) Standard deviation

\( N = \) Number of replicate measurements

\( t = \) Student t values

\( \mu = \) Confidence interval (90%)

\( \bar{X} = \) Average (mean)

\[
S = \sqrt{\frac{\sum(X_i - \bar{X})^2}{N-1}}
\]

\[
\mu = \bar{X} \pm \frac{tS}{\sqrt{N}}
\]

\( \bar{X} = 138.51 \)

\( S = 18.57 \)

\( N = 10 \)

\( t = 1.83 \)

\( K_{\text{rel}} = 138 \pm 11 \)

(58 relative to 24)
Table 2.4  c) 25 vs. 24 Competition for LiClO₄ in CD₃CN.

<table>
<thead>
<tr>
<th>Integration #</th>
<th>25</th>
<th>25 Li⁺</th>
<th>K_{rel} (25 relative to 24)</th>
<th>(X_i - X)^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.7435</td>
<td>9.7896</td>
<td>173.37</td>
<td>32.72</td>
</tr>
<tr>
<td>2</td>
<td>0.7932</td>
<td>9.8838</td>
<td>155.271</td>
<td>153.20</td>
</tr>
<tr>
<td>3</td>
<td>0.8055</td>
<td>9.9071</td>
<td>151.27</td>
<td>268.15</td>
</tr>
<tr>
<td>4</td>
<td>0.7767</td>
<td>9.9330</td>
<td>163.55</td>
<td>16.79</td>
</tr>
<tr>
<td>5</td>
<td>0.7727</td>
<td>9.9227</td>
<td>164.907</td>
<td>7.51</td>
</tr>
<tr>
<td>6</td>
<td>0.7560</td>
<td>9.8731</td>
<td>170.555</td>
<td>8.45</td>
</tr>
<tr>
<td>7</td>
<td>0.7757</td>
<td>9.8982</td>
<td>162.826</td>
<td>23.25</td>
</tr>
<tr>
<td>8</td>
<td>0.7690</td>
<td>9.9751</td>
<td>168.260</td>
<td>0.37</td>
</tr>
<tr>
<td>9</td>
<td>0.7386</td>
<td>9.8963</td>
<td>179.526</td>
<td>141.08</td>
</tr>
<tr>
<td>10</td>
<td>0.7534</td>
<td>9.8263</td>
<td>170.11</td>
<td>6.06</td>
</tr>
<tr>
<td>11</td>
<td>0.6744</td>
<td>9.9227</td>
<td>216.483</td>
<td>2384.84</td>
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<tr>
<td>12</td>
<td>0.8053</td>
<td>9.8388</td>
<td>149.269</td>
<td>337.80</td>
</tr>
<tr>
<td>13</td>
<td>0.8023</td>
<td>9.9572</td>
<td>154.028</td>
<td>185.51</td>
</tr>
</tbody>
</table>

S = Standard deviation
N = Number of replicate measurements
\( t \) = Student t values
\( \mu \) = Confidence interval (90%)

\[ \bar{X} = \text{Average (mean)} \]

\[ S = \sqrt{\frac{\sum(X_i - \bar{X})^2}{N-1}} \]

\[ \mu = \bar{X} \pm tS/\sqrt{N} \]

\( \bar{X} = 167.6 \)
\( S = 17.24 \)
\( N = 13 \)
\( t = 1.78 \)

\( K_{rel} = 168 \pm 8 \) (25 relative to 24)
Table 2.5  

<table>
<thead>
<tr>
<th>Integration #</th>
<th>25</th>
<th>25+Li⁺</th>
<th>K\text{rel} (25 relative to 24)</th>
<th>(Xᵢ - \bar{X})²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.7845</td>
<td>9.8352</td>
<td>157.17</td>
<td>846.34</td>
</tr>
<tr>
<td>2</td>
<td>0.6085</td>
<td>9.8868</td>
<td>263.99</td>
<td>6041.33</td>
</tr>
<tr>
<td>3</td>
<td>0.7064</td>
<td>9.8781</td>
<td>195.54</td>
<td>86.08</td>
</tr>
<tr>
<td>4</td>
<td>0.7400</td>
<td>9.7747</td>
<td>174.48</td>
<td>138.93</td>
</tr>
<tr>
<td>5</td>
<td>0.7400</td>
<td>9.9054</td>
<td>179.18</td>
<td>50.27</td>
</tr>
<tr>
<td>6</td>
<td>0.7323</td>
<td>9.7971</td>
<td>178.99</td>
<td>53.01</td>
</tr>
<tr>
<td>7</td>
<td>0.6875</td>
<td>9.9012</td>
<td>207.41</td>
<td>447.07</td>
</tr>
<tr>
<td>8</td>
<td>0.7687</td>
<td>9.8386</td>
<td>163.82</td>
<td>504.05</td>
</tr>
<tr>
<td>9</td>
<td>0.7323</td>
<td>9.9250</td>
<td>183.69</td>
<td>6.64</td>
</tr>
<tr>
<td>10</td>
<td>0.7235</td>
<td>9.7963</td>
<td>183.34</td>
<td>8.59</td>
</tr>
<tr>
<td>11</td>
<td>0.7780</td>
<td>9.8816</td>
<td>161.32</td>
<td>622.15</td>
</tr>
</tbody>
</table>

S = Standard deviation  
N = Number of replicate measurements  
t = Student t values  
μ = Confidence interval (90%)  

\( \bar{X} = \text{Average (mean)} \)  
\( \bar{X} = 186.3 \)  
S = 29.67  
N = 11  
t = 1.81  

\( K\text{rel} = 186 \pm 16 \)  
(25 relative to 24)
d) 5 8 vs. 2 5 Competition for Sodium-(NaBPh₄)

Method of detection: $^{13}$C {$^1$H} NMR

Signal:Noise = 42:1

Concentration of a single amine component: 0.16 M

<table>
<thead>
<tr>
<th>Compound</th>
<th>Peak Integrated (uncomplexed): ppm</th>
<th>Peak Integrated (sodium complex): ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 8</td>
<td>43.63</td>
<td>45.53</td>
</tr>
<tr>
<td>2 5</td>
<td>42.96</td>
<td>50.68</td>
</tr>
</tbody>
</table>

In this case, multiple integrations were not performed in the determination of $K_{rel}$. The carbons chosen were not necessarily corresponding, but were the only few which were clearly resolved and were not obscured due to overlap. The results of this competition are shown below. In this competition the equilibrium constant of 2 5 relative to 5 8 was calculated to be $K_{rel} = 12 \pm 3$. The calculated free energy of competition is $\Delta G^° = -1.47 \pm 0.37$.
Kcal/mol. The error analysis was performed by measuring the intensities of the $^{13}\text{C}$ NMR signals and reporting the result ± the intensity of the noise.

\[
K_{\text{rel}} = \frac{[\text{Na} \cdot 25^+] [58]}{[\text{Na} \cdot 58^+] [25]} = \frac{[2.70] [1.00]}{[0.71] [0.33]} = 11.5
\]

\[
K_{\text{rel}} = 12 \pm 3
\]

e) 58 vs. 25 Competition for Protonation by TFA

A competition between ligands 58 and 25 for a proton provided by CF$_3$CO$_2$H (TFA) was performed and quantified by $^{13}\text{C}$ {1H}NMR. For this competition carbon resonances corresponding to the free ligands and their complexes were clearly resolved. This allowed the integration of representative resonances from which the competition equilibrium constant could be determined. A $^{13}\text{C}$NMR
spectrum of the 1:1 molar mixture of free 5\(\text{8}\) and 2\(\text{5}\) is shown in Figure 2.16A. The \(^1\text{H}\) NMR spectrum of a 1:1 mixture of 5\(\text{8}\) and 2\(\text{5}\) after the addition of one equivalent of TFA is shown in Figure 2.16B. The peaks corresponding to the free ligands and their conjugate acids are labelled in an expansion of the upfield portion of the \(^{13}\text{C}\) \(^1\text{H}\)NMR spectrum (Figure 2.16C). The peaks chosen for the integration were the upfield methylene peaks (CH\(_2\)CH\(_2\)CH\(_2\)) which should have similar \(T_1\) and NOE values. The peak at 28.00 ppm arises from ligand 5\(\text{8}\) and corresponds to one carbon. The peak at 28.57 ppm arises from ligand 2\(\text{5}\) and corresponds to two carbons. The peak at 22.82 ppm arises from the conjugate acid of ligand 5\(\text{8}\) and corresponds to one carbon. The peak at 24.95 ppm arises from the conjugate acid of ligand 2\(\text{5}\) and corresponds to two carbons. These clearly resolved carbon resonances corresponding to all four species were integrated. The total concentration of a single amine component (either 5\(\text{8}\) or 2\(\text{5}\)) was 0.15 M. The signal to noise ratio in this example was 50:1. Multiple integrations of these resonances were performed and the results of this study are shown in Table 2.6. The calculated competition equilibrium constant (5\(\text{8}\) relative to 2\(\text{5}\)) was calculated to be \(K_{\text{rel}} = 21 \pm 1\) and the \(\Delta pK_a\) (5\(\text{8}\)-2\(\text{5}\)) = 1.33 \(\pm\)
58 vs. 25 Basicity
Competition in CD3CN

Figure 2.16A

1:1 2 5:5 8
(No CF3CO2H)

Figure 2.16B

1:1:1 2 5:5 8:CF3CO2H

Figure 2.16C

25·CF3CO2H

58·CF3CO2H

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From these results it is clear that 58 is a stronger base than 25. The free energy of competition is $\Delta G^\circ = -1.80 \pm 0.09$ Kcal/mol. A TFA protonation competition between 25 and 24 (performed by Rogers), showed that 25 was a stronger base than 24. Given this result, 58 is the strongest base encountered so far in this series (58, 25, and 24).

Table 2.6  e) 58 vs. 25 Proton Competition

<table>
<thead>
<tr>
<th>Integration #</th>
<th>25*</th>
<th>58</th>
<th>25-TFA*</th>
<th>58-TFA</th>
<th>$K_{rel}$ (58 relative to 25)</th>
<th>$(X_r - X)^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.0667</td>
<td>0.4227</td>
<td>0.4742</td>
<td>2.0889</td>
<td>21.54</td>
<td>0.3638</td>
</tr>
<tr>
<td>2</td>
<td>1.9963</td>
<td>0.4148</td>
<td>0.4931</td>
<td>2.1465</td>
<td>20.95</td>
<td>1.537e-4</td>
</tr>
<tr>
<td>3</td>
<td>1.9693</td>
<td>0.4343</td>
<td>0.4896</td>
<td>2.1524</td>
<td>19.94</td>
<td>1.002</td>
</tr>
<tr>
<td>4</td>
<td>1.9853</td>
<td>0.4249</td>
<td>0.4945</td>
<td>2.1244</td>
<td>20.07</td>
<td>0.7432</td>
</tr>
<tr>
<td>5</td>
<td>1.9763</td>
<td>0.4018</td>
<td>0.5085</td>
<td>2.1212</td>
<td>20.20</td>
<td>0.5421</td>
</tr>
<tr>
<td>6</td>
<td>1.9881</td>
<td>0.4223</td>
<td>0.4754</td>
<td>2.1082</td>
<td>20.88</td>
<td>3.329e-3</td>
</tr>
<tr>
<td>7</td>
<td>2.0919</td>
<td>0.4301</td>
<td>0.4803</td>
<td>2.2392</td>
<td>22.67</td>
<td>3.0213</td>
</tr>
<tr>
<td>8</td>
<td>2.0225</td>
<td>0.4209</td>
<td>0.4901</td>
<td>2.0859</td>
<td>20.45</td>
<td>0.2360</td>
</tr>
<tr>
<td>9</td>
<td>2.0003</td>
<td>0.4268</td>
<td>0.4896</td>
<td>2.0518</td>
<td>19.64</td>
<td>1.6744</td>
</tr>
<tr>
<td>10</td>
<td>2.0141</td>
<td>0.4343</td>
<td>0.4846</td>
<td>2.0899</td>
<td>20.00</td>
<td>0.8776</td>
</tr>
<tr>
<td>11</td>
<td>1.9712</td>
<td>0.4372</td>
<td>0.4798</td>
<td>2.0802</td>
<td>20.27</td>
<td>0.4476</td>
</tr>
<tr>
<td>12</td>
<td>2.0840</td>
<td>0.4092</td>
<td>0.4944</td>
<td>2.3069</td>
<td>23.76</td>
<td>7.9863</td>
</tr>
<tr>
<td>13</td>
<td>2.0100</td>
<td>0.4324</td>
<td>0.4967</td>
<td>2.2206</td>
<td>20.78</td>
<td>0.0234</td>
</tr>
<tr>
<td>14</td>
<td>1.9861</td>
<td>0.4163</td>
<td>0.4717</td>
<td>2.1705</td>
<td>21.95</td>
<td>1.0316</td>
</tr>
</tbody>
</table>
Integral values shown have been divided by 2 for the determination of $K_{rel}$.

\[ S = \text{Standard deviation} \]
\[ N = \text{Number of replicate measurements} \]
\[ t = \text{Student t values} \]
\[ \mu = \text{Confidence interval (90\%)} \]
\[ \bar{X} = \text{Average (mean)} \]
\[ S = \sqrt{\frac{\sum(X_i - \bar{X})^2}{N-1}} \]
\[ \mu = \bar{X} \pm t\frac{S}{\sqrt{N}} \]

$X = 20.94$

$S = 1.175$

$N = 14$

$t = 1.77$

$K_{rel} = 21 \pm 1$

(58 relative to 25)

$\Delta pK_a = 1.32 \pm 0.06$

$pK_a = 26.2 \text{ (calc)}$

4. Li$^+$/Na$^+$ Competition with 58.

![Diagram of 58](image)

The above competitions were cases where two ligands compete for a single metal cation. In this case, Na$^+$ (NaBPh$_4$) and Li$^+$ (LiClO$_4$) ions competed for a single ligand (58). This experiment determined the selectivity of the ligand for Li$^+$ over Na$^+$. The method of data analysis was much simpler in this case and involved measuring the
line intensities of the observed complexes. The chemical shifts of the upfield methylene carbon (C₆) in the Na⁺ (25.49 ppm) and Li⁺ (22.82 ppm) complex differ by 2.67 ppm and are easily resolved in the ¹³C NMR spectrum. The sodium complex in this case was not observed experimentally. An estimation of the relative equilibrium constant was obtained by assuming that the height of the peak corresponding to the sodium complex was less than or equal to the height of the noise. The signal to noise ratio for this example was 270:1. The total concentration of tetraamine 5 8 was 0.21 M. The calculation of the lower limit $K_{rel}$ is shown below.

$$K_{rel} \geq \frac{[Li^+ \cdot 58]^2}{[Na^+ \cdot 58]^2} = \frac{[130]^2}{[0.48]^2} = 3.5 \times 10^4$$

$$K_{rel} \geq 4 \times 10^4$$

Other ion selectivity studies performed by Rogers⁵⁰c,d are presented alongside this result in Table 2.7.

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Table 2.7

Li⁺ Selectivity: LiClO₄/NaBPh₄ Competitions

\[ \text{LNa}^+ + \text{Li}^+ \rightleftharpoons \text{LLi}^+ + \text{Na}^+ \]

\(^{13}\text{C} \) NMR-slow exchange; CD₃CN

<table>
<thead>
<tr>
<th>Ligand</th>
<th>( K_{\text{Li}^+} / K_{\text{Na}^+} )</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Ligand 1" /></td>
<td>(1.2-5.0) \times 10^2</td>
</tr>
<tr>
<td><img src="image2" alt="Ligand 2" /></td>
<td>&gt; 4 \times 10^4</td>
</tr>
<tr>
<td><img src="image3" alt="Ligand 3" /></td>
<td>&gt; 10^4</td>
</tr>
</tbody>
</table>

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5. Attempted Determination of the Relative pKₐ values of 5 8 and 8 8.

Method of detection: $^{13}$C $\{^1$H$\}$NMR

Signal:Noise = 130:1

Total concentration of a single amine component: 0.21 M

In the competition between 5 8 and 8 8 for the proton of TFA only a trace of the free ligand 5 8 was detectable by $^{13}$C NMR. When an additional equivalent of 8 8 was added to the 1:1:1 mixture of 5 8:8 8:CF₃CO₂H no significant change in the amount of observable free 5 8 occurred. As a result, this experiment did not allow the determination of the $\Delta$pKₐ (5 8 relative to 8 8). The result only qualitatively shows that 5 8 is a much stronger base and cannot be accurately compared to 8 8. However, the pKₐ of 5 8 was calculated relative to 2 5 which was previously determined relative to 8 8 by Rogers.⁴⁹ The results of the competition between 5 8 and 2 5 for the
proton of TFA was presented earlier.

IV. Conclusions

A. N,N’-Dimethyl Cross-bridged Tetraamines

An efficient approach to the synthesis of N,N’-dimethyl cross-bridged cyclam (25) has been extended to the synthesis of two other N,N’-dimethyl cross-bridged tetraamines (24 and 58), which have been completely characterized.

All three ligands (24, 25, and 58) are strong Li$^+$ binders and fast equilibrating proton sponges. Their protonation properties and metal ion selectivities, have been compared.

B. Synthesis of Parent Cross-bridged Tetraamines.

The previously established Rogers$^{50a}$ procedure for the preparation of 29 was successfully scaled up to a multi-gram scale.
The synthesis of two new cross-bridged tetraamines 6 7 and 7 2 was achieved by utilizing this procedure. Functionalization of macrocycle 2 9 with ligating groups containing heteroatoms will be discussed in Chapter III.

\[
\begin{align*}
7 2 & \\
6 7 & \\
2 9 & 
\end{align*}
\]

In the future, macrocycles 6 7 and 7 2 should be functionalized in the same manner as 2 9.

C. Synthesis of N-Methyl Cross-bridged Tetraamines.

The attempts made at preparation of monofunctionalizable cross-bridged cyclam and cyclen has led to the full characterization of 8 1. The characterization of 8 7 was completed but the final debenzylazation step to 8 4 does not go cleanly.
Purification of tetraamine 84 was problematic and will require further work. The synthesis of monofunctionalized derivatives of 81 has yet to be attempted.

Monoalkylation of 56 will undoubtedly lead to two possible products 89 and 90 as shown in Scheme 2.29.

Scheme 2.29

If a single isomer does not predominate in this alkylation the two products may be separable by fractional recrystallization. If a single isomer can be isolated or prepared then the possibility of carrying it through the sequence described in this chapter may permit the synthesis of either 91 or 92 in the future.
The hydrolyses of 85 and 80 have yet to be attempted. This might provide a new method for the monoprotection of cyclam and cyclen as shown in Scheme 2.30.

Scheme 2.30

The successful preparation of 93 and 94 would permit functionalization of the remaining secondary amine groups to give selectively functionalized cyclams and cyclens.
D. Complexation Studies

Both ligands 5 8 and 2 5 are strong complexers of Li⁺ compared to 2 4 as shown in Table 2.8. Ligands 2 4 and 5 8 are very selective for Li⁺ over Na⁺ as shown in Table 2.7. Ligand 2 5 is selective for Li⁺ over Na⁺ but is at least a factor of 100 less selective than ligands 2 4 and 5 8. Ligand 5 8 is unique in that it is a strong complexer of Li⁺ like 2 5, but is very selective for Li⁺ over Na⁺. The comparison of the basicity of these three ligands revealed 5 8 as the strongest base, which is consistent with the pKₐ determinations of tetraamines 2 4 and 2 5 relative to DBU done previously by Rogers.⁵⁰a The results of pKₐ determinations and proton competitions performed by Rogers⁵⁰a are summarized in Tables 2.9 and 2.10.

More attempts should be made to prepare pure 6 5 or 6 6.

It would be useful to compare isomers 6 6 and 6 5 to ligands 2 4, 5 8,
Table 2.8

Ligand/Ligand' Competitions for Li⁺ (CD₃CN)

\[ L + ML⁺ \rightleftharpoons L' + ML⁺ \]

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Ligand'</th>
<th>( K/K' )</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Ligand 1" /></td>
<td><img src="image2" alt="Ligand' 1" /></td>
<td>1.22 ± 0.04</td>
<td>(^1\text{H} \text{ Integration} )</td>
</tr>
<tr>
<td><img src="image3" alt="Ligand 2" /></td>
<td><img src="image4" alt="Ligand' 2" /></td>
<td>168 ± 8</td>
<td>(^1\text{H} \text{ Integration} )</td>
</tr>
<tr>
<td></td>
<td></td>
<td>186 ± 16</td>
<td>(^1\text{H} \text{ Integration} )</td>
</tr>
<tr>
<td><img src="image5" alt="Ligand 3" /></td>
<td><img src="image6" alt="Ligand' 3" /></td>
<td>138 ± 11</td>
<td>(^{13}\text{C} \text{ Integration} )</td>
</tr>
</tbody>
</table>
Table 2.9
Protonation Competition Experiments
1:1:1 Base 1:Base 2:CF$_3$COOH

(Monitored by $^1$H and $^{13}$C NMR)

<table>
<thead>
<tr>
<th>Base 1</th>
<th>Base 2</th>
<th>Solvent</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Image 1]</td>
<td>![Image 2]</td>
<td>CD$_3$CN</td>
<td>65% protonated 2 5</td>
</tr>
<tr>
<td>pK$_a$ = 24.9*</td>
<td>pK$_a$ = 24.2 (calc)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Base 1</th>
<th>Base 2</th>
<th>Solvent</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Image 3]</td>
<td>![Image 4]</td>
<td>CD$_3$CN</td>
<td>19% protonated 2 5</td>
</tr>
<tr>
<td>pK$_a$ = 24.9*</td>
<td>pK$_a$ = 26.2 (calc)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* calculated from DBU competition
Table 2.10

Protonation Competition Experiments
1:1:1 Base 1:Base 2:CF₃COOH

(Monitored by $^1$H and $^{13}$C NMR)

<table>
<thead>
<tr>
<th>Base 1</th>
<th>Base 2</th>
<th>Solvent</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Base 1" /></td>
<td><img src="image" alt="Base 2" /></td>
<td>CD₃CN</td>
<td>34% protonated DBU</td>
</tr>
<tr>
<td><img src="image" alt="Base 1" /></td>
<td><img src="image" alt="Base 2" /></td>
<td>CD₃CN</td>
<td>trace free 5B</td>
</tr>
<tr>
<td><img src="image" alt="Base 1" /></td>
<td><img src="image" alt="Base 2" /></td>
<td>CDCl₃</td>
<td>no free 25</td>
</tr>
<tr>
<td><img src="image" alt="Base 1" /></td>
<td><img src="image" alt="Base 2" /></td>
<td>CDCl₃</td>
<td>no free 25</td>
</tr>
</tbody>
</table>

pKₐ = 24.32 (lit)
and 25 with respect to metal ion selectivity and proton affinity.

It would be interesting to compare the metal selectivities and protonation properties of the cross-bridged ligands synthesized in this laboratory to the bridged tetraamine macrocycles synthesized by Ciampolini47,67-72 and coworkers. The length of the chain used to bridge non-adjacent nitrogens should play a significant role in determining protonation characteristics and alkali metal selectivities.
Chapter III

Functionalized Tetraamines

I. Introduction

Over the past twenty years the area of research including the synthesis of N-functionalized tetraamine macrocycles has grown several fold. Several reviews have been written regarding this subject.\textsuperscript{83} The properties of functionalized macrocyclic polyamines are unique with respect to their ability to coordinate with metal ions. Unfunctionalized tetraamine macrocycles can coordinate with metal ions but cannot fill the coordination sphere of a five- or six-coordinate transition metal. N-functionalization of tetraamine macrocycles with one or two pendant arms containing heteroatoms leads to macrocycles capable of completing the coordination spheres of such metal cations. Furthermore, functionalization allows the lipophilicity to be adjusted by changing the identity of the pendant arms. The incorporation of lipophilic groups may also allow metal cations to be transported across lipid membranes, as was discussed in Chapter I.
Some functionalized macrocycles are useful for NMR imaging,\textsuperscript{84} radiopharmaceutical applications,\textsuperscript{85} removal of toxic metal ions (lead, cadmium, chromium) from the environment,\textsuperscript{86} and for the treatment of heavy metal poisoning.\textsuperscript{87}

II. Background

Before discussing the results obtained in this laboratory, the topic of functionalized tetraamine macrocycles will be briefly reviewed. Some representative tetra-N-functionalized cyclams are shown in Figure 3.1.
Figure 3.1

\[ R = \]

- \( \text{O} \)\( \text{Et} \)
- \( \text{O} \)\( \text{H} \)
- \( \text{O} \)\( \text{NH}_2 \)
- \( \text{SH} \)
- \( \text{CN} \)
- \( \text{OMe} \)
- \( \text{OH} \)
- \( \text{NH}_2 \)
- \( \text{OH} \)
- \( \text{S} \)
- \( \text{NH}_2 \)
- \( \text{OH} \)
- \( \text{NO} \)
- \( \text{P} \)\( \text{Me} \)
- \( \text{O} \)\( \text{Me} \)
- \( \text{H} \)
- \( \text{N} \)\( \text{Me} \)

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A. Functionalized Tetraamine Macrocycles

The synthesis of 95 by Stetter and Frank\textsuperscript{88} in 1976 was accomplished by the reaction of cyclam (8) with chloroacetic acid in the presence of aq NaOH. The inorganic salts were separated from the ligand using ion exchange chromatography to give tetafunctionalized macrocycle 95 (Scheme 3.1).

Scheme 3.1

In a similar fashion Stetter and Frank tetrafunctionalized cyclen (11) to give macrocycle 96 (DOTA).\textsuperscript{88}

The Cu(II) complex of macrocycle 96 was synthesized by Kaden\textsuperscript{89} and
an X-ray crystal structure indicated that two non-adjacent pendant acetato groups coordinate through axial positions with the metal ion lying within the macrocyclic cavity. The macrocyclic complex exhibited octahedral geometry. Much work has been done on the coordination chemistry of cyclam and cyclen tetraacetic acids (95 and 96). For example, the protonation constants (pKₐ's) have been determined by Ascenso⁹⁰a and Clarke⁹⁰b. Several other workers have studied other transition metal complexes,⁹¹a-f heavy metal complexes,⁹²a-f lanthanide complexes,⁹³a-f,⁹⁴ and alkaline earth metal complexes,⁹⁵ of 95 and 96. The terbium(III) complex of 95 was studied by Spirlet.⁹⁶ The thorium complex of 96 was recently synthesized by Jaques.⁹⁷

A high yield synthesis of tetraester 97 was accomplished by Weiss.⁹⁷ Ligand 97 was prepared by stirring a suspension of cyclam (8) and methyl acrylate for two days at room temperature (Scheme 3.2).
The α,β-unsaturated esters in this example functioned as the solvent; excess was removed by reduced pressure distillation to give pure 97. Tetraester 97 was hydrolyzed to the tetraacid 98 (as the tetrahydrochloride) in 20% aqueous HCl at room temperature (Scheme 3.3).

Tetranitrile derivative 99 was first reported by Wainwright, who prepared 99 by the conjugate addition of 8 in neat acrylonitrile (Scheme 3.4).
Macrocycle 99 coordinated with Ni(II) in a tetradeutate fashion binding through the tertiary amine nitrogens alone. Reduction of 99 with sodium and ethanol in toluene gave octaamine 100 (Scheme 3.5).

Attempts to make the mononuclear Ni(II) complex of 100 resulted in a complex where the four tertiary amine nitrogens and a single pendant amino group coordinated to the metal. The coordination chemistry of ligand 100 was reported by several different
Murase and coworkers\textsuperscript{101} reported the synthesis of tetrakis(2-aminoethyl) cyclam \textit{101}. The reaction of cyclam with tosylaziridine gave \textit{102}. The deprotection of \textit{102} in 48\% HBr/HOAc (1:1) gave pure octaamine \textit{101} as the octahydrobromide salt (Scheme 3.6).

Scheme 3.6

\[ \begin{array}{c}
\text{8} \\
\text{Ts} \\
\text{MeCN} \quad 85\% \\
\rightarrow \\
\text{102}
\end{array} \]

Kida\textsuperscript{101} expanded this methodology to prepare macrocycles \textit{103-105} (Figure 3.2).
Kida discovered by X-ray crystallography that octaamine ligands 103-105 formed binuclear complexes with Ni(II). When ligand 101 was protonated\textsuperscript{103a} or complexed with Cu(II)\textsuperscript{103b} it formed strong association complexes with anions. The Ni(II)\textsuperscript{103c} and Cr(II)\textsuperscript{103d} complexes of 101 have also been studied.

The synthesis of 106 was reported by Moore\textsuperscript{104a} and coworkers. The reaction of cyclam (8) and 2-(chloromethyl)pyridine proceeded in a mixture of dichloromethane and aq NaOH (Scheme 3.7).

Scheme 3.7
Attempts to make a mononuclear Cu(II) complex were unsuccessful. A dinuclear Cu(II) complex was obtained according to the X-ray crystal structure. Each Cu(II) center was five-coordinate involving two adjacent pyridyl nitrogens, two tertiary amine nitrogens from one of the NCH$_2$CH$_2$N bridges, and one bromide ion. Magnetic susceptibility measurements showed that there is no interaction between the two copper centers. The coordination chemistry of 106 with Ni(II) has also been studied by other research groups.$^{104b,c}$

Hancock and coworkers$^{105}$ reported the tetra(2-hydroxyethyl) cyclam derivative (107), which was synthesized by the addition of excess ethylene oxide to a stirred solution of 8 in ethanol (Scheme 3.8).

Scheme 3.8

![Scheme 3.8](image)

The ligand 107 more rapidly complexes Co(II), and Ni(II) as compared to tetramethyl cyclam (41).
It was postulated that the hydroxyethyl groups of macrocycle 107 increase the rate of incorporation of metal ions by providing initial points of attachment for the incoming metal. Other groups have studied metal complexation of ligand 107 with Hg(II) and Pb(II). The Eu(III) and La(III) complexes of tetra(2-hydroxyethyl)cyclen (108) have been studied by Morrow. Lincoln and coworkers have studied the Li+, Na+, K+, and Cs+ complexes of ligand 108.

Several derivatives of 8 and other tetraamines were reported by Tsukube. The ligands were prepared directly from the tetraamines and N,N'-diethylchloroacetamide, ethyl chloroacetate, chloroacetonitrile, phenacyl bromide, or benzoyl chloride to give
tetrafunctionalized ligands 109, 110, and 111 as shown in Figure 3.3.

Figure 3.3

Pyrazole groups were attached to 8 by a method developed by Malachowki. Treatment of a solution of 8 in acetonitrile with 1-(hydroxymethyl)-3,5-dimethylpyrazole gave crude 112, which was purified by conversion to the copper (II) complex, followed by treatment with EDTA to yield free ligand 112. Ligand 112 was prepared as shown in Scheme 3.9.

Scheme 3.9

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This same functional group was attached to 11 by DiVarin\textsuperscript{110,111} and coworkers.

The homologous ligand 113 was synthesized by a different route by Malachowki\textsuperscript{109} as shown in Scheme 3.10.

Scheme 3.10

Compounds 112 and 113 formed stable dinuclear complexes with Cu(II), both of which catalyzed the oxidation of catechol to quinone.

The initial rate of oxidation was much faster with the Cu(II) complex of 112, demonstrating the influence of arm length on catalytic activity.

The synthesis of two cyclam-based chelators containing hydroxamate and acetylacetone pendant arms have recently been reported by Koshti and coworkers.\textsuperscript{112} The ligands 114 and 115 were designed for the potential binding of plutonium (IV) through the pendant arms.
Lehn and Ziesel\textsuperscript{113} reported the synthesis of a variety of 2,2'-bipyridyl functionalized ligands derived from acyclic and macrocyclic polyamines. For example, cyclam (8) was tetrafunctionalized by reaction with 6-(bromomethyl)-2,2'-bipyridine in a mixture of MeOH and aqueous NaOH to give 116 (Scheme 3.11).
Tsukube\textsuperscript{114} and coworkers reported the synthesis of a tetrafunctionalized cyclam bearing furan moieties (1\textsuperscript{17}). The ligand was prepared by the addition of furoyl chloride to a solution of cyclam (8) and pyridine in THF (Scheme 3.12).

The resulting amide was reduced with borane in THF, affording 1\textsuperscript{17} following the standard workup. As demonstrated by salt extraction
experiments, 117 discriminated between NH$_4^+$ ion and K$^+$ effectively.

The functionalization of cyclen with alkylphosphinic and arylphosphinic substituents was reported by Parker.\textsuperscript{115} The ligands were synthesized by condensation of cyclen (11) with paraformaldehyde in THF to yield an intermediate hydroxymethyl species 118 (not isolated) which was reacted with various dialkoxy phosphines (119) to give polymethylene phosphinate esters (120) (Scheme 3.13).

**Scheme 3.13**

\[
\begin{align*}
&\text{11} \quad \text{118} \\
&\quad \text{120}
\end{align*}
\]

The ligands were designed to complex \textsuperscript{90}Y$^+$ and Gd$^{3+}$ which are metal ions commonly used for MRI (NMR) contrast agents. Some of...
the ligands described in this paper are bifunctional and can conceivably be attached to antibodies through a remote protected amine moiety. The functionalization of cyclam\textsuperscript{116} and cyclen\textsuperscript{117} with coordinating arms containing phosphorus has been examined by several research groups.

Parker\textsuperscript{118} and coworkers studied the complexation of 121 with Li\textsuperscript{+}, Na\textsuperscript{+}, and Ca\textsuperscript{2+} by spectroscopic methods (\textsuperscript{13}C NMR, IR, fast atom bombardment mass spectrometry (FABMS). The compound was synthesized by the alkylation of cyclen (11) with N,N'-diethyl bromoacetamide in ethanol using Cs\textsubscript{2}CO\textsubscript{3} as the base (Scheme 3.14).

Scheme 3.14

Ligand 121 forms a very strong complex with Ca\textsuperscript{2+} in water.

Tsukube et al\textsuperscript{118b,c} have synthesized other macrocycles containing amide functionalities.
B. Functionalization of Aza-lariat Ether 122.

Diazacrown ether 122 is a related macrocycle that can be functionalized at nitrogen with pendant arms in much the same way as cyclam. Functionalization enables further coordination by pendant-arm donor groups when a metal ion is complexed in the macrocyclic cavity as discussed in Chapter I. Tsukube\textsuperscript{119} has synthesized macrocycle 123 by reaction of 122 with 2-thiophenecarbonyl chloride in THF (Scheme 3.15).

Scheme 3.15

\begin{center}
\includegraphics[width=0.5\textwidth]{image}
\end{center}

The resulting amide was reduced with borane in THF and following the standard workup gave 123.

De-Fen\textsuperscript{120} and coworkers synthesized lariat ethers 124 and 125 by treating an acetonitrile solution of 122 with 2-chloroethyl alkyl sulfides or 2-chloroethyl allyl sulfide (Scheme 3.16).
Anhydrous sodium carbonate was used to scavenge the HCl formed in the reaction. Monofunctionalized lariat ethers 1 2 6 and 1 2 7 were also prepared by this method.

The high pressure addition of 2-vinylpyridine to 1 2 2 was accomplished by Nolte121, who prepared macrocycle 1 2 8 (Scheme 3.17).

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Compound 128 forms a 1:2 coordination complex with Cu(II) in which both Cu(II) ions are five-coordinate. Each Cu(II) ion is coordinated by two nitrogen atoms (one pyridyl and one macrocyclic nitrogen) and two oxygen atoms from the macrocycle. The fifth coordination site is a hydroxo group which bridges the two Cu(II) centers. The redox behavior of the Cu(II) complex of 128 was studied by cyclic voltametry in acetonitrile. The reduction of this complex was irreversible due to possible ligand oxidation or decomposition.

The preceding overview of the different types of functional groups attached to the tetraamines cyclam (8), cyclen (1 1), and diaza crown ether 122 is not comprehensive. Some of the examples presented are representative of the successful attempts made at functionalization of ethylene cross-bridged cyclam (29). The
overview also points out some examples of functionalization which may hold future potential.

The procedure used to synthesize cross-bridged cyclam 29 was successfully scaled up as discussed in Chapter II to give multigram quantities of product. This enabled several attempts at functionalization since the secondary amine portion of 29 is well suited for many of the arming reactions mentioned in the background section of this Chapter. Functionalization resulted in tetraamines containing ligating arms useful for coordination. Some of these functionalized polyamines have ionizable groups in which metal ion coordination might be controlled by pH adjustment.

III. Results and Discussion

A. Synthesis of Diester 129 and Related Derivatives.

Cross-bridged cyclam 29 can be alkylated effectively with ethylbromoacetate in acetonitrile using a procedure similar to that of Parker122 and coworkers (Scheme 3.18).
Scheme 3.18

1H NMR evidence strongly suggested that the crude product 129 (after step 1) was monoprotonated and as a result was not amenable to chromatography. The yellow oil that resulted from evaporation of acetonitrile was dissolved in aq KOH and extracted with CHCl₃. All of the reagents used for this extraction were cooled to 0°C immediately prior to use and all manipulations were performed quickly so as to prevent possible hydrolysis of the ester groups. The product was >99% pure (Yield 85-100%) and was characterized by IR, high resolution mass spectrometry (HRMS), ¹H, and ¹³C NMR.

Cross-bridged cyclen (72) was functionalized using this procedure to give 130 in 69% yield. Compound 130 was not fully characterized, but the ¹H and ¹³C NMR indicate it was >98% pure. The IR and high resolution mass spectra (HRMS) still need to be obtained to fully characterize 130.
1. Diester Hydrolysis Leading to Diacid 131.

Diester 129 was hydrolyzed in 6N HCl to give the acid derivative 131 (Scheme 3.19).

Scheme 3.19

\[
\begin{align*}
\text{EtO} & \quad \text{OEt} \\
& \quad \text{O} \\
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{H} \\
\text{H} & \quad \text{H} \\
\end{align*}
\]

\[129 \xrightarrow{6 \text{ N HCl}} 100^\circ \text{C} 48 \text{ h (95\%)} \]

\[131 \cdot 4\text{HCl}\cdot 1.5\text{H}_2\text{O}

Compound 129 was very slow to hydrolyze and required heating at 100°C for 48 hours. The 6N HCl was removed under reduced pressure to give a solid. Diacid 131 was characterized by IR, \(^1\)H NMR, \(^{13}\)C NMR, and elemental analysis as the hygroscopic tetrahydrochloride hydrate (1.5 H\text{2O}). \(^1\)H NMR evidence indicated the hydrolysis was incomplete after 24 hours at 100°C.
2. Attempted Aminolysis of Diester 129.

Several attempts to prepare the diamide 132 from diester 129 failed. The procedure for aminolysis is quite general and required refluxing the ester in conc. aqueous NH₃.

The first attempt at the preparation of diamide 132 involved heating 129 in conc. aq NH₃ for 3 hours at 80°C. The isolated product was actually 131, which was obtained in 51% yield. When the reaction was run in a pressure tube (3 hours, 100°C) no change in the outcome of the reaction was observed and 131 was isolated in 62% yield. ¹H and ¹³C NMR indicated the isolated product was very pure, however it was not 132. The low and high resolution mass spectra (HRMS) indicated a molecular ion at 343 (M+1, Cl, NH₃). This molecular ion is consistent with 4,11-bis-(carboxymethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (131) as shown in 133.
3. Reduction of Diester 129 with NaBH₄.

Diester 129 was reduced with excess NaBH₄ in 95% EtOH (4 hours at 100°C) as shown in Scheme 3.21.

Decomposition of excess NaBH₄ by slow addition of 3M HCl followed by workup with aq KOH and extraction with toluene gave pure product. The modest yield of 133 (22%) might be improved by employing a stronger reducing agent such as LiAlH₄. Compound 133
was characterized by IR, high resolution mass spectrometry (HRMS), $^1$H, and $^{13}$C NMR. The 360 MHz $^1$H NMR spectrum of 1 3 3 in CDCl$_3$ is shown in Figure 3.4A. A D$_2$O exchange experiment identified the hydroxyl protons at 2.85-3.00 ppm, which were overlapped with a portion of the AA'XX' of the NCH$_2$CH$_2$N bridge (Figure 3.4B). The multiplet at 3.5 ppm changes drastically upon addition of D$_2$O. This change in CH$_2$OH fine structure is due to the loss of coupling after D$_2$O exchange.

B. Conjugate Additions with 2 9.

Conjugate additions of cross-bridged cyclam to acrylonitrile and methyl acrylate have resulted in diester 1 3 4 (Scheme 3.22) and dinitrile 1 3 5 (Scheme 3.24) respectively.

1. Conjugate Addition using Methyl Acrylate.

A general procedure for the addition of 8 to methyl acrylate was demonstrated previously by Weiss.$^{98}$ In our example, 2 9 was stirred for 24 hours with excess methyl acrylate (Scheme 3.22).
Figure 3.4A

CH$_2$OH

Figure 3.4B

CH$_2$OD
Excess methyl acrylate was removed under reduced pressure to give 134 as an oil. The crude product was >96% pure and used in the subsequent hydrolysis without purification. Compound 134 was characterized by IR, high resolution mass spectrometry (HRMS), $^1$H, and $^{13}$C NMR. In one attempt to distill the product (80°C, 0.04 mmHg) elimination of the pendant arms was observed. A $^1$H NMR of the distillate indicated 29, 134 and a one-armed version of 134 were present.

The hydrolysis of 134 was first attempted by heating in 3M aq HCl for 24 hours at 100°C. The 3M HCl was removed under reduced pressure to give solid product (136) which was approximately 80% pure as indicated by $^{13}$C NMR. However, lowering the temperature (4 days, room temperature) increased the purity of 136 to >90% (Scheme 3.23).
It is very possible that the loss of one arm occurs, which is consistent with the impurities observed in the $^{13}$C NMR spectrum. It may be that hydrolysis was complete after only a few hours at room temperature. The characterization of compound 1 3 6 is incomplete at this point ($^1$H and $^{13}$C NMR only).

2. Conjugate Addition using Acrylonitrile.

A procedure for the Conjugate addition of acrylonitrile to amines was reported by Mülhaupt and Wörler, who utilized an addition/reduction sequence for dendrimer synthesis. Compound 2 9 was stirred for 24 hours with a large excess of acrylonitrile (Scheme 3.24).
After the excess acrylonitrile was removed under reduced pressure, the residue was dissolved in toluene, dried over Na$_2$SO$_4$, and filtered. The solvent was removed to give an oil which crystallized when stored at 10°C for 14 hours. The solidified product (1,3,5) was >98% pure (79-91% yield) and characterized by IR, elemental analysis, $^1$H, and $^{13}$C NMR.

Hydrogenolysis of 1,3,5 in 95% EtOH (1.4 M in NaOH) with W-2 Raney Ni at moderate pressure (32 psi H$_2$) gave a mixture of products (Scheme 3.25).
Investigation of the $^{13}$C NMR spectrum of the distilled product revealed 24 major peaks. This is consistent with the formation of the desired product 137 and one-armed macrocycle 138. In addition to the 24 major peaks in the NMR spectrum, several small peaks were also observed. The mass spectrum (El, CH$_4$) indicated two molecular ions at 283 and 240, corresponding to the two products 137 and 138 respectively. The relative proportions (~1:1) of the two products were estimated on the basis of the $^{13}$C NMR line intensities of the macrocyclic upfield methylene carbon (CHCHCH$_2$) peaks. It is clear that elimination was competing with reduction. The basicity of 135 and related tetraamines strongly suggested that
even in very strong base (1.4 M NaOH in 95% EtOH) 135 is partially monoprotonated and as a result, subsequent elimination of one of the pendant arms occurs. Utilization of sodium in ethanol has been successful for the reduction of similar nitriles as demonstrated by Wainwright.99 However, this procedure has yet to be attempted with nitrile 135.

Another approach toward the preparation of 137 might involve the DCC coupling of 29 with Boc protected β-alanine (Scheme 3.26).

Scheme 3.26

Successful coupling would lead to an amide which could be reduced with borane to give hexamine 137 after workup as shown above.


Acylation of 29 with methoxyacetyl chloride (Scheme 3.27) was achieved following a procedure analogous to one used by
Gokel$^{124a}$ Vachon$^{124b}$ had also used a similar procedure for the acylation of cyclen.

Scheme 3.27

Both the $^1$H and $^{13}$C NMR spectra of chromatographed material are complex. We believe that the spectral complexity is due to the fact that 139 is a mixture of conformational isomers which interconvert on the NMR time scale by slow amide bond rotation at room temperature. This effect is well known and has been previously reported.$^{125a-d}$ The $^{13}$C NMR spectra can be interpreted in terms of three conformational isomers which are interconverted by slow amide bond rotation. The four resonances observed in the upfield portion of the $^{13}$C NMR spectrum (CH$_2$CH$_2$CH$_2$) are consistent with three conformational isomers, two C$_2$ symmetric rotamers and one C$_1$ rotamer. Compound 139 was characterized by IR, high resolution
Several attempts at LiAlH₄ reduction of 139 resulted in reductive cleavage of armed material and recovery of 29. In one example an old batch of LiAlH₄ was used and resulted in product which was a mixture (~1:1) of 29 and monomethoxyethyl-armed cyclam. When the reaction was repeated with fresh LiAlH₄ pure 29 was recovered quantitatively from the reaction mixture. Diamide 139 was successfully reduced with BH₃·THF using a procedure similar to that of Vachon¹²⁴b to give 2-methoxyethyl armed cyclam 140 in 60% crude yield (Scheme 3.28). This reagent (BH₃·THF) was originally shown by Brown¹²⁴c to be quite effective for the reduction of tertiary amides under mild conditions.

Scheme 3.28

139 → 140

1) BH₃/THF
2) aq HCl, MeOH
3) aq NaOH
4) CHCl₃ Extraction
5) Distillation (60-73%)

140 (~95% pure)

Purification was achieved by the conversion of crude 140 to the

143

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monoprotonated tetraphenylborate salt 141 (Scheme 3.29).

Scheme 3.29

When 140 was dissolved in MeOH an equilibrium is established as shown in the top of Scheme 3.29. A solution of NaBPh4 in MeOH was added to this solution and resulted in the precipitation of a solid product, which is very likely 141. This solid (141) was isolated by filtration, dissolved in aq KOH and extracted with toluene. This resulted in 56% recovery of pure 140 giving an overall yield of 34%.
from 139. Compound 140 was characterized by IR, elemental analysis, $^1$H, and $^{13}$C NMR.

D. Attempted Preparation of Ligand 142.

Some progress was made toward the synthesis of ligand 142.

![Chemical structure of ligands 142 and 143]

Wieghardt\textsuperscript{126} has previously functionalized cyclic triamines with this same functional group.

The starting material necessary for our approach to the synthesis 142 was protected alkylation agent 144, which was obtained by Karlin's method.\textsuperscript{127} The acylation of o-cresol 145 with acetic anhydride/pyridine (1:1 v/v) gave 146 (Scheme 3.30) in 81\% yield.
The bromination of 2-methylphenyl acetate 146 with NBS in CCl₄, however, did not go as cleanly as described by Karlin¹²⁷ (Scheme 3.30). The crude product was distilled to give a clear liquid which was a mixture of monobrominated (144) and dibrominated products (147) (4:1-determined by ¹H NMR integration). Compounds 144 and 147 were separated by fractional distillation. The total yield of products was 70% (56% of 144, and 14% of 147) which was much better than that reported by Karlin who obtained a 33% yield of 144.

Several attempts to alkylate bisaminal 16 with 144 failed. In the first attempt 16 (0.015 M) and 144 (0.03 M) were combined in stoichiometric proportions in CH₃CN. After one week at room temperature, the alkylation had proceeded very little as indicated by ¹H NMR of the crude reaction mixture. A small amount (1-2%) of

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very pure 148 crystallized out of an NMR sample (CDCl₃) containing
an aliquot of this crude mixture. The next several alkylation
ttempts were performed by stirring an excess of 144 with 16 in
DMSO at various temperatures (room temperature to 100°C). In
every case a small amount of the desired product 148 was formed,
as indicated by ¹H and ¹³C NMR. Heating of the reaction mixture
usually accelerated the formation of decomposition products,
further complicating isolation of pure 148. Compound 16 (0.037 M)
cleanly dialkylated in MeCN at room temperature after 3 weeks. In
the presence of a large excess of alkylating agent (144, 0.377 M) the
desired product (148) crystallized and was isolated by filtration
(Scheme 3.31).

Scheme 3.31

Several washings with Et₂O and CH₂Cl₂ were necessary to remove
excess 144. Elemental analysis identified 148 as the monohydrate
(71-74% yield). The IR, $^1$H, and $^{13}$C NMR were consistent with the formation of 148.

A few attempts were made to reduce 148 with NaBH$_4$ in 95% EtOH (Scheme 3.32) using the same procedure to prepare cross-bridged dibenzyl cyclam 28 (described in Chapter II).

Scheme 3.32

The $^1$H NMR spectrum of the reduced product was complex, indicative of a mixture. Cross-bridged cyclam derivatives usually have a distinct downfield multiplet in the range of 3.50-4.00 ppm in the $^1$H NMR spectrum. The $^1$H NMR of the crude product exhibited a characteristic multiplet at 4.50 ppm. A $^1$H NMR of this reduced material was very similar to the $^1$H NMR spectra of other related
functionalized cross-bridged cyclam ligands. Acetyl groups were cleaved in the reduction step (the peak at 172.97 was absent in the $^{13}$C NMR of the reduced product). The mass spectrum (EI, CH$_4$) identified two molecular ions at 332 and 438. These two molecular ions do not correspond to the sodium salts 1 4 9 and 1 4 2, instead correspond to the free ligands. The presence of water in the sample submitted for a mass spectrum may explain why the free ligands were observed in the mass spectrum. The downfield region of the $^1$H NMR spectrum showed that two ortho-disubstituted aromatic rings were present. All of these results agree with the interpretation that the reduced material is a ~1:1 mixture of 1 4 9 and 1 4 2. The combined yield of the two products was over the theoretical amount. Ion exchange chromatography might be utilized to separate 1 4 2 from 1 4 9.

E. Synthesis of Trityl-protected N,N'-Bis(2-mercaptoethyl) Cross-bridged Cyclam (1 5 0).

Trujillo et al$^{128}$ published a procedure describing the preparation of 2-(chloroethyl) trityl sulfide (1 5 1). Compound 1 5 1 was synthesized by stirring equal molar amounts of trityl chloride
(152) and thiirane (153) in CH₂Cl₂ (Scheme 3.33).

Scheme 3.33

\[
\begin{align*}
\text{Ph} & \quad \text{Ph} \\
\text{Cl} & \quad \text{S} \\
152 & \quad 153 \quad \text{r.t. 4 h} \\
\text{CH₂Cl₂} & \quad 151 \\
\end{align*}
\]

Compound 151 crystallized after removal of residual solvent.

Alkylation of 29 (1 equivalent) with 151 (2 equivalents) in MeCN in the presence of K₂CO₃ or Na₂CO₃ was slow at room temperature. The mixture was then refluxed for 3 days after the addition of two times the stoichiometric amount of KI. The desired product 150, isolated in 37% yield, was about 80-85% pure. The use of DMF or N,N'-dimethylpropyleneurea (DMPU)¹²⁹ have improved this procedure since these are good solvents for S_n2 alkylations. In one attempt, 151 (4 equivalents) was stirred with 29 (1 equivalent) in DMF. The product (150) was isolated in 50% yield and was ~93% pure (Scheme 3.34).
An attempt was made to chromatograph the crude product on basic alumina, which resulted in partial removal of the trityl groups as indicated by a $^1$H NMR of chromatographed material. A preparative HPLC attempt to purify 150, and alternative suggestions for the preparation of protected thiol ligands are discussed in Chapter V.

Compound 150 was characterized by IR, high resolution mass spectrometry (HRMS), $^1$H, and $^{13}$C NMR.


Compound 154 was synthesized by heating 3 equivalents of 151 and 1 equivalent of 29 in DMF at 80 °C for 3 hours in the presence of KI (2 x stoichiometric) and K$_2$CO$_3$ (2 x stoichiometric). The resulting solution was stirred overnight at room temperature.
After 24 hours a component of the mixture crystallized, so the solution and solid were separated. The crystals and the mother liquors were worked up separately with aq KOH and each extracted with toluene. The material that had originally crystallized overnight was very pure 154 (21%). The mother liquors portion ended up being impure 150 (~54% yield, ~85% pure). Compound 154 was characterized by IR, elemental analysis, $^1$H, and $^{13}$C NMR.
G. Results of Ligand/Ligand Competitions with 140 and 25.

1. Table 3.1: $^{13}$C NMR Chemical Shifts of 140 and the Li$^+$ and Na$^+$ Complexes (CD$_3$CN).

<table>
<thead>
<tr>
<th>Host (δ)</th>
<th>LiClO$_4$ complex (δ)</th>
<th>NaBPh$_4$ complex (δ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>140 (0.11 M)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>28.95 (C$_6$,13)</td>
<td>25.87 (C$_6$,13)</td>
<td>26.32 (C$_6$,13)</td>
</tr>
<tr>
<td>51.57</td>
<td>50.37</td>
<td>50.29</td>
</tr>
<tr>
<td>54.90</td>
<td>53.79</td>
<td>51.61</td>
</tr>
<tr>
<td>55.84</td>
<td>59.46</td>
<td>59.28</td>
</tr>
<tr>
<td>57.75</td>
<td>59.79</td>
<td>59.49</td>
</tr>
<tr>
<td>58.31</td>
<td>59.94</td>
<td>59.65</td>
</tr>
<tr>
<td>58.71</td>
<td>60.43</td>
<td>60.25</td>
</tr>
<tr>
<td>61.06</td>
<td>62.27</td>
<td>61.44</td>
</tr>
<tr>
<td>72.69 (CH$_2$CH$_2$OCH$_3$)</td>
<td>70.63 (CH$_2$CH$_2$OCH$_3$)</td>
<td>69.98 (CH$_2$CH$_2$OCH$_3$)</td>
</tr>
</tbody>
</table>

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1. **140 vs. 25** Competition for (NaBPh₄)

Method of detection: $^{13}\text{C} \{^1\text{H}\}$ NMR

Signal:Noise = 60:1

Concentration of a single amine component: 0.10 M

Peak Integrated (uncomplexed 140): 28.95 ppm (C₆)

Peak Integrated (complexed 140): 26.32 ppm (C₆)

In this case, multiple integrations of these resonances were not performed in the determination of $K_{rel}$. The competition equilibrium constant (140 relative to 25) is $K_{rel} = 1.0 \pm 0.07$ The free energy of competition was calculated (58 relative to 24) $\Delta G_{298}^° = 0.0 \pm 0.07$ kcal/mol at 25°C. It is clear that 140 is just as effective as 25 in the complexation of NaBPh₄.
**2. 1 4 0 vs. 2 5 Competition for (LiClO₄)**

Method of detection: $^{13}$C $^{1}$H NMR

Signal:Noise = 50:1

Concentration of a single amine component: 0.11 M

Peak Integrated (uncomplexed 1 4 0): 28.95 ppm (C₆)

Peak Integrated (complexed 1 4 0): 25.87 ppm (C₆)

In this case, multiple integrations of these resonances were not performed in the determination of $K_{re1}$. The competition equilibrium constant (1 4 0 relative to 2 5) is $K_{re1} = 32 \pm 2.2$ The free energy of competition was calculated (1 4 0 relative to 2 5) $\Delta G^0_{298 K} = 2.05 \pm 0.14$ kcal/mol at 25° C. It is clear that 1 4 0 is a better complexer of Li⁺ than 2 5.
**IV. Conclusions**

The efforts at functionalization of cross bridged cyclam have led to several new functionalized tetraamines. A list of characterized ligands shown below in Figure 3.5 highlights the successful attempts at functionalization.

Figure 3.5

![Functionalized Ligands Diagram](image)

Some of the functionalized ligands synthesized contain ionizable groups which would form neutral complexes with divalent metals. Figure 3.1 indicates that there are several other possible functional groups which might be attached to 29 in an analogous fashion to that of 8.
Chapter IV

Selectively Functionalized Tetraamines

I. Introduction

Selectively protected tetraamines are frequently sought-after reagents for the preparation of selectively N-functionalized tetraamines. There are many examples of selectively functionalized tetraamines,\textsuperscript{130} several of which form thermodynamically stable coordination complexes with Gd(III).\textsuperscript{131} Some of these Gd(III) complexes are suitable paramagnetic relaxation agents for Nuclear Magnetic Resonance Imaging (MRI).

Various techniques for the selective functionalization of cyclen 1,\textsuperscript{132} cyclam 8,\textsuperscript{133} and isocyclam 4 7\textsuperscript{134} have been demonstrated. Selective protection of non-adjacent nitrogens in tetraamines such as cyclam and cyclen would allow functionalization of non-adjacent nitrogens to give 1,8-difunctionalized cyclam and 1,7-difunctionalized cyclen. The functionalization of non-adjacent nitrogens in a tetraamine would produce a ligand capable of complexing with a metal ion in an
octahedral fashion. The ligating arms enable the ligand to completely “wrap up” the coordination sphere of a metal cation. The expected geometry would involve coordination of the four macrocyclic nitrogens equatorially while the pendant arms coordinate through axial positions. For example, Chen$^{133a}$ and coworkers have synthesized the Cu(II), Ni(II), and Co(II) complexes of 155.

The complexes exhibited trans-octahedral geometry with the four macrocyclic nitrogens in a plane while the two carboxylate arms coordinate through axial positions.

II. Background

The selective 1,7-diprotection of cyclen has been achieved by Desreux$^{135}$ and coworkers by a method in which the protecting groups are positioned prior to macrocyclization. The starting 158

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diprotected triamine 156 was prepared as described by Martin (Scheme 4.1).\textsuperscript{136}

Scheme 4.1

Compound 156 was mesylated to give 157. Compound 157 was cyclized with 158 by the method of Richman and Atkins\textsuperscript{137} to give 1,7-dimesyl-4,10-ditosylcyclen (159). The selective removal of the tosyl groups was achieved by heating 8 in concentrated H\textsubscript{2}SO\textsubscript{4} by the general method of Raymond.\textsuperscript{138} Basic workup gave 1,7-dimesylcyclen (160) which was functionalized in the 4 and 10 positions and demesylated to give various 1,7-difunctionalized cyclens. The strong reducing conditions necessary to remove the mesyl groups often caused reaction of the newly introduced
functional groups. For instance, amide arms were easily reduced during demesylation.

A highly regioselective method for the preparation of 1,7-ditosyl cyclen \( 1 \times 1 \) has been reported by Desreux.\(^{135} \) The addition of a pyridine solution of cyclen (1 1) to two molar equivalents of tosyl chloride in pyridine gave \( 1 \times 1 \) in 90\% yield (Scheme 4.2).

Scheme 4.2

When one equivalent of tosyl chloride was used, only the 1,7-regioisomer \( 1 \times 1 \) was obtained in 45\% yield. Unreacted cyclen was recovered from the reaction mixture by aqueous extraction.

Attempts to tosylate cyclen in \( \text{CHCl}_3/\text{Et}_3\text{N} \) gave some very different results.\(^{135} \) For a 1:1 ratio (TsCl:cyclen) 63\% of \( 1 \times 2 \) and 16\% of \( 1 \times 1 \) were obtained (Scheme 4.3).
Scheme 4.3

For a 2:1 ratio (TsCl:cyclen) 15% of 162, 24% of 161, and 52% of tritosylcyclen (163) were isolated. The products of this reaction were easily separated due to solubility differences.

Selective protection of cyclen was also achieved using diethylphosphate to give the 1,7-regioisomer 164 (Scheme 4.4).135

Scheme 4.4

The reaction of cyclen (1 eq. in CCl₄) with diethylphosphate (2 eq. in CCl₄) in a two phase system gave 164 in 90% yield. The N-P bonds
were readily cleaved with 6M aq HCl.

Another synthesis of 1,7-diprotected cyclens has been achieved by Avelli\textsuperscript{139} and coworkers. The monoalkylation of cyclen has been achieved using a variety of alkylating agents (Scheme 4.5).

Scheme 4.5

Monoalkylated products 165 have been treated with dimethylformamide diethylacetal to quantitatively yield tricyclic orthoamides 166, which can be hydrolyzed in EtOH/H\textsubscript{2}O to give 1-alkyl-7-formyl cyclen derivatives 167. These disubstituted macrocycles were functionalized on the remaining 4 and 10 positions to give selectively functionalized cyclens. Micheloni\textsuperscript{140} and coworkers have previously reported the synthesis of 168.
Their macrocyclization technique requires several protection/deprotection steps giving a rather low overall yield (8%) of 168.

A synthesis of 1,8-difunctionalized cyclams has been reported by Parker. This was achieved by tosylation of cyclam (8) with 1.5 equivalents of TsCl, which generates the statistical products as shown in Scheme 4.6.

Scheme 4.6

The desired product 169 can be separated by chromatography in 30%
yield. A small amount of $170$ ($3.5\%$) was isolated along with the tritosylated derivative of cyclam ($172$), which was the major product of the reaction ($54\%$). A small amount of $171$ ($>3.5\%$) apparently forms in this reaction as indicated by $^1$H NMR, but could not be isolated. The remaining secondary amine groups of $169$ were then functionalized to give $173$ as shown in Scheme 4.7.

Scheme 4.7

The deprotection step also hydrolyzed the ester functionality to give $1,8$-difunctionalized hexadentate macrocycle $174$. The low selectivity of tosylation makes this sequence undesirable for large scale preparation due to the low yield of the desired $1,8$-diprotected cyclam.

North$^{142}$ previously synthesized several $1,8$-dialkyl cyclams by a different route. The tricyclic bis-formal adduct $175$ is easily prepared from cyclam 8 and a formalin solution (Scheme 4.8).
The conformation of 175 is such that there are two intraannular nitrogen lone pairs and two extraannular nitrogen lone pairs (Figure 4.1(a)).

Figure 4.1

The X-ray crystal structure\textsuperscript{143} and solution conformation\textsuperscript{144} are both consistent with a rectangular [2323] diamond lattice conformation for the 10-membered ring of 175 (Figure 4.1(b)). The tricyclic bis-aminal can undergo a topomerization via sequential inversion of all
four nitrogens. Weisman\textsuperscript{144} demonstrated by dynamic $^{13}$C NMR that the topomerization barrier is $\Delta G^\ddagger = 9.1$ kcal/mol (Figure 4.2).

**Figure 4.2**

The dialkylation of tricyclic bis-formal adduct 175 with benzyl bromide gave selectively alkylated bis-quaternary dibromide salt 176 (Scheme 4.8). In the presence of an alkylating agent, the first alkylation occurs at one of the extraannular nitrogen lone pairs, which locks the conformation, forcing the second alkylation to occur at the other extraannular nitrogen lone pair (Figure 4.3).

**Figure 4.3**

This regioselectivity is very similar to that observed for the alkylation of cyclam bis-aminal 16 as discussed in Chapter II.
III. Results

North showed that NaBH₄ reduction of 176 leads to 1,8-dibenzyl-4,11-dimethylcyclam 177. The hydrolysis of 176 in 6 M NaOH followed by benzene extraction gave 1,8-dibenzylcyclam 178 (Scheme 4.9).
We repeated the benzylation/hydrolysis sequence using the procedure outlined by North in an attempt to fully characterize 178. The benzylation procedure involved heating a mixture of 175 and excess benzyl bromide in acetonitrile at 100 °C (steam bath) for 4 h. The product precipitated as the dibromide salt 176, however an impurity was evident by 1H NMR. When the reaction was repeated at room temperature for 24 h, instead of 4 h at 100 °C, the amount of this impurity was significantly decreased. Isolation by filtration gave a pure white solid 176 in 87% yield. 176 was hydrolyzed in 3M NaOH.
aq NaOH to give pure \(1\) 78 in 88% yield.

Rogers\(^{145}\) had previously demonstrated that bisaminal dimethiodide \(3\) 1 (preparation described in Chapter II) could be hydrolyzed (20% KOH) to give \(1\) 68 in 79% yield (Scheme 4.10).

Scheme 4.10

\[
\begin{array}{c}
\text{3} 1 \\
\text{3)} \text{Distill}
\end{array} \quad \begin{array}{c}
\text{1} 68 \\
\text{2)} \text{PhH extraction} \\
\text{Na}_2\text{SO}_4 \\
\text{1)} \text{20% KOH, 4 h}
\end{array}
\]

This method for the preparation of \(1\) 68 is more efficient than the Micheloni\(^{11}\) route, which requires several additional steps and gives a low overall yield (8%).

Using the general Rogers procedure, related salt \(7\) 0 was hydrolyzed to give \(1\) 79 in 80% yield (Scheme 4.11). Compounds \(8\) 2 and \(8\) 6 were also hydrolyzed in a similar fashion to give tetraamines \(1\) 80 and \(1\) 81, respectively (Schemes 4.12 and 4.13).
The synthesis of dihalide salts 82 and 86 is discussed in Chapter II. Bis-quaternary dibromide salt 70 was synthesized previously by Rogers as discussed in Chapter II, but only partially...
characterized. The dihalide salts 70, 82, and 86 were hydrolyzed in 20% aq KOH at room temperature for 4 h. The two step yields for the synthesis of 180 from 80 and 181 from 85, are reported (Schemes 4.12 and 4.13) since the dihalide salts were not completely characterized (IR, 1H and 13C NMR only).

**IV. Discussion**

We have developed a highly efficient synthesis of 1,8-diprotected cyclams and 1,7-diprotected cyclens. It should be possible to functionalize the remaining secondary amine nitrogens with ligating arms. The removal of the benzyl groups by hydrogenolysis would give 1,8-difunctionalized cyclams. In a similar fashion, 1,7-difunctionalized cyclen derivatives could also be synthesized. North has referred to these di-armed ligands as "fullbacks" since the ligand could adopt a conformation in which the arms complex in an over-under fashion (Figure 4.4), as previously discussed in the Introduction.
The non-adjacent functionalized polyamines 168, 179, 180 and 181 could conceivably be functionalized with arms in the same way as cross-bridged cyclam (see Chapter III).

The most interesting aspect of this method is the ability to functionalize cyclam or cyclen with two different ligating groups. For example, 1-benzyl-8-methylcyclam (180) could be functionalized with two ligating groups to give 182 (Scheme 4.14).
Scheme 4.14

182 could be debenzylated by hydrogenolysis to give 183, which could then be alkylated and hydrolyzed to give 184 (Scheme 4.14). The synthesis of functionalized macrocycle 184 would certainly be challenging by typical macrocyclization methods. This same type of sequence could also be applied to cyclen derivatives, thus providing a route to selectively functionalized cyclens.
Chapter V

$^{99m}$Technetium Labelling Studies with Functionalized Tetraamine Ligands

1. Introduction

In collaboration with The Dupont/Merck Pharmaceutical Company Imaging Agents Research and Development Division, $^{99m}$Technetium radiolabelling experiments were performed on several of the ligands previously discussed in this thesis. Radiolabelling has become a valuable diagnostic tool in nuclear medicine and continues to grow as a relatively new area of research. The fundamental principle of radiolabelling relies on the combination of an organic ligand and a radionuclide, ($^{99m}$Technetium, for example), forming a complex which can be injected into the bloodstream, typically in a saline solution. The complex can then be metabolized or accumulated in a particular organ in the body. At this point, the radionuclide will then undergo gamma decay and the emission can be detected using a gamma camera.

Radiopharmaceuticals are typically divided into two classes,
according to the mode of distribution. For one type the biological
distribution is dependent on the interaction between the
radiolabelled complex and the tissue it adheres to (perfuses into)
while traveling through the blood stream. For instance, some
labelled complexes are better suited for imaging the brain, while
some are well suited for imaging the heart. The other type is
dependent on specific biochemical or receptor binding interactions.
For instance, monoclonal and polyclonal antibodies have been
labelled with $^{99m}$Tc while maintaining their immunoreactivity.

Injections of radiolabelled agents into humans allows
visualization (imaging) of internal organs without the need for
investigative surgery. The size and lipophilicity of radiolabelled
ligands are determining factors that affect where the complex is
accumulated or metabolized. Research has led to the development of
novel ligands whose $^{99m}$Tc complexes target such organs as the
heart, brain, liver and the kidneys. Several reviews have
discussed radiolabelling with $^{99m}$Tc. Labelled $^{99m}$Tc complexes of
the ligands discussed herein were designed to be pharmaceuticals
for imaging. One of these labelled $^{99m}$Tc complexes was used for a
biodistribution study in mice to determine where (in what organs) it
accumulates.

$^{99m}$Technetium generators were eluted daily on site at The DuPont Merck Pharmaceutical Company Radiopharmaceutical Research and Development Organization Discovery Division at the Billerica site in MA to supply Na$^{99m}$TcO$_4^-$ in a saline solution for labelling studies. The half-life of $^{99m}$Tc (6.01 h) makes it very convenient for in-vivo studies.

$^{99m}$Tc originates from $^{99}$Mo which is generated by irradiation of $^{98}$Mo with thermal neutrons in a nuclear reactor (Scheme 5.1).

Scheme 5.1

\[ ^{98}\text{Mo} \xrightarrow{n,\gamma} ^{99}\text{Mo} \xrightarrow{\beta^-} ^{99m}\text{Tc} \]

The form of $^{98}$Mo is usually molybdenum trioxide, ammonium molybdate, or molybdenum metal. Na$_2$$^{99m}$MoO$_4^-$ undergoes beta decay to generate Na$^{99m}$TcO$_4^-$. Na$^{99m}$TcO$_4^-$ is isolated by placing $^{99}$Molybdenum (Na$_2$$^{99m}$MoO$_4$) on an alumina column, which is then eluted with saline solution every 24 hours. The more highly charged $^{99}$MoO$_4^{-2}$ is retained while the $^{99m}$TcO$_4^-$ is eluted from the column due to its lower charge. The labelling procedures employed for preliminary testing of the macrocyclic polyamine ligands synthesized for this...
$^{99m}$Tc can occupy oxidation states from -III to +VII, the most common being V, III, II, IV, and I. The following examples are representative of the oxidation states typically found in $^{99m}$Tc complexes. $^{99m}$Tc(V)O-glucoheptonate$^{152}$ 185 (Glucoscan, DuPont Merck; TechneScan Gluceptate, Mallinckrodt) is an example of a radiopharmaceutical believed to be a Tc(V) species as shown. Glucoscan has been approved by the Food and Drug Administration (FDA) for kidney and brain imaging to determine renal and brain perfusion.

\[
\begin{align*}
\text{Glucoscan:} & \\
\text{Tc(V)} & \text{O-glucoheptonate} \\
185
\end{align*}
\]

$^{99m}$Tc-penetate$^{153}$ (AN-DTPA (DTPA = diethylenetriaminepentaacetic acid), Syncor International; MPI DTPA Kit, MediPhysics; Techniplex, Bristol-Myers Squibb; DTPA, DuPont Merck) is an example of a radiopharmaceutical believed to be either a Tc(IV) 186 or a Tc(V) 187 species as shown below. DTPA is FDA approved for imaging of
the kidneys, brain, and to evaluate kidney function.

\[ \text{186} \quad + \quad \text{187} \]

\[ ^{99m}\text{Tc-HIDA}^{154a} \text{ (HIDA = hepatobiliary iminodiacetic acid) is an example of a Tc(III) complex.}^{99m}\text{Tc-HIDA is FDA approved for imaging of the liver, gall bladder, bile duct, and intestines. The actual structure of this complex is unknown, but FAB mass spectral analysis reported by Davison}^{155} \text{ are consistent with the proposed 2:1 complex 188 originally postulated by Loberg}^{154a}. \]

\[ \text{188} \]

\[ ^{99m}\text{Tc-sestamibi}^{154b} \text{ (Cardiolite®, Dupont Merck) is an example of a} \]
Tc(I) complex (188a). 99mTc-sestamibi is an octahedral complex in which the 99mTc is surrounded by isonitrile ligands. 99mTc-sestamibi is FDA approved for imaging of the heart.

\[ \text{188a} \]

II. Results and Discussion

A. General

Our investigation of 99mTc "labelling" included studies of the ligands 29, 72, 131, 189, and 190.
"Labelling" refers to the technique used to combine a ligand with $^{99m}$Tc to form a radiolabelled coordination complex. Typically, a reducing agent (for example SnCl$_2$) and $^{99m}$Tc (Na$^{99m}$TcO$_4$) are combined in an aqueous medium such as saline in the presence of the desired ligand (Scheme 5.2).

Scheme 5.2

$$\text{Na}^{99m}\text{TcO}_4 + \text{Ligand} \xrightarrow{\text{SnCl}_2, \text{Saline}} \text{Complex}(^{99m}\text{Tc}(n))$$

$n =$ oxidation state
$n =$ V, III, II, IV, I

Under these conditions the $^{99m}$Tc(VII) is reduced to a lower oxidation state ($^{99m}$Tc(V), $^{99m}$Tc(III), $^{99m}$Tc(II), $^{99m}$Tc(IV), $^{99m}$Tc(I)) species
which combines with the ligand to form a complex. The solvent for this reaction should be either saline or a buffered aqueous solution, however small amounts of EtOH would be acceptable. Organic solvents cannot be used because injection into the test subject (mouse) may result in accidental death and thus interfere with crucial biodistribution data. Ultimately, solvents are avoided since the complex may eventually be used in human subjects.

All of our attempts to label ligands 29, 72, and 131 with $^{99m}$Tc failed or resulted in the formation of very polar $^{99m}$Tc containing species according to HPLC analysis. Labelling conditions including identity of reducing agent, pH, temperature, and reaction time were all varied separately without success. Four reducing agents: stannous (II) chloride (SnCl$_2$), stannous (II) tartrate, TPPTS$^{156}$ (triphenylphosphine-$m$-trisulfonate) and Na$_2$S$_2$O$_3$ (sodium thiosulfate) were tried, since they are water soluble and are frequently used at DuPont Merck for labelling new ligands. Several attempts to label 29 in the pH range 7-11 using SnCl$_2$ or Sn(tartrate) as reducing agents failed. Attempts to label 72 at pH 11 using either Na$_2$S$_2$O$_3$ or SnCl$_2$ following this same procedure did not result in labelling. Attempts to label 131 at pH 5 and pH 11
failed. A few representative procedures of the unsuccessful attempts are included in the experimental part of this thesis. Compounds 189 and 190 were successfully labelled with $^{99m}$Tc but not cleanly.

B. Preparation of Thiol Ligands


Ligand precursor 154 used for labelling studies was completely characterized in the laboratory at UNH and was >98% pure. Ligand precursor 150 was synthesized in the laboratory at UNH and was no greater than 93% pure. Thiol-containing ligands 189 and 190, were prepared by detritylation of 154 and 150 respectively using trifluoroacetic acid (TFA) and triethylsilane ($\text{Et}_3\text{SiH}$) just prior to labeling experiments (Schemes 5.3 and 5.4). Triethylsilane reduces the trityl cation to triphenylmethane, thus making the deprotection sequence irreversible. Solid triphenylmethane is easily removed by filtration.
Both ligand exchange and direct reduction of generator eluent in the presence of ligand led to complex formation in each case, proving that ligands 189 and 190 label ⁹⁹ᵐTc. Unfortunately, the labelling experiments resulted in mixtures of ⁹⁹ᵐTc-containing products, one of which was isolated in the case of ligand 190.

2. Preparative HPLC of 150

Several attempts in the laboratory at UNH to purify 150 to

183
purity greater than 93% were unsuccessful (chromatography and recrystallization). Reverse-phase preparative HPLC was attempted on site at Dupont Merck in Billerica, MA. A chromatogram is shown in Figure 5.1. Each of the solids resulting from lyophilization of fractions #1-4 were submitted for high resolution mass spectrometry (HRMS) analysis. The desired ligand precursor (150) was found in fraction #4 while fractions #1, 2, and 3 contained mixtures of 154 and 150. A $^1$H NMR of fraction #4 indicated that 150 was present but appears to have decomposed considerably under the chromatography conditions used for separation (Figure 5.2).

3. Attempted Analytical HPLC of 154.

Reverse phase analytical HPLC was attempted on compound 154 to compare the results to those obtained for the preparative HPLC of 150. The result indicated that compound 154 appeared to have also decomposed considerably under the HPLC conditions used. The fact that compound 154 would be protonated under the chromatographic conditions used may also help explain why chromatography was unsuccessful.
Preparative Reverse Phase HPLC of 150

Data: 
- Date: Tue, Jan 31, 1995 1:35 PM
- Data: Dhillim2-31JAN95-001
- Sample: Cyclam Bis thiol- Gradient elution
- Method: Dhillim2
- Inject Vol: 1.0
- Internal Std: 10.0
- Sampling Int: 0.1 Seconds

HPLC Chromatogram

<table>
<thead>
<tr>
<th>Fraction #</th>
<th>Collection interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3-6 min</td>
</tr>
<tr>
<td>2</td>
<td>16-21 min</td>
</tr>
<tr>
<td>3</td>
<td>22-26 min</td>
</tr>
<tr>
<td>4</td>
<td>26-32 min</td>
</tr>
</tbody>
</table>

Analytic Channel A

<table>
<thead>
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<th>Peak No.</th>
<th>Time</th>
<th>Type</th>
<th>Height(µV)</th>
<th>Area(µV-sec)</th>
<th>Area%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.701</td>
<td>N</td>
<td>46097</td>
<td>1284223</td>
<td>69.820</td>
</tr>
<tr>
<td>2</td>
<td>17.780</td>
<td>N1</td>
<td>3900</td>
<td>77402</td>
<td>4.208</td>
</tr>
<tr>
<td>3</td>
<td>18.311</td>
<td>N2</td>
<td>8803</td>
<td>111670</td>
<td>6.085</td>
</tr>
<tr>
<td>4</td>
<td>18.725</td>
<td>N3</td>
<td>22041</td>
<td>366115</td>
<td>19.905</td>
</tr>
<tr>
<td>Total Area</td>
<td></td>
<td></td>
<td></td>
<td>1839310</td>
<td>99.898</td>
</tr>
</tbody>
</table>

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Figure 5.2

1H NMR of HPLC fraction #4 (expansion, 1.00-7.50 ppm)

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C. Techniques used for the Labelling of Bis(thiol-armed) Ligand 190.

The first method used to label 190 was ligand exchange. The $^{99m}$Tc(V)O (glucoheptonate) complex 188 was synthesized and to this was added the deprotected thiol ligand 190. The ligand exchange reaction is shown in Scheme 5.5.

Scheme 5.5

Ligand 190 + $^{99m}$Tc(V)O(glucoheptonate) $\rightarrow$ $^{99m}$Tc(Ligand 190) Complex + glucoheptonate

A general $^{99m}$Tc labelled species is indicated in Scheme 5.5. The actual structure, oxidation state, and charge of the labeled complex is not known. The initial glucoheptonate ligand exchange experiments provided some interesting information about the labelling potential of 190. HPLC chromatograms (radiodetection of $^{99m}$Tc, a $\gamma$-emitter) showed that two major components were formed, with retention times of 7.0 and 10.0 minutes respectively. The chromatograms indicated the rapid formation of a complex (retention time = 7.0 minutes) two minutes after addition of ligand 190. This complex was converted into a new complex (retention time = 10.0 minutes) after heating for 30 minutes or more. The
intensity of the peak at 7.0 minutes slowly decreased while the intensity of the peak at 10.0 increased. When the reaction mixture was buffered to pH 8 and heated for 1 h at 80 °C, the major component had a retention time of 7.0 minutes (Figure 5.3). When the reaction mixture was buffered to pH 10 the major product after 1 h at 80 °C had a retention time of 10.0 minutes (Figure 5.4). The broad peak between 8.0-9.5 minutes seems to indicate there are several components in the reaction mixture. Successful labelling was accomplished with 4.00-0.04 mg/mL (5 x 10⁻³ M-5 x 10⁻⁵ M) of ligand and labelling still occurred using as little as 8.0 μg/mL (10⁻⁵ M) of ligand. The concentration of Na⁹⁹mTcO₄ in the reaction mixture was typically in the range of 10⁻⁶ to 10⁻⁸ M. There was usually very little labelling when the pH of the reaction mixture was below 6.0. The percent of colloid (⁹⁹mTcO₂) formed on the labelling reactions was typically less than 10%. An important feature of this ligand is the fact that labelling occurred rapidly at such low concentration (8.0 μg/mL). It is very possible that the ligand could be used in conjunction with a peptide based receptor. The resulting radiopharmaceutical would contain a chelating portion (used for labelling ⁹⁹mTc) and a peptide portion (used for targeting a specific
Figure 5.3

Labelling Procedure 1

Tc(VI)O (glucoheptonate) ligand exchange,
pH 8, 20 μL injection.

HPLC-TFA method

A) 80 °C, 1 min
B) 80 °C, 30 min
C) 80 °C, 60 min
Figure 5.4

Labelling Procedure 1

Tc(V)O (glucoheptonate) ligand exchange,

pH 10, 20 μL injection.

HPLC-TFA method

A) R.T., 1 min
B) 80 °C, 30 min
C) 80 °C, 90 min

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organ or area of the body).

An alternative method for labelling 190 by ligand exchange utilized a tricine complex. Tricine 191 was first labelled with 

\[
\begin{align*}
\text{HO} & \quad \text{N} \\
\text{HO} & \quad \text{OH} \\
\text{HO} & \quad \text{HO}
\end{align*}
\]

191

\[{}^{99m}\text{Tc} \text{ (retention time } {}^{99m}\text{Tc} \text{ complex } = 3.3 \text{ min) using } \text{SnCl}_2 \text{ as the reducing agent. The freshly deprotected 190 was then added displacing tricine 191 (Scheme 5.6).}

Scheme 5.6

\[
\text{Ligand 190 + } {}^{99m}\text{Tc(tricine Complex)} \longrightarrow
\]

\[{}^{99m}\text{Tc(Ligand 190) Complex + Tricine (191)}
\]

HPLC chromatograms (Figure 5.5) indicated successful labelling of 190 by this method, but a complex mixture of products. Two major peaks (retention times 7.0 and 10.0 minutes) were observed under these labelling conditions. A broad peak between 8.0-9.5 minutes accompanied the two sharp peaks described above. This same peak pattern was also observed in the labelling attempts with 191.
Figure 5.5

Labelling Procedure 2

SnCl₂, Tricine

pH 10, 20 μL injection.

HPLC-TFA method

A) R.T., 1 min

B) R.T., 30 min

C) R.T., 30 min, then 50 °C, 40 min
glucoheptonate, suggesting comparable products are formed using this method. Again, a general $^{99m}$Tc species is shown in Scheme 5.6. The actual structure and oxidation state of the labeled complex is not known.

Another method used for labeling of 190 was ligand exchange with a $^{99m}$Tc complex of ethylenediaminetetraacetic acid (EDTA). EDTA was first labeled with $^{99m}$Tc using SnCl$_2$ as the reducing agent. The deprotected 190 was then added displacing EDTA (Scheme 5.7).

![EDTA](image)

Scheme 5.7

\[
\text{Ligand 190} + ^{99m}\text{Tc}^{(IV)}(\text{EDTA}) \rightarrow ^{99m}\text{Tc}(\text{Ligand 190}) \text{ Complex} + \text{EDTA (192)}
\]

The HPLC chromatograms indicate that labeling occurred with this method as well (Figure 5.6) again giving a complex mixture of
Labelling Procedure 3

SnCl₂, EDTA

pH 7, 20 μL injection.

HPLC-TFA method

A) R.T., 2 min

B) R.T., 35 min

C) R.T., 35 min, then 50 °C, 30 min
products. One major sharp peak (retention time 7.0 minutes) and one very broad peak (retention time 8.0-9.5 minutes) was observed in the reaction mixture after one hour of heating at 50 °C. This same peak pattern was also observed in $^{99m}$Tc labelling attempts with glucoheptonate, suggesting similar products were formed using this method. Again, the structure and oxidation state of the $^{99m}$Tc ligand 190 complex (Scheme 5.7) are unknown.

The labelling method used for the biodistribution study involved direct reduction of Na$^{99m}$TcO$_4$ with triphenylphosphine-$m$-trisulfonate (TPPTS) in the presence of ligand 190. A mixture of Na$^{99m}$TcO$_4$, TPPTS, and freshly prepared ligand 190 was heated to give a labelled complex of unknown structure and oxidation state as suggested in Scheme 5.8.

Scheme 5.8

A chromatogram (Figure 5.7A) of the crude reaction mixture
Labelling Procedure 4

Figure 5.7

TPPTS, pH 7, 80 °C, 1 h.

HPLC-Phosphate method

A) Crude reaction mixture (300 µL injection)

B) Isolate collected between 14.5-16.0 min dissolved in 0.1% (w/w) solution of Tween 80 in saline (0.4 mg deprotected ligand present).

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indicates that there are many products from the reaction. The major peak at 15.3 minutes (retention time) comprised about 40-60% of the activity depending on the run. The major peak found at 15.3 minutes using the phosphate HPLC method corresponds the major peak found at 10.0 minutes using the TFA HPLC method. The 15.3 minute component was isolated by collection of the eluent between 14.5 and 16.0 minutes. Solvents (H₂O, CH₃CN) were removed under reduced pressure and the residue was dissolved in saline. This solution was analyzed by HPLC; the chromatogram of the purified material is shown in Figure 5.7B. The radiochemical purity was acceptable, so the reaction was scaled up for a biodistribution study. One problem encountered with the isolation of the 15.3 minute component was that labelled material adhered quite strongly to the glass flasks used for collection of the HPLC eluent. This created serious losses during transfer since labelled material could not be effectively rinsed out with saline solution. This problem was solved by rinsing the flask and transfer syringes with a surfactant (Tween 80). The transfer of labelled material increased from 46-57% using saline to 97-98% using a 0.1% (w/w) solution of Tween 80 in saline.
D. Labelling Technique used for Thiol-armed Ligand 189.

Successful labelling of ligand 189 was accomplished by ligand exchange with $^{99m}$Tc(V)O (glucoheptonate) (Scheme 5.9).

Scheme 5.9

\[
\text{Ligand 189} + ^{99m}\text{Tc(V)O(glucoheptonate)} \rightarrow ^{99m}\text{Tc(Ligand 189)} \text{ Complex} + \text{glucoheptonate}
\]

Ligand 189 did label (Scheme 5.9) but the complexity of the crude product mixture discouraged further attempts to study this ligand. The chromatograms are shown in Figure 5.8.

E. Biodistribution Study on the Major Component of Labelled 190.

Once a suitable method for separation of the major labelled component of 190 had been established (Figure 5.7A), the procedure was scaled up to supply enough material for a biodistribution study in mice. The results of this study and the scaleup procedure are discussed below.

The chromatograms of the crude reaction mixture and of the isolated material are shown in Figure 5.9. The results are
Labelling Procedure 5

Tc\(\text{V})\)O glucoheptonate ligand exchange,
pH 10, 25 \(\mu\text{L}\) injection.
HPLC-TFA method

A) R.T., 1 min
B) 80 °C, 60 min
C) 80 °C, 105 min

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Labelling Procedure 6

Figure 5.9

TPPTS, pH 7, 80 °C, 1 h.

HPLC-Phosphate method

A) Crude reaction mixture (500 µL injection)

B) Isolate collected between 14.5-16.0 min dissolved in 0.1% (w/w) solution of Tween 80 in saline (0.4 mg deprotected ligand present).

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consistent with that observed for the small scale run. The recovery of activity was calculated by measurement of the amount of activity in each vial or syringe with a Capintec radioisotope calibrator (model CRC-12). The results of several isolation attempts are shown in Table 5.1. The typical recovery or yield for radiolabelling ranged from as low as 9% to as high as 68% depending on the run.

Table 5.1

<table>
<thead>
<tr>
<th>Isolation Attempt</th>
<th>Injection Volume (µL)</th>
<th>Injected Activity (mCi)</th>
<th>Collected Activity (mCi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>#41a</td>
<td>300</td>
<td>16.0</td>
<td>9.5</td>
</tr>
<tr>
<td>#41b</td>
<td>350</td>
<td>8.0</td>
<td>5.4</td>
</tr>
<tr>
<td>#42a</td>
<td>500</td>
<td>91.2</td>
<td>10.9</td>
</tr>
<tr>
<td>#42b</td>
<td>500</td>
<td>138.0</td>
<td>16.9</td>
</tr>
<tr>
<td>#44a</td>
<td>300</td>
<td>60.0</td>
<td>7.0</td>
</tr>
<tr>
<td>#44b</td>
<td>500</td>
<td>100.2</td>
<td>9.8</td>
</tr>
<tr>
<td>#44c</td>
<td>600</td>
<td>120.0</td>
<td>10.3</td>
</tr>
</tbody>
</table>

The biodistribution results are shown in Tables 5.2 and 5.3. The biodistribution studies using the purified isolate were carried out by DuPont Merck personnel. Ten mice chosen for the study were weighed and injected with known amounts of the labelled compound (250-303 µCi). The distribution of activity in the carcass, organs,
Table 5.2

To-99m Labeled Ligand #HIL DMP 3307-45 tested 11/29/94

<table>
<thead>
<tr>
<th>Organ uptake</th>
<th>Blood uptake</th>
<th>Femur uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organ activity / total activity x 100</td>
<td>Blood activity / blood wt. x 0.05 x mouse wt. / total activity x 100</td>
<td>Femur activity / femur wt. x 0.1 x mouse wt. / total activity x 100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>15 min. in-vivo</th>
<th>Red 1</th>
<th>Blue 1</th>
<th>Purple 1</th>
<th>Yellow 1</th>
<th>Orange 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse Wt.</td>
<td>37 g</td>
<td>37 g</td>
<td>34 g</td>
<td>38 g</td>
<td>40 g</td>
</tr>
<tr>
<td>Femur Wt.</td>
<td>0.119 g</td>
<td>0.115 g</td>
<td>0.112 g</td>
<td>0.121 g</td>
<td>0.110 g</td>
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<tr>
<td>Tube Wt.</td>
<td>13.04 g</td>
<td>13.07 g</td>
<td>13.15 g</td>
<td>13.08 g</td>
<td>12.99 g</td>
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<td>Blood-Tube Wt.</td>
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<td>14.16 g</td>
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<td>Activities</td>
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<tr>
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<td>7.0 μCi</td>
<td>9.0 μCi</td>
<td>9.0 μCi</td>
<td>7.0 μCi</td>
<td>8.0 μCi</td>
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<tr>
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<td>0.0 μCi</td>
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<tr>
<td>Stomach</td>
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<td>3.0 μCi</td>
<td>4.0 μCi</td>
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<td>4.0 μCi</td>
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<tr>
<td>Kidneys</td>
<td>20.0 μCi</td>
<td>19.0 μCi</td>
<td>18.0 μCi</td>
<td>22.0 μCi</td>
<td>19.0 μCi</td>
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<tr>
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<td>44.0 μCi</td>
<td>42.0 μCi</td>
<td>42.0 μCi</td>
<td>41.0 μCi</td>
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<tr>
<td>Lungs</td>
<td>3.0 μCi</td>
<td>4.0 μCi</td>
<td>3.0 μCi</td>
<td>3.0 μCi</td>
<td>3.0 μCi</td>
</tr>
<tr>
<td>Heart</td>
<td>1.0 μCi</td>
<td>0.5 μCi</td>
<td>0.5 μCi</td>
<td>0.6 μCi</td>
<td>0.6 μCi</td>
</tr>
<tr>
<td>Femur</td>
<td>0.5 μCi</td>
<td>0.6 μCi</td>
<td>0.6 μCi</td>
<td>0.5 μCi</td>
<td>0.4 μCi</td>
</tr>
<tr>
<td>Bladder/Urine</td>
<td>68.0 μCi</td>
<td>57.0 μCi</td>
<td>53.0 μCi</td>
<td>56.0 μCi</td>
<td>72.0 μCi</td>
</tr>
<tr>
<td>Carcass</td>
<td>159.0 μCi</td>
<td>155.0 μCi</td>
<td>151.0 μCi</td>
<td>167.0 μCi</td>
<td>147.0 μCi</td>
</tr>
<tr>
<td>Total Activity</td>
<td>302.5 μCi</td>
<td>292.1 μCi</td>
<td>281.1 μCi</td>
<td>300.1 μCi</td>
<td>295.0 μCi</td>
</tr>
</tbody>
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<table>
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Average
Table 5.3

Tc-99m Labeled Ligand #HIL DMP 3307-45 tested 11/29/84

Organ uptake = organ activity / total activity \times 100

Blood uptake = blood activity / blood wt. \times 0.05 \times mouse wt. / total activity \times 100

Femur uptake = femur activity / femur wt. \times 0.1 \times mouse wt. / total activity \times 100

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Activities:

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Uptake:

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Average:

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blood, and bones was measured by sacrificing half of the animals at 15 minutes and the other half at 60 minutes after injection. The two time points were selected to determine if the labelled ligand was retained or excreted over time. After 15 minutes, the average organ uptake was significant in the carcass (53%), liver (14%), kidneys (7%), and femurs (6%). The high level detected in the bladder/urine (22%) indicates that the complex was readily metabolized or excreted. At the one hour time point, average organ uptake values were: carcass (44%), liver (13%), kidneys (9%), and femurs (5%). The level of activity in the bladder/urine increased to 30%. This higher level at the later time point is consistent with the fact that labelled compound was being excreted.

The actual structure of the $^{99m}$Tc ligand 190 complex is still unknown since the isolated labelled material cannot be characterized by typical methods ($^{13}$C and $^1$H NMR, IR, MS/HRMS, elemental analysis). The HPLC analysis suggests that the material is very lipophilic, as does the fact that transfer of the material is ineffective without the use of a surfactant (Tween 80). This evidence also points to the fact that the $^{99m}$Tc labelled complex (190) is not highly charged, if at all.
F. Molecular Modeling of Technetium Complexes.

To investigate possible structures of the labelled $^{99m}$Tc bis(thiol-armed) complex molecular modeling calculations were performed on a Silicon Graphics workstation using the Spartan molecular modelling package (version 2.0) which uses the Tripos force field. The purpose of the modelling studies was to determine if the geometry of $^{99m}$Tc complexes are reasonable with respect to steric strain, bond distances, and bond angles. Some possible structures for the labelled dithiol ligand are shown in Figures 5.10 and 5.11; the hydrogen atoms are omitted for clarity. The Tripos force field does not take metal oxidation states and charge into account. The oxidation state of technetium in these structures is (III). The structure of Figure 5.10 would carry a positive charge, while that of 5.11 would be a neutral species. The Tc-N and the Tc-S bond distances are noted in Å. The bond lengths are not distorted and the structures a actually quite reasonable with respect to their geometry. Some other possible structures that were not modeled are shown in Figure 5.12.
IV. Conclusions

Unfortunately, ligands 29, 72, and 131 were not labelled by $^{99m}$Tc under the conditions described. Successful labelling was observed with ligands 189 and 190. Both ligands 189 and 190 labelled but gave mixtures of products according to HPLC analysis. This suggests the possibility that a variety of products may result from ligand oxidation or from oligomerization further complicating the reaction mixture. In each labelling method (direct reduction vs. ligand exchange), the same major component was observed regardless of the reducing agent. In all successful $^{99m}$Tc labelling experiments with ligand 190 a broad peak (retention time 8.0-9.5 minutes) was observed. This broad peak may be due to a complex mixture of products which are rapidly interconverting on the timescale of the separation. A major component of the labelling
mixture of 190 was isolated and was stable at room temperature for over 2 h as long as excess ligand was present. The necessity of having excess ligand present during isolation suggests that the complex is readily oxidized, possibly by O₂. The excess “free thiol” added to the isolate serves as an antioxidant preserving the isolate so it can be used for biodistribution studies. The labelled complex could disproportionate which would result in complex dissociation. Isolation difficulties were encountered when transferring the major component. This problem was solved by rinsing transfer syringes and flasks with a surfactant.

A biodistribution study in mice was performed on the purified isolate resulting from labelling of 190. The major organs targeted were the liver, kidneys, and the bladder. A small amount of the labelled material ended up in the bone (femur).

Suggestions for further study might include an investigation of alternative protecting groups for sulfur. The method used to prepare the desired trityl-protected dithiol ligand gave 190 in 50 % yield (91-93% pure). Alternative protecting groups commonly used for thiol protection are S-(acetylamino)methyl (acm), S-(benzoylamino)methyl (Bcm), and S-benzyl (Bzl) (Figure 5.13).
Davison and coworkers have developed a method in which the deprotection of all three types of the protecting groups shown in Figure 5.13 occur during labelling. A proposed metal-assisted deprotection occurs giving $^{99}$technetium thiolate complexes in high yields.

An alternative procedure for the preparation of pure dithiol ligand 190 might utilize S-protected thioglycolic acid derivative 193. The 1,3-dicyclohexylcarbodiimide (DCC) coupling of 193 and 29 would give 194, which could be reduced with borane, for example, to give S-protected dithiol ligand 195 (Scheme 5.10).
Attempts should be made to prepare other dithiol ligands. For example, 1,8-dimethyl cyclam could be selectively functionalized to give 196.

The successful results of the labelling studies should promote further work on thiol functionalized tetraamine macrocycles.
PART II: SYNTHESIS AND DYNAMIC NMR STUDIES OF BICYCLIC UREAS
Chapter I

Bicyclic Ureas

1. Introduction

There has been a great deal of interest concerning an attractive transannular interactions between mutually compatible functional groups in medium size (8-10 membered) rings.\textsuperscript{159a,b} Typically, transannular attractions result from the positioning of electron-donor and electron-acceptor groups transannular to one another in a medium size ring. The extent of such transannular interactions has been studied by investigation of CD spectra,\textsuperscript{159c} EPR spectra,\textsuperscript{159c} photoelectron spectra,\textsuperscript{159c} UV spectra,\textsuperscript{159c} visible spectra,\textsuperscript{159d} IR spectra,\textsuperscript{159e} and NMR spectra.\textsuperscript{159f}

One example of a transannular interaction examined by Dunitz\textsuperscript{160} is the attraction between an amine and a ketone carbonyl which are situated transannular to one another in a medium size ring. X-ray structural analysis revealed that amino ketones with short transannular N...C=O distances had longer C=O bond lengths than amino ketones with long N...C=O distances. This deviation in the C=O
bond distance was determined to be directly related to the N–C=O distance in bicyclic amino ketones.

Several research groups have studied intramolecular reactions\textsuperscript{161-167} which are typically much faster than their analogous intermolecular reactions. However, the determining factors which qualitatively and quantitatively describe observed rate enhancements for transannular reactions are still very controversial. A few of these opposing viewpoints will be briefly discussed.

Menger has postulated that the rate of a bimolecular reaction depends on the time two reactants spend at a critical distance,\textsuperscript{161a,c} a theory known as the "spatiotemporal postulate." Strategically placing two reactive functional groups transannular to one another holds them at a "critical distance." For this reason, Menger believes, intramolecular reactions are often much faster than their intermolecular counterparts.\textsuperscript{161a,c}

Earlier, Koshland\textsuperscript{168} proposed that fast intramolecular and enzymatic reactions are a result of "orbital steering." According to his theory, improper alignment of reactive functional groups by 10° will cause a 10^4 decrease in reaction rate. However, Bruice\textsuperscript{169}
refuted this argument, claiming that at room temperature these slight misalignments are overshadowed by vibrational and rotational motions of the molecule. Jencks\textsuperscript{170}, Page\textsuperscript{171}, and Mandolini\textsuperscript{172} strongly believe the observed rate enhancements for intramolecular reactions are due to entropic factors. Analogous intramolecular and intermolecular reactions show different degrees of entropy loss as the transition state of the rate determining step is approached. In a bimolecular reaction there is a loss of translational entropy as two molecules come together in the transition state. However, in an intramolecular cyclization reaction there is only rotational entropy loss.

Weisman realized that bicyclic urea 197 is analogous to the amino ketones studied by Dunitz\textsuperscript{160} and might be expected to exhibit a transannular interaction. It was anticipated that this transannular interaction might facilitate a degenerate transamidation (topomerization) that could be studied by dynamic NMR.
Wang proved the existence of the transannular interaction experimentally by analysis of IR spectra.\(^{173}\) and performed dynamic NMR experiments on bicyclic urea 197 that demonstrated a remarkably fast, acid catalyzed, degenerate transannular transamidation reaction (Scheme 1.1).

Scheme 1.1

II. Background

A. Topomerization of Bicyclic Urea 197.

Wang's work showed that the absence of an acid catalyst, three sharp lines were observed for the methylene carbons of 197 in the \(^1\)H-decoupled \(^{13}\)C NMR spectrum (CDCl\(_3\)).
Upon addition of mesitoic acid, the methylene resonances broadened, and substantially coalesced into a single peak. In the $^1$H NMR spectrum of 197 in CDCl$_3$ the methylene resonances (consistent with $C_s$ symmetry) also simplified as catalyst was added, to an AA'XX' pattern (consistent with $C_{3v}$ symmetry) as a result of the topomerization. This observation rules out the mechanistic intervention of guanidinium ion 198 ($D_{3h}$ symmetry) along the reaction pathway, which would average the methylene resonances in the $^1$H NMR spectrum into a singlet.

The $^1$H NMR results also show that the urea carbonyl cannot "tuck through" the ring during topomerization (Scheme 1.2) as this process would also average the $^1$H NMR spectrum into a singlet (time...
averaged $D_{3h}$ symmetry).

Scheme 1.2

In this work bicyclic urea 199 was synthesized in order to determine if it would exhibit a transannular attraction and undergo a similar acid catalyzed dynamic process.

Compound 199 was synthesized by a procedure similar to that used by Wang\textsuperscript{173} in the preparation of 197. Dynamic NMR studies of 199 using $^1$H and $^{13}$C NMR spectroscopy were used to probe the dynamic process in solvents such as $D_2O$ and $C_6D_6$. The results of these studies strongly suggest a dynamic process is taking place as will be detailed.
B. Acid Catalyzed Amide Aminolysis

The reaction of an amide with an amine is defined as an amide aminolysis. When the reaction products are another amine and amide, the reaction is a transamidation. The acid-catalyzed mechanism of a transamidation reaction is thought to involve initial O-protonation of the amide. The secondary amine then attacks the electropositive carbonyl carbon to produce a tetrahedral intermediate. After proton transfer, the intermediate can break down in either of two ways to reform an amine and an O-protonated amide (Scheme 1.3).
Several research groups have studied base-catalyzed intramolecular transamidations using bases such as 3-aminopropyl amide\textsuperscript{176} (KAPA), quinoline\textsuperscript{177}, KOtBu\textsuperscript{178}, and several others\textsuperscript{179}.

Alternatively, the reaction of an amide with an amine can produce an amidine\textsuperscript{180} as shown in Scheme 1.4.

Scheme 1.4

\[
\text{R'NH} + \text{R'CONR''} \rightarrow \text{R'NR''} + \text{H}_2\text{O}
\]
C. Bicyclic Ureas and Topomerization of 197.

Compound 197 contains an amine and a urea functional group. The reactions of amines and ureas are usually extremely slow.\textsuperscript{174,181} However, in 197, the amine moiety is transannular to the urea functional group, and as a result readily reacts with the carbonyl upon O-protonation to form the tetrahedral intermediate 200. This tetrahedral intermediate can break down in any of three ways to reform aminourea 197 (Scheme 1.5).
The basicity of a secondary amine is much greater than a urea oxygen (pKa of tetramethylurea conjugate acid in H₂O is about 2.02¹⁸²). In the presence of an acid catalyst the secondary amine is preferentially protonated, and must be deprotonated to bring about
topomerization. O-protonation of ureas was demonstrated by Steward and Munster\textsuperscript{183} in the case of N,N'-dicyclohexyl urea (201).

![Chemical Structure](image)

201

The CO stretching frequency is shifted from 1628 cm\(^{-1}\) to 1699 cm\(^{-1}\) upon protonation. The formation of a resonance stabilized conjugate acid in the case of O-protonation is responsible for the shifted IR frequency. Others\textsuperscript{184} have verified the results of Steward and Munster. O-protonation of urea 197 is expected to bring about topomerization.

Mowlam\textsuperscript{185,186} synthesized aminoureas 202 and 204, which are stable in solvents such as CDCl\(_3\) and DMSO-d\(_6\), but readily convert to guanidinium salts 203 and 205 respectively in protic solvents such as methanol-d\(_4\) or D\(_2\)O (Scheme 1.6). The equilibria are pH-dependent, low pH favoring the guanidinium ions (with protonation of OH\(^-\)).
A mechanism proposed by Mowlam for the formation of guanidinium ion of 203 is shown in Scheme 1.7.

The intramolecular attack of the amine on the protonated...
transannular carbonyl carbon forms a tetrahedral intermediate, which undergoes two nitrogen inversion as shown above. Upon oxygen protonation the C-O bond is broken to give guanidinium ion (An uncatalyzed mechanism involving attack on unactivated carbonyl and final expulsion of OH\textsuperscript{-} is no doubt operative at higher pH\textsuperscript{185}).

D. Acyl Transfer Studies by DNMR

Molecules which exhibit intramolecular reactions have conveniently been studied by dynamic NMR techniques. The principles and techniques of DNMR have been extensively reviewed in the literature\textsuperscript{187} and several books have been written\textsuperscript{188}. A few examples of intramolecular acyl transfer reactions studied by this technique are discussed.

Calder\textsuperscript{189} and coworkers have used variable temperature NMR to demonstrate that a rapid intramolecular exchange occurs in the case of the mono- and diacetates of the napthazarin system. The dynamic situation in the case of the monoacetate 206 is shown in Scheme 1.8.
$^1$H NMR evidence revealed that at temperatures below 100° C the topomerization of the monoacetate was slow with respect to the $^1$H NMR timescale (100 MHz) as indicated by the sharp peaks resulting from two separate AB systems (quinonoid protons) observed in the aromatic region of the $^1$H NMR spectrum. As the temperature was raised to 190° C the peaks broadened into the baseline, clearly indicating a dynamic process. The $\Delta G^\ddagger$ and rate constant for the topomerization of monoacetate 206 were determined to be 21.8 kcal/mol and 37 sec$^{-1}$ respectively at a temperature of 140° C. The results of this experiment were consistent with the intramolecular exchange of acetyl groups between peri-oxygen atoms.

Masamune$^{190}$ and coworkers clearly demonstrated by $^{13}$C NMR that tropolone acetate 207 exhibited a rapid degenerate rearrangement at room temperature (Scheme 1.9).
At 50 °C the proton decoupled (22.63 MHz) $^{13}$C NMR spectrum of 207 exhibited three peaks which broadened as the sample was cooled and eventually decoalesced into 9 singlets at -70 °C. This observation is consistent with a two-site dynamic exchange process resulting from an acyl migration. The $\Delta G^\ddagger$ for the migration process was calculated at several different temperatures between -70° C and -10° C, the average being 10.8 Kcal/mol.

III. Results and Discussion

A. Synthesis of Bicyclic Urea 199.

Tritosyltriamide 212 was prepared by the Kellogg$^{75,76}$ Cs$_2$CO$_3$ modification of the method of Richman and Atkins$^{77}$ as shown in Scheme 1.10 and subsequently converted to medium-ring triamine 208.
Starting material 210 was prepared by reaction of 1,3-propanediamine 209 with 2 equivalents of p-toluenesulfonyl chloride by the method of Koyama.\textsuperscript{191} Compound 210 was converted to the dicesium salt with two equivalents of Cs\textsubscript{2}CO\textsubscript{3} and cyclized with one equivalent of tritosyl diethanolamine 211 in DMF at 100 °C. This gave 1,4,7-tri(p-toluenesulfonyl)-1,4,7-triazacyclodecane 212 in 59% yield. Removal of the tosyl groups was accomplished by the conc. H\textsubscript{2}SO\textsubscript{4} method.\textsuperscript{77,78} Heating a solution of 212 in 97% H\textsubscript{2}SO\textsubscript{4} gave the desired triamine 208 after workup with NaOH.

The reaction of 1,4,7-triazacyclodecane 208 and N,N'-carbonyldiimidazole\textsuperscript{192} (CDI) 213 in THF was used to prepare bicyclic...
urea 199 (Scheme 1.11).

Scheme 1.11

\[
\begin{align*}
\text{213} + \text{208} & \xrightarrow{\text{THF, 50°C (30%)}} \text{199}
\end{align*}
\]

Wang\textsuperscript{173} used this procedure to prepare bicyclic urea 197. It was based upon Wright's procedure\textsuperscript{193} for conversion of 1,2-diamines into five-membered ureas. Column chromatography was necessary to completely separate imidazole from the desired bicyclic urea 199. The chromatographed material was further purified by sublimation and then recrystallized (EtO/pentane) to give pure 199. Compound 199 was characterized by IR, elemental analysis, mass spectrometry, \textsuperscript{1}H, and \textsuperscript{13}C NMR. In one run, a twofold excess of N,N'-carbonyldiimidazole (213) was used with respect to triamine 208. This resulted in the isolation of N-imidazolyl bicyclic urea 214 in 75\% yield after chromatography. Compound 214 was characterized by high resolution mass spectroscopy (HRMS), IR, \textsuperscript{1}H, and \textsuperscript{13}C NMR.
B. Identification of Isomer 199 by NMR Spectroscopy

The $^{13}$C NMR spectrum ($\text{C}_6\text{D}_6$, 90.56 MHz) of 199 exhibited eight sharp lines, which is consistent with bicyclic urea 199 rather than the alternative bicyclic urea 215 since the latter would be expected to exhibit five sharp lines in the $^{13}$C NMR as result of $C_5$ symmetry.

![Structures 199 and 215]

The upfield methylene ($\text{C}_6$-26.44 ppm) and carbonyl carbon ($\text{C}_1$-171.05 ppm) are readily assignable but further assignments required additional experiments. COSY and HETCOR spectra of 199 are shown in Figures 1.1, 1.2, and 1.3. The $^1$H NMR of 199 in $\text{C}_6\text{D}_6$ is complex but interpretable (see experimental section-preparation of 199). The 2D-NMR analysis (COSY, HETCOR) allowed the assignment of carbons $\text{C}_5$ and $\text{C}_7$. The remaining carbons could be assigned in pairs.
Figure 1.1

C₆D₆
as shown in Table 1.

Table 1.1: $^{13}$C NMR Assignments of Urea 199.

<table>
<thead>
<tr>
<th>Carbon #</th>
<th>$^{13}$C Chemical Shifts (δ, ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₂, C₃</td>
<td>42.33, 48.76</td>
</tr>
<tr>
<td>C₉, C₁₀</td>
<td>45.89, 52.10</td>
</tr>
<tr>
<td>C₅, C₇</td>
<td>46.10, 51.20</td>
</tr>
</tbody>
</table>

C. Dynamic NMR Experiments

A series of NMR experiments were performed in order to better understand the behavior 199 exhibited in solution and in the presence of an acid catalyst.

1. Enantiomerization Study of Urea 199 in D₂O.

A sample of 199 was dissolved in D₂O and the pD of the solution was measured using universal pH paper (pD 8-9). The $^1$H and $^{13}$C NMR spectra of this sample were acquired immediately, after 48 hours, 1 week, and 3 weeks. The pD of the mixture was measured (pH electrode) immediately, after 48 hours, 1 week, and 3 weeks. Figure 1.4 shows the $^1$H NMR spectrum of the mixture soon after mixing. It is clear that a significant amount of guanidinium salt 2 formed.

234
initially.

\[
\begin{align*}
216 \ X &= \text{OH}^- \\
217 \ X &= \text{BPh}_4^-
\end{align*}
\]

The two multiplets corresponding to the upfield methylene in 199 remained sharp (peaks at \(-1.30\) ppm and \(-1.80\) ppm) while dynamic broadening was observed between \(2.50\) and \(4.00\) ppm. The dynamic broadening is due the rapid enantiomerization shown in Scheme 1.12.

Scheme 1.12

The two interconverting species are in fact enantiomers, since the urea functionality cannot "tuck through" the 9-membered ring (on the NMR timescale). The pD of the sample was measured after two hours (pD 13.8) using an pH meter (pH = pD + 0.4194) equipped with a microelectrode as described in the experimental section. The dramatic change in pD after two hours is most likely due to the formation of guanidinium salt 216. The counterion in this case is
hydroxide, which would raise the pH should a significant amount of 2 1 6 form. Figure 1.5 shows the 1H NMR spectrum of the mixture after 48 hours at room temperature.

The 1H NMR spectrum of this mixture after 1-3 weeks (Figures 1.6 and 1.7) show little change except for the formation of a hydrolysis product which is triamine 2 0 8 (labelled in Figure 1.7).

Figure 1.8 shows the 13C NMR spectrum of the mixture of 1 9 9 and D2O soon after mixing. It is worthy to note that the upfield methylene C₆ and the carbonyl carbon C₁₁ in 1 9 9 remained sharp while the remaining six carbons were significantly broadened. The relative proportions (~1:1) of urea 1 9 9 and guanidinium ion 2 1 6 are based upon 13C NMR line intensity measurements of C₃ in 2 1 6 and C₆ in 1 9 9. The 13C NMR spectrum, acquired 48 hours (Figure 1.9) after mixing the solution, also indicated that the guanidinium ion was the major component after 48 h. After 1-3 weeks (Figures 1.10 and 1.11) 13C NMR spectrum showed the appearance of a small amount of hydrolysis product 2 0 8 (Figure 1.11). Guanidinium salt 2 1 7 was isolated by addition of one equivalent of NaBPh₄ (in 1 mL MeOH) to the NMR sample, causing the precipitation of the salt 2 1 7.
Figure 1.7

- Compound in D$_2$O
- 3 weeks
- pD 13.9

Figure 1.8

- Compound in D$_2$O
- pD 8-9

Figure 1.9

- Compound in D$_2$O
- 48 h
- pD 13.8

PPM

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Figure 1.10

![Chemical structure](image1)

- N_{2}N_{2}
- in D_{2}O
- 1 week
- pD 13.6

Figure 1.11

![Chemical structure](image2)

- N_{2}N_{2}
- in D_{2}O
- 3 weeks
- pD 13.9
- 208
Tetraphenylborate salt 217 was isolated by filtration and recrystallized from acetonitrile.

Table 1.2: $^{13}$C and $^1$H NMR Assignments of 217 (CD$_3$CN).

<table>
<thead>
<tr>
<th>$^{13}$C Chemical Shifts (δ, ppm)</th>
<th>$^1$H Chemical Shifts (δ, ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>23.22 (C$_3$)</td>
<td>2.08 (p, 2H, $J = 5.7$ Hz)</td>
</tr>
<tr>
<td>42.90 (C$_2$, C$_4$)</td>
<td>3.22 (t, 4H, $J = 5.8$ Hz)</td>
</tr>
<tr>
<td>47.86 (C$<em>6$, C$</em>{10}$)</td>
<td>3.48 (t, 4H, $J = 7.5$ Hz)</td>
</tr>
<tr>
<td>55.57 (C$_9$, C$_7$)</td>
<td>3.91 (t, 4H, $J = 7.5$ Hz)</td>
</tr>
<tr>
<td>166.02 (C$_{11}$)</td>
<td></td>
</tr>
</tbody>
</table>

Alder et al.$^{186}$ has reported the preparation of mesylate salt 218 by a route shown in Scheme 1.13.

Scheme 1.13

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1H and 13C NMR spectral evidence was consistent with the formation of 217 by comparison to the 1H and 13C spectra of 218.

2. Enantiomerization of Urea 199 in D2O-A pH Study

In this experiment, 199 was dissolved in NaOD (12 M, 40 wt. % in D2O, pH 14), the pH was measured with a micro pH meter (pH = pH + 0.4), and the 1H and 13C NMR spectra were recorded. The pH of this solution was then adjusted with DCI/D2O (20% DCI solution in D2O) and 1H and 13C NMR spectra were recorded. The pH was measured with a micro pH meter before and after each NMR experiment. There were a total of five different NMR experiments performed in this study (Figures 1.12-1.22). The results of the pH measurements are summarized in Table 1.3.

Table 1.3

<table>
<thead>
<tr>
<th>Exp. #</th>
<th>pH before</th>
<th>pH after</th>
<th>1H, 13C NMR figs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13.45</td>
<td>13.41</td>
<td>Fig. 1.12,1.18</td>
</tr>
<tr>
<td>2</td>
<td>12.56</td>
<td>12.89</td>
<td>Fig. 1.13,1.19</td>
</tr>
<tr>
<td>3</td>
<td>9.67</td>
<td>12.76</td>
<td>Fig. 1.14,1.20</td>
</tr>
<tr>
<td>4</td>
<td>8.23</td>
<td>7.70</td>
<td>Fig. 1.15,1.21</td>
</tr>
<tr>
<td>5</td>
<td>1.00</td>
<td>1.12</td>
<td>Fig. 1.16,1.22</td>
</tr>
</tbody>
</table>
Figures 1.12 and 1.13 (1H NMRs, high pD) show the formation of guanidinium salt 216 and dynamic exchange broadening of urea 199. The NMR spectra shown in Figures 1.13, 1.14 and 1.15 indicate the magnet of the instrument was poorly shimmed, and as a result do not provide an accurate indication of broadening. However, it is obvious that as the pD was lowered, the amount of guanidinium salt 216 increased. At pH below 8, 216 (X = Cl-) is the dominant component in the mixture (Figures 1.15 and 1.16).

Both the 1H (Figure 1.12) and 13C NMR (Figure 1.18) spectra indicated that enantiomerization of 199 was occurring and that guanidinium salt 216 formed in D2O even at pD>12. At pD values lower than 12 guanidinium salt 216 predominates (Figures 1.20, 1.21, and 1.22). The small amount of remaining 199 is not exchange
Figure 1.15

Figure 1.16

Figure 1.17

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broadened since N-protonation shuts off enantiomerization. This NMR sample was then adjusted to pH14 with aq NaOD which caused a phase separation in the NMR tube. Removal of the organic phase was accomplished by extraction with C$_6$H$_6$. Combined organic extracts were dried (Na$_2$SO$_4$) and concentrated under reduced pressure to give pure 199 (50% recovery from original NMR sample, $^1$H NMR-Figure 1.17). The observed phase separation seemed odd since urea 199 was shown to be soluble in D$_2$O at pH 13.5. The observed phase separation was probably due to the high concentration of NaCl in the NMR sample, since 12 M NaOD/D$_2$O was titrated with 20% DCI/D$_2$O. In other words, “salting out” occurred.


$^1$H and $^{13}$C NMR spectra of 199 in C$_6$D$_6$ gave no indication of any dynamic broadening ($^1$H NMR-Figure 1.23, and $^{13}$C NMR-Figure 1.25). Figure 1.26 shows the $^{13}$C NMR spectrum of this mixture immediately after the addition of mesitoic acid (2,4,6-trimethylbenzoic acid). Unfortunately, the $^1$H NMR spectrum of sample 199 directly after the addition of acid was not obtained. Mesitoic acid was chosen as a proton source, since it is soluble in...
benzene and since the conjugate base is a poor nucleophile and should not react with bicyclic urea 199. The spectrum shows peaks arising from 199 and 221. The upfield methylene C6 (26.47 ppm) and the carbonyl carbon C11 (171.05 ppm) of 199 remained sharp while the remaining six carbons were broadened (13C NMR-Figure 1.26).

Carbons C6 and C11 in 199 remained sharp after addition of acid due to the fact that these two nuclei do not exchange during the enantiomerization. The broadening of the other six nuclei of 199 indicates that the enantiomerization process was in intermediate slow exchange with respect to the NMR timescale. At the fast exchange limit the six broad peaks should coalesce into three peaks which result from time averaged C6 symmetry. However, this was not observed. The peaks corresponding to guanidinium salt 221 all remained sharp. After one week at room temperature the 1H and 13C
NMR spectra indicated that there was a very small amount of the urea left (1H NMR-Figure 1.24, and 13C NMR-Figure 1.27). The addition of more mesitoic acid eventually converted the remaining 199 into guanidinium salt 221.

D. Dynamic NMR Simulations.

The appearance of broad peaks in the 13C NMR spectrum shown in Figure 1.28 (an expansion of the spectrum of Fig.1.26) allowed the estimation of a rate constant for the observed enantiomerization by computer simulation of the DNMR spectrum. In order to simulate the spectrum, the 13C chemical shifts of urea 199 in the slow exchange limit must be known. Upon enantiomerization, the following nuclear positions will exchange in compound 199: C2 and C10, C3 and C9, C7 and C5. Only C5 and C7 could unequivocally be assigned, as discussed earlier. So, these two 13C NMR peaks (C5 and C7) were chosen for simulation as a two-site mutual exchange system for compound 199. A first order rate constant of 90 ± 15 s⁻¹ (0.22 M mesitoic acid concentration) was calculated for the enantiomerization, which was simulated by the Complete Band Shape Analysis195 (CBS) method using NMREX196 a dynamic multisite NMR simulation program (Figure 251.)
Figure 1.28

Expansion of $^{13}$C NMR spectrum of Figure 1.26

Simulated

Chemical Shift | Population | T2   | k12 |
---            |           | ---  | ---  |
4168.84       | 0.5        | 0.08095 | 90   |
4628.59       | 0.5        | 0.08095 | 90   |

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1.28, bottom). The population of each site is $1/2$, which for the
two-site exchange system utilizes the algorithm developed by
Sandstrom. The effective transverse relaxation time was
estimated from the reciprocal of the product of $\pi$ and the full width
at half height ($\Delta \nu = 3.9$ Hz) of the methyl signal from mesitoic acid.

E. Infrared Analysis of Bicyclic Ureas.

The carbonyl stretching frequency of cyclic ureas 222198 and
223 are 1685 cm$^{-1}$ (neat), and 1681 (neat) cm$^{-1}$, respectively. By
constraining a urea functional group (199) in a bicyclic framework,
the degree of frequency lowering due to resonance is decreased,
since the urea functional group is forced to adopt a non-planar
geometry. The stretching frequency of the carbonyl in bicyclic
urea 224 is 1700 cm$^{-1}$ (KBr). The greater stretching frequency
observed for 224 is a result of poorer OCN conjugation.
The bicyclic framework forces the urea functional group in 224 to adopt a non-planar geometry. Compound 199 has a carbonyl stretching frequency of 1689 cm\(^{-1}\) (KBr) probably at least partly reflecting the resonance contribution of the transannular nitrogen, as was also observed with compound 197 (CO stretch at 1675 cm\(^{-1}\) (KBr)).\(^{173}\)

F. Semiempirical Calculations

Computer models are valuable for the investigation of interactions between atoms in a molecule.\(^{200-205}\) AM1\(^{206}\) and PM3\(^{207}\) semi-empirical calculations were used to model A and potential reaction intermediates in the observed acid catalyzed topomerization of compound A. Compound A has some unique
properties that make it a good candidate for study. The observed IR stretching frequency of the carbonyl in A is shifted towards lower frequency when compared to its bicyclic analog I. This is largely due to the partial bonding of the amine nitrogen transannular to the urea carbonyl as discussed previously. The interaction decreases the \( \pi \) bond order of the carbonyl. The goal of these calculations was to determine the extent of this attraction using computational chemistry.

1. Results of AM1 and PM3 Calculations

Calculations on O-protonated and N-protonated bicyclic urea A, and guanidinium ion F or G were performed with geometry optimization. The heats of formation, C=O bond vibrational frequencies, NCCN torsional angles, and N\ldots C=O transannular distances (where applicable) for all calculated compounds are shown in Tables 1.4 and 1.5. The structures shown in Figure 1.29 were built on a Silicon Graphics workstation using the Spartan molecular modelling package (version 2.0) which uses the standard geometrical parameters of the Tripos force field. Force-field minimized structures served as starting points for the AM1 and PM3 geometry
optimizations. The calculations were carried out without solvation and must be considered "gas phase" results. The intent of this study is merely an overview and a detailed treatment of the data will not be pursued. The results of the AM1 calculations are shown in Table 1.4 and the results of the PM3 calculations are shown in Table 1.5. A list of the calculated structures is shown below.
Figure 1.29

Structure Key to Figure 1.29

A-Bicyclic urea (Cs)
B-Protonation of A on oxygen (Cs)
C-Protonation of A on urea nitrogen (C₁)
D-Protonation of A on amine nitrogen (C₁)
E-Protonation of pseudo $C_3$ symmetric J ($C_1$)

F-Guanidinium ion + $\Delta H^\circ f$ of $H_2O$ (flat nitrogens sp$^2$ hybridized-$C_3h$,

note: The $\Delta H^\circ f$ calculated by AM1 was -59.24 Kcal/mol,

the $\Delta H^\circ f$ calculated by PM3 was -53.43 Kcal/mol)

G-Guanidinium ion + $\Delta H^\circ f$ of $H_2O$ (pyramidalized nitrogens-$C_3$)

H-Ketene iminium ion (9-membered ring in starting structure was in a [333] conformation)

I-Bicyclic urea without transannular nitrogen

J-Hydroxyorthoamide

K-(sp$^2$ hybridized nitrogens were flat)

L-N-Methyl bicyclic urea ($C_5$)

M-N-Methyl bicyclic urea (alkylated at oxygen) $C_1$

N-Methoxyorthoamide (pseudo $C_3$ symmetric)

O-N-Methyl bicyclic urea (alkylated at oxygen-CHOCN = gauche,

Me bisects NCN.
Table 1.4

Summary of Semiempirical (AM1) Calculations

<table>
<thead>
<tr>
<th>Input structure #</th>
<th>Heat of formation (kcal/mol)</th>
<th>CO bond vib. frequency (cm⁻¹)</th>
<th>N--CO dist. in Å (trans-annular)</th>
<th>NCCN torsional angles*</th>
<th>Final structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>-5.057</td>
<td>1728</td>
<td>2.741</td>
<td>-55.3/+55.3</td>
<td>A</td>
</tr>
<tr>
<td>B</td>
<td>151.971</td>
<td>1696</td>
<td>2.468</td>
<td>-45.9/+48.4</td>
<td>B</td>
</tr>
<tr>
<td>C</td>
<td>147.709</td>
<td>2112</td>
<td>2.654</td>
<td>-58.0/+46.1</td>
<td>C</td>
</tr>
<tr>
<td>D</td>
<td>151.464</td>
<td>2056</td>
<td>2.83</td>
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<tr>
<td>E</td>
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<td>-</td>
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<td>G</td>
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<td>-</td>
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<td>H</td>
<td>154.627</td>
<td>1974</td>
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<td>similar to A</td>
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<td>K</td>
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<tr>
<td>M</td>
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<td>1724</td>
<td>2.514</td>
<td>-47.1/+50.5</td>
<td>O</td>
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<tr>
<td>N</td>
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<td>1.527</td>
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<tr>
<td>O</td>
<td>165.484</td>
<td>1796</td>
<td>1.644</td>
<td>-16.3/+16.4</td>
<td>N</td>
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</table>

* The NCCN torsional angles given are $\phi_N$(urea)-C-C-N(amo) or the corresponding torsional angles in related structures.
Table 1.5

Summary of Semiempirical (PM3) Calculations

<table>
<thead>
<tr>
<th>Input structure #</th>
<th>Heat of formation (kcal/mol)</th>
<th>CO bond vib. frequency cm⁻¹</th>
<th>N--CO distance in Å (transannular)</th>
<th>NCCN torsional angles*</th>
<th>Final structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>-31.94</td>
<td>1696</td>
<td>2.824</td>
<td>-59.2/+59.2</td>
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<tr>
<td>C</td>
<td>131.52</td>
<td>1729</td>
<td>2.663</td>
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<td>1.502</td>
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<td>O</td>
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<td>1965</td>
<td>1.599</td>
<td>-19.2/+19.2</td>
<td>O</td>
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</tbody>
</table>

* The NCCN torsional angles given are φN(urea)-C-C-N(amino) or the corresponding torsional angles in related structures.

2. Discussion of AM1 and PM3 Calculations

a. AM1 Calculations. With AM1 the protonation of either the
oxygen or the urea nitrogen of A resulted in a change in the N4 to C10 transannular distance indicating a transannular attraction. Ketene imine H is also a possible intermediate in the topomerization pathway. An AM1 geometry optimization of input structure H did not significantly change the transannular N···C=O distance. Compounds F or G are very high in energy due to strain according to AM1. N-methyl urea L was also modeled in a similar fashion to that of A. In this example L was methylated at oxygen to give M which was then calculated. The resulting structure exhibited shortening of the N···C=O transannular distance.

b. PM3 Calculations. The results of PM3 reflect a significant transannular interaction between atoms N4 and C10. Upon protonation at oxygen structure B closed up to structure E. Upon protonation at nitrogen structure C also closed up to structure E however the transannular distance was longer by 1.0Å compared to the O-protonation result. Calculation of amine protonated urea D actually increased the transannular N···C=O. Calculation of ketene imine intermediate H results in a minimized structure that also closely resembles closed structure E. Compound F and G is very high

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in energy due to strain according to PM3. Analogous bicyclic urea without the transannular nitrogen exhibits no interaction (C\cdash C=O is 3.0Å). N-methyl urea L was modeled in a similar fashion to that of A. In this example L was methylated at oxygen to give structure M which was calculated to give a structure (C_s symmetry) in which the methyl group bisects the NCN linkage. The resulting structure O had a short N\cdash C=O transannular distance and very closely resembled closed structure N.

G. Ab Initio Calculations: Conformational Studies of A.

Ab initio calculations\textsuperscript{208} were used to begin to evaluate the relative energies of conformations of urea A. Three main conformations were constructed: boat-chair A\textsubscript{1} (Figure 1.30), boat-boat A\textsubscript{2} (Figure 1.31), and the chair-chair A\textsubscript{3} (Figure 1.32) or crown conformation. These three conformations were built on a Silicon Graphics workstation using the Spartan molecular modelling package (version 2.0) which uses the Tripos force field. HF/3-21G//3-21G calculations were performed on these conformations and some of the important results are shown in Table 1.6. The ring tautomer bicyclic urea J (Figure 1.33) was also calculated in this study.
Figure 1.33
3-21G Geometry Optimized Structure J

Figure 1.32
3-21G Geometry Optimized Structure A3

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Table 1.6: Results of Ab Initio Calculations

<table>
<thead>
<tr>
<th>Compound Number</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>J</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative Energy kcal/mol</td>
<td>_</td>
<td>27.74</td>
<td>0.00</td>
<td>7.98</td>
</tr>
<tr>
<td>Point Group</td>
<td>C1</td>
<td>Cs</td>
<td>Cs</td>
<td>C1</td>
</tr>
<tr>
<td>Dihedral O-C$_1$-N-C$_1$</td>
<td>_</td>
<td>-118.3°</td>
<td>-171.5°</td>
<td>_</td>
</tr>
<tr>
<td>N...C=O distance</td>
<td>_</td>
<td>2.29Å</td>
<td>2.63Å</td>
<td>_</td>
</tr>
<tr>
<td>Mulliken (Lowdin)</td>
<td>_</td>
<td>1.88(2.12)</td>
<td>1.74(1.98)</td>
<td>_</td>
</tr>
<tr>
<td>C=O</td>
<td>_</td>
<td>0.08 (0.12)</td>
<td>0.03 (0.36)</td>
<td>_</td>
</tr>
</tbody>
</table>

Conformer A2 was 28 kcal higher in energy than A3. Ring tautomer J was 8 kcal higher in energy than conformer A3 and 20 kcal lower in energy than conformer A2. The amine nitrogen in A2 is essentially flat. The amine nitrogen in conformer A3 is inverted and the hydrogen points toward the carbonyl oxygen. The Mulliken bond order matrix gave values of 1.88 and 1.73 for the carbonyl in A2 and
A3 respectively. The Mulliken bond order values for the amine nitrogen/carbonyl carbon interaction were 0.08 for A2 and 0.03 for A3. The interatomic distances between N4 and C10 were 2.29Å and 2.63Å for conformers A2 and A3. An attempt to submit the boat-chair conformer A1 for a geometry optimization HF/3-21//HF/3-21G was unsuccessful (convergence was not achieved after two days so the calculation was stopped).

Of the two conformers calculated A3 was lower in energy by 28 kcal/mol than A2. The short transannular distance observed for A2 strongly suggests that a transannular attraction exists and that intermediate J is certainly plausible for the topomerization reaction A undergoes as observed experimentally.

The Mulliken bond order results are consistent with the rehybridization of urea carbonyl. The lone pair electrons on the amine nitrogen change the sp\(^2\) hybridized CO towards sp\(^3\) hybridization. The detectable Mulliken bond order values between atoms N4 and C10 support a transannular interaction. The interatomic distances between the urea carbonyl carbon and the amine nitrogen were well within the accepted range for typical transannular interactions (2.0-2.5 Å).
The results of these calculations further support the existence of \textit{J} as a likely intermediate for the topomerization observed experimentally by Wang.
PART II. SYNTHESIS AND DYNAMIC NMR STUDIES OF BICYCLIC UREAS

By

Daniel C. Hill

B.S. University of Southern Maine, 1990

DISSERTATION

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Chapter II

Experimental Section

I.UNH

A. General Methods

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

Infrared Spectra (IR) were run on a Nicolet FT IR spectrometer model MX-1.

$^1$H NMR Spectra were taken on a Brüker AM360 FT-NMR spectrometer operating at 360.134 MHz. Chemical shifts are reported in parts per million (ppm) relative to internal Me$_4$Si unless otherwise noted and coupling constants (J values) are in Hertz (Hz).

$^{13}$C NMR Spectra were taken on a Brüker AM360 FT-NMR spectrometer operating at 90.556 MHz. Chemical shifts are reported in parts per million (ppm) relative to internal Me$_4$Si unless otherwise noted.

Low-Resolution Mass Spectra (MS) were obtained on a Hewlett Packard 5988A GC/MS (using the direct insertion probe) at the
University Instrumentation Center of UHN.

**High-Resolution Mass Spectra** (HRMS) were obtained from either the Dupont/Merck Experimental Station in Wilmington, Delaware or the Mass Spectroscopy Center at the University of Nebraska, Lincoln. **CHN Analysis** were obtained on a Perkin-Elmer Series II CHNS/O elemental analyzer model 2400 at the University Instrumentation Center of UNH.

**B. Solvents**

**Absolute ethanol** (100% EtOH) was obtained from AAPER Alcohol and Chemical Co.

**Acetonitrile** (CH₃CN) was obtained from EM Science. It was distilled from CaH₂ and stored over 3Å molecular sieves.

**Benzene** (C₆H₆) was obtained from J.T. Baker and was distilled prior to use.

**Carbon tetrachloride** (CCl₄) was obtained from Eastman Kodak Co. and distilled prior to use.

**Chloroform** (CHCl₃) was obtained from EM Science and distilled prior to use.

**Deuterated NMR Solvents** were used as is from Cambridge Isotope

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Laboratories and stored over 3Å molecular sieves. DMSO-d$_6$ was
distilled from CaH$_2$ and stored over 3Å molecular sieves.

**Diethyl ether** (Et$_2$O) was obtained from EM Science. It was distilled
from purple sodium benzophenone ketyl prior to use.

**Dimethylformamide** (DMF) was obtained from J.T. Baker. It was
distilled from CaH$_2$ and stored over 3Å molecular sieves.

**Ethanol** (95% EtOH) was obtained from AAPER Alcohol and Chemical
Co.

**Glacial acetic acid** (HOAc) was obtained from Fisher Chemical Co.

**Methanol** (MeOH) was obtained from Fisher Chemical Co. It was
distilled and stored over 3Å molecular sieves.

**Methylene chloride** (CH$_2$Cl$_2$) was obtained from J.T. Baker. It was
distilled from CaH$_2$ and stored over 3Å molecular sieves.

**Pyridine** (py) was obtained from Fisher Chemical Co. It was distilled
from CaH$_2$ and stored over 3Å molecular sieves.

**Tetrahydrofuran** (THF) was obtained from EM Science. It was
distilled from purple sodium benzophenone ketyl prior to use.

**Toluene** (PhCH$_3$) was obtained from EM Science. It was distilled from
Na° and stored over 3Å molecular sieves.
C. Reagents

Acetic Anhydride (AcOAc) was obtained from Fisher Chemical Co. It was distilled prior to use.

Acrylonitrile was obtained from Aldrich Chemical Co. and distilled prior to use.

Benzophenone was obtained from Aldrich Chemical Co.

Benzyl bromide (PhCH₂Br) was obtained from Aldrich Chemical Co.

Borane-tetrahydrofuran complex (BH₃·THF, 1.0 M solution in tetrahydrofuran) was obtained from Aldrich Chemical Co.

N-Bromosuccinimide (NBS) was obtained from Eastman Kodak Co.

Calcium hydride (CaH₂) was obtained from Alfa Products Inc.

1,1'-Carbonyldiimidazole was obtained from Aldrich Chemical Co.

Celite was obtained from VWR Scientific.

Cesium carbonate (Cs₂CO₃) was obtained from Cabot Chemical Co.

o-Cresol was obtained from Eastman Kodak Co. It was purified by reduced pressure distillation.

1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) was obtained from Aldrich Chemical Co. It was purified by reduced pressure distillation prior to use.
Ethylbromoacetate was obtained from Aldrich Chemical Co.

Glyoxal (40 wt. % solution in H₂O) was obtained from Aldrich Chemical Co.

Hydrochloric acid (12 M HCl) was obtained from Fisher Chemical Co.

Lithium perchlorate (LiClO₄) was obtained from J.T. Baker.

Methoxyacetic acid was obtained from Fluka Chemie AG.

Methyl acrylate was obtained from Aldrich Chemical Co. It was distilled prior to use to remove inhibitor.

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

10% Palladium on carbon (10% Pd/C) was obtained from Engelhard Minerals and Chemical Corp.

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

Potassium iodide (KI) was obtained from Fisher Chemical Co.

Raney nickel (W-2) was prepared by the method of Monzingo from Raney Nickel catalyst powder 2813 obtained from Grace Davison Chemical.

Sea Sand was obtained from Fisher Chemical Co.

Sodium borohydride (NaBH₄) was obtained from Alfa Products Inc.

Sodium carbonate (Na₂CO₃) was obtained from Fisher Chemical Co.

Sodium hydroxide (NaOH) was obtained from Fisher Chemical Co.
Sodium tertaphenylborate (NaBPh₄) was obtained from J.T. Baker. Sulfuric acid (97% H₂SO₄) was obtained from J.T. Baker.

1.4.7.10-Tetraazacyclododecane (cyclen) was graciously prepared by Fagan. The tetra-p-toluenesulfonyl derivative was prepared by the Kellogg Cs₂CO₃ modification⁷⁵,⁷⁶ of the cyclization procedure of Richman and Atkins.⁷⁷ The detosylation procedure was based upon that of Raymond.⁷⁷,⁷⁸

1.4.8.11-Tetraazacyclotetradecane (cyclam) was obtained from Strem Chemical Co.

1.4.7.11-Tetraazacyclotetradecane (isocyclam) was graciously prepared by Fagan. The tetra-p-toluenesulfonyl derivative was prepared by the Kellogg Cs₂CO₃ modification⁷⁵,⁷⁶ of the cyclization procedure of Richman and Atkins.⁷⁷ The detosylation procedure was based upon that of Raymond.⁷⁷,⁷⁸

1.4.7.10-Tetraazacyclotridecane was prepared as described in the experimental procedures section of this chapter.

1.4.8.11-Tetraazatricyclo[9.3.1.1⁴,⁸]hexadecane was prepared previously by North.¹⁴² The product was purified by sublimation before use.

Thiirane (ethylene sulfide) was obtained from Aldrich Chemical Co.
Thionyl chloride (SOCl₂) was obtained from J.T. Baker. It was distilled prior to use.

Triethylamine (Et₃N) was obtained from Fisher Chemical Co. It was distilled from CaH₂ and stored over 3Å molecular sieves.

2,4,6-Trimethylbenzoic acid (mesitoic acid) was recrystallized from CCl₄ before use.

Trityl chloride was obtained from Aldrich Chemical Co.

D. Column Chromatography Solid Supports.

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

Florisil: Florisil (100-200 mesh) “suitable for chromatography” was obtained from Fisher Chemical Co.

E. Experimental Procedures

1,4,7-Tri-p-toluenesulfonyl-1,4,7-triazacyclodecane (212). 1,4,7-Tri-p-toluenesulfonyl-1,4,7-triazacyclodecane was prepared by the Kellogg Cs₂CO₃ modification⁷⁵,⁷⁶ of the cyclization procedure of Richman and Atkins.⁷⁷ The crude product was

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recrystallized from CH₂Cl₂/hexane to yield white powder (59%): mp 233-236 °C; (lit. mp 237 °C)

1,4,7-Triazacyclodecane (208). Compound 208 was prepared by a detosylation procedure based upon that of Raymond. A stirred solution of 1,4,7-tri-p-toluene-sulfonyl-1,4,7-triazacyclodecane (9.772 g, 16.10 mmol) in 97% H₂SO₄ (50 mL) was heated to 100 °C under N₂ atmosphere for 112 h. To the cooled solution (0-5 °C), 80 mL of absolute EtOH was slowly added, and followed by 120 mL of anhydrous Et₂O. The precipitated product was filtered under nitrogen, dissolved in H₂O (50 mL), adjusted to pH 14 with solid KOH (with cooling), and extracted with CHCl₃ (6 x 75 mL). The combined extracts were dried (Na₂SO₄), and solvent was removed under reduced pressure. The crude product was kugelrohr distilled (0.25 mmHg, 120 °C air bath temperature) to give an oil (1.95 g, 85%); ¹H NMR (360 MHz, CDCl₃) δ 1.60 (s, 2H, J = 5.5 Hz, CH₂CH₂CH₂), 2.35 (br s, 3H, NH), 2.68-2.77 (m, 4H), 2.78-2.86 (m, 4H), 2.91 (t, 4H, J = 5.4 Hz); ¹³C NMR (90.56 MHz, CDCl₃) δ 26.16 (CH₂CH₂CH₂), 46.05, 47.40, 48.70. Compound 208 was prepared by
Johnson. The trihydrobromide salt of 208 has previously been reported.

**1,4,8-Triaza-(11-oxo)-bicyclo[6.2.1]undecane (199).** A 1,1-carbonyldiimidazole solution (2.32 g, 14.31 mmoL in 50 mL THF) was added dropwise over 2.5 h, under a nitrogen atmosphere, to a solution of 1,4,7-triazacyclononane (2.05 g, 14.3 mmoL) in THF (25 mL) at 65 °C. The reaction mixture was then heated an additional 1.5 h at 65 °C. Solvent was removed under reduced pressure to give an oil which was flash chromatographed on basic alumina (2% EtOH/CH₂Cl₂). The fractions containing primarily one spot by TLC (R_f=0.59) were combined and flash chromatographed again on florisil (2% EtOH/CH₂Cl₂, R_f=0.63). Purified product was sublimed (0.02 mmHg, 70 °C oil bath temperature) and then recrystallized from Et₂O/Pentane (1:1) to give white crystals (0.729 g, 30%): mp 56-58°C; ¹H NMR (360 MHz, C₆D₆) δ -0.15 (br s, 1H, NH), 0.78 (dtt, 1H, J = 15.0, 4.0, 1.6 Hz, H₅-equ), 1.36 (dtdd, 1H, J = 15.0, 12.5, 4.3, 2.2 Hz, H₅-ax), 2.25 (ddd, 1H, J = 13.9, 9.6, 7.0 Hz), 2.50-2.60 (m, 2H), 2.64 (ddt, 1H, J = 14.3, 4.3, 1.6 Hz), 2.69 (ddd, 1H, J = 13.6, 12.3, 1.4 Hz),
2.86 (dd, 1H, \( J = 9.8, 8.4, 4.7 \) Hz), 2.89-3.00 (m, 2H), 3.08 (dd, 1H, \( J = 11.2, 8.3, 7.0 \) Hz), 3.24 (td, \( J = 9.5, 7.0 \) Hz), 3.58 (dd, 1H, \( J = 13.9, 7.1, 1.6 \) Hz), 4.22 (dd, 1H, \( J = 14.2, 12.8, 3.9 \) Hz); \(^{13}\)C NMR (90.56 MHz, \( \mathrm{C}_6\mathrm{D}_6 \)) \( \delta \) 26.44 (CH\(_2\)CH\(_2\)CH\(_2\)), 42.34, 45.90, 46.10, 48.76, 51.20, 52.09, 171.05 (NCON); IR (KBr) 3360 (NH), 2950, 2925, 2905, 2840, 1689 (C=O), 1480, 1455, 1440, 1405, 1255, 1235, 1170, 1110, 775 cm\(^{-1}\); MS, \( m/z \) 169 (M\(^+\)); Anal Calcd for \( \mathrm{C}_8\mathrm{H}_{15}\mathrm{N}_3\mathrm{O} \): C, 56.78; H, 8.93; N, 24.83. Found: C, 56.50; H, 9.13; N, 24.70.

4-Imidazoly1-11-oxo-1,4,8-triazabicyclo[6.2.1]-undecane (214). A solution of 1,1-carbonyldiimidazole (0.161 mg, 0.990 mmol) in THF (3.0 mL) was added dropwise over 2.5 h, under a nitrogen atmosphere, to a solution of 1,4,7-triazacyclodecane (67.1 mg, 0.469 mmol) in THF (7.0 mL). The reaction mixture was then heated for 3 h at 65 °C. Solvent was removed under reduced pressure to give a solid which was chromatographed on 75 g basic alumina (2% EtOH/\( \mathrm{CH}_2\mathrm{Cl}_2 \), \( R_f=0.35 \)). The purified product was sublimed (0.15 mmHg, 80 °C oil bath temperature) to give white crystals (93.6 mg, 75%): mp 147-148°C; \(^1\)H NMR (360 MHz, CDCl\(_3\)) \( \delta \)
1.20-1.90 (m, 2H, CH$_2$CH$_2$CH$_2$), 2.72-2.91 (dm, 1H, $J = 14.6$ Hz), 3.11-3.24 (m, 1H). 3.25-3.36 (m, 1H), 3.37-3.46 (m, 2H), 3.53 (dd, 1H, $J = 15.7$, 11.0 Hz) 3.63-3.90 (m, 4H), 4.01 (ddd, 1H, $J = 14.7$, 12.5, 3.7 Hz), 4.07-4.18 (ddm, 1H, $J = 15.6$, 6.0 Hz), 7.10-7.20 (m, 1H), 7.31 (t, 1H, $J = 1.4$ Hz), 7.87-8.01 (m, 1H, NCHN); $^{13}$C NMR (90.56 MHz, CDCl$_3$) δ 23.98 (CH$_2$CH$_2$CH$_2$), 42.86, 45.85, 46.98, 47.81, 51.55 (br), 53.29 (br), 117.28 (NCHN), 130.11, 136.36, 152.07 (NCOIm), 168.75 (NCON); MS, $m/z$ 263 (M$^+$); IR (KBr) 3137, 3114, 3097, 2991, 1680 (CO), 1675 (CO), 1491, 1458, 1418, 1350, 1334, 1296, 1275, 1244, 764 cm$^{-1}$; HRMS exact mass calcd for C$_{12}$H$_{17}$N$_5$O$_2$ 263.1382, found 263.1381.

cis-13-1,4,7,10-Tetraazatetracyclo-
[5.5.2.0$^4$,14.0$^1$0.$^1$3]$\text{tetradecane}$ (30). Aqueous glyoxal (1.64 mL 40% (w/w); 11.3 mmol) was added dropwise over 20 min under nitrogen to a stirred solution of 1,4,7,10-tetraazacyclododecane (1.50 g, 8.70 mmoles) in MeCN (90 mL). The reaction mixture was then heated to 50 °C for 16 h. Solvent was removed to give a brown oil which crystallized upon the removal of residual solvent. The crude product was purified by sublimation (80 °C, 0.02 mmHg) to give white powder (1.34 g, 79%): mp 90-92 °C; $^1$H NMR (360 MHz, 282

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CDCl$_3$ $\delta$ 2.52-2.60 (m, 4H), 2.70 (br s, 4H), 2.93-3.02 (m, 8H), 3.14 (s, 2H, CHCH); $^{13}$C NMR (90.56 MHz, CDCl$_3$) $\delta$ 50.39, 51.18, 77.51 (CHCH); IR (CCl$_4$) 2940, 2890, 2830, 2800, 2760, 1315, 1270, 1185, 1070, 850 cm$^{-1}$; HRMS exact mass calcd for C$_{10}$H$_{18}$N$_4$ 194.1522, found 194.1525.

$(1R,7S,13S,14S)$-1,7-Dimethyl-4,10-diaza-1,7-diazeniatetracyclo[5.5.2.0$^{4,1}$.1$^{4,13}$]tetradecane diiodide (31). To a stirred solution of cis-13-1,4,7,10-tetraazatetracyclo-[5.5.2.0$^{4,1}$.1$^{4,13}$]tetradecane (1.45 g, 7.47 mmol) in MeCN (20 mL) at room temperature, was added Mel (10.60 g, 74.69 mmol) all in one portion. The reaction flask was tightly stoppered, shielded from light, and the solution was stirred for 14 days. Precipitated product was collected by suction filtration and washed with MeCN (2 x 5 mL), then CH$_2$Cl$_2$ (2 x 15 mL). Residual solvent was removed under high vacuum to give 3.23 g of white powder (90%): mp 230-240 °C (dec); $^1$H NMR (360 MHz, D$_2$O, MeCN secondary ref. set at 2.06 ppm) $\delta$ 3.02-3.20 (m, 4H), 3.31-3.45 (m, 2H + overlapping s, 6H), 3.60 (td, 2H, $J = 8.6, 4.2$ Hz), 3.65 (dm, 2H, $J = 10.8$ Hz), 3.88 (ddd, 2H, $J = 8$ Hz, $J = 4.2$ Hz, $J = 10.8$ Hz)
12.2, 10.8, 4.2 Hz), 3.95-4.10 (m, 4H), 4.49 (s, 2H, CHCH); $^{13}$C NMR (90.56 MHz, D$_2$O, MeCN secondary ref. set at 1.7 ppm) δ 47.67, 51.10, 51.34 (CH$_3$), 63.73, 69.59, 82.67 (CHCH); IR (KBr) 2995, 2950, 2920, 2900, 2875, 1460, 1450, 1390 cm$^{-1}$. Anal Calcd for C$_{12}$H$_{24}$N$_4$I$_2$: C, 30.14; H, 5.06; N, 11.72. Found: C, 30.43; H, 5.32; N, 12.02.

**4,10-Dimethyl-1,4,7,10-tetraazabicyclo[5.5.2]-tetradecane (24).** To a stirred solution of (1RS,7RS,13SR,14SR)-1,7-dimethyl-4,10-diaza-1,7-diazoniatetracyclo[5.5.2.0$^4$.1$^4$.0$^1$.1$^3$.]tetradecane diiodide (1.30 g, 2.72 mmol) in 95% EtOH (50 mL) was added NaBH$_4$ (1.40 g, 37.00 mmol) in small portions over 20 minutes. The reaction mixture was stirred at room temperature for 4 days. Excess NaBH$_4$ was then decomposed by slow addition of 30 mL 3 M HCl. Evaporation of solvent under reduced pressure gave a white solid which was dissolved in H$_2$O (30 mL), adjusted to pH 14 with solid KOH (with cooling), and extracted with benzene (6 x 15 mL). The combined extracts were dried (Na$_2$SO$_4$), solvent was removed under reduced pressure, and crude product was kugelrohr distilled (80 °C air bath temp, 0.04 mmHg) to give 0.60 g (96%) of a clear oil:

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$^1$H NMR (360 MHz, C$_6$D$_6$) δ 2.27 (s, 6H, CH$_3$), 2.36-2.48 (m, 4H), 2.65-2.85 (m, 12H), 3.05 (s, 4H, NCH$_2$CH$_2$N); $^{13}$C NMR (90.56 MHz, C$_6$D$_6$) δ 44.27 (CH$_3$), 56.98 (NCH$_2$CH$_2$N), 57.35, 60.58; MS, m/z 226 (M$^+$); IR (neat) 2951, 2910, 2848, 2812, 2793, 2747, 1445, 1366, 1105, 1062 cm$^{-1}$; Anal Calcd for C$_{12}$H$_{26}$N$_4$: C, 63.67; H, 11.58; N, 24.75. Found: C, 63.61; H, 11.85; N, 24.36.

1,4,7,10-Tetra-p-toluenesulfonyl-1,4,7,10-tetrazacyclotridecane (53). 1,4,7,10-Tetra-p-toluenesulfonyl-1,4,7,10-tetrazacyclotridecane was prepared by the Kellogg Cs$_2$CO$_3$ modification$^{75,76}$ of the cyclization procedure of Richman and Atkins.$^{77}$ The crude product was triturated with acetone twice to yield white powder (57%): mp 208-210°C (lit. mp 194-197°C); $^1$H NMR (360 MHz, CDCl$_3$) δ 2.05 (~p, 2H, J = 6.5 Hz, CH$_2$CH$_2$CH$_2$), 2.43 (s, 6H, ArCH$_3$), 2.44 (s, 6H, ArCH$_3$) 3.16 (t, 4H, J = 6.5 Hz), 3.28-3.42 (m, 12H), 7.27-7.40 (m, 8H), 7.40-7.55 (m, 8H); $^{13}$C NMR (90.56 MHz, CDCl$_3$) δ 21.50 (4C), 27.04 (1C), 47.48 (2C), 48.95 (2C), 51.60 (4C), 127.49 (1C), 127.77 (1C), 129.79 (1C), 129.85 (1C), 133.58 (1C), 134.31 (1C), 143.74 (1C), 144.02 (1C).
1,4,7,10-Tetraazacyclotridecane (46). Compound 43 was prepared by a detosylation procedure based upon that of Raymond.77

A stirred solution of 1,4,7,10-tetra-p-toluenesulfonyl-1,4,7,10-tetraazacyclotridecane (20.25 g, 25.20 mmol) in 97% H$_2$SO$_4$ (150 mL) was heated to 100 °C under N$_2$ atmosphere for 96 h. To the cooled solution (0-5 °C), 180 mL of absolute EtOH was slowly added, and followed by 510 mL of anhydrous Et$_2$O. The precipitated product was filtered under nitrogen, dissolved in H$_2$O (130 mL), adjusted to pH 14 with solid KOH (with cooling), and extracted with CHCl$_3$ (6 x 50 mL, 2 x 30 mL). The combined extracts were dried (Na$_2$SO$_4$), and solvent was removed under reduced pressure. The crude product was purified by sublimation (0.02 mmHg, 80 °C) to give hydroscopic needles (3.81 g, 81%): mp 36-38 °C (lit. mp 40 °C);$^7$7 $^1$H NMR (360 MHz, CDCl$_3$) δ 1.67 (~p, 2H, J = 5.4 Hz, CH$_2$CH$_2$CH$_2$), 1.80-2.10 (br s, 4H, NH), 2.55-2.85 (m, 16H); $^{13}$C NMR (90.56 MHz, CDCl$_3$) δ 29.01 (CH$_2$CH$_2$CH$_2$), 47.43, 47.64, 48.90, 49.91.

cis-14-1,4,8,11-tetraazatetracyclo-
pentadecane (56). Aqueous glyoxal (3.7 mL 40% (w/w); 25.1 mmol) was added dropwise over 1 h under nitrogen to a stirred solution of 1,4,7,10-tetraazacyclotridecane (3.42 g, 18.36 mmols) in MeCN (100 mL). The reaction mixture was then heated to 60 °C for 2.5 h. Solvent was removed to give a yellow oil, which was dissolved in CHCl₃ and dried (Na₂SO₄). After concentration of the solution, the crude product was purified by flash chromatography on basic alumina (10% EtOH/Et₂O, Rₙ 0.29) to give a clear oil which crystallized upon storage at -10 °C. (1.91 g, 50%): mp 28-30°C; ¹H NMR (360 MHz, CDCl₃) δ 1.20-1.32 (d, 1H, J = 8.1 Hz) 1.90-3.70 (br m, 19H) 2.88 (d, J = 2.8 Hz, CH) 3.37 (d, J = 2.8 Hz, CH)(Note: the ¹H NMR spectrum exhibited severe dynamic exchange broadening.); ¹³C NMR (22.5 MHz, CDCl₃, -39 °C) δ 19.79 (CH₂CH₂CH₂), (Note: the following three pairs of peaks (labelled A, B, and C) each broadened upon warming and coalesced to a singlet by 55 °C; see below) 45.51 and 53.18 (A), 49.35 and 51.82 (B), 51.82 and 54.76 (C), 49.93, 75.39 (CH), 77.74 (CH); ¹³C NMR (22.5 MHz, CDCl₃, 55 °C) δ 20.35, (Note: the following peaks (labelled A, B, and C) correspond to three pairs of coalesced resonances.) 49.82 (br) (A), 50.97 (B), 53.83
(C), 50.41, 76.13 (CH), 78.17 (CH); MS, \( m/z \) 208 (M+); IR (CCl₄) 2930, 2838, 2842, 2795, 2790, 1320, 1278, 1161, 1130, 1112, 1095 cm⁻¹. Anal Calcd for \( C_{n}H_{20}N_{4} \): C, 63.43; H, 9.68; N, 26.90. Found: C, 63.17; H, 9.98; N, 27.02. A minor isomer was also isolated (0.20 g, 5%, \( R_f \) 0.07 10% EtOH/Et₂O); \(^1\)H NMR (360 MHz, CDCl₃) δ 1.40 (dtt, 1H, \( J = 15.0, 5.6, 2.0 \) Hz) 2.02 (dtt, 1H, \( J = 15.0, 10.7, 2.9 \) Hz) 2.45-2.85 (m, 8H) 2.92-3.08 (m, 3H) 3.13 (ddd, 2H, \( J = 13.7, 5.7, 2.6 \) Hz) 3.27-3.40 (m, 2H) 3.59 (s, 2H, CHCH); \(^{13}\)C NMR (90.56 MHz, CDCl₃) δ 25.11, 49.33, 51.31, 51.63, 52.31, 80.94.

\((1RS,8SR,14SR,15SR)-1,8\)-Dimethyl-4,11-diaza-1,8-diazeniatetrayclo[6.5.2.0⁴,15.0¹¹,1⁴]pentadecane diiodide \((62)\). To a stirred solution of \( cis-14-1,4,8,11\)-tetraazatetrayclo-[6.5.2.0⁴,15.0¹¹,1⁴]pentadecane (0.358 g, 1.72 mmol) in MeCN (5 mL) at room temperature, was added Mel (5.7 g, 40 mmol) all in one portion. The reaction flask was tightly stoppered, shielded from light, and the solution was stirred for 13 days. Precipitated product was collected by suction filtration and washed with MeCN (2 x 5 mL). Residual solvent was removed under vacuum to give 0.74 g of white
powder (87%): mp 220-224 °C (dec); $^1$H NMR (360 MHz, D$_2$O, MeCN secondary ref. set at 2.06 ppm) $\delta$ 1.90-2.03 (dm, 1H, $J = 15.3$ Hz, H$_{12}$-eq), 2.37 (dtt, 1H, $J = 15.3$, 13.8, 4.6 Hz, H$_{12}$-ax), 2.71 (td, 1H, $J = 12.3$, 3.0 Hz), 3.01-3.24 (m, 4H), 3.46 (tm, 2H, $J = 11.8$ Hz), 3.35 (s, 3H, CH$_3$), 3.42 (s, 3H, CH$_3$), 3.44-3.64 (m, 2H), 3.69 (td, 1H, $J = 13.5$, 3.8 Hz), 3.81-3.91 (m, 2H), 3.95-4.12 (m, 2H), 4.41 (m, 1H), 4.65 and 4.69 (AB, 2H, $J = 2.1$ Hz, CHCH); $^{13}$C NMR (90.56 MHz, D$_2$O, MeCN secondary ref. set at 1.7 ppm) $\delta$ 19.43, 42.71, 46.34, 46.74, 47.33, 49.12, 50.87, 52.04, 56.95, 64.59, 65.53, 76.98, 78.49; IR (KBr) 2994, 2990, 2965, 2950, 2927, 2909, 2862, 2852, 2843, 2831, 2826, 1418, 1121 cm$^{-1}$. Anal Calcd for C$_{13}$H$_{26}$N$_4$I$_2$: C, 31.73; H, 5.32; N, 11.38. Found: C, 31.66; H, 5.50; N, 11.34. In a separate example when the reaction was run for 3 days an 85% yield of pure 6 2 was isolated.

4,11-Dimethyl-1,4,8,11-tetraazabicyclo[6.5.2]-pentadecane (58). To a stirred solution of (1R S,8S R,14S R,15S R)-1,8-dimethyl-4,11-diaza-1,8-diazoniatetracyclo[6.5.2.0$^4$15.0$^1$11.14]-pentadecane diiodide (0.624 g, 1.27 mmol) in 95% EtOH (20 mL) was added NaBH$_4$ (1.64 g, 43.3 mmol) in small portions. The reaction was
stirred at room temperature for 7 days. Excess NaBH₄ was decomposed by slow addition of 3 M HCl (25 mL). Solvent removal gave a white solid which was dissolved in H₂O (20 mL), adjusted to pH 14 with solid KOH (with cooling), and extracted with toluene (6 x 15 mL). The combined extracts were dried (Na₂SO₄), solvent was removed under reduced pressure, and crude product was kugelrohr distilled (100-130 °C air bath temp, 0.03 mmHg) to give 0.291 g (96%) of a clear viscous oil: ¹H NMR (360 MHz, C₆D₆) δ 1.47 (tt, 2H, J = 6.7, 4.9 Hz, CH₂CH₂CH₂), 2.06 (dt, 1H, J = 12.1, 4.9 Hz), 2.14 (s, 3H, CH₃), 2.29 (s, 3H, CH₃), 2.23-2.37 (m, 2H), 2.39-2.53 (m, 2H), 2.54-2.93 (m, 12H), 2.96 (ddd, 1H, J = 13.6, 5.6, 2.4 Hz), 3.22-3.32 (m, 1H), 3.43 (dt, 1H, J = 12.1, 6.8 Hz); ¹³C NMR (90.56 MHz, C₆D₆) δ 27.91 (CH₂CH₂CH₂), 43.38, 43.88, 54.00, 54.05, 54.69, 55.33, 55.44, 56.78, 57.52, 58.41, 60.57, 61.11; IR (neat) 2949, 2916, 2874, 2854, 2854, 2788, 2784, 2745, 1447, 1363, 1117, 1053 cm⁻¹; HRMS exact mass calcd for C₁₃H₂₉N₄ 241.2392, found 241.2383.

cis-15-1,4,8,12-tetraazatetracyclo-
[6.6.2.0⁴,¹⁶.0¹²,¹⁵]hexadecane (57). Aqueous glyoxal (0.63 mL
40% (w/w); 4.34 mmol) was added dropwise over 20 minutes under nitrogen to a stirred solution of 1,4,7,11-tetraazacyclotetradecane (0.66 g, 2.97 mmol) in MeCN (45 mL). The reaction mixture was then heated to 55 °C for 1.5 h. Solvent was removed under reduced pressure to give a yellow oil which was dissolved in CHCl₃ and dried (Na₂SO₄). After concentration of the solution, the crude product was purified by flash chromatography on basic alumina (10% EtOH/Et₂O, Rf 0.32) to give a clear oil. This oil was kugelrohr distilled (100 °C air bath temperature, 0.03 mmHg) to give pure product, which solidified upon standing (0.353 g, 49%): mp 50-52°C; ¹H NMR (360 MHz, CDCl₃) δ 1.32-1.42 (m, 1H), 1.58 (dddt, 1H, J = 13.6, 7.5, 5.1, 2.5 Hz), 1.87-2.18 (m, 3H), 2.27 (td, 1H, J = 11.7, 2.7 Hz), 2.39-2.70 (m, 6H), 2.77 (ddd, 1H, J = 12.0, 6.8, 1.8 Hz), 2.84 (ddd, 1H, J = 14.0, 7.1, 3.5 Hz), 2.88-2.99 (m, 3H), 2.91 (d, 1H, J = 3.8 Hz, CH), 3.06 (ddd, 1H, J = 10.3, 9.2, 6.8 Hz), 3.13 (ddd, 1H, J = 12.0, 7.1, 2.3 Hz), 3.48 (d, 1H, J = 3.8 Hz, CH), 3.58 (td, 1H, J = 10.5, 6.8 Hz); ¹³C NMR (90.56 MHz, CDCl₃) δ 23.93, 30.65, 47.96, 50.15, 52.33, 52.71, 53.00, 55.55, 55.89, 56.97, 81.90 (CH), 84.44 (CH); MS, m/z 222 (M⁺); IR (KBr) 2958, 2935, 2926, 2893, 2875, 2850, 2813, 2792, 2771, 2757,
2749, 1439, 1377, 1299, 1177, 1141, 1123, 1097, 1035, 965 cm$^{-1}$.
Anal Calcd for $C_{12}H_{22}N_4$: C, 64.83; H, 9.97; N, 25.20. Found: C, 65.13; H, 10.23; N, 25.56.

(1RS,7RS,13SR,14SR)-1,7-Dibenzyl-4,10-diaza-1,7-
diazoniatetracyclo[5.5.2.0$^{4,1}$.0$^{10,13}$]tetradecane dibromide (70). To a stirred solution of cis-13-1,4,7,10-tetraazatetracyclo-
[5.5.2.0$^{4,1}$.0$^{10,13}$]tetradecane (260 mg, 1.34 mmol) in MeCN (10 mL) at room temperature, was added benzyl bromide (1.44 g, 8.41 mmol), all in one portion. The reaction mixture was stirrred for 3 days. Precipitate was collected by suction filtration, and washed with MeCN (3 x 5 mL). Residual solvent was removed under vacuum. This gave 640 mg of product as white powder (89%): mp 208-212°C (dec); $^1$H NMR (360 MHz, D$_2$O, MeCN secondary ref. set at 2.06 ppm) $\delta$ 3.10-3.25, (m, 2H), 3.40-3.55 (m, 4H) 3.58-3.75 (m, 6H), 3.80-3.95 (m, 2H), 4.25-4.90 (m, 2H), 4.84 (s, 2H, CH), 4.82 and 4.98 (AB, 2H, $J = 13.2$ Hz, CH$_2$Ph), 7.50-7.75 (m, 10H, CH$_2$Ph); $^{13}$C NMR (90.56 MHz, D$_2$O, MeCN secondary ref. set at 1.70 ppm) $\delta$ 43.55, 46.82, 55.55, 61.68, 61.83, 78.37 (CH$_2$Ar), 126.97, 130.52, 132.11, 133.21; IR (KBr) 3063,
3039, 3006, 2956, 2941, 2917, 2893, 2842, 1459, 1442, 1340, 1292, 1202, 941, 761, 704 cm$^{-1}$. Anal Calcd for C$_{24}$H$_{33}$N$_4$Br$_2$: C, 53.75; H, 6.01; N, 10.45. Found: C, 53.56; H, 6.20; N, 10.35.

4,10-dibenzyl-1,4,7,10-tetraazabicyclo[5.5.2]-tetradecane (71). To a stirred solution of (1RS,7RS,13SR,14SR)-1,7-dibenzyl-4,10-diaza-1,7-diazoniattetracyclo[5.5.2.0$^4$.0$^10$.0$^4$.0$^1$.0$^3$.0$^4$.0$^5$.0$^6$.0$^7$.0$^3$.0$^4$.0$^1$.0$^2$.0$^4$.Oio,i3]-tetradecane dibromide (3.83 g, 7.14 mmol) in 95% EtOH (135 mL) was added NaBH$_4$ (10.09 g, 0.267 mol) in small portions over 20 minutes. The reaction was stirred at room temperature for 4 days. Excess NaBH$_4$ was then decomposed by slow addition of 100 mL 3 M HCl. Evaporation of solvent under reduced pressure gave a white solid which was dissolved in H$_2$O (40 mL), adjusted to pH 14 with solid KOH (with cooling), and extracted with benzene (6 x 50 mL). The combined extracts were dried (Na$_2$SO$_4$), and solvent was removed under reduced pressure to yield 2.89 g (~100%) of a viscous oil; $^1$H NMR (360 MHz, C$_6$D$_6$) $\delta$ 2.50-2.90 (m, 16H), 3.17 (s, 4H), 3.59 (s, 4H), 7.05-7.33 (m, 10H); $^1^3$C NMR (90.56 MHz, C$_6$D$_6$) $\delta$ 57.00, 57.88, 58.75, 61.35, 126.97, 128.42, 129.17, 140.89; MS, m/z 378 (M$^+$); IR (KBr)
3080, 3055, 3025, 2915, 2865, 2820, 2800, 1493, 1450, 1365, 1093, 720, 690 cm⁻¹; HRMS exact mass calcd for C₁₀H₁₈N₄ 379.2862, found 379.2849. The ¹³C and ¹H NMR showed the material to be >99% purity. This material was debenzylated without further purification.

1,4,7,10-Tetraazabicyclo[5.5.2]tetradecane (72).

Hydrogenolysis of 4,10-dibenzy1-1,4,7,10-tetraazabicyclo[5.5.2]-tetradecane was carried out in a glass apparatus designed for the exclusion of O₂ and for measurement of H₂ uptake with maintainance of constant pressure. The catalyst (0.79 g, 10% Pd/C) and solvent (250 mL glacial acetic acid) were added to a 500 mL hydrogenation flask which was connected to the hydrogenator. The system was evacuated (by means of a water aspirator) and flushed with nitrogen four times. After another evacuation the system was filled with hydrogen. The mixture of 10% Pd/C and glacial acetic acid was equilibrated with stirring under H₂ (766 mmHg) for 1.25 h. To this was added 4,10-dibenzy1-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane (2.89 g, 7.64 mmol) dissolved in glacial acetic acid (10 mL). The mixture was stirred for 40 minutes under H₂ (759 mmHg). The theoretical H₂ uptake for this reaction was 399 mL. The actual
uptake was 460 mL, which is 115% of the theoretical value. The hydrogenation apparatus was evacuated and flushed with nitrogen four times. The reaction flask was removed from the apparatus, the contents were filtered through celite, and the catalyst and celite were washed with glacial acetic acid (3 x 10 mL). The filtrate and washings were combined and concentrated under reduced pressure to give a light yellow oil, which was dissolved in H₂O (50 mL), adjusted to pH 14 with solid KOH (with cooling), and extracted with benzene (6 x 40 mL). The combined extracts were dried (Na₂SO₄) and solvent was removed under reduced pressure to give 1.68 g of crude solid product, which was kugelrohr distilled (100 °C air bath temperature, 0.03 mmHg). The collection bulb for the distillation was changed after the first 20 minutes and the distillation was continued. The first fraction (1.10 g) was a white solid and the second fraction (0.308 g) was a clear liquid. The distillation residue (0.150 g), the sublimation residue (0.110 g), and the second fraction collected from the distillation (0.308 g) contained 4-benzyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane and 4,10-dibenzyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane. An attempt to obtain more product by hydrogenolysis of these mixtures was unsuccessful. The
first fraction collected from the distillation was further purified by sublimation (80 °C, 0.03 mmHg) to yield 0.91 g of white crystals (55%): mp 102-104°C; 1H NMR (360 MHz, C6D6) δ 2.15 (s, 2H, NH), 2.45-2.75 (m, 20H), 2.66 (s, NCH2CH2N bridge); 13C NMR (90.56 MHz, C6D6) δ 50.58, 52.63, 55.40; MS (Cl, CH4), m/z 199 (M+1); IR (KBr) 3390, 2940, 2935, 2910, 2890, 2870, 2850, 2825, 2805, 1580, 1462, 1365, 1340, 1155, 804 cm⁻¹; Anal Calcd for C10H22N4: C, 60.57; H, 11.18; N, 28.25. Found: C, 60.61; H, 11.31; N, 28.30. The crude product could also be purified by an alternate method. The crude solid product (2.00 g) was dissolved in absolute EtOH (4 mL) containing (2 mL) concentrated HCl. Mixing resulted in precipitation of the hydrochloride salt which was filtered, washed with cold (0-5 °C) absolute EtOH (2 x 1 mL), and air-dried. The hydrochloride salt was recrystallized from CH3CN (10 mL) containing enough H2O to totally dissolve all of the solid. The recrystallized material was dissolved in H2O (10 mL), adjusted to pH 14 with solid KOH (with cooling), and extracted with benzene (5 x 20 mL). The combined extracts were dried (Na2SO4), and solvent was removed under reduced pressure to give crystalline material identical to that
mentioned above. The yield for this purification method was 50% (0.99 g).

(1RS,8SR,14SR,15SR)-1,8-Dibenzyl-4,11-diaza-1,8-diazoniatetracyclo[6.5.2.04,15.011,14]pentadecane dibromide hydrate (68). To a stirred solution of cis-14-1,4,8,11-tetraaza-tetracyclo[6.5.2.04,15.011,14]pentadecane (1.49 g, 7.17 mmol) in MeCN (35 mL) at room temperature, was added benzyl bromide (12.40 g, 72.50 mmol), all in one portion. The reaction mixture was stirred at room temperature for 3 weeks. Precipitate was collected by suction filtration, washed with MeCN (2 x 30 mL), then CH$_2$Cl$_2$ (3 x 15 mL). Residual solvent was removed under vacuum. This gave 3.60 g of product as white powder (90%): mp 222-225 °C (dec); $^1$H NMR (360 MHz, D$_2$O, MeCN secondary ref. set at 2.06 ppm) δ 1.86-1.97 (dm, 1H, $J$ = 15.0 Hz, H$_6$-eq) 2.15-2.33 (m, 1H, H$_6$-ax) 2.78 (td, 1H, $J$ = 12.2, 2.9 Hz) 3.09-3.92 (m, 13H) 4.21-4.39 (m, 2H), 4.78 and 5.09 (AB, 2H, $J$ = 13.3 Hz, CH$_2$Ph) 4.87 and 5.18 (AB, 2H, $J$ = 13.0 Hz, CH$_2$Ph), 4.95 and 5.12 (AB, 2H, $J$ = 1.1 Hz, CHCH), 7.30-7.90 (m, 10H, Ph); $^{13}$C NMR (90.56 MHz, D$_2$O, MeCN secondary ref. set at 1.70 ppm) δ 19.40
(CH₂CH₂CH₂), 43.37, 46.34, 47.68, 48.94, 51.25, 52.84, 61.05, 61.16, 61.37, 63.34, 76.81 (CH), 78.82 (CH), 125.56 (1 carbon), 126.62 (1 carbon), 130.28 (2 carbons), 130.52 (2 carbons), 132.18 (2 carbons), 133.18 (2 carbons), 134.10 (2 carbons); IR (KBr) 3062, 3050, 3026, 3018, 2996, 2957, 2915, 2886, 2864, 2844, 2821, 2807, 1480, 1456, 1437, 1283, 1212, 1146, 1110, 1055, 769, 711, 706 cm⁻¹. Anal Calcd for C₂₅H₃₄N₄Br₂·0.5H₂O: C, 53.68; H, 6.31; N, 10.02. Found: C, 53.94; H, 6.10; N, 9.97.

4,11-dibenzyl-1,4,8,11-tetraazabicyclo[6.5.2]-pentadecane (69). NaBH₄ (13.10 g, 0.340 mol) was added in small portions over 20 minutes to a stirred solution of (1RS,8SR,14SR,15SR)-1,8-dibenzyl-4,11-diaza-1,8-diazonia-tetracyclo[6.5.2.04,15.011,14]pentadecane dibromide (3.05 g, 5.55 mmol) in 95% EtOH (75 mL). The reaction mixture was stirred at room temperature for 10 days. Excess NaBH₄ was then decomposed by slow addition of 150 mL 3 M HCl. Evaporation of solvent under reduced pressure gave a white solid which was dissolved in H₂O (100 mL), adjusted to pH 14 with solid KOH (with cooling), and extracted...
with benzene (6 x 50 mL). The combined extracts were dried (Na$_2$SO$_4$), and solvent was removed under reduced pressure to yield 1.44 g (~100%) of a viscous oil; $^1$H NMR (360 MHz, C$_6$D$_6$) δ 1.37-1.60 (m, 2H, CH$_2$CH$_2$CH$_2$), 2.26-2.49 (m, 5H), 2.53-2.73 (m, 7H), 2.78 (ddd, 1H, $J$ = 13.7, 4.3, 2.6 Hz), 2.82-3.04 (m, 5H), 3.09-3.21 (m, 1H), 3.26 and 3.64 (AB, 2H, $J$ = 13.4 Hz, CH$_2$Ph), 3.60 and 3.62 (AB, 2H, $J$ = 14.4 Hz, CH$_2$Ph), 3.88 (ddd, 1H, $J$ =12.7, 8.2, 4.9 Hz), 7.07-7.41 (m, 10H, CH$_2$Ph); $^{13}$C NMR (90.56 MHz, C$_6$D$_6$) δ 28.59 (CH$_2$CH$_2$CH$_2$), 52.39, 53.78, 55.43, 55.84, 56.36, 56.43, 57.04, 57.93, 59.04, 59.48, 60.82, 60.99, 126.93, 126.98, 128.41, 128.42, 129.08, 129.39, 140.92, 141.03; MS, m/z 392 (M$^+$); IR (neat) 3077, 3053, 3020, 2919, 2863, 2800, 2784, 2745, 2721, 2702, 1448, 1364, 1110, 1046, 1025, 729, 696 cm$^{-1}$; HRMS exact mass calcd for C$_{25}$H$_{37}$N$_4$ 393.3018, found 393.3012. The $^{13}$C and $^1$H NMR showed the material to be >98% purity. This material was debenzylated without further purification.

1,4,8,11-Tetraazabicyclo[6.5.2]pentadecane (67).

Hydrogenolysis of 4,11-dibenzyl-1,4,8,11-tetraazabicyclo[6.5.2]-pentadecane was carried out in a glass apparatus designed for the
exclusion of O₂ and for measurement of H₂ uptake with maintenance of constant pressure. 10% Pd/C (0.20 g) glacial HOAc (60 mL) were added to a 125 mL hydrogenation flask which was connected to the apparatus. The system was evacuated (by means of a water aspirator) and flushed with nitrogen four times. The system was then evacuated, filled with hydrogen, and catalyst was equilibrated under H₂ (767 mmHg) for 1.5 h. To this was added a solution of 4,11-dibenzyl-1,4,8,11-tetraazabicyclo[6.5.2]pentadecane (1.11 g, 2.83 mmol) in glacial HOAc (5 mL). The mixture was stirred for 19 h under H₂ (767 mmHg). (The theoretical H₂ uptake for this reaction was 138 mL. The actual uptake was 144 mL, which is 104% of the theoretical value.) The apparatus was evacuated and flushed with nitrogen four times, the reaction flask was removed from the apparatus, the contents were filtered through celite, and the catalyst and celite were washed with glacial acetic acid (2 x 5 mL). The filtrate and washings were concentrated under reduced pressure to give a light yellow oil which was dissolved in H₂O (50 mL), adjusted to pH 14 with KOH, and extracted with benzene (5 x 50 mL). The combined extracts were dried (Na₂SO₄), and solvent was
removed under reduced pressure to give an oil which was kugelrohr distilled (90-120 °C air bath temperature, 0.03 mmHg). This oil solidified upon standing to give 0.494 g (82% crude yield) of solid product. The material contained minor impurities and was further purified. This solid was dissolved in absolute EtOH (5 mL) containing (1 mL) concentrated HCl. Mixing resulted in precipitation of a hydrochloride salt which was filtered, washed with cold (0-5 °C) absolute EtOH (2 x 1 mL), and air-dried. This solid was dissolved in H$_2$O (10 mL), adjusted to pH 14 with solid KOH (with cooling), and extracted with benzene (6 x 15 mL). The combined extracts were dried (Na$_2$SO$_4$), and solvent was removed under reduced pressure to give an oil. This oil was kugelrohr distilled (100 °C air bath temperature, 0.03 mmHg) and solidified upon standing to give 0.283 g (47%) of white solid: mp 28-30°C; $^1$H NMR (360 MHz, C$_6$D$_6$) δ 1.26-1.51 (m, 2H CH$_2$CH$_2$CH$_2$), 2.10-2.90 (m, 21H), 3.30 (ddd, 1H, $J = 12.1, 7.5, 2.4$ Hz); $^{13}$C NMR (90.56 MHz, C$_6$D$_6$) δ 27.14 (CH$_2$CH$_2$CH$_2$), 47.68, 48.56, 48.89, 49.02, 52.85, 53.27, 53.59, 54.20, 55.97, 59.41; MS, m/z 212 (M$^+$); IR (KBr) 3474, 2948, 2929, 2907, 2894, 2845, 2699, 1577, 1464, 1399, 1356, 1342, 812, 612, 523 cm$^{-1}$; HRMS exact
mass calcd for C₁₁H₂₅N₄ 213.2079, found 213.2078.

\[(1RS,8RS,15RS,16RS)-1,8-Dibenzyl-4,11-diaza-1,8-
diaziotetracyclo[6.6.2.0⁴.16.0¹¹.15]hexadecane dibromide
monohydrate\] (27). Benzyl bromide (100 g, 0.585 mol) was added
in one portion to a stirred solution of cis-15-1,4,8,11-tetraaza-
tetracyclo[6.6.2.0⁴.16.0¹¹.15]hexadecane (9.04 g, 40.60 mmol) in MeCN
(200 mL) at room temperature under nitrogen. The reaction mixture
was stirrred for 14 days. Precipitate was collected by suction
filtration and washed with MeCN (2 x 20 mL), followed by CH₂Cl₂ (2
x 50 mL). Residual solvent was removed under vacuum. This gave
21.35 g of product as white powder (93%): mp 147-151 °C (dec); \(^{1}H
NMR (360 MHz, D₂O, MeCN secondary ref. set at 2.06 ppm) \(\delta\) 1.86-1.96
(dm, 2H, \(J = 15.2\) Hz, H₆,1₃-eq) 2.20-2.39 (qm, 2H, \(J = 15.2\) Hz, H₆,1₃-ax)
2.81 (td, 2H, \(J = 12.3, 2.8\) Hz) 3.17-3.30 (dm, 4H, \(J = 12.3\)), 3.39-3.65
(m, 6H) 3.74 (td, 2H, \(J = 13.2, 3.4\) Hz), 4.43 (td, 2H, \(J = 13.1, 4.0\) Hz),
4.75 and 5.27 (AB, 4H, \(J = 13.0\) Hz, CH₂Ph) 5.08 (s, 2H, CH), 7.40-7.80
(m, 10H CH₂Ph); \(^{13}C NMR (90.56 MHz, D₂O, MeCN secondary ref. set at
1.70) \(\delta\) 18.77, 46.66, 47.52, 51.89, 61.14, 63.07, 77.49, 125.45,
130.24, 132.15, 134.02; IR (KBr) 3051, 3030, 3003, 2995, 2960, 2943, 2869, 2853, 2844, 2812, 1456, 1356, 1137, 1056, 871, 722, 706 cm\(^{-1}\). Anal Calcd for C\(_{26}\)H\(_{36}\)N\(_4\)Br\(_2\)•H\(_2\)O: C, 54.36; H, 6.67; N, 9.75. Found: C, 54.71; H, 6.79; N, 9.40.

4,11-Dibenzy1-1,4,8,11-tetraazabicyclo[6.6.2]-hexadecane (28). NaBH\(_4\) (66.70 g, 1.76 mol) was added in small portions over 1 h to a stirred solution of (1RS,8RS,15RS,16RS)-1,8-dibenzyl-4,11-diaza-1,8-diazoniatetracyclo[6.6.2.0\(^4.1\)6.0\(^1.1\)5]hexadecane dibromide monohydrate (20.25 g, 35.90 mmol) in 95% EtOH (900 mL). The reaction mixture was stirred at room temperature for 16 days. Excess NaBH\(_4\) was then decomposed by slow addition of 700 mL 3 M HCl (with cooling). Evaporation of solvent under reduced pressure gave a white solid which was dissolved in H\(_2\)O (400 mL), adjusted to pH 14 with solid KOH (with cooling), and extracted with benzene (6 x 250 mL). The combined extracts were dried (Na\(_2\)SO\(_4\)) and solvent was removed under reduced pressure to yield 12.44 g (88%) of solid product: mp 89-91 °C; \(^1\)H NMR (360 MHz, CDCl\(_3\)) \(\delta\) 1.31-1.45 (m, 2H, CH\(_2\)CH\(_2\)CH\(_2\)), 1.50-1.65 (m,
2H, CH₂CH₂CH₂), 2.26-2.53 (m, 12H), 2.38-2.54 (XX' of AA'XX', 2H, 
NCH₂CH₂N bridge), 2.85 (ddd, 2H, J = 13.0, 11.2, 4.0 Hz), 3.11-3.29 
(AA' of AA'XX', 2H, NCH₂CH₂N bridge), 3.17 and 3.78 (AX, 4H, J = 13.5 
Hz, CH₂Ph), 3.96 (ddd, 2H, J = 12.1, 10.8, 4.3 Hz), 7.15-7.45 (m, 10H, 
CH₂Ph); ¹³C NMR (90.56 MHz, CDCl₃) δ 28.06 (CH₂CH₂CH₂), 52.00, 
54.29, 56.57, 57.22, 57.61, 59.98, 126.49, 127.99, 128.94, 141.01; 
MS, m/z 406 (M⁺); IR (KBr) 3050, 3025, 3000, 2960, 2945, 2915, 
2900, 2860, 2800, 2770, 2720, 2860, 2655, 1445, 1435, 1345, 
1093, 710, 660 cm⁻¹. Anal Calcd for C₂₆H₃₈N₄: C, 76.80; H, 9.42; N, 
13.78. Found: C, 76.72; H, 9.82; N, 13.82. The ¹³C and ¹H NMR showed 
the material to be >99% purity. This material was debenzylated 
without further purification.

1,4,8,11-Tetraazabicyclo[6.6.2]hexadecane (29).

Hydrogenolysis of 4,11-dibenzyl-1,4,8,11-tetraazabicyclo[6.6.2]- 
hexadecane was carried out in a glass apparatus designed for 
exclusion of O₂ and for measurement of H₂ uptake with maintenance 
of constant pressure. 10% Pd/C (0.80 g) and glacial HOAc (125 mL) 
were added to a 500 mL hydrogenation flask which was connected to
the apparatus. The system was evacuated (by means of a water aspirator) and flushed with nitrogen four times. The system was then evacuated, filled with hydrogen, and catalyst was equilibrated under $H_2$ (759 mmHg) for 1 h. To this was added a solution of 4,11-dibenzyl-1,4,8,11-tetraazabicyclo[6.6.2]-hexadecane (4.54 g, 11.17 mmol) in glacial HOAc (5 mL). The mixture was stirred for 21 h under $H_2$ atmosphere (759 mmHg). (The theoretical $H_2$ uptake for this reaction was 542 mL. The actual uptake was 525 mL, which is 97% of the theoretical value.) The apparatus was evacuated and flushed with nitrogen four times, the reaction flask was removed from the apparatus, the contents were filtered through celite, and the catalyst and celite were washed with glacial acetic acid (3 x 10 mL). The filtrate and washings were concentrated under reduced pressure to give a light yellow oil which was dissolved in $H_2O$ (20 mL), adjusted to pH 14 with solid KOH (with cooling), and extracted with benzene (6 x 25 mL). The combined extracts were dried ($Na_2SO_4$), and solvent was removed under reduced pressure to give an oil (2.35 g, 93% crude yield). This oil was dissolved in Et$_2$O (17 mL) and then crystallized at -78 °C (acetone/dry ice bath) in a fritted
Schlenck tube to give 2.02 g (80%) of white solid: mp 48-49°C; ¹H NMR (360 MHz, C₆D₆) δ 1.16 (dtt, 2H, J = 15.1, 6.8, 2.7 Hz, H₆,₁₃-eq), 1.53 (dtt, 2H, J = 15.0, 9.0, 2.7 Hz, H₆,₁₃-ax), 2.07-2.26 (m, 6H), 2.31 (ddd, 2H, J = 13.0, 6.7, 2.6 Hz), 2.42-2.64 (m, 6H), 2.74-2.86 (m, 4H), 3.21 (ddd, 2H, J = 12.6, 8.8, 2.7 Hz), 3.59 (br s, 2H, NH); ¹³NMR (90.56 MHz, C₆D₆) δ 24.89 (CH₂CH₂CH₂), 47.37, 50.73, 52.40, 56.13, 59.04; MS, m/z 226; IR (CHCl₃) 3260, 2955, 2925, 2890, 2870, 2825, 1490, 1352, 1248, 1135, 1090, 652 cm⁻¹; HRMS exact mass calcd for C₁₂H₂₆N₄ 226.2157, found 226.2151.

(1RS,13SR,14RS)-1-Benzyl-4,7,10-triaza-1-azoniatetracyclo[5.5.2.0⁴,1⁵.0¹⁰,1³]tetradecane bromide (85). To a stirred solution of cis-13-1,4,7,10-tetraazatetracyclo-[5.5.2.0⁴,1⁵.0¹⁰,1³]tetradecane (0.373 g, 2.17 mmol) in toluene (30 mL) at room temperature, was added benzyl bromide (0.360 g, 2.10 mmol) all in one portion. The reaction mixture was stirred for 18 h. Precipitated product was collected by suction filtration and washed with toluene (3 x 15 mL). Residual solvent was removed under vacuum to give 0.63 g of white powder (84%): mp 215-225 °C (dec);
$^1$H NMR (360 MHz, D$_2$O, MeCN secondary ref. set at 2.06 ppm) δ 2.43-2.58 (m, 2H), 2.74-2.96 (m, 4H), 3.05-3.35 (m, 5H), 3.41-3.63 (m, 4H), 3.74 (d, 1H, J = 2.5 Hz, CH), 4.02 (d, 2H, J = 2.5 Hz, CH), 4.14-4.24 (m, 2H), 4.69 and 4.85 (AB, 2H, J = 13.3 Hz, CH$_2$Ar), 7.50-7.70 (m, 5H); $^{13}$C NMR (90.56 MHz, D$_2$O, MeCN secondary ref. set at 1.7 ppm) 44.53, 48.32, 48.36, 48.93, 49.09, 52.06, 57.90, 62.05, 62.24, 72.43, 83.43, 127.67, 130.40, 131.85, 133.31; IR (KBr) 3059, 3050, 3034, 3024, 2980, 2880, 2848, 2838, 2817, 2803, 1443, 1185 cm$^{-1}$; Anal. Calcd for C$_{17}$H$_{25}$N$_4$Br: C, 55.89; H, 6.90; N, 15.34. Found: C, 55.63; H, 7.08; N, 15.21.

(1RS,7RS,13SR,14SR)-1-Benzyl-7-methyl-4,10-diaza-1,7-diazoniatriacyclo[5.5.2.0$^4$.1$^4$.0$^{10}$.1$^3$]tetradecane dihalide (86). To a stirred solution of (1RS,13SR,14RS)-1-benzyl-4,7,10-triaza-1-azoniatriacyclo[5.5.2.0$^4$.1$^4$.0$^{10}$.1$^3$]tetradecane bromide (0.125 g, 0.342 mmol) in MeCN (5 mL) at room temperature, was added MeI (0.460 g, 3.24 mmol), all in one portion. The reaction flask was tightly stoppered, shielded from light, and the solution was stirred for 5 days. Precipitated product was collected by suction filtration and washed with MeCN (2 x 2 mL). Residual
solvent was removed under vacuum to give 144 mg of white powder: mp 195-200°C (dec); $^1$H NMR (360 MHz, D$_2$O, MeCN secondary ref. set at 2.06 ppm) δ 3.00-3.23 (m, 3H), 3.33-3.51 (m, 3H), 3.43 (s, 3H, CH$_3$), 3.52-3.72 (m, 5H), 3.79-3.99 (m, 2H), 4.01-4.16 (m, 2H), 4.28 (td, 1H, $J$ = 10.9, 3.5 Hz), 4.62 and 4.68 (AB, 2H, $J$ = 2.7 Hz, CHCH), 4.75 and 4.94 (AB, 2H, $J$ = 13.7 Hz, CH$_2$Ph), 7.50-7.70 (m, 5H, CH$_2$Ph); $^{13}$C NMR (90.56 MHz, D$_2$O, MeCN secondary ref. set at 1.7 ppm) δ 43.54, 43.80, 46.87, 47.17, 47.24, 55.61, 59.86, 61.67, 61.94, 65.70, 78.19, 79.01, 126.92, 130.50, 132.11, 133.20; IR (KBr) 3042, 3024, 3012, 2999, 2948, 2906, 2843, 2837, 1463, 1454, 1283, 1200, 770, 707 cm$^{-1}$. This salt was carried on without further characterization because of its mixed halide nature.

1-Benzyl-7-methyl-1,4,7,10-tetraazabicyclo[5.5.2]-tetradecane (87). NaBH$_4$ (2.91g, 76.92 mmol) was added in small portions over 20 minutes to a stirred solution of (1RS,7RS,13RS,14RS)-1-benzyl-7-methyl-4,10-diaza-1,7-diazoniatetracyclo[5.5.2.0$_4^4$.1$_4^4$.0$_1^1$.0$_3^3$]tetradecane dihalide (0.86 g) in 95% EtOH (80 mL). The reaction mixture was stirred at room
temperature for 5 days. Excess NaBH$_4$ was then decomposed by slow addition of 50 mL 3 M HCl. Evaporation of solvent under reduced pressure gave a white solid which was dissolved in H$_2$O (30 mL), adjusted to pH 14 with solid KOH (with cooling), and extracted with benzene (6 x 25 mL). The combined extracts were dried (Na$_2$SO$_4$), and solvent was removed under reduced pressure to give an oil. The crude product was kugelrohr distilled (100 °C air bath temperature, 0.04 mm Hg) to give pure product as a clear oil (0.415 g). The yield was 85% for two steps from (1RS,13SR,14RS)-1-benzyl-4,7,10-triaza-1-azoniatetracyclo[5.5.2.0$^{4,13}$.0$^{10,10}$]tetradecane bromide; $^1$H NMR (360 MHz, C$_6$D$_6$) δ 2.26 (s, 3H, CH$_3$), 2.36-2.48 (m, 2H), 2.58-2.85 (m, 14H), 3.02-3.22 (AB part of ABXY, 4H), 3.58 (s, 2H, CH$_2$Ph), 7.07-7.33 (m, 5H, CH$_2$Ph); $^{13}$C NMR (90.56 MHz, C$_6$D$_6$) δ 44.14, 56.97, 57.28, 57.88, 58.71, 60.46, 61.45, 126.96, 128.42, 129.17, 140.97; MS m/z 302 (M$^+$); IR (neat) 3106, 3085, 3061, 3026, 2958, 2921, 2805, 2747, 1676, 1493, 1452, 1372, 1108, 729, 697 cm$^{-1}$; HRMS exact mass calcd for C$_{18}$H$_{30}$N$_4$ 303.2549, found 303.2550.

1-Methyl-1,4,7,10-tetraazabicyclo[5.5.2]tetradecane

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The hydrogenolysis was carried out in a glass apparatus designed for the exclusion of \( \text{O}_2 \) and for measurement of \( \text{H}_2 \) uptake with maintainance of constant pressure. Catalyst (25 mg, 10\% Pd/C) and solvent (10 mL glacial acetic acid) were added to a 50 mL hydrogenation flask which was connected to the apparatus. The system was evacuated (by means of a water aspirator) and flushed with nitrogen four times. After another evacuation the system was filled with hydrogen. The mixture of 10\% Pd/C and glacial acetic acid was equilibrated under \( \text{H}_2 \) (744 mmHg) for 40 minutes. A solution of 4-benzyl-10-methyl-1,4,7,10-tetraazabicyclo[5.5.2]-tetradecane (99.3 mg, 0.328 mmol) in glacial acetic acid (2 mL) was then added and the mixture was stirred for 21 minutes under \( \text{H}_2 \) (22 °C, 744 mmHg). The hydrogenolysis was stopped because no noticeable \( \text{H}_2 \) uptake was observed after the first five minutes. (The theoretical \( \text{H}_2 \) uptake for this reaction was 8.1 mL. The actual uptake was 5.8 mL, which is 71% of the theoretical value.) The apparatus was evacuated and flushed with nitrogen four times, the reaction flask was removed from the apparatus, the contents were filtered through celite, and the catalyst and celite were washed with glacial acetic acid (3 x 2 mL). The filtrate and washings were

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concentrated under reduced pressure to give a light yellow oil which was dissolved in H₂O (2 mL), adjusted to pH 14 with solid KOH (with cooling), and extracted with benzene (6 x 10 mL). The combined extracts were dried (Na₂SO₄), solvent was removed under reduced pressure, and the crude product was kugelrohr distilled (100 °C air bath temperature, 0.04 mmHg) to give 65.1 mg of an oil which solidified upon standing (93% crude yield, >85% pure by ¹³C NMR).

An attempt to sublime the material was unsuccessful. This material could not be purified by conversion to the hydrochloride salt using the same procedure described for the purification of 1,4,7,10-tetraazabicyclo[5.5.2]tetradecane; mp 53-61°C; ¹H NMR (360 MHz, C₆D₆) δ 2.21-2.66 (m, 17H), 2.27 (s, CH₃), 2.68-2.85 (m, 4H), 4.07 (s, 1H, NH); ¹³C NMR (90.56 MHz, C₆D₆) δ 44.05, 47.55, 51.39, 51.80, 51.93, 56.21; MS, m/z 212 (M⁺); IR (CCl₄) 3213, 3037, 2963, 2933, 2914, 2890, 2841, 2803, 1675, 1550, 1448, 1372, 1164, 1131, 793, 784, 761 cm⁻¹.

(1RS,15RS,16SR)-1-Benzyl-4,8,11-triaza-1-azoniatetracyclo[6.6.2.⁰⁴,1⁶.⁰¹¹,¹⁵]hexadecane bromide
hydrate (80). Benzyl bromide (0.79 g, 4.62 mmol) was added in one portion to a stirred solution of cis-15-1,4,8,11-tetraaza-tetracyclo[6.6.2.0^{4,16}.0^{11,15}]hexadecane (0.75 g, 3.37 mmol) in toluene (25 mL) at room temperature. The reaction mixture was stirred for 11 days. Precipitated product was collected by suction filtration and washed with toluene (3 x 15 mL). Residual solvent was removed under vacuum to give 0.82 g of white powder (62%): mp 137-140 °C (dec); ¹H NMR (360 MHz, D₂O, MeCN secondary ref. set at 2.06 ppm) δ 1.40-1.51 (dm, 1H, J = 14.1 Hz, H₆ or H₁₃-eq), 1.70-1.83 (dm, 1H, J = 14.9 Hz, H₆ or H₁₃-eq), 2.09-2.34 (m, 3H), 2.41-2.53 (m, 2H), 2.65 (td, 1H, J = 12.2, 2.8 Hz), 2.92-3.18 (m, 7H), 3.20-3.27 (dm, 1H, J = 13.6 Hz), 3.28-3.36 (dm, 1H, J = 13.7 Hz), 3.43-3.61 (m, 2H), 3.68 (s, 1H, CH slightly broadened by small gauche coupling), 4.19 (td, 1H, J = 13.1, 3.5 Hz), 4.36 (s, 1H, CH slightly broadened by small gauche coupling), 4.77 and 5.05 (AB, 2H, J = 13.3 Hz, CH₂Ar), 7.45-7.68 (m, 5H); ¹³C NMR (90.56 MHz, D₂O, MeCN secondary ref. set at 1.7 ppm) δ 18.77 (CH₂CH₂CH₂), 19.14 (CH₂CH₂CH₂), 42.73, 47.37, 49.31, 52.09, 52.72, 54.05, 54.70, 60.56, 63.31, 70.29, 82.64, 126.45, 130.11, 131.84, 134.08; IR (KBr) 3063, 3034, 3025, 3006, 2947,
Anal. Calcd for C$_{19}$H$_{29}$N$_4$Br$\cdot$1.5H$_2$O: C, 54.28; H, 7.67; N, 13.32. Found: C, 53.95; H, 7.81; N, 13.13.

(1RS,8RS,15RS,16RS)-1-Benzyl-8-methyl-4,11-diaza-1,8-diazoniatetracyclo[6.6.2.0$^{4.16}.0^{11.15}$]hexadecane dihalide (82). To a stirred solution of (1RS,15RS,16SR)-1-benzyl-4,8,11-triaza-1-azoniatetracyclo[6.6.2.0$^{4.16}.0^{11.15}$]hexadecane bromide hydrate (0.59 g, 1.51 mmol) in MeCN (15 mL) at room temperature, was added Mel (1.07 g, 7.54 mmol), all in one portion. The reaction flask was tightly stoppered, shielded from light, and the solution was stirred for 7 days. Precipitated product was collected by suction filtration and washed with MeCN (2 x 2 mL). Residual solvent was removed under vacuum to give 0.753 g of white powder: mp 150-155 °C (dec); $^1$H NMR (360 MHz, D$_2$O, MeCN secondary ref. set at 2.06 ppm) δ 1.80-1.90 (dm, 1H, $J$ = 15.4 Hz, H$_6$ or 13-eq), 1.93-2.02 (dm, 1H, $J$ = 15.2 Hz, H$_6$ or 13-eq), 2.12-2.30 (m, 1H, H$_6$ or 13-ax), 2.37-2.53 (m, 1H, H$_6$ or 13-ax), 2.72 (td, 1H, $J$ = 12.4, 3.3 Hz), 2.93 (td, 1H, $J$ = 12.5, 3.4 Hz), 3.07-3.48 (m, 9H), 3.44 (s, 3H, CH$_3$), 3.54
(td, 1H, $J = 13.2, 3.6$ Hz), 3.67 (td, 1H, $J = 13.3, 3.9$ Hz), 3.73-3.87 (m, 2H), 4.37 (td, 1H, $J = 13.2, 4.6$ Hz), 4.44-4.58 (m, 1H), 4.71 and 5.21 (AB, 2H, $J = 13.2$ Hz, CH$_2$Ph), 4:84 (br s, 1H, CH), 5.03 (br s, 1H, CH), 7.50-7.68 (m, 5H, CH$_2$Ph); $^{13}$C NMR (90.56 MHz, D$_2$O, MeCN secondary ref. set at 1.7 ppm) δ 18.74, 19.13, 47.15, 47.20, 47.22, 48.99, 50.32, 51.82, 51.91, 60.10, 63.22, 65.94, 77.31, 77.50, 125.43, 130.27, 132.19, 134.02; IR (KBr) 3059, 3029, 2996, 2956, 2940, 2912, 2869, 2842, 2807, 1443, 1277, 1152, 1125, 1049, 881, 704 cm$^{-1}$. This salt was carried on without further characterization because of its mixed halide nature.

4-Benzyl-11-methyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (83). NaBH$_4$ (3.00 g, 79.32 mmol) was added in small portions over 20 minutes to a stirred solution of (1RS,8RS,15RS,16RS)-1-benzyl-8-methyl-4,11-diaza-1,8-diazoniatetracyclo-[6.6.2.0$^4$.0$^6$.0$^{11}$.0$^{15}$]hexadecane dihalide (0.753 g) in 95% EtOH (45 mL). The reaction mixture was stirred at room temperature for 7 days under nitrogen. Excess NaBH$_4$ was then decomposed by slow addition of 60 mL 3 M HCl. Evaporation of solvent under reduced pressure gave a white solid which was
dissolved in H₂O (75 mL), adjusted to pH 14 with solid KOH (with cooling), and extracted with benzene (5 x 30 mL). The combined extracts were dried (Na₂SO₄), and solvent was removed under reduced pressure. The crude product was kugelrohr distilled (120° C air bath temperature, 0.03 mmHg) to give a clear oil (340 mg, 68% for two steps from (1RS,15RS,16SR)-1-benzyl-4,8,11-triazol-1-azoniatetracyclo[6.6.2.0⁴.₁⁶.⁰¹¹.₁⁵]hexadecane bromide hydrate); ^1^H NMR (360 MHz, C₆D₆) δ 1.26-1.59 (m, 4H, CH₂CH₂CH₂), 2.12 (s, 3H, CH₃), 2.15-2.63 (m, 13H), 2.67-2.82 (m, 2H), 2.97 (td, 1H, J = 12.1, 4.0 Hz), 3.07 (X of AX, 1H, J = 13.5 Hz, CH₂Ph), 3.20 (td, 1H, J = 12.2, 3.8 Hz), 3.34 (td, 1H, J = 12.2, 3.8 Hz), 3.66 (td, 1H, J = 11.5, 4.6 Hz), 3.77 (A of AX, 1H, J = 13.5 Hz, CH₂Ph), 4.09 (td, 1H, J = 11.7, 3.8 Hz), 7.09-7.25 (m, 3H, CH₂Ph), 7.35-7.40 (m, 2H, CH₂Ph); ^1^C NMR (90.56 MHz, C₆D₆) δ 28.29 (CH₂CH₂CH₂), 28.81 (CH₂CH₂CH₂), 42.28, 51.71, 51.93, 54.82, 56.43, 57.16, 57.41, 57.59, 57.89, 60.34, 61.66, 126.94, 128.39, 129.26, 141.34; MS, m/z 330 (M⁺); IR (neat) 3080, 3060, 3025, 2960, 2915, 2880, 2835, 2745, 1455, 1365, 1125, 1110, 728, 690 cm⁻¹; HRMS exact mass calcd for C₂₀H₃₅N₄ 331.2862, found 331.2851.
1-Methyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (81). Hydrogenolysis of 1-benzyl-4-methyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane was carried out in a glass apparatus designed for the exclusion of O₂ and for measurement of H₂ uptake with maintenance of constant pressure. 10% Pd/C (35 mg) and glacial HOAc (15 mL) were added to a 50 mL hydrogenation flask which was connected to the apparatus. The system was evacuated (by means of a water aspirator) and flushed with nitrogen four times. The system was then evacuated and filled with hydrogen. The catalyst was equilibrated under H₂ (26 °C, 752 mmHg) for 1.5 h. 1-Benzyl-4-methyl-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (0.158 g, 0.478 mmol) in glacial acetic acid (2 mL) was added and the mixture was stirred for 21 h under H₂ (752 mmHg). (The theoretical H₂ uptake for this reaction was 11.9 mL. The actual uptake was 12.3 mL, 103% of the theoretical value.) The apparatus was evacuated and flushed with nitrogen four times. The reaction flask was removed from the apparatus, the contents were filtered through celite, and the catalyst and celite were washed with glacial acetic acid (2 x 4 mL). The filtrate and washings were concentrated.
under reduced pressure to give a light yellow oil which was
dissolved in H₂O (4 mL), adjusted to pH 14 with solid KOH (with
cooling), and extracted with benzene (6 x 10 mL). The combined
extracts were dried (Na₂SO₄), and solvent was removed under
reduced pressure. The crude product was kugelrohr distilled (100 °C
air bath temperature 0.04 mmHg) to give 114 mg (99%) of product as
an oil; ¹H NMR (360 MHz, C₆D₆) δ 1.21-1.37 (m, 2H, CH₂CH₂CH₂), 1.42-
1.58 (m, 2H, CH₂CH₂CH₂), 1.93 (ddd, 1H, J = 13.5, 10.0, 1.9 Hz), 2.02
(s, 3H, CH₃), 2.05-2.85 (m, 15H), 2.97-3.12 (m, 3H) 3.41-3.52 (m,
1H), 4.67-4.82 (m, 1H, NH); ¹³C NMR (90.56 MHz, C₆D₆) δ 25.81, 28.07,
41.26, 49.17, 49.38, 49.47, 49.75, 55.21, 56.40, 56.63, 57.59, 59.41,
62.13; MS m/z 240 (M⁺); IR (neat) 3232, 2918 (br), 2792 (br), 2918 (br), 1487,
1452, 1355, 1112 cm⁻¹; HRMS exact mass calcd for C₁₃H₂₉N₄
241.2392, found 241.2390.

4,11-Bis-(carboethoxymethyl)-1,4,8,11-tetraaza-
bicyclo[6.6.2]hexadecane (129). To a solution of 1,4,8,11-
tetraaza-bicyclo[6.6.2]hexadecane (0.831 g, 3.67 mmol) in
acetonitrile (30 mL) was added anhydrous sodium carbonate (0.78 g,
7.3 mmol) and ethyl bromoacetate (1.23 g, 7.39 mmol). The mixture was stirred under N\textsubscript{2} and heated to 50°C for 22.5 h. Solvent was then removed under reduced pressure and residual material was dissolved in 20% aq NaOH (30 mL) at ice bath temperature (0-5 °C). This aqueous solution was extracted with cold (0-5 °C) CHCl\textsubscript{3} (6 x 50 mL), combined extracts were dried (Na\textsubscript{2}SO\textsubscript{4}), and solvent was removed under reduced pressure to give a solid (1.520 g, essentially quantitative): mp 57-59°C; \textsuperscript{1}H NMR (360 MHz, C\textsubscript{6}D\textsubscript{6}) 2.17 (ddd, 2H, J = 13.4, 3.7, 1.8 Hz), 2.29 (ddd, 2H, J = 13.0, 4.7, 2.5 Hz), 2.43-2.57 (XX' of AA'XX', 2H, NCH\textsubscript{2}CH\textsubscript{2}N bridge), 3.08 and 3.20 (AB, 4H, J = 16.7 Hz, NCH\textsubscript{2}COOEt), 3.21-3.35 (AA' of AA'XX', 2H, NCH\textsubscript{2}CH\textsubscript{2}N bridge), 3.69 (td, 2H, J = 11.9, 4.4 Hz), 3.96 and 3.97 (AB of ABX\textsubscript{3}, J = 7.1 Hz, CH\textsubscript{2}CH\textsubscript{3}); \textsuperscript{13}C NMR (90.56 MHz, C\textsubscript{6}D\textsubscript{6}) 14.36 (CH\textsubscript{2}CH\textsubscript{3}), 28.23 (CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{3}), 50.91, 53.26, 54.71, 56.37, 57.32, 59.84, 60.23, 171.60 (COOEt); IR (KBr) 2969, 2945, 2926, 2907, 2851, 2833, 2819, 2784, 1743 (CO), 1461, 1444, 1379, 1365, 1301, 1184, 1162, 1137, 1124 cm\textsuperscript{-1}; MS, m/z 398 (M\textsuperscript{+}); HRMS exact mass calcd for C\textsubscript{20}H\textsubscript{39}N\textsubscript{4}O\textsubscript{4} 399.2971, found 399.2963.
4,10-Bis-(carboethoxymethyl)-1,4,7,10-tetraaza-bicyclo[5.5.2]tetradecane (130). To a solution of 1,4,7,10-tetraaza-bicyclo[5.5.2]tetradecane (53.7 mg, 0.271 mmol) in acetonitrile (4 mL) was added anhydrous sodium carbonate (54.7 mg, 0.516 mmol) and ethyl bromoacetate (90.4 mg, 0.541 mmol). The mixture was stirred under N\textsubscript{2} and heated to 55°C for 3 h. Solvent was then removed under reduced pressure and residual material was dissolved in 40\% aq KOH (3 mL) at ice bath temperature (0-5 °C). This aqueous solution was immediately extracted with cold (0-5 °C) CHCl\textsubscript{3} (5 x 5 mL), combined extracts were dried (Na\textsubscript{2}SO\textsubscript{4}), and solvent was removed under reduced pressure to give an oil (69.3 mg, 69\%): \textsuperscript{1}H NMR (360 MHz, CDCl\textsubscript{3}) \delta 1.27 (t, X\textsubscript{3} of A\textsubscript{2}X\textsubscript{3}, 6H, J = 7.1 Hz, CH\textsubscript{2}CH\textsubscript{3}), 2.70-2.86 (m, 12H), 2.97 (s, 4H, NCH\textsubscript{2}CH\textsubscript{2}N bridge), 2.93-3.05 (m, 4H), 3.39 (A\textsubscript{2}, 4H, NCH\textsubscript{2}COOEt), 4.17 (q, A\textsubscript{2} of A\textsubscript{2}X\textsubscript{3}, J = 7.1 Hz, CH\textsubscript{2}CH\textsubscript{3}); \textsuperscript{13}C NMR (90.56 MHz, CDCl\textsubscript{3}) \delta 14.23 (CH\textsubscript{2}CH\textsubscript{3}), 56.31, 56.50, 56.84, 57.79, 60.02, 172.59 (COOEt); IR (neat) 1736 (CO) cm\textsuperscript{-1}.

4,11-Bis-(carboxymethyl)-1,4,8,11-tetraaza-
bicyclo[6.6.2]hexadecane tetrahydrochloride hydrate (131).

4,11-Bis-(carboethoxymethyl)-1,4,8,11-tetraazabicyclo-
[6.6.2]hexadecane (0.635 g, 1.593 mmol) was dissolved in 6 N HCl (30 mL), and heated to 100 °C with stirring under N₂ for 48 h. Solvent was removed under reduced pressure to give an oil which crystallized after removal of residual solvent under vacuum (0.777 g, 95%): mp 155-160°C; ¹H NMR (360 MHz, D₂O, CH₃CN as reference set at 2.06 ppm) δ 1.63-1.89 (dm, 2H, J = 16.6 Hz, H₆₁₃-eq), 2.23-2.53 (m, 2H, H₆₁₃-ax), 2.93-3.05 (tm, 4H, J = 13.4 Hz), 3.04-3.10 (m, 4H), 3.24-3.43 (m, 4H), 3.45-3.73 (m, 8H), 3.50 and 3.90 (AB, 4H, J = 17.8 Hz, NCH₂COOH); ¹³C NMR (90.56 MHz, CH₃CN as reference set at 1.7 ppm) δ 19.69 (CH₂CH₂CH₂), 47.36, 47.60, 53.33, 55.21, 57.70, 60.04, 172.92 (COOH); IR (KBr) 3411, 3291, 3177, 2936, 2860, 2714, 2600, 2528, 1709 (CO), 1461, 1429, 1232, 989, 664 cm⁻¹: Anal Calcd for C₁₆H₃₀N₄O₄•4HCl•1.5H₂O: C, 37.29; H, 7.24; N, 10.87. Found: C, 37.20; H, 7.41; N, 11.21.

Attempted preparation of 4,11-bis-(acetamido)-
1,4,8,11-tetraazabicyclo[6.6.2]hexadecane/Actual
preparation of 4,11-bis-(carboxymethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (131). 4,11-Bis-(carboethoxymethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (69.8 mg, 0.175 mmol) was dissolved in conc. aq NH₃ (2 mL). The mixture was heated to 100 °C for 3 days in a pressure tube. The mixture was cooled for 15 minutes (0-5 °C), and solvent was removed under reduced pressure to give an oil which crystallized after removal of residual solvent (37.2 mg, 62%): mp 270-275°C (dec); ¹H NMR (360 MHz, D₂O, CH₃CN as reference set at 2.06 ppm) δ 1.70-1.77 (dm, 2H, J = 16.7 Hz, H₆,₁₃-eq), 2.20-2.45 (dtt, 2H, J = 16.0, 12.9, 3.2 Hz, H₆,₁₃-ax ), 2.56 (dd, 2H, J = 13.6, 2.2 Hz), 2.80-3.79 (m, 17H), 3.31 (XX' of AA'XX', 2H, NCH₂CH₂N), 4.14 (AA' of AA'XX', 2H, NCH₂CH₂N), 3.69 (td, 2H, J = 14.5, 3.5 Hz); ¹³C NMR (90.56 MHz, D₂O, CH₃CN as reference set at 1.7 ppm) δ 20.51 (CH₂CH₂CH₂), 49.14, 49.62, 52.60, 57.76, 58.77, 59.11, 171.51; MS (Cl, NH₃), m/z 343 (M+1); IR (KBr) 3409, 2999, 2991, 2957, 2898, 2838, 2810, 1629, 1485, 1404, 1392, 1101, 1062, 791, 740 cm⁻¹. HRMS exact mass calcd for C₁₆H₃₁N₄O₄ 343.2345, found 343.2349. This molecular ion is consistent with 4,11-bis-(carboxymethyl)-1,4,8,11-tetraaza-
bicyclo[6.6.2]hexadecane.

4,11-Bis-(2-hydroxyethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (133). NaBH₄ (0.50 g, 13.30 mol) was added in small portions over 20 minutes to a stirred solution of 4,11-bis-(carboethoxymethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (0.110 g, 0.276 mmol) in 95% EtOH (10 mL). The reaction mixture was heated to 100 °C and stirred for 4 h. Excess NaBH₄ was then decomposed by the slow addition of 6.0 mL 3 M HCl (with cooling). Evaporation of solvent under reduced pressure gave a white solid which was dissolved in H₂O (20 mL), adjusted to pH 14 with solid KOH (with cooling), and extracted with PhCH₃ (6 x 15 mL). The combined extracts were dried (Na₂SO₄), and solvent was removed under reduced pressure to give an oil. This oil was kugelrohr distilled (0.02 mmHg, 110 °C air bath temperature) yielding 19.4 mg (22%) of a clear viscous oil; ¹H NMR (360 MHz, CDCl₃) δ 1.38-1.68 (m, 4H, CH₂CH₂CH₂), 2.25-2.78 (m, 20H), 2.80-3.02 (m, 2H, OH + overlapping AA' of AA'XX', 2H, NCH₂CH₂N bridge), 3.41-3.65 (m, 4H, CH₂CH₂OH), 3.97 (ddd, 2H, J = 13.4, 9.0, 5.1 Hz); ¹³C NMR (90.56 MHz,
CDCl$_3$ $\delta$ 27.64 ($\text{CH}_2\text{CH}_2\text{CH}_2$), 52.87, 53.50, 56.15, 56.28, 57.79, 58.93, 59.20; IR (neat) 3342, 2948, 2914, 2902, 2842, 2820, 2800, 1652, 1461, 1363, 1289, 1124, 1058, 1038 cm$^{-1}$; MS (Cl, NH$_3$), $m/z$ 315 (M+1); HRMS exact mass calcd for C$_{16}$H$_{35}$N$_4$O$_2$ 315.2760 found 314.2757.

4,11-(2-Carbomethoxyethyl)-1,4,8,11-tetraaza-bicyclo[6.6.2]hexadecane (134). 1,4,8,11-Tetraazabicyclo[6.6.2]hexadecane (0.331 g, 1.46 mmol) was dissolved in 35 mL freshly distilled methyl acrylate (33 g, 0.39 mol) under a nitrogen atmosphere. The reaction mixture was shielded from light and stirred at room temperature for 24 h. Removal of excess methyl acrylate under reduced pressure gave an oil (0.644 g, essentially quantitative); $^1$H NMR (360 MHz, C$_6$D$_6$) $\delta$ 1.18-1.31 (m, 2H, CH$_2$CH$_2$CH$_2$), 1.46-1.58 (m, 2H, CH$_2$CH$_2$CH$_2$ bridge), 2.10-2.40 (m, 16H), 2.35-2.46 (XX' of AA'XX', 2H, NCH$_2$CH$_2$N), 2.64-2.82 (m, 4H), 2.86-2.97 (m, 2H), 3.05-3.17 (AA' of AA'XX', 2H, NCH$_2$CH$_2$N bridge), 3.41 (s, 6H, COOCH$_3$), 3.64 (td, 2H, $J = 12.1, 3.7$ Hz); $^{13}$C NMR (90.56 MHz, C$_6$D$_6$) $\delta$ 28.50 (CH$_2$CH$_2$CH$_2$), 33.68 (NCH$_2$CH$_2$COOMe), 50.83,
50.97, 51.28, 55.00, 57.33, 57.82, 59.39, 172.74 (COOMe); MS, m/z 398 (M+); IR (neat NaCl plates) 2970, 2945, 2904, 2831, 2805, 2797, 2716, 1736 (CO), 1455, 1432, 1359, 1190, 1171, 1119, 692 cm⁻¹; HRMS exact mass calcd for C₂₀H₃₉N₄O₄ 399.2971, found 399.2956.

Trace impurities (<2%) are evident by close examination of the ¹H and ¹³C NMR spectra. Attempts to distill and crystallize the product were unsuccessful.

4,11-Bis-(2-carboxyethyl)-1,4,8,11-tetraaza-bicyclo[6.6.2]hexadecane hydrochloride (136). 4,11-Bis-(2-carbomethoxyethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (0.132 g, 0.331 mmol) was dissolved in 2 mL 20% aq HCl. The mixture was stirred at room temperature under N₂ for 4 days. Solvent was removed under reduced pressure to give a solid (0.096 g, 56%): mp 155-160 °C; ¹H NMR (360 MHz, D₂O, HOD set at 4.80 ppm) δ 1.78 (dm, 2H, J = 15.0 Hz, H₆₁₁₃·eq), 2.22-2.45 (m, 2H), 2.50-2.72 (m, 2H), 2.75-3.42 (m, 10H), 3.54 (td, 2H, J = 12.5, 4.1 Hz), 3.63-3.94 (m, 4H); ¹³C NMR (90.56 MHz, D₂O, CH₃CN as ref. set at 1.7 ppm) δ 19.71, 27.91, 49.14, 49.51, 54.63, 54.45, 57.78, 175.08 (CO). Material
appears to be >90% pure by $^{13}$C NMR. Attempts to recrystallize this material (CH$_3$CN, EtOH) were unsuccessful (oiling out occurred).

4,11-Bis-(2-cyanoethyl)-1,4,8,11-tetrazabicyclo-[6.6.2]hexadecane (135). 1,4,8,11-Tetraazabicyclo[6.6.2]-hexadecane (0.170 g, 0.751 mmol) was dissolved in 2.0 mL acrylonitrile (1.6 g, 30 mmol) under a nitrogen atmosphere. The reaction mixture was shielded from light and stirred at room temperature for 24 h. Removal of excess acrylonitrile under reduced pressure gave an oil which was dissolved in toluene (15 mL), dried (Na$_2$SO$_4$), and filtered. Evaporation of solvent under reduced pressure gave an oil which crystallized upon storage at -10 °C to a pale yellow solid (0.196 g, 79%): mp 62-64°C; $^1$H NMR (360 MHz, C$_6$D$_6$) $\delta$ 1.05-1.35 (m, 4H, CH$_2$CH$_2$CH$_2$), 1.45-1.68 (m, 4H), 1.81-1.98 (m, 4H), 2.10-2.35 (m, 12H), 2.39-2.57 (XX' of AA'XX', 2H, NCH$_2$CH$_2$N), 2.53 (ddd, 2H, $J = 13.2, 11.0, 3.2$ Hz), 2.77 (td, 2H, $J = 12.0, 4.2$ Hz), 3.01-3.19 (AA' of AA'XX', 4H, NCH$_2$CH$_2$N), 3.51 (td, 2H, $J = 12.0, 4.2$ Hz); $^{13}$C NMR (90.56 MHz, C$_6$D$_6$) $\delta$ 16.44 (CH$_2$CH$_2$CN), 28.31 (CH$_2$CH$_2$CH$_2$), 50.28, 51.15, 53.90, 57.20, 57.76, 58.98, 119.10
(CH₂CH₂CN); MS, m/z 332 (M⁺); IR (KBr) 2946, 2901, 2889, 2825, 2797, 2786, 2704, 2238 (CN), 1455, 1358, 1117 cm⁻¹. Anal Calcd for C₁₈H₃₂N₆: C, 65.02; H, 9.70; N, 25.28. Found: C, 65.14; H, 10.01; N, 25.54.

**Hydrogenation of 4,11-Bis-(2-cyanoethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane.** The hydrogenation was carried out using a Parr shaker equipped with a 250 mL hydrogenation flask (tested to 120 psig). To 50 mL of a 1.4 M solution of NaOH in EtOH/H₂O (95:5), was added 4,11-bis-(2-cyanoethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (0.207 g, 0.623 mmol), followed by W-2 Raney nickel (1.25 g slurry in 2.5 mL H₂O). The system was evacuated (by means of a water aspirator) and flushed with nitrogen twice, then evacuated and filled with hydrogen twice. The mixture was hydrogenated with constant agitation at 2.4 barr H₂ pressure for 1 week at 25 °C. The system was evacuated (by means of a water aspirator) and flushed with nitrogen twice. The mixture was filtered through celite, and washed with 95% EtOH (2 x 10 mL). The catalyst was deactivated with 25 mL 6M aq HCl (H₂ effervescence). Filtrate and washings were concentrated under...
reduced pressure to give an oil which was dissolved in H₂O (25 mL) and extracted with CHCl₃ (6 x 15 mL). The combined extracts were dried (Na₂SO₄) and solvent was removed under reduced pressure to give 0.208 g of crude product, which was kugelrohr distilled (160°C air bath temperature, 0.03 mmHg) to yield 0.145 g of a clear viscous oil. The ¹³C NMR spectrum and the mass spectrum (EI, CH₄) are consistent with a mixture of 4-(3-aminopropyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (0.0650 g, 37%) and 4,11-bis-(3-aminopropyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (0.0792 g, 37%) contaminated with 10% of unidentified material. The relative proportions of the two major products are based upon ¹³C NMR line intensities; ¹H NMR (360 MHz, CDCl₃) δ 1.25-1.72 (m), 2.05-2.92 (m), 2.93 (dt, J = 12.2, 4.4 Hz), 3.02-3.14 (m), 3.20 (td, J = 12.5, 3.9 Hz), 3.26-3.33 (m), 3.76 (ddd, J = 12.0, 10.8, 4.6 Hz), 5.01 (m, NH); ¹³C NMR (90.56 MHz, CDCl₃) δ 25.72 (4,11-bis-(3-aminopropyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane), 27.37 (4-(3-aminopropyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane), 27.75 (4-(3-aminopropyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane), 32.30, 32.43, 40.66, 48.53, 48.67, 49.01, 49.15, 51.68, 52.49, 52.86, 54.14,
54.50, 54.72, 56.40, 57.14, 57.73, 57.91, 58.07, 58.56, 58.98, 59.75; MS, m/z 340 (M+), 283 (M+); IR (neat NaCl plates) 3370, 3237, 2949, 2928, 2914, 2854, 2810, 2798, 2722, 2145, 1672, 1598, 1459, 1364, 1117 cm⁻¹.

**Methoxyacetyl chloride.** The acid chloride was prepared by the method of Rothstein in 85% yield. bp 113-115 °C; (lit. bp 112-113 °C).²⁰⁹

4,11-Bis(methoxyacetyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (139). 1,4,8,11-Tetraazabicyclo[6.6.2]-hexadecane (0.582 g, 2.57 mmol) and 8.0 mL triethylamine (distilled from CaH₂) were dissolved in dry THF (40 mL). A solution of methoxyacetyl chloride (0.590 g, 5.47 mmol) in 10 mL of dry THF was added dropwise over a period of 40 minutes. The solution was stirred under nitrogen at room temperature for 2 days. The mixture was filtered, the solids were washed with THF, and filtrate and washings were combined and evaporated under reduced pressure to give an oil. The crude product was chromatographed on 50 g of basic alumina (20% EtOH/Et₂O, Rf 0.27) to yield 0.849 g (essentially
quantitative, purity >96%) of a yellow viscous oil: $^1$H NMR (360 MHz, C$_6$D$_6$; Note: the $^1$H NMR spectrum exhibits multiple resonances consistent with slow amide rotation, therefore the $^1$H integrations are not given.) $\delta$ 1.27-1.64 (m), 1.65-1.90 (m), 2.00-2.77 (m), 2.96-3.15 (m), 3.25 (s, CH$_3$), 3.39 (d, $J = 4.5$ Hz), 3.54-3.73 (m), 3.75-4.09 (m), 4.10-4.20 (m); $^{13}$C NMR (90.56 MHz, C$_6$D$_6$; Note: the $^{13}$C NMR exhibits multiple resonances which are grouped according to their intensities. The four resonances observed in the upfield region of the $^{13}$C NMR spectrum are consistent with three conformational isomers, one C$_1$ rotamer and two different C$_2$ rotamers. $\delta$ 29.13 (B or C)(CH$_2$CH$_2$CH$_2$), 29.25 (B or C)(CH$_2$CH$_2$CH$_2$), 29.39 (A)(CH$_2$CH$_2$CH$_2$), 29.60 (B or C)(CH$_2$CH$_2$CH$_2$), 43.54 (B or C), 44.20 (B or C), 45.94 (A), 46.24 (B or C), 46.41 (B or C), 50.09 (B or C), 50.38 (A), 52.09 (A), 52.42 (B or C), 52.79 (B or C), 53.13 (A), 53.23 (B or C), 57.65 (B or C), 57.83 (B or C), 57.89 (B or C), 58.50 (B or C), 58.91 (A), 59.27 (B or C), 71.91 (B or C), 71.97 (B or C), 72.98 (A), 73.04 (B or C), 169.26 (B or C)(CO), 169.44 (A)(CO), 169.62 (B or C)(CO); IR (neat) 2955, 2910, 2880, 2860, 2790, 2770, 1630, 1455, 1405, 1350, 1300, 1275, 1255, 1180, 1055, 1045, 985, 915 cm$^{-1}$; MS, $m/z$ 370 (M$^+$);
HRMS exact mass calcd for C$_{18}$H$_{39}$N$_4$O$_4$ 371.2658, found 371.2655.
The $^1$H and $^{13}$C NMR suggest minor impurities are present. The resulting yellow oil was reduced without further purification.

4,11-Bis-(2-methoxyethyl)-1,4,8,11-tetrazabicyclo-[6.6.2]hexadecane (140). A borane solution (22 mL of 1.0 M BH$_3$·THF complex in THF) was added dropwise over 30 minutes, under a nitrogen atmosphere, to a cooled solution (0-5 °C) of 4,11-bis-(2-methoxyacetyl)-1,4,8,11-tetrazabicyclo[6.6.2]hexadecane (0.608 g, 1.641 mmol) in THF (75 mL). The reaction mixture was warmed to room temperature and refluxed for 3 h. Excess borane was decomposed by the addition of H$_2$O (10 mL). THF was removed under reduced pressure to give a white solid which was dissolved in 90 mL HCl·H$_2$O-MeOH (6:9:30) and refluxed for 4 h. MeOH removal gave a solid which was dissolved in H$_2$O, adjusted to pH 14 with NaOH, and extracted with CHCl$_3$ (5 x 20 mL). Combined extracts were dried (Na$_2$SO$_4$) and evaporated to afford an oil which was kugelrohr distilled (120 °C air bath temp, 0.04 mmHg) to give 0.337 g (60%) of solid crude product. A solution of NaBPh$_4$ (0.025 g, 0.730 mmol) in
MeOH (1 mL) and a solution of crude 4,11-bis(2-methoxyethyl)-1,4,8,11-tetrazabicyclo[6.6.2]hexadecane (0.025 g, 0.730 mmol) in MeOH (1 mL) were mixed to give a white precipitate which was isolated by filtration. The precipitate was dissolved in H2O, basified to pH 14 with NaOH, and extracted with benzene (5 x 2 mL). The combined extracts were dried (Na2SO4) and solvent was removed under reduced pressure to give an oil. This oil was kugelrohr distilled (120 °C air bath temp, 0.04 mmHg) to give 0.014 g (56% recovery) of product, which solidified on standing. The overall yield for this reaction was 34% (0.189 g): mp 47-49 °C; 1H NMR (360 MHz, C6D6) δ 1.22-1.38 (m, 2H), 1.41-1.58 (m, 2H), 2.25-2.42 (m, 6H), 2.44-2.65 (m, 8H), 2.68-2.85 (m, 4H), 2.92 (td, 2H, J =12.6, 4.0 Hz), 3.14 (s, 3H, CH3), 3.22-3.46 (m, 6H), 3.68 (td, 2H, J =11.9, 4.0 Hz); 13C NMR (90.56 MHz, C6D6) δ 28.77 (CH2CH2CH2), 51.12, 54.62, 55.48, 57.45, 57.90, 58.46, 60.90, 72.51 (CH2CH2OCH3); MS, m/z 342 (M+); IR (neat) 2950, 2897, 2861, 2841, 2780, 2760, 1441, 1346, 1182, 1145, 1111 cm⁻¹. Anal Calcd for C18H36N4O2: C, 63.12; H, 11.18; N, 16.36. Found: C, 63.13; H, 11.54; N, 16.29.
2-(Methyl)phenyl acetate. The ester 146 was prepared by the method of Karlin\textsuperscript{127}, a modification of the procedure published by Range\textsuperscript{6}. The crude product was purified by reduced pressure distillation to give a colorless liquid (81%): bp 88 °C / 11 mmHg (lit. 83 °C / 20 mmHg).

2-(Bromomethyl)phenyl acetate. Compound 144 was prepared in 56% yield by the method described by Karlin\textsuperscript{127} and coworkers. The crude product was fractionally distilled in vigreux column twice to obtain pure material: bp 77 °C / 0.005 mmHg (lit. 88 °C / 0.03 mmHg); \textsuperscript{1}H NMR (360 MHz, CDCl\textsubscript{3}) \( \delta \) 2.29 (s, 3H, CH\textsubscript{3}), 4.73 (s, 2H, CH\textsubscript{2}Br), 7.06-7.10 (dm, 1H, \( J = 8.2 \) Hz, ArH), 7.11-7.18 (tm, 1H, \( J = 7.5 \) Hz, ArH), 7.28 (td, 1H, \( J = 7.7,1.6 \) Hz, ArH), 7.34 (dd, 1H, \( J = 7.6,1.5 \) Hz, ArH); \textsuperscript{13}C NMR (90.56 MHz, CDCl\textsubscript{3}) \( \delta \) 20.65 (COCH\textsubscript{3}), 27.49 (CCH\textsubscript{2}Br), 122.84, 125.97, 129.30 (CCH\textsubscript{2}Br), 129.57, 130.55, 148.69 (COAc), 168.55 (OCOCH\textsubscript{3}); MS \textit{m/z} 229 (M\textsuperscript{+}). The authors did not mention that a minor dibrominated side product (o-dibromomethyl-phenyl acetate) is formed. This higher boiling component was isolated (>85% pure) in ~14% yield: bp 82 °C / 0.005 mmHg; \textsuperscript{1}H NMR
(360 MHz, CDCl$_3$) δ 2.29 (s, 3H, COCH$_3$), 6.83 (s, 1H, CCHBr$_2$), 7.06 (dd, 1H, $J = 7.9$, 1.1 Hz, ArH), 7.22 (td, 1H, $J = 7.7$, 1.1 Hz, ArH), 7.29 (td, 1H, $J = 7.5$, 1.5 Hz, ArH), 7.79 (dd, 1H, $J = 7.7$, 1.6 Hz, ArH); $^{13}$C NMR (90.56 MHz, CDCl$_3$) δ 20.78 (COCH$_3$), 34.19 (CCHBr$_2$), 122.64, 126.19, 129.30, 130.55, 132.68 (CCHBr$_2$), 145.19 (COAc), 168.12 (OCOCH$_3$); MS $m/z$ 308 (M$^+$).

(1RS,8RS,15RS,16RS)-1,8-Bis-(2-acetoxybenzyl)-4,11-diaza-1,8-diazoniatriacyclo[6.6.2.0$^{4,16}.0^{11,15}$]hexadecane dibromide monohydrate (148). To a stirred solution of cis-15-1,4,8,11-tetraazatricyclo[6.6.2.0$^{4,16}.0^{11,15}$]hexadecane (0.268 g, 1.21 mmol) in MeCN (8 mL) at room temperature, was added 2-(bromomethyl)-phenyl acetate (2.60 g, 11.3 mmol), all in one portion. The reaction mixture was stirred for 18 days. Precipitated product was collected by suction filtration, washed with MeCN (2 mL), then Et$_2$O (2 x 10 mL). Residual solvent was removed under vacuum. This gave 0.59 g of product as white powder (70%): mp 135-140 °C (dec); $^1$H NMR (360 MHz, D$_2$O, MeCN secondary ref. set at 2.06 ppm) δ 1.84-1.94 (dm, 2H, $J = 14.9$)
Hz, H$_6$ (eq), 2.14-2.34 (q, 2H, J = 14.3 Hz, H$_6$ (ax)), 2.50 (s, 6H, COCH$_3$), 2.95 (td, 2H, J = 12.1, 2.8 Hz), 3.16-3.27 (m, 4H), 3.37-3.66 (m, 6H), 3.74 (td, 2H, J = 13.1, 3.3 Hz), 4.36 (td, 2H, J = 12.7, 3.4 Hz), 4.71 (A of AX, 2H, J = 13.2 Hz, CH$_2$Ar), 5.42 (X of AX, 2H, J = 13.2 Hz, CH$_2$Ar), 5.46 (s, 2H, CH), 7.36 (d, 2H, J = 8.3 Hz), 7.43 (t, 2H, J = 7.6 Hz), 7.61-7.66 (m, 4H); $^{13}$C NMR (90.56 MHz, D$_2$O, MeCN secondary ref. set at 1.7 ppm) δ 18.81, 22.12, 46.52, 47.83, 51.84, 57.92, 60.83, 78.29, 118.10, 124.79, 127.98, 133.97, 135.61, 151.44, 172.97; IR (KBr) 3423, 3067, 3057, 3053, 3045, 3024, 2995, 2955, 2938, 2902, 2855, 2851, 2811, 1764 (C=O), 1454, 1370, 1181, 903 cm$^{-1}$. Anal. Calcd for C$_{30}$H$_{40}$N$_4$O$_4$Br$_2$.H$_2$O: C, 51.59; H, 6.06; N, 8.02. Found: C, 51.97; H, 6.22; N, 7.95.

**Attempted reduction of (1RS,8RS,15RS,16RS)-1,8-bis-(2-acetoxybenzyl)-4,11-diaza-1,8-diazoniatriocyclo-[6.6.2.0$^{4,16}$.0$^{11,15}$]hexadecane dibromide monohydrate (148) with NaBH$_4$.** To a stirred solution of (1RS,8RS,15RS,16RS)-1,8-bis-(2-acetoxybenzyl)-4,11-diaza-1,8-diazoniatriocyclo-[6.6.2.0$^{4,16}$.0$^{11,15}$]hexadecane dibromide monohydrate (0.105 g, 0.150
mmol) in 95% EtOH (6.0 mL), was added NaBH₄ (0.26 g, 6.87 mol) in small portions over 10 minutes. The reaction mixture was stirred at room temperature for 8 days. Excess NaBH₄ was decomposed by the slow addition of 3 M HCl (3.0 mL). Evaporation of solvent under reduced pressure gave a white solid which was dissolved in H₂O (2.0 mL), adjusted to pH 14 with solid KOH (with cooling), and extracted with CHCl₃ (4 x 5 mL). The combined extracts were dried (Na₂SO₄) and solvent was removed under reduced pressure to yield 0.092 g of an oil. The ¹³C NMR spectrum of this oil clearly indicates that the ester had been reduced (peak at 172.97 ppm absent). The aromatic region of the ¹³C NMR spectrum is consistent with two o-hydroxy phenyl rings resulting from two products. A multiplet in the ¹H NMR at 4.5 ppm is very characteristic of functionalized cross-bridged cyclam derivatives. The mass spectrum (El, CH₄) identifies two molecular ions at 332 and 438. The spectra suggest the product of this reaction is most likely a mixture of 4-(2-hydroxybenzyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane and 4,11-bis-(2-hydroxybenzyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane; ¹H NMR (360 MHz, CDCl₃) δ 1.15-1.30 (m), 1.50-1.85 (m), 2.00-3.20 (m),

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3.41-3.65 (m), 3.85-4.00 (d, \( J = 13.9 \) Hz), 4.45 (dt, \( J = 13.0, 6.3 \) Hz)
6.65-6.84 (m), 6.96 (tm, \( J = 5.6 \) Hz), 7.10-7.20 (m); \(^{13}\)C NMR (90.56 MHz, CDCl\(_3\)) \( \delta \) 19.57, 22.52, 25.54 (br), 29.65, 44.76, 47.60, 49.22, 49.71, 51.84, 52.48, 52.80, 53.23, 53.61, 54.00, 54.37, 56.05, 56.28, 56.90, 58.04, 60.12, 115.83, 116.87, 118.23, 118.81, 122.24, 123.21, 128.40, 128.58, 128.64, 129.74, 158.12, 158.20; MS, \( m/z \) 438 (228), 332 (1405).

2-Chloroethyl trityl sulfide. Compound 151 was prepared by the method of Trujillo\(^{128}\) and coworkers (75-95% yield). The \(^1\)H and \(^{13}\)C NMR spectra are consistent with the literature report.

4-(S-trityl-2-mercaptoethyl)-1,4,8,11-tetraaza-bicyclo[6.6.2]hexadecane (154). To a solution of 1,4,8,11-tetraaza-bicyclo[6.6.2]hexadecane (0.270 g, 1.192 mmol) in anhydrous DMF (7.0 mL) was added anhydrous potassium carbonate (0.67 g, 4.85 mmol), potassium iodide (0.80 g, 4.83 mmol), and 2-chloroethyl trityl sulfide (0.809 g, 2.387 mmol). The mixture was stirred under \( \text{N}_2 \) and heated to 80°C for 2 h, then stirred for 16 h at

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room temperature. Crystallized material was collected by filtration and residual DMF was removed under vacuum. Solid was dissolved in H2O (5 mL), adjusted to pH 14 with solid KOH (with cooling), and extracted with PhCH3 (6 x 5 mL). The combined extracts were dried (Na2SO4) and solvent was removed under reduced pressure to give an oil which crystallized after removal of residual solvent (0.134 g, 21%): mp 124-126°C; 1H NMR (360 MHz, C6D6) δ 1.15-1.33 (m, 2H, CH2CH2CH2), 1.35-1.53 (m, 2H, CH2CH2CH2), 1.80 (br s, H2O), 1.94 (dm, 1H, J = 13.6 Hz), 2.02-2.60 (m, 17H), 2.11 (s, residual PhCtf3), 2.69-2.82 (m, 2H), 2.98 (td, 1H, J = 10.3, 5.7 Hz), 3.04 (td, 1H, J = 12.1, 4.0 Hz), 3.14 (td, 1H, J = 12.5, 3.8 Hz), 3.37-3.47 (m, 1H), 4.58-4.72 (m, 1H, NH), 6.90-7.40 (m, 9H), 7.51-7.68 (m, 6H); 13C NMR (90.56 MHz, C6D6) δ 25.89 (CH2CH2CH2), 28.32 (CH2CH2CH2), 31.10 (NCH2CH2S), 48.81, 48.90, 48.92, 50.11, 54.03, 54.51, 55.46, 57.70, 59.04, 59.41, 60.39, 67.23 (SCPh3), 126.79, 128.13, 130.11, 145.76; MS (Cl, CH4) m/z 529 (M+1), 557 (M+C2H5); IR (KBr) 3240, 3088, 3057, 2991, 2956, 2940, 2921, 2862, 2804, 2722, 1491, 1464, 1444, 1363, 1117, 763, 743, 700 cm⁻¹. Anal Calcd for C33H44N4S·0.5H2O: C, 73.69; H, 10.42; N, 8.43. Found: C, 73.41; H, 8.36; N, 10.33.
4,11-Bis-(S-trityl-2-mercaptoethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (150). To a solution of 1,4,8,11-tetraazabicyclo[6.6.2]hexadecane (79.7 mg, 0.352 mmol) in anhydrous DMF (4.0 mL) was added anhydrous potassium carbonate (0.202 g, 1.46 mmol), potassium iodide (0.250 g, 1.51 mmol), and 2-chloroethyl trityl sulfide (0.483 g, 1.43 mmol). The mixture was stirred under N₂ and heated to 80 °C for 3 h. The solvent was evaporated under reduced pressure and the residue extracted with benzene (25 mL). Insoluble solids were removed by filtration and the benzene solution was evaporated under reduced pressure to give a yellow oil. Trituration of this oil with Et₂O (35 mL) caused precipitation of a light yellow solid, which was filtered and washed with Et₂O (2 x 3 mL). This solid was dissolved in H₂O (1.0 mL). The resulting solution was adjusted to pH 14 with solid KOH (with cooling) and extracted with PhCH₃. The combined extracts were dried (Na₂SO₄) and solvent was removed under reduced pressure to give an oil which solidified under vacuum (0.172 g, 60% crude). ¹³C NMR indicated that the material was 91-93% desired product, (contaminated with 4-(S-trityl-2-mercaptoethyl)-1,4,8,11-
tetraazabicyclo[6.6.2]hexadecane (154): mp 135-140 °C (dec); \(^1\)H NMR (360 MHz, C\(_6\)D\(_6\)) \(\delta\) 1.11-1.27 (tm, 2H, \(J = 10.6\) Hz, CH\(_2\)CH\(_2\)CH\(_2\)CH\(_2\)CH\(_2\)) 1.30-1.45 (tm, 2H, \(J = 10.6\) Hz, CH\(_2\)CH\(_2\)CH\(_2\)), 2.03-2.55 (m, 20H), 2.64 (td, 2H, \(J = 12.5\), 3.8 Hz), 2.80 (td, 2H, \(J = 12.1\), 3.3 Hz), 3.13-3.27 (AA' of AA'XX', 2H, NCH\(_2\)CH\(_2\)N bridge), 3.56 (td, 2H, \(J = 11.6\), 3.2 Hz), 6.93-7.02 (m, 6H, CPh\(_3\)), 7.03-7.13 (m, 12H, CPh\(_3\)), 7.57-7.65 (m, 12H, CPh\(_3\)); \(^{13}\)C NMR (90.56 MHz, C\(_6\)D\(_6\)) \(\delta\) 28.45 (CH\(_2\)CH\(_2\)CH\(_2\)), 31.29 (CH\(_2\)CH\(_2\)S), 51.33, 54.02, 54.40, 57.10, 57.78, 59.14, 66.98 (CH\(_2\)SCPh\(_3\)), 126.61, 127.99, 130.00, 145.69; IR (KBr) 3085, 3057, 3052, 3026, 2940, 2930, 2923, 2880, 2850, 2838, 2827, 2820, 2809, 2776, 1670, 1487, 1444, 1133, 1118, 1080, 1034, 743, 701 cm\(^{-1}\); MS (Cl, NH\(_3\)), \(m/z\) 831 (M\(^+\)); HRMS exact mass calcd for C\(_{54}\)H\(_{63}\)N\(_4\)S\(_2\) 831.4494, found 831.4506. The low resolution mass spectrum (Cl, NH\(_3\)) indicates that minor impurities other than 4-(S-trityl-2-mercaptoethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecane are also present. Attempted chromatography on alumina (20% MeOH/Et\(_2\)O) resulted in partial deprotection (removal of trityl groups) of the ligand as indicated by \(^1\)H NMR of the eluted fractions. Several two solvent recrystallization systems (CH\(_2\)Cl\(_2\)/Hexane;
C₆H₆/Et₂O; CH₃CN/Et₂O; EtOH/Et₂O) were tried without success (oiling out occurred).

(1RS,8SR)-1,8-Dibenzyl-4,11-diaza-1,8-diazoniatrioclo[9.3.1.14,8]hexadecane dibromide (176).
Benzyl bromide (388 mg, 2.268 mmol) was added in one portion to a stirred solution of 1,4,8,11-tetraazatrioclo[9.3.1.14,8]hexadecane (131 mg, 0.580 mmol) in MeCN (12 mL) at room temperature, and the reaction mixture was stirred for 17 h. Precipitate was collected by suction filtration, washed with MeCN (2 x 2 mL), and residual solvent was removed under vacuum to give 288 mg of product as white powder (87%): mp 200-206°C (dec); ¹H NMR (360 MHz, D₂O, MeCN secondary ref. set at 2.06 ppm) δ 1.80-1.95 (dm, 2H, J = 13.9 Hz, H₆,1₃-ax), 2.30-2.49 (m, 2H, H₆,1₃-eq), 2.57 (td, 2H, J = 12.1, 3.4 Hz), 2.85-3.05 (dm, 4H, J = 12.9 Hz), 3.17 (dd, 2H, J = 11.2, 4.8 Hz), 3.25-3.40 (m, 2H), 3.42 (td, 2H, J = 12.8, 4.0 Hz), 3.59 (d, 2H, J = 9.6 Hz, NCH₂N), 3.57-3.70 (tm, 2H, J = 13.8 Hz), 4.36-4.49 (tm, 2H, J = 13.1 Hz), 4.55 (B of AB, 2H, J = 13.4 Hz, NCH₂Ph), 4.75 (A of AB, 2H, J = 13.4 Hz, NCH₂Ph) (Note: The A part of AB is obscured by the HDO
signal. An analysis of the signal intensities of the B part was used to calculate δA), 5.51 (d, 2H, J = 9.6 Hz, NCH₂N), 7.40-7.68 (m, 10H, Ph); ¹³C NMR (90.56 MHz, D₂O, MeCN as secondary ref. set at 1.7 ppm) δ 19.99 (CH₂CH₂CH₂), 47.92, 48.17, 51.78, 60.10, 63.45, 77.22, 126.44, 129.78, 131.39, 133.44; IR (KBr) 3042, 3028, 3014, 2977, 2963, 2942, 2902, 2813, 2766, 1487, 1459, 1442, 1184, 1132, 1006, 769, 717 cm⁻¹: Anal Calcd for C₂₆H₃₈N₄Br₂: C, 55.13; H, 6.76; N, 9.89. Found: C, 54.75; H, 6.86; N, 10.17.

1,8-Dibenzy1-1,4,8,11-tetraazacyclotetradecane (178). (1RS,8RS)-1,8-Dibenzyl-4,11-diaza-1,8-diazone-tricyclo[9.3.1.1⁴,8]hexadecane dibromide (101 mg, 0.178 mmol) was dissolved in 20 mL 3 M KOH. The solution was heated to 50°C and stirred for 20 minutes under N₂, after which it was extracted with CHCl₃ (6 x 15 mL). The combined extracts were dried (Na₂SO₄) and solvent was removed under reduced pressure to yield an oil (59.6 mg, 88%) which was kugelrohr distilled (150-170 °C air bath temperature, 0.04 mmHg); ¹H NMR (360 MHz, C₆D₆) δ 1.75-2.95 m(~p), 4H, J = 5.5 Hz, CH₂CH₂CH₂), 2.52 (t, 4H, J = 5.3 Hz) 2.55-2.60 (m, 4H),
2.67-2.76 (m, 8H), 2.70-3.00 (br s, 2H, NH), 3.73 (s, 4H, N\textsubscript{CH\textsubscript{2}}Ph),
7.11-7.38 (m, 10H, Ph); \textsuperscript{13}C NMR (90.56 MHz, C\textsubscript{6}D\textsubscript{6}) \delta 26.55, 48.19,
49.76, 51.70, 54.33, 58.37, 127.11, 128.53, 129.71, 138.76; IR (neat)
3301, 3083, 3060, 3027, 3002, 2949, 2924, 2869, 2859, 2829,
2809, 2799, 2660, 1496, 1456, 1333, 1265, 1126, 1076, 759, 729,
699, 631 cm\textsuperscript{-1}. HRMS exact mass calcd for C\textsubscript{24}H\textsubscript{37}N\textsubscript{4} 381.3018, found
381.3008.

1-Benzyl-8-methyl-1,4,8,11-tetraazacycloadecane (180). (1RS,8RS,15RS,16RS)-1-Benzyl-8-methyl-
4,11-diaza-1,8-diazoniatetra[6.6.2.0.4.160\textsuperscript{11}.15]hexadecane
dihalide (0.488 g) was dissolved in 10 mL 20% aq KOH. The solution
was heated to 60 °C and stirred for 4 h under N\textsubscript{2}, after which it was
cooled to 0 °C and extracted with benzene (6 x 20 mL). The combined
extracts were dried (Na\textsubscript{2}SO\textsubscript{4}), and solvent was removed under
reduced pressure to give an oil. This oil was kugelrohr distilled
(100-130°C air bath temperature, 0.04 mmHg) to yield 0.211 g of
product. The yield for two steps from (1RS,15RS,16SR)-1-benzyl-
4,8,11-triaza-1-azoniatetra[6.6.2.0.4.16.0\textsuperscript{11}.15]hexadecane was
73%; \( ^1 \text{H NMR (360 MHz, C}_6\text{D}_6 \) } \delta 1.55-1.65 (\sim \text{p, 2H, } J = 6.3 \text{ Hz, CH}_2\text{CH}_2\text{CH}_2), \ 1.66-1.75 (\sim \text{p, 2H, } J = 6.3 \text{ Hz, CH}_2\text{CH}_2\text{CH}_2), \ 2.17 (\text{s, 3H, CH}_3), \ 2.29 (\text{t, 2H, } J = 5.5 \text{ Hz}) \ 2.32-2.43 (\text{m, 6H}), \ 2.44-2.70 (\text{m, 8H}), \ 2.74 (\text{br s, 2H, NH}), \ 3.39 (\text{s, 2H, CH}_2\text{Ph}), \ 7.09-7.18 (\text{m, 1H, CH}_2\text{Ph}), \ 7.19-7.27 (\text{m, 2H, CH}_2\text{Ph}), \ 7.36-7.43 (\text{m, 2H, CH}_2\text{Ph}); \ ^{13}\text{C NMR (90.56 MHz, C}_6\text{D}_6 \) } \delta 26.70 (\text{CH}_2\text{CH}_2\text{CH}_2), \ 26.76 (\text{CH}_2\text{CH}_2\text{CH}_2), \ 42.84, \ 47.91, \ 48.11, \ 50.05, \ 51.13, \ 53.39, \ 54.41, \ 57.71, \ 58.22, \ 58.83, \ 127.00, \ 128.41, \ 129.42, \ 139.86; \text{ MS (Cl, NH}_3), \ m/z \ 305 (\text{M}+1); \text{ IR (neat) 3295, 3081, 3056, 3022, 2930, 2915, 2868, 2820, 2798, 2726, 2652, 1455, 1130, 1058, 726, 696 cm}^{-1}; \text{ HRMS exact mass calcd for C}_{18}\text{H}_{33}\text{N}_4 \ 305.2705, \text{ found 305.2717.}

1,7-Dibenzyl-1,4,7,10-tetraazacyclododecane (179).

\((1\text{RS,7RS,13SR,14SR)-1,7-Dibenzyl-4,10-diaza-1,7-diazone-tetracyclo[5.5.2.0^4,140^10,13]tetradecane dibromide (1.54 g, 2.87 mmol) was dissolved in 45 mL 20% aq KOH. The solution was heated to 50°C and stirred for 5 h under N}_2. \text{ The reaction mixture was then cooled to 0°C, extracted with benzene (6 x 50 mL), and the combined extracts were dried (Na}_2\text{SO}_4). \text{ Solvent was removed under reduced
pressure to yield 0.780 g (80%) of solid product which was kugelrohr distilled (130-150°C air bath temperature, 0.03 mmHg); m.p. 88-89°C; \(^1\)H NMR (360 MHz, C\(_6\)D\(_6\)) \(\delta\) 2.10-2.80 (m, 18H), 3.39 (s, 4H, CH\(_2\)Ar); 7.06-7.43 (m, 10H); \(^{13}\)C NMR (90.56 MHz, C\(_6\)D\(_6\)) \(\delta\) 45.73, 52.20, 60.01, 127.24, 128.54, 129.30, 139.95; MS, \(m/z\) 352 (M\(^+\)); IR (KBr) 3304, 3061, 3017, 3001, 2933, 2921, 2873, 2857, 2822, 2794, 2726, 2718, 2698, 1447, 1260, 1112, 1045, 925, 770, 726, 694 cm\(^{-1}\): Anal Calcd for C\(_{22}\)H\(_{32}\)N\(_4\): C, 74.96; H, 9.15; N, 15.89. Found: C, 74.79; H, 9.40; N, 15.88; HRMS exact mass calcd for C\(_{22}\)H\(_{32}\)N\(_4\) 353.2705, found 353.2704.

1-Benzyl-7-methyl-1,4,7,10-tetraazacyclododecane (181). (1RS,7RS,13SR,14SR)-1-Benzyl-7-methyl-4,10-diaza-1,7-diazaonia-tetracyclo[5.5.2.0\(^{4.10}\).\(^{10.13}\)]tetradecane dihalide (73.2 mg) was dissolved in 10 mL 20% aq KOH. The mixture was heated to 50 °C and stirred for 4 h under N\(_2\). The reaction mixture was cooled to 0 °C, and extracted with benzene (6 x 15 mL). The combined extracts were dried (Na\(_2\)SO\(_4\)), and solvent was removed under reduced pressure to give an oil, which was kugelrohr distilled (100-130 °C
air bath temperature, 0.02 mmHg). The yield was 68% (39.3 mg) for two steps from (1RS,13SR,14RS)-1-benzyl-4,7,10-triaza-1-azoniatetracyclo[5.5.2.0.4.10.13]tetradecane bromide; $^1$H NMR (360 MHz, C$_6$D$_6$) $\delta$ 2.10 (s, 3H, CH$_3$), 2.26-2.34 (m, 4H), 2.37-2.42 (m, 4H), 2.48-2.57 (m, 10H), 3.39 (s, 2H, CH$_2$Ph), 7.02-7.09 (m, 1H, CH$_2$Ph), 7.12-7.19 (m, 2H, CH$_2$Ph), 7.24-7.29 (m, 2H, CH$_2$Ph); $^{13}$C NMR (90.56 MHz, C$_6$D$_6$) $\delta$ 44.16, 45.74, 45.81, 52.22, 54.85, 60.09, 127.14, 128.49, 129.34, 139.61; MS (Cl, NH$_3$), $m/z$ 277 (M+1); IR (neat) 3308, 3085, 3061, 2938, 2919, 2879, 2835, 2816, 2802, 1495, 1354, 1341, 1120, 1053, 929, 731, 699 cm$^{-1}$; HRMS exact mass calcd for C$_{16}$H$_{39}$N$_4$ 277.2392, found 277.2381.

F. Experimental Procedures for Ion, Ligand, and Proton Competition Experiments.

1. Competitions (General Procedure)-Ligand/Ligand for Metal Cations

Equal molar amounts (~25-40 mg each) of the two ligands used for each study were weighed out into separate 1 dram vials and transferred into a 5 mm Wilmad 528 NMR tube with the aide of 0.50-
1.00 mL CD$_3$CN. The volume of the solution in the NMR tube was determined by comparison to an identical tube containing acetone. The volume of the acetone was determined using a Hamilton syringe. The sample tube was sealed with a teflon cap and parafilm. The concentration of a single amine component was typically between 0.09 and 0.27 M. The $^1$H (360.13 MHz) and $^{13}$C (90.56 MHz) NMR spectra were recorded on an AM-360A Bruker NMR Spectrometer. One equivalent of the desired metal salt was added directly to the NMR tube with the aid of a glassine paper funnel. The solution was agitated until all solids had dissolved. The $^1$H and $^{13}$C NMR spectra were recorded and the appropriate resonances were integrated. No account was taken for slight differences in NOE.

2. Competitions (General Procedure)-Ligand/Ligand Competition for Trifluoroacetic acid (TFA).

Equal molar amounts (~25-40 mg each) of the two ligands used for each study were weighed out in separate 1 dram vials and transferred into a 5 mm Wilmad 528 NMR tube with the aide of 0.50-1.00 mL CD$_3$CN. The volume of the solution in the NMR tube was determined by comparison to an identical tube containing acetone.

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The volume of the acetone was determined using a Hamilton syringe. The sample tube was sealed with a teflon cap and parafilm. The \(^1\)H (360.13 MHz) and \(^{13}\)C (90.56 MHz) NMR spectra were recorded on an AM-360A Bruker NMR Spectrometer. One equivalent of TFA (trifluoroacetic acid) was added to the NMR tube via a µL syringe and the solution was agitated. The \(^1\)H and \(^{13}\)C NMR spectra were recorded and the appropriate resonances were integrated. No account was taken for slight differences in NOE.

3. \(P_{k_a}\) Determinations of 58 Relative to DBU.

Into a 1 dram vial was weighed DBU (22.5 mg, 0.148 mmol) which was transferred to a Wilmad 528 NMR tube with the aide of \(~0.5\) mL CD\(_3\)CN. The sample tube was sealed with a teflon cap and parafilm. The \(^1\)H (360.13 MHz) and \(^{13}\)C (90.56 MHz) NMR spectra were acquired on an AM-360A Bruker NMR Spectrometer. Trifluoroacetic acid (TFA, 11.3 µL, 0.148 mmol) was added via a Hamilton 20 µL syringe. The solution was mixed and the \(^1\)H (360.13 MHz) and \(^{13}\)C (90.56 MHz) NMR spectra were recorded on an AM-360A Bruker NMR Spectrometer. Ligand 58 (35.5 mg, 0.148 mmol) was weighed out in a 1 dram vial and added to the NMR tube with the aide of \(~0.5\) mL.

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CD₃CN. The tube was resealed as described above. The solution was mixed and the ¹H (360.13 MHz) and ¹³C (90.56 MHz) NMR spectra were acquired on an AM-360A Brüker NMR Spectrometer.

4. Li+/Na+ Ion Selectivity Determination of Ligand 58

Equal molar amounts NaBPh₄ (29.2 mg, 8.53 x 10⁻⁵ mol), and LiClO₄ (9.1 mg, 8.55 x 10⁻⁵ mol) used for this study were transferred into a 5 mm Wilmad 528 NMR tube with the aide of a glassine paper funnel. Ligand 58 (20.5 mg, 8.54 x 10⁻⁵ mol) was weighed out in a 1 dram vial and transferred to the NMR tube with the aide of ~0.5 mL of CD₃CN. The volume of the solution in the NMR tube was determined by comparison to an identical tube containing acetone. The volume of the acetone was determined using a Hamilton syringe. The sample tube was sealed with a teflon cap and parafilm. The ¹H (360.13 MHz) and ¹³C (90.56 MHz) NMR spectra were acquired on an AM-360A Brüker NMR Spectrometer. The ¹H and ¹³C NMR spectra were recorded and the appropriate resonances were integrated. No account was taken for slight differences in NOE.
II. Dupont/Merck

A. HPLC Instrumentation Used for Ligand Radiolabelling Experiments.

The analytical reverse phase HPLC work was performed on a Hewlett Packard 1050 HPLC in conjunction with a Hewlett Packard integrator recorder-model 3392A unless otherwise noted. A Ludlum survey meter (model 177 or equivalent) equipped with a NaI probe (model 44-2 or equivalent) was used for radiodetection ($^{99m}$Tc is a $\gamma$-emitter with a half of 6.01 h). The column (4.6 x 250 mm) was a C$_{18}$ Vydac reverse phase column, manufactured by West (part # 218 TP 54). A mixture of the ligand, reducing agent, and Na$^{99m}$TcO$_4$ in a buffered solution was put into a sealed vial. The vial was put into a lead container and heated using a water bath. The contents were analyzed by reverse phase analytical HPLC. Aliquots of the reaction mixture (10-25 $\mu$L) were removed using a leaded-glass syringe, injected onto the HPLC column, and eluted with a solvent gradient that started very polar (75-90% aqueous buffer) and was ramped to a higher percentage of CH$_3$CN (70-85%) over time. Most of the labelling attempts resulted in the formation of very polar

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components that eluted at or near the void volume (void volume = volume of the sample loop + volume of the column + volume of the connecting tubing).

Buffered solutions were prepared and adjusted to the correct pH (with 1.0 M HCl or 1.0 M NaOH) by measurement with an Orion Ross® electrode combination (model 81-02) in conjunction with a Corning pH ion analyzer (model 335).

B. Reagents:

Ammonium Acetate (NH₄OAc) was obtained from Aldrich Chemical Co.

Ethylene diamine tetraacetate (disodium salt) was obtained from Fisher Scientific.

Glucoscan® (gluceptate) was obtained from Dupont/Merck Pharmaceutical.

Sodium Pertechnitate (Na⁹⁹ᵐTcO₄⁻) was obtained from a DuPont Merck Technelite® ⁹⁹ᵐTechnetium generator by elution with saline.

Sodium phosphate-monobasic (HNa₂PO₄) was obtained from Fisher Scientific.

Sodium phosphate-dibasic (H₂NaPO₄) was obtained from Fisher Scientific.
Sodium thiosulfate (Na$_2$S$_2$O$_3$) was obtained from Aldrich Chemical Co.

Stannous (II) chloride (SnCl$_2$) was obtained from Aldrich Chemical Co.

Tricine (N-[tris(Hydroxymethyl)methyl]glycine) was obtained from Sigma Chemical Co.

Triethylsilane (Et$_3$SiH) was obtained from Aldrich Chemical Co.

Trifluoroacetic acid (TFA) was obtained from Aldrich Chemical Co.

Triphenylphosphine-m-trisulfonate (TPPTS) was prepared previously by the method of Bartik$^{1}$ at Dupont/Merck (sample # DMP 112994-DEMO).

Tween 80® (polyoxyethylene (20) sorbitan monooleate) was obtained from Aldrich Chemical Co.

C. Solvents:

Acetonitrile (CH$_3$CN) was obtained from Fisher Scientific (HPLC grade).

Saline Solution was manufactured for Dupont/Merck for use with $^{99m}$Tc generators (5.8 mL crimp top bottles, 9 mg/mL NaCl solution).
D. Experimental Procedures:

1. Preparative HPLC of 150.

The reverse phase HPLC analysis for compound 150 was performed on a C\textsubscript{18} Vydac column (22.1 x 250 mm). The instrument consisted of a Rainin Rabbit solvent delivery system in conjunction with Knauer variable wavelength monitor set at 254 nm. A Dynamax HPLC method manager installed in a Macintosh IIci was used to control the instrument and printout data. A sample of 150 (20.0 mg) was dissolved in 2.0 mL of 1:1 solution of 0.05 M NH\textsubscript{4}OAc (pH 7.0) and CH\textsubscript{3}CN. This solution was then injected into the HPLC and four fractions were collected. The gradient and solvents are summarized below.

**HPLC Conditions**

| Column: | 22.1 x 250 mm C\textsubscript{18} Vydac |
| Flow: | 12.0 mL/min |
| Solvents: | A-0.05 M NH\textsubscript{4}OAc, pH 7.0 |
| Ramp: | 30 sec |
| Gradient: Time (minutes) | %B |
| 0 | 35% |
| 25 | 95% |
| 35 | 95% |
| 40 | 35% |

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Table 2.1 shows the amounts of each fraction collected after lyophilization.

Table 2.1

<table>
<thead>
<tr>
<th>Fraction #</th>
<th>Collection time (minutes)</th>
<th>Dupont/Merck Notebook#</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3-6</td>
<td>DMP-4701</td>
<td>1.0 mg</td>
</tr>
<tr>
<td>2</td>
<td>16-21</td>
<td>DMP-4702</td>
<td>1.0 mg</td>
</tr>
<tr>
<td>3</td>
<td>22-26</td>
<td>DMP-4703</td>
<td>1.0 mg</td>
</tr>
<tr>
<td>4</td>
<td>26-32</td>
<td>DMP-4704</td>
<td>2.0 mg</td>
</tr>
</tbody>
</table>

The total weight of fractions #1-4 after lyophilization was 5.0 mg giving a weight recovery of 25%.

E.\textsuperscript{99m}Tc Labelling of 1\textsuperscript{89} and 1\textsuperscript{90}.

1. Preparation of 1\textsuperscript{90} by Deprotection of Tritylated Monothiol

Ligand 1\textsuperscript{50}.

The ligand precursor (1\textsuperscript{50}) used in this procedure was ~93% pure according to $^{13}$C NMR. A solution of 1\textsuperscript{50} (0.4-4.0 mg) in CF$_3$CO$_2$H (0.5 mL) was stirred at room temperature in a stoppered flask for 10 minutes. To this solution was added Et$_3$SiH (0.5 mL) all

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in one portion and the stoppered flask was stirred for 10 minutes. CF₃CO₂H and Et₃SiH were removed under reduced pressure to give an oil which was dissolved in saline. This solution was used directly in labelling studies without purification.

2. Preparation of 189 by Deprotection of Tritylated Precursor 154. The (154) used in this procedure was >98% pure according to ¹³C NMR. A solution of 154 (4.0 mg) in CF₃CO₂H (0.5 mL) was stirred at room temperature in a stoppered flask for 10 minutes. To this was added triethylsilane (0.5 mL) all in one portion. The stoppered flask was stirred for 10 minutes. CF₃CO₂H and triethylsilane were removed under reduced pressure to give an oil which was dissolved in saline. This solution was used directly in labelling studies without purification.

3. Labelling Procedure 1: Ligand Exchange with ⁹⁹ᵐTc(V)O Glucoheptonate (General).

Into a 10 cc crimped vial was injected 0.2 mL of a glucoscan® kit (reconstituted with 2.0 mL distilled H₂O) and 20-50 millicuries (mCi) Na⁹⁹ᵐTcO₄ in saline. In some cases a phosphate buffer was
added at this point to control pH. Deprotected ligand 1 9 0 dissolved in saline (0.5 mL), was added to the mixture which was heated at 80 °C (water bath temperature) for 20 minutes and analyzed by HPLC. The total volume of the reaction mixture ranged from 1.0 mL to 2.3 mL. The presence of labelled ⁹⁹ᵐTc glucoheptonate was checked by TLC and HPLC prior to the addition of ligand.

HPLC Conditions

<table>
<thead>
<tr>
<th>Column:</th>
<th>4.6 x 250 mm C₁₈ Vydac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow:</td>
<td>1.0 mL/min</td>
</tr>
<tr>
<td>Solvents:</td>
<td>A-0.1% TFA in H₂O</td>
</tr>
<tr>
<td></td>
<td>B-9:1 CH₃CN:H₂O (0.1% TFA)</td>
</tr>
<tr>
<td>Gradient:</td>
<td>Time (minutes)</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>35</td>
</tr>
</tbody>
</table>

4. Labelling Procedure 2: Ligand Exchange Using Tricine and SnCl₂

A 10 cc vial was charged with 22.2 mg of Tricine and sealed with a crimp top. To this was added 4.0 mg of deprotected ligand 1 9 0 (weight before deprotection step) dissolved in 0.2 mL saline. The solution was adjusted to pH 10 using 0.03 mL of phosphate buffer (0.05 M, pH 12.5). A 10.0 μL solution of SnCl₂ (20.0 mg/mL in

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0.1 M HCl) was added to the mixture followed by 1.00 mL Na\textsuperscript{99m}TcO\textsubscript{4} solution (60 mCi/mL, Generator # 9438-6D-19). The mixture was left for 30 minutes at room temperature, then heated to 50 °C (water bath). The mixture was analyzed by HPLC at 20-30 minute intervals after addition of deprotected ligand. The total volume of the reaction mixture was 1.5 mL and the pH was 10.

**HPLC Conditions**

| Column: | 4.6 x 250 mm C\textsubscript{18} Vydac |
| Flow:   | 1.0 mL/min |
| Solvents: | A-0.1% TFA in H\textsubscript{2}O |
|         | B-9:1 CH\textsubscript{3}CN:H\textsubscript{2}O (0.1% TFA) |
| Gradient: | Time (minutes) | %B |
|          | 0             | 5% |
|          | 20            | 85% |
|          | 30            | 85% |
|          | 35            | 5% |

5. **Labelling Procedure 3: SnCl\textsubscript{2} and EDTA at pH 7.0.**

A 10 cc vial was charged with 15.3 mg of EDTA and sealed with a crimp top. To this was added 0.4 mg of deprotected ligand 190 (weight before deprotection step) dissolved in 0.2 mL saline. The solution was adjusted to pH 7.0 using 0.02 mL of phosphate buffer (0.05 M, pH 10.5). A 10.0 μL solution of SnCl\textsubscript{2} (20.0 mg/mL in 0.1 M HCl) was added to the mixture, followed by 1.00 mL Na\textsuperscript{99m}TcO\textsubscript{4}.
solution (55 mCi/mL, Generator # 9439-6D-17). The mixture was left for 30 minutes at room temperature, then heated to 50 °C (water bath). The mixture was analyzed by HPLC at 20-30 minute intervals after addition of deprotected ligand. The total volume of the reaction mixture was 1.4 mL and the pH was 7.

**HPLC Conditions**

- **Column:** 4.6 x 250 mm C<sub>18</sub> Vydac
- **Flow:** 1.0 mL/min
- **Solvents:**
  - A-0.1% TFA in H<sub>2</sub>O
  - B-9:1 CH<sub>3</sub>CN:H<sub>2</sub>O (0.1% TFA)
- **Gradient:**
<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5%</td>
</tr>
<tr>
<td>20</td>
<td>85%</td>
</tr>
<tr>
<td>30</td>
<td>85%</td>
</tr>
<tr>
<td>35</td>
<td>5%</td>
</tr>
</tbody>
</table>

6. **Labelling Procedure 4: Reduction with TPPTS on a Small Scale.**

A 10 cc vial was charged with 2.5 mg of sodium triphenylphosphine-m-trisulfonate (TPPTS) and sealed with a crimp top. To this was added 0.5 mg of deprotected ligand 190 (weight before deprotection step) dissolved in 0.2 mL saline. A 0.50 mL Na<sup>99m</sup>TcO<sub>4</sub> solution (198 mCi/mL, Generator #9443-6D-21) was
added to the mixture which was then heated to 80 °C (water bath). The mixture was analyzed by HPLC at 20-30 minute intervals. The total volume of the reaction mixture was 0.7 mL and the pH was 7.

**HPLC Conditions**

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<thead>
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<th>Column:</th>
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</thead>
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<td>Flow:</td>
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<tr>
<td>Solvents:</td>
<td>A-0.05 M Phosphate, pH=7.0</td>
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<tr>
<td></td>
<td>B-9:1 CH3CN:H2O</td>
</tr>
<tr>
<td>Gradient:</td>
<td>Time (minutes)</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>25</td>
</tr>
</tbody>
</table>

7. Labelling Procedure 5: Ligand Exchange with $^{99m}$Tc(V)O4 Glucoheptonate.

Into a 10 cc crimped vial was injected 0.2 mL of a glucoscan® kit (reconstituted with 2.0 mL distilled H2O) and 0.5 mL Na$^{99m}$TcO4 (100 mCi/mL) solution in saline. Ligand 189 dissolved in phosphate buffer (0.6 mL, 0.05 M, pH 10.5), was added to the mixture which was heated at 80 °C (water bath) for 20 minutes and analyzed by HPLC. The total volume of the reaction mixture was 1.3 mL and the pH was 10.0.

**HPLC Conditions**
Column: 4.6 x 250 mm C_{18} Vydac  
Flow: 1.0 mL/min  
Solvents: A-0.1% TFA in H_{2}O  
B-9:1 CH_{3}CN:H_{2}O (0.1% TFA)  
Gradient: Time (minutes) %B  
<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5%</td>
</tr>
<tr>
<td>20</td>
<td>85%</td>
</tr>
<tr>
<td>30</td>
<td>85%</td>
</tr>
<tr>
<td>35</td>
<td>5%</td>
</tr>
</tbody>
</table>

8. Labelling Procedure 6: Reduction with TPPTS for Bio-
distribution Scaleup.

A 10 cc vial was charged with 3.0 mg of sodium triphenylphosphine-m-trisulfonate (TPPTS) and sealed with a crimp top. To this was added 0.6 mg of deprotected ligand 190 (weight before deprotection step) dissolved in 0.2 mL saline. A 1.00 mL Na^{99m}TcO_{4} solution (366 mCi/mL, Generator # 9447-6D-18) was added to the mixture which was then heated to 80 °C (water bath) for 20 minutes. A 500 μL portion of the reaction mixture was injected into the HPLC. The method used for HPLC analysis and isolation of the 15.56 (elution time) minute component was the same as that used in labelling procedure 4. It was necessary to have excess ligand present in the collection flask for the isolation.
procedure. Excess ligand often helps improve the stability of the complex.

F. Analytical HPLC of 1 8 9

The reverse phase HPLC analysis for compound 1 8 9 was performed on a C₁₈ Vydac column (4.6 x 250 mm). The instrument consisted of a Hewlett Packard 1050 HPLC equipped with an autoinjector and UV wavelength monitor set at 254 nm. The Hewlett Packard 3D Software installed on a Hewlett Packard Vectra 486/66XM was used to control the instrument and printout data. A sample of 1 8 9 (1.0 mg) was dissolved in 0.5 mL of a 1:1 solution of 0.05 M NH₄OAc (pH 7.0) and CH₃CN. A portion of this solution (20 μL) was then injected into the HPLC. The gradient and solvents are summarized below.

**HPLC Conditions**

<table>
<thead>
<tr>
<th>Column:</th>
<th>4.6 x 250 mm C₁₈ Vydac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow:</td>
<td>1.0 mL/min</td>
</tr>
<tr>
<td>Solvents:</td>
<td>A-0.05 M NH₄OAc, pH 7.0</td>
</tr>
<tr>
<td>Ramp:</td>
<td>1 minute</td>
</tr>
<tr>
<td>Gradient:</td>
<td>Time (minutes)</td>
</tr>
<tr>
<td>0</td>
<td>35%</td>
</tr>
<tr>
<td>25</td>
<td>85%</td>
</tr>
</tbody>
</table>

360
G. Unsuccessful Labelling Attempts of \textit{2 9, 7 2, and 1 3 1}.

1. Ligand 7 2.

A 10 cc vial was charged with 5.0 mg of 7 2 and 3.0 mg Na$_2$S$_2$O$_3$ and then sealed with a crimp top. To this was added 1.0 mL of phosphate buffer (0.10 M, pH 11.0) and 1.0 mL Na$^{99m}$TcO$_4$ solution (100 mCi/mL, Generator \# 9418-D-19). The mixture was then heated to 80 °C (water bath). The mixture was analyzed by HPLC at 20-30 minute intervals after addition of ligand 7 2. The total volume of the reaction mixture was 2.0 mL and the pH was 11.0.

\textbf{HPLC Conditions}

\begin{center}
\begin{tabular}{|c|c|}
\hline
Column & 4.6 x 250 mm C$_{18}$ Vydac \\
Flow: & 1.0 mL/min \\
Solvents: & A-0.1\% TFA in H$_2$O \\
& B-9:1 CH$_3$CN:H$_2$O (0.1\% TFA) \\
Gradient: & Time (minutes) \%B \\
0 & 5\% \\
20 & 85\% \\
30 & 85\% \\
35 & 5\% \\
\hline
\end{tabular}
\end{center}


A 5 cc vial was charged with 3.0 mg of 2 9 and 3.0 mg Na$_2$S$_2$O$_3$
and then sealed with a crimp top. To this was added 1.0 mL of phosphate buffer (0.10 M, pH 7.0) and 0.5 mL Na$_{99}$mTcO$_4$ solution (50 mCi/mL, Generator # 2). The mixture was then heated to 100 °C (boiling water). The mixture was analyzed by HPLC at 20-30 minute intervals after addition of ligand 2.9. The total volume of the reaction mixture was 1.5 mL and the pH was 7.0.

**HPLC Conditions**

Column: 4.6 x 250 mm C$_{18}$ Vydic
Flow: 1.0 mL/min
Solvents: A-0.1% TFA in H$_2$O
B-9:1 CH$_3$CN:H$_2$O (0.1% TFA)
Gradient: Time (minutes) %B

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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<tr>
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<td>85%</td>
</tr>
<tr>
<td>30</td>
<td>85%</td>
</tr>
<tr>
<td>35</td>
<td>5%</td>
</tr>
</tbody>
</table>

3. Ligand 131.

A 5 cc vial was charged with 10.0 mg of 131 and 3.0 mg Na$_2$S$_2$O$_3$ and then sealed with a crimp top. To this was added 1.0 mL of phosphate buffer (0.10 M, pH 11.0). A 1.0 mL Na$_{99}$mTcO$_4$ solution (100 mCi/mL, Generator # 9418-D-19) was injected into the mixture which was analyzed by HPLC at 20-30 minute intervals (Note: no heat). The total volume of the reaction mixture was 2.0 mL.
and the pH was 11.0.

**HPLC Conditions**

- **Column:** 4.6 x 250 mm C$_{18}$ Vydec
- **Flow:** 1.0 mL/min
- **Solvents:**
  - A: 0.1% TFA in H$_2$O
  - B: 9:1 CH$_3$CN:H$_2$O (0.1% TFA)
- **Gradient:**
<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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<td>85%</td>
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<tr>
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<td>5%</td>
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Nicolot D4 Sample File

14 May 95 14:08:09

CCl₄ solution

30

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Nicolet DX: Sample File 28 Jun 94 16.08.51

WAVENUMBER (CM\(^{-1}\))

XTRANSMITTANCE

4000.0 3550.0 3100.0 2650.0 2200.0 1750.0 1300.0 850.0 400.0

WAVENUMBER (CM⁻¹) 800 600

ABSCISSA
EXPANSION _________ I________ EXPANSION _________ I_________
SUPPRESSION _______ % T. _______ ABS _______

ORDINATE
EXPANSION _______ ___ EXPANSION _______ ___

SCAN TIME _________ 12.4 mT RESPONSE _______ ( )
SLIT PROGRAM _______ ( ) OPERATOR _______ D.: 4/27/70

RESPONSE _______ ( ) TIME DRIVE _______ PRE SAMPLE CHQK

140° Neat
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