Expanding the Structural Scope of Supramolecular Assemblies and their Applications as Mechanistic Probes

by

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Abstract

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Chapter 1. A brief background and perspective is provided for the field of supramolecular chemistry. Justification for the continued expansion of the field as well as the work described in this dissertation are presented.

Chapter 2. A new synthetic strategy for the rapid diversification of $\text{M}_4\text{L}_6$ host structures is described. The outlined approach consists of two components: the first is the late-stage functionalization of a ligand precursor to access structural variation while preserving favorable self-assembly properties, and the second is the post-synthetic modification of these functional groups after host assembly. Through this approach, new amine-, azide-, and carboxylate-functionalized hosts are described with preliminary work and outlook for future applications.

Chapter 3. A novel supramolecular mechanistic probe is introduced, which serves as an experimental platform for isolating and evaluating the role of host charge in supramolecular catalysis. The probe consists of two isostructural metal–ligand catalysts of $\text{M}_4\text{L}_6$ stoichiometry with a significant variation in overall anionic charge: 12$^-$ versus 8$^-$. Together, they enable a unique experimental investigation that allows supramolecular structural features to be connected to specific mechanisms of reactivity. Though the importance of charge and electrostatic effects have been highlighted in enzymes and other supramolecular catalysts, this is the first example in which these effects have been experimentally defined in a synthetic microenvironment.

Chapter 4. An unusual enzyme-like mechanism of host–guest binding is described in a new metal–ligand host of $\text{Ga}_4\text{L}_4$ stoichiometry. The introduction of a sufficiently large and tightly bound guest enforces a configurational isomerization in the host from an $S_4$-symmetric conformation to a $T$-symmetric conformation with a proposed larger internal volume. Detailed mechanistic investigations reveal that this configurationally adaptive binding phenomenon proceeds via a conformational selection mechanism, a unique enzymatic mechanism that has
never been definitively recapitulated in a synthetic system prior to this work. This comprehensive study shows that a simple chemical system can stand as a model for analogous behavior in biological systems that are often too challenging to experimentally deconvolute and speaks to the symbiotic relationship between the fields of enzymology and supramolecular chemistry.
Expanding the Structural Scope of Supramolecular Assemblies and their Application as Mechanistic Probes

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Chapter 1
A Brief Introduction to the History and Aspirations of Supramolecular Chemistry
1.1 A Brief Introduction to Supramolecular Chemistry

The pursuit of knowledge and understanding has been a defining feature of humankind since its evolution, and over time, has yielded innumerable advances in the theories, methods, and technologies which accelerate these endeavors. Now, more than ever, we are witnessing a transition in which the stark lines that once segregated scientific fields such as chemistry, physics, and biology are being challenged and diminished.\(^1\) The increasingly collaborative and interdisciplinary nature of modern cutting-edge research speaks of this transformation. Perhaps this is owed to the fact that as we gain a stronger grasp on the governing principles of each field, these principles are progressively shown to be intimately tied together to constitute the defining properties of the universe. Thus, lessons which are at the core of one scientific field can be translated and applied to give new insights in a completely separate area of study.\(^2\)

At the time of its inception, supramolecular chemistry was a pioneer in expanding the notion of what constitutes synthetic chemistry.\(^3\) Synthetic chemistry was initially dominated by the study of molecules built by strong covalent and ionic bonds and the transformations which could be performed on these molecular species. Supramolecular chemistry, however, is primarily focused on the phenomena which arise from the accumulation of relatively weak interactions between multiple molecular components, or as one may phrase it, “chemistry beyond the molecule.”\(^4\)–\(^6\) Indeed, the importance of the field was recognized when the 1987 Nobel Prize was awarded to Professors Donald J. Cram, Jean-Marie Lehn, and Charles J. Pedersen for their advancements in molecular recognition.

Supramolecular chemistry began with systems as simple as the selective complexation of a wide range of metal cations to different, suitably sized crown ethers.\(^7,8\) This notion of a circular framework which circumscribes a binding site was common in other examples of early supramolecular frameworks. Key structures included cucurbiturils, calixarenes, and cyclodextrins (Figure 1.1).\(^9\)–\(^11\) These host frameworks, which were all composed of covalent linkages, could loop around molecular guests with measurably strong binding affinities. Very quickly, examples in the literature demonstrated that these abilities for molecular recognition and supramolecular interactions could be exploited to promote or catalyze simple biomimetic reactions.\(^12\)–\(^14\)

\begin{figure}
\centering
\includegraphics[width=\textwidth]{crown ethers.png}
\caption{Figures 1.1. Examples of covalent supramolecular hosts: crown ether, cyclodextrin, calixarene.}
\end{figure}
As supramolecular chemists sought more advanced applications and greater feats of catalysis, they continued to find inspiration from the active sites of proteins and enzymes. Nature, boasting of wildly successful and creative uses of microenvironments to promote life-sustaining reactions, had always captured the awe and envy of synthetic chemists. The beauty of enzymes is that their complex, tertiary structures and well-defined active sites are all generated by the carefully preprogrammed folding of linear peptide chains. Similarly, supramolecular chemists began to increasingly rely on the self-assembly of many molecular components to efficiently generate better defined and spatially segregated microenvironments. Often, these self-assembled microenvironments were more proficient at discriminating their interior from the bulk solution, relative to the circular structures that once defined the field.

1.2 A Brief Introduction to Self-Assembled Molecular Architectures

Self-assembly as a means to host synthesis can be achieved through a number of recognition motifs such as hydrogen bonding, hydrophobic association, and metal–ligand coordination (Figure 1.2). For brevity, hosts which rely on dynamic covalent chemistry are not included in this discussion. In analogy to proteins and nucleic acids, whose final tertiary structure is determined by numerous specific intramolecular interactions along the peptide framework, self-assembled systems are governed by the interactions between the simple components that generate them. Because of this, all self-assembled supramolecular hosts must obey certain restrictions in the molecular components incorporated and reaction conditions that they can be subjected to. For example, hosts which rely on hydrophobic association require the inclusion of a hydrophobic guest molecule and high concentrations of water for complexation. Similarly, hydrogen bonded hosts also have solvent requirements which must be met for the assembly to remain intact.

Figure 1.2. Examples of self-assembled hosts: hydrogen bonded and metal–ligand coordination motifs.
Metal–ligand coordination is often a favored motif for self-assembly, because the bonds which hold the host together are considerably stronger and tolerate a wider variety of reaction conditions than those utilized in other approaches. Additionally, these coordination cages tend to feature some degree of overall charge as a consequence of cationic metal centers and neutral or anionic ligand moieties. Electrostatic effects are well established as important in supramolecular and enzymatic catalysis, and metal–ligand coordination cages offer direct access to these features. Metal–ligand bonds are also highly directional, with predictable and defined geometries around metal centers. The combination of these features has resulted in a plethora of discrete self-assembled polyhedra in the literature, including but not limited to octahedra, cubes, cylinders, and tetrahedra.

The work presented in this dissertation focuses on the chemistry of tetrahedral metal–ligand coordination hosts. Though each chapter focuses on different variants of these hosts, the assembly developed by Raymond and coworkers is the representative structure of interest (Figure 1.3). Self-assembly of ligand with Ga(III) ions at the four vertices affords, which features Ga$_4$ stoichiometry. Each Ga(III)–catecholate vertex is pseudooctahedral in geometry and carries a trianionic formal charge. This gives an overall dodecaanionic charge and renders the host soluble in water and polar organic solvents. In contrast to the environment of the bulk solution, the microenvironment inside the host cavity is significantly hydrophobic due to the aromatic walls. 1 has been demonstrated to be a highly competent catalyst in a wide range of chemical transformations. In addition to diversity in reactivities, 1 can effect rate accelerations that are in the enzymatic regime (up to $10^7$-fold).

![Diagram of [Ga$_4$2]$_{12}^-$ tetrahedron 1. Only one ligand 2 is shown of six and counterions are omitted for clarity. Blue lines represent 2 and spheres represent metal ions.](image)

Figure 1.3. The [Ga$_4$2]$_{12}^-$ tetrahedron 1. Only one ligand 2 is shown of six and counterions are omitted for clarity. Blue lines represent 2 and spheres represent metal ions.

1.3 Why Should We Care?

Supramolecular chemistry affords scientists the unique opportunity to imitate nature at the molecular level. Lessons learned from enzymatic catalysis and molecular recognition in biological systems often provide the inspiration for the reactions and applications that are explored. However, the relationship between supramolecular chemistry and biology need not be
strictly unidirectional; rather, as the field matures, examples of supramolecular hosts which can stand as model systems for biological phenomena and platforms for dissecting fundamental physical organic phenomena are demonstrated more frequently.\textsuperscript{50} Progressively sophisticated systems can now imitate signal transduction,\textsuperscript{51} perform chemistry in conjunction with enzymes,\textsuperscript{52} and even act as synthetic enzymes for chemistry that has never been broached by nature.\textsuperscript{53} As science becomes more interdisciplinary in the coming years, fundamental insights and applications developed in the field of supramolecular chemistry will surely grow in number and in importance.
1.4 References


Chapter 2
A Synthetic Strategy for Generating Diversity in Self-Assembled Tetrahedral Hosts
2.1 Preface

This chapter outlines a new synthetic strategy to expand the library of self-assembled M₄L₆ tetrahedral hosts. One of the most significant challenges in studies of supramolecular catalysis is the restricted range of available hosts. This issue is exacerbated by the limited ability to rationally design new ligands for self-assembly. Moreover, the synthesis of a new ligand does not guarantee that the self-assembled host will be useful as a catalyst. The outlined strategy attempts to circumvent this issue by preserving the geometry and binding motif of a well-established supramolecular catalyst ligand in a precursor molecule. Late-stage functionalization of this precursor grants rapid access to structurally unique ligands which self-assemble as predicted. The resulting supramolecular hosts can be further diversified by post-synthetic modification of these functional groups after host assembly. A scope of amine-, azide-, and carboxylate-functionalized hosts are described with some outlook for their applications.

2.2 Introduction to the Challenges in Supramolecular Catalyst Diversification

In many ways, supramolecular catalysis is a unique subset of homogeneous catalysis, featuring a variety of interesting advantages as well as challenges in its application and study. ¹ Perhaps the most defining characteristic of this field is the promotion of reactions within well-defined microenvironments.² Naturally, enzymes and their active sites serve as a plentiful source of guiding principles as well as inspiration for understanding and developing supramolecular catalysis.³ Like enzymes, these catalysts utilize spatially defined microenvironments to promote reactions with rate accelerations and product selectivities that vary drastically from those in bulk solution. These microenvironments often have excellent solvent exclusion properties and recognize substrates through interactions such as hydrogen bonding, π–π stacking, cation–π and anion–π interactions, constrictive, hydrophobic, and electrostatic effects.⁴

While active sites in enzymes are formed by the precisely programmed folding of proteins, microenvironments in synthetic supramolecular catalysts must be constructed either by careful manipulation of covalent architectures or by efficient self-assembly of many simple components.⁵–⁷ The former presents significant synthetic challenges, while the latter can be achieved through a number of self-assembly motifs, including hydrogen bonding,⁸–¹¹ hydrophobic association,¹²,¹³ and metal–ligand coordination.¹⁴–¹⁷ Similar to proteins, which have their final tertiary structure determined by numerous non-covalent interactions along the peptide framework, self-assembled systems are governed by the interactions of simple components that generate them.¹⁸,¹⁹ A myriad of equilibria dictated by subtle intermolecular interactions between these components must align to yield the supramolecular host.²⁰,²¹ As a result, relatively minor changes to reaction conditions or constituents can have deleterious effects on self-assembly.

This aspect of catalyst synthesis leads to a daunting challenge in the study of supramolecular catalysis. Minor changes to catalyst components such as the introduction of electron withdrawing/donating groups, hydrogen bonding moieties, altered ligand sterics, and modified ligand geometries often impact the delicate series of equilibria and lead to intractable
mixtures of products. Unfortunately, this often precludes structural variation of the catalyst, which limits exploration for modified reactivity and rigorous mechanistic investigations. For instance, the field of transition metal catalysis, another subset of homogeneous catalysis, has been developed largely through incremental ligand modifications and the insights gained from those. These modular ligand platforms for transition metals also enable rapid diversification and are often the most valued.

Given the challenge of designing, modifying, and accessing supramolecular catalysts, much of the field has grown around a relatively small number of key host structures. Of these, the tetrahedral metal–ligand coordination host 1 developed by Raymond and coworkers is notable (Figure 2.1). Six rigid naphthalene-based ligands 2 (1,5-bis(2,3-dihydroxybenzoylaminonaphthalene)) form discrete M₄L₆ stoichiometry tetrahedral cages when complexed to four Ga(III) ions under basic conditions. Each Ga(III)–catecholate vertex carries a trianionic formal charge, giving 1 an overall dodecaanionic character and rendering the host soluble in water and polar organic solvents such as DMSO, methanol, and DMF. In contrast, the cavity within the assembly is highly hydrophobic. Since its appearance in the literature, host 1 has been well characterized and studied in a number of catalytic reactions. In addition to boasting a wide scope of catalytic transformations, host 1 is understood to drive reactivity through multiple modes, including but not limited to constrictive binding effects, substrate pKₐ shifts, and solvent exclusion. The success of 1 as a catalyst motivates structural diversification of this system, which would greatly benefit from structure–activity relationships and possibly enable improved catalyst structures.

![Figure 2.1. The [Ga₄L₆]₁²⁻ tetrahedron 1. Only one ligand 2 is shown of six and counterions are omitted for clarity. Blue lines represent ligand 2 and spheres represent Ga(III) ions.](image)

To this end, Raymond and co-workers have modified assembly 1 by variation of ligand 2. Specifically, the introduction of homochiral directing groups at the distal ends of the chelators has allowed a vertex-directed diastereoselective synthesis of enantiopure host 3, facilitating studies with enantioselective catalysis and an unusual S_N₂ reaction with stereochemical retention (Figure 2.2). Furthermore, modification of the naphthalene linker at the core of ligand 2 to a pyrene linker gives rise to host 4, which leads to a larger interior cavity. Finally, host 5 was developed from the incorporation of both a pyrene linker and homochiral directing groups to generate an analogous enantiopure pyrene-host. This enabled the dissection of constrictive
binding and size exclusion effects in a catalytic Prins cyclization reaction. In parallel, these hosts have been applied as probes for understanding the role of the cavity in catalysis (Figure 2.2).

Figure 2.2. The M₄L₆ host 1 and related variations to its structure: an enantiopure host 3 which enables enantioselective catalysis, an expanded pyrene-based host 4 which enables constrictive binding and size exclusion studies in catalysis, and the expanded, enantiopure host 5.

The advancements gained by modifications to size and chirality demonstrate that there is much left to be explored with structural variations of 1. Of particular interest are variations in the overall host charge for an improved understanding of electrostatic effects in catalysis, especially with substrates that experience an increase in charge throughout the course of the reaction. Another point of interest is the variation of electron withdrawing/donating abilities of the host ligands, which may provide insight into proposed interactions of the substrate with the aromatic walls of the host. Other interests include studying hosts with different solvation properties, as well as hosts with pendent functional groups. These functional groups may influence reactivity despite being spatially removed, much like an allosteric binding effect in enzymatic catalysis. They may also act as secondary, external active sites for catalysis, secondary sites for guest binding, or sites for post-synthetic modification of the hosts. These new hosts may serve as useful probes for studying known catalytic systems, as shown above, or open avenues for developing new interesting applications. Described here is a two-component strategy for accessing such new hosts, which target rapid access to structural diversification while maintaining favorable and predictable self-assembly properties of ligands.

2.3 A Two-Component Approach to Diversified M₄L₆ Hosts

The first component of this diversification strategy aims to minimize synthetic steps that must be optimized for each new ligand, while maintaining predictable self-assembly properties. The proposed method of achieving these goals is to establish a ligand precursor based on ligand 2, where late-stage functionalization can rapidly generate diversified ligands. It is known that the favorable self-assembly properties of ligand 2 are endowed by its 1,5-substituted naphthalene core, as well as the amide linkage between the core and the catecholate moieties. Amide bond rotation allows for flexibility in the host, and the offset angle of the 1,5-substituted naphthalene
core is an ideal match for forming a tetrahedron with octahedral metal vertices.\textsuperscript{41} Therefore, the late-stage ligand precursor should maintain the core and amide connections of ligand 2. While this strategy limits diversification of the arene core, this is not a significant limitation, as very little diversification at this site is tolerated. Modest modification of 1 or 4 from a naphthalene or pyrene core to an anthracene core leads to selective formation of the helicate over the tetrahedron.\textsuperscript{42} It is evident that the unique offset of the 1,5-naphthalene core is important for reliable self-assembly to a tetrahedron.

The self-assembly behavior of host 3 inspired the strategy for diversifying the host at the distal amide bond moiety of a terephthalic acid-based ligand precursor (Figure 2.3A). In previous work, homochiral alkyl directing groups were installed \textit{via} the terephthalamide motif to direct diastereoselective self-assembly.\textsuperscript{38} In this work, a one-step amidation of a terephthalic acid ligand precursor generates a new protected ligand, which can be deprotected and tested for self-assembly. As long as the installed functionalities are not too sterically demanding, these ligands should reasonably be expected to self-assemble into $M_4L_6$ tetrahedra.

The second component of the strategy entails further modification of the hosts formed from divergent ligand synthesis. This can be achieved by derivatizing the ligands after self-assembly, from here on referred to as post-synthetic modification (PSM). This approach is advantageous in a number of ways over traditional host assembly, where self-assembly is the last synthetic manipulation. For example, sensitive moieties that are incompatible with prior
synthetic steps or self-assembly conditions may be incorporated via PSM. In addition, one could imagine that formed hosts may be immobilized on or tethered to surfaces and other chemical entities. Lastly, PSMs which take advantage of the spatial arrangement of the ligands could be of interest, such as the construction of covalent frameworks which are difficult to access without the preorganization offered by metal–ligand self-assembly.

2.4 Design and Synthesis of a Ligand Precursor for Late-State Diversification

The first component of this strategy requires a simple and robust synthetic route to a late-stage ligand precursor. To this end, conditions were optimized to access methyl ether-protected ligand precursor 6 on multi-gram scale with high yields and no chromatographic purification steps (Scheme 2.1). 1,5-diaminonaphthalene was acylated with the previously published lithium terephthalate salt 7 via a HATU-mediated amidation. The resulting permethylated ligand precursor 8 could be isolated by filtration and then subjected to nucleophilic carboxylate demethylation with potassium trimethylsilanolate to give 6 in high yields. The low solubility of 8 in non-halogenated solvents necessitates this unusual approach to an effective hydrolysis of the methyl esters; high temperatures and biphasic conditions did not afford 6 in usable quantities.

After diversification of precursor 6, the aryl methyl ether protecting groups can be cleaved by treatment with an excess of boron tribromide to reveal the catecholate binding moieties. While these conditions are tolerant to interesting functional groups such as amines, saturated hydrocarbons, ketones, and carboxylate acids, many others are not compatible. In the interest of accessing milder deprotection conditions, a related ligand precursor 9 that employs benzyl ethers was prepared in a manner analogous to that used for precursor 6 (Scheme 2.2). Benzyl ethers were chosen as a protecting group to enable hydrogenation or acidic deprotection strategies. Known benzyl ether-protected terephthalate intermediate 10 was used to acylate 1,5-diaminonaphthalene via an acid chloride intermediate. Issues with the solubility of 11 again led to the use of potassium trimethylsilanolate in a nucleophilic demethylation strategy to reveal the carboxylic acid functional handles in precursor 9.
Scheme 2.2. Synthetic procedure to access to benzyl ether-protected ligand precursor 9.

2.5 Amine-Functionalized Ligands and Their Self-Assemblies and Post-Synthetic Modifications

To demonstrate the viability of this approach for ligand diversification, three new amine-functionalized ligands were targeted (Figure 2.4). Ligands 12, 13, and 14 were synthesized to explore a range of methods targeting primary amines for PSM. These include nucleophilic addition of the pendent amines into activated esters to form amides, addition into isocyanates and isothiocyanates to form urea and thiourea moieties, as well as reductive amination with aldehyde partners. Varying amine linker lengths to ethyl, butyl, and hexyl moieties was anticipated to provide different degrees of flexibility and freedom about the highly charged, sterically congested vertices of the hosts. This is an important consideration for PSM, given that these effects may attenuate the reactivities of pendent amines in the PSM manifolds described.

Figure 2.4. Amine-functionalized ligands 12, 13, and 14 with varying linker lengths.
All three ligands were synthesized via a general synthetic strategy where a HATU-mediated di-amidation of 6 with mono-N-Boc-protected diamine prepares the protected ligand. Global deprotection of the Boc groups and methyl ethers was realized via standard boron tribromide treatment (Scheme 2.3). Interestingly, synthesis of ligands 12, 13, and 14 from the benzyl ether-protected precursor 9 led to mixtures of products which could not be separated.

Scheme 2.3. A general approach to amine-functionalized ligands from precursor 6.

Initial attempts to synthesize amine-functionalized hosts using the established conditions for host 1 led to the formation of precipitates, even in the presence of the strongly binding template tetraethylphosphonium. Upon screening a variety of host forming conditions (order of addition, stoichiometries, basic salts, gallium source, reaction times, and temperature), an optimized procedure to generate hosts Ga₄12₆ and Ga₄13₆ from ligands 12 and 13 was established (Scheme 2.4). It should be noted that excess base, predissolving of ligand and guest, and use of Ga(NO₃)₃ as the Ga(III) source are very important for host formation. It is speculated that a large excess of base is required for two reasons. First, it is possible the pendant amines are protonated by residual hydrobromic acid from deprotection conditions, and the initial equivalent of base is consumed to neutralize them. Second, the pendant amines may experience a shift in effective pKa upon catecholate deprotonation, which may cause issues with solubility and subsequent precipitation. An excess of base may disfavor this effect.

As observed previously with host 1, the inclusion of a strongly bound guest molecule (tetraethylphosphonium) inside Ga₄12₆ is confirmed by chemical shifts that are moved significantly upfield (Figure 2.5). The small number of aromatic signals observed demonstrates that the host is overall T-symmetric, identical to that of 1. Interestingly, the methylene protons of the ethyl-linker in the pendant amine are diastereotopically discriminated by the chiral vertices of the host, resulting in the complex splitting observed. This is analogous to the splitting observed in tightly bound guest molecules. It is also noted that the unusually low integration values of aromatic host signals at 8.12 and 7.77 ppm are likely due to long T₁ times, which are characteristic of large molecules that tumble slowly in solution. Host Ga₄13₆ also forms under these conditions and exhibits similar spectroscopic properties (Figure 2.6).
Scheme 2.4. Synthetic conditions for the self-assembly of amine-functionalized hosts.

Figure 2.5. The $^1$H NMR spectrum of PEt$_4^+ \subset $Ga$_4$12$_6$. Diastereotopic discrimination of otherwise identical protons are observed in the guest as well as the pendent ethylamine linkers.

Figure 2.6. The $^1$H NMR spectrum of PEt$_4^+ \subset $Ga$_4$13$_6$. The spectroscopic properties of PEt$_4^+ \subset $Ga$_4$13$_6$ are similar to those observed for PEt$_4^+ \subset $Ga$_4$12$_6$. 
While this strategy was successful for self-assembly with ligands 12 and 13 to form clean host-guest complexes, all attempts to synthesize the Ga₄₁₄₆ host were unsuccessful. Ligand 14 could not be readily solubilized, and treatment with aqueous bases and tetraethylphosphonium bromide consistently formed a thick yellow precipitate upon addition of any Ga(III) source. The yellow precipitate was insoluble in water and persisted through a wide range of pH values. Furthermore, attempts to improve reaction homogeneity in water/DMSO mixtures yielded various oligomeric species that do not encapsulate tetraethylphosphonium, even after several days of heating at 60 °C. Given that the ethylamine and butylamine functionalized ligands 12 and 13 readily form hosts, it was hypothesized that the added hydrophobicity of the hexylamine functionality reduces solubility of intermediate Ga(III)–ligand oligomers, kinetically trapping out any intermediate species as precipitate before PEt₄⁺ ⋅ Ga₄₁₄₆ forms. It is also possible that the increased linker length leads to enough flexibility within the ligand to enable intramolecular hydrogen bonds that contribute to reduced solubilities.

With two new amine-functionalized hosts in hand, systematic screening of solvent and pH were tested to understand solubility and stability, respectively. This was particularly important in the context of exploring the second component of the described synthetic strategy, PSM. Several of the targeted strategies for amine PSM reaction conditions are not compatible with basic pH. Thus, it was crucial to establish the hosts’ stability and solubilities under neutral to mildly acidic conditions and in organic solvents. Surprisingly, it was found that a thick yellow suspension formed from an aqueous solution of PEt₄⁺ ⋅ Ga₄₁₄₆ at pH values lower than 10. Removing the water in vacuo left a yellow residue that was insoluble in hot DMSO, DMF, and methanol. Furthermore, this opaque solution was not easily centrifuged or isolated via filtration. Altogether, the evidence suggested that the host was not suffering from decomposition, given that no free or decomposed ligand was observed.

These observations suggest instead that the pendent amines experience effective pKₐ shifts, become protonated, and the observed precipitate results from protonation of the host to produce dangling ammonium moieties. The generation of an overall lower charged host would lower the enthalpic driving force for solvation, reducing host solubility in water. Furthermore, this charge variation across the surface of the protonated host could also limit solubility in other solvents. To test this, a variety of conditions known to break and solubilize aggregated macromolecules with high surface charge variation were explored. Attempts included various solvent mixtures, the addition of high concentrations of magnesium salts in water, and various chaotropic agents such as urea to disrupt hydrogen bonding networks. Unfortunately, all of these were inefficient at dissolving enough precipitate to enable host detection by ¹H NMR. Another possible strategy to solubilize these hosts is to deprotonate the ammoniums with a non-nucleophilic organic base. Screening various organic amine bases led to the discovery that tetramethylguanidine was competent at solubilizing detectable amounts of host. By adding a large excess of tetramethylguanidine, PEt₄⁺ ⋅ Ga₄₁₂₆ could be dissolved in DMSO-d₆ to obtain an ¹H NMR spectrum (Figure 2.7).
With conditions in-hand to prepare samples with soluble host, PSM of hosts Ga$_4$12$_6$ and Ga$_4$13$_6$ were investigated. Upon treatment of host PEt$_4^+$ $\subseteq$ Ga$_4$12$_6$ with an excess of benzyl isocyanate, new resonances in the $^1$H NMR spectrum in the region appropriate for benzylic protons ($4 - 5$ ppm) grew in. These resonances are consistent with nucleophilic addition of the host amine to generate putative urea adducts. Unfortunately, the reaction was very exothermic and the reaction mixture was heterogeneous. Isolation of the functionalized host from the reaction mixture proved difficult and detrimental reactivity from the exotherm seemed likely. Many products were observed and impeded direct confirmation of host functionalization by $^1$H NMR. However, the expected di-functionalized ligands were successfully detected by mass spectrometry upon quenching and acidic decomposition of the host, suggesting that some degree of functionalization had indeed occurred.

Though an encouraging initial hit, conditions for PSM with a milder electrophile were sought to achieve more selective reactivity and minimize byproduct formation. A series of reagents were screened against PEt$_4^+$ $\subseteq$ Ga$_4$12$_6$ under a variety of reaction conditions including reaction temperature, addition techniques, and substrate concentrations. Reaction pH was varied as well, ranging from homogenous under basic conditions to heterogenous reactions under neutral conditions. Additionally, reactivity in DMSO-$d_6$ was investigated under three different conditions. The first was homogeneous PEt$_4^+$ $\subseteq$ Ga$_4$12$_6$ (isolated under basic conditions), followed by heterogeneous PEt$_4^+$ $\subseteq$ Ga$_4$12$_6$ (isolated under neutral conditions), as well as homogeneous PEt$_4^+$ $\subseteq$ Ga$_4$12$_6$ (isolated under neutral conditions and solubilized with tetramethylguanidine as an additive) in DMSO-$d_6$. Beyond reactivity conditions, a variety of reactivity modes were investigated including addition into milder electrophiles such as isothiocyanates, as well as condensation with aldehydes known to favor imine formation, and amidation with activated esters (Figure 2.8). Unfortunately, most attempts resulted in either no reactivity or host decomposition.
2.6 An Azide-Functionalized Ligand and its Self-Assembly and Post-Synthetic Modifications

The challenges encountered in demonstrating useful PSMs with amine-functionalized hosts inspired the development of azide-functionalized ligand 15. Azide was selected as an ideal functional group because it is a very weak Brønsted base and therefore should not be susceptible to detectable effective pKₐ shifts. Additionally, azides have known modes of reactivity under aqueous conditions and have limited sensitivity to basic pH. Furthermore, literature on the robust chemistry of Cu(I)-catalyzed alkyne–azide cycloadditions is extensive.⁴⁵–⁴⁷ Though the preservation of azides through the harsh conditions necessary for aryl methyl ether and benzyl ether cleavages initially posed a barrier to this strategy, this challenge was overcome by a simple late-stage modification of ligand 12. Imidazole-1-sulfonyl azide salts are known to efficiently perform Cu(II)-catalyzed di-azo transfers onto nucleophilic amines under mild conditions.⁴⁸

Gratifyingly, with minor reaction modifications ligand 15 was obtained from ligand 12, albeit in low yields and with variable purity (Scheme 2.5). The successful installation of an azide functionality was supported by the characteristic azide stretch observed by IR (2142 cm⁻¹) (Figure 2.9). The yield and product purity were improved by recrystallizing the sulfonyl azide reagent and performing the reaction under air-free conditions. It was also found that the reaction yield degrades rapidly with copper catalyst loadings beyond 5%, presumably due to undesired oxidation and degradation of the ligand by Cu(II). To overcome the air sensitivity of the product, rapid acid work-up is required. The optimized work up requires quenching the excess potassium carbonate by dropwise addition of 1M hydrochloric acid to pH 4 to ensure product stability in air. Although, Ni(II), Co(II), and Zn(II) halide salts are competent catalysts, the best yields and purities are achieved with Cu(II) sulfate hydrate.

Figure 2.8. A selection of the PSM reagents investigated with host PEt₄⁺ ⊂ Ga₄12₆."
Scheme 2.5. Protecting group-free synthesis of ligand 15 from ligand 12.

Figure 2.9. IR spectrum of ligand 15 with the characteristic azide stretch at 2142 cm$^{-1}$.

Interestingly, the optimized self-assembly of host with ligand 15 required Ga(acac)$_3$ as a Ga(III) source rather than Ga(NO$_3$)$_3$. To achieve clean formation, free ligand, Ga(acac)$_3$, and tetraethylphosphonium bromide must be incubated at 60 °C before slow addition of excess potassium hydroxide as an aqueous solution. The $^1$H NMR spectrum of PEt$_4^+\subset$Ga$_4$15$_6$ displays some unique properties, including severe line-broadening of the aromatic proton signals of the host. There is no clear cause for this broadening, and these effects precluded a reliable baseline correction of the spectrum and thus accurate integration of the peaks. There is also diastereotopic splitting of the methylene protons in the azide linker, similar to that observed in the amine-functionalized hosts (Figure 2.10).
Figure 2.10. $^1$H NMR spectrum of of PEt$_4^+$ ⊂ Ga$_4$15$_6$. Though there are severe line broadening effects, host formation and encapsulation are observed.

Preliminary PSM efforts were directed at functionalization of host PEt$_4^+$ ⊂ Ga$_4$15$_6$ with alkynes such as propargylamine and propargyl alcohol. Initial screens under a variety Cu(I)-catalyzed alkyn–azide cycloaddition conditions revealed that Cu(II) salts and an excess of a reductant led to host decomposition. While Cu(I) salts were viable as catalysts, catalytic loadings greater than 10% resulted in precipitation of the PEt$_4^+$ ⊂ Ga$_4$15$_6$ with no reaction progress. Therefore, ligated sources of Cu(I) were investigated. Heating a solution of PEt$_4^+$ ⊂ Ga$_4$15$_6$ in various water/t-butanol/acetonitrile solvent mixtures with (Cu(I)-TBTA)I and excess propargyl alcohol overnight provided detectable quantities of modified ligand 15, which was verified by MALDI-TOF analysis (see the Supporting Information). Despite this promising hit, it was unfortunately very difficult to quantitatively functionalize all twelve azide groups on PEt$_4^+$ ⊂ Ga$_4$15$_6$. While the development of ligands 12 and 15 has enabled the first demonstration of PSM in this series of tetrahedral hosts, subsequent efforts were focused on a new carboxylate-functionalized host described below.

2.7 A Carboxylate-Functionalized Ligand and its Properties Upon Self-Assembly

Carboxylic acid-functionalized ligand 16 could be accessed from either precursor 6 or 9 upon global deprotection of the ester and aryl ether moieties with boron tribromide (Scheme 2.6). The self-assembly of this ligand was anticipated to generate a host that, upon deprotonation of the carboxylic acids, would double the dodecaanionic charge of the host and possibly facilitate studies regarding the influence of host charge on catalysis and guest binding. Furthermore, ligand 16 benefits from additional stability to oxidation, even when deprotonated. This is in contrast to other Raymond self-assemblies, which are reported to decompose via oxidation of the catecholates by air. While the free ligands are air stable under acidic or neutral conditions, deprotonated catecholates oxidize rapidly, even when complexed to Ga(III). Indeed, it was observed that ligand 16 under basic aqueous conditions will resist oxidation over a period of several days, whereas ligand 2 under identical conditions decomposes within hours.
Scheme 2.6. Deprotection of either 6 or 9 to access ligand 16.

Following standard conditions for self-assembly, ligand 16 was suspended in degassed H$_2$O with Ga(acac)$_3$ and tetraethylphosphonium bromide, followed by addition of excess aqueous potassium phosphate tribasic. Strongly encapsulated tetraethylphosphonium was observed, as evidenced by $^1$H NMR with the appearance of characteristic peaks in the far upfield region and the simple aromatic region (Figure 2.11). Broad upfield peaks suggested that excess tetraethylphosphonium was associating with the exterior of PEt$_4^+ \subset$ Ga$_4$16$_6$.

Figure 2.11. $^1$H NMR spectrum of PEt$_4^+ \subset$ Ga$_4$16$_6$. The spectrum was collected in H$_2$O, which enables observation of the amide proton peak.

Although the cavity of PEt$_4^+ \subset$ Ga$_4$16$_6$ is anticipated to be isostructural to that of host 1, the host’s catalysis and guest interactions may be significantly different due to the large anionic charge generated upon deprotonation of the carboxylic acids of host Ga$_4$16$_6$. Since charge is known to strongly affect solvation of the host, as well as electrostatic binding properties, variations on this charge are expected to perturb encapsulation properties as well. Currently, all known Raymond assemblies reside at a maximum overall charge of 8$^-$ or 12$^-$. Full deprotonation of PEt$_4^+ \subset$ Ga$_4$16$_6$ would give rise to a 24$^-$ charge structure.

Unexpectedly, PEt$_4^+ \subset$ Ga$_4$16$_6$ is only soluble in water under basic conditions (pD > 8), forming a clumpy yellow precipitate upon further acidification. Given the persistence of Ga(III)--catecholates and other Raymond hosts in solutions at pD > 6, decomposition of the PEt$_4^+ \subset$ Ga$_4$16$_6$ seemed to be an unlikely cause for the precipitate observed. This yellow precipitate was
isolated via filtration and found to be readily soluble in DMSO-$d_6$. $^1$H NMR analysis of this residue revealed that indeed, PEt$_4^+$ \textit{Ga}_{416}_6 persists, despite formation of precipitate (Figure 2.12). An interesting feature of this $^1$H NMR spectrum is a new peak at 14.93 ppm, consistent with quantitative protonation of the pendent carboxylate in PEt$_4^+$ \textit{Ga}_{416}_6. In contrast, PEt$_4^+$ \textit{Ga}_{416}_6 isolated by removal of water \textit{in vacuo} at pD 13 was only sparingly soluble in DMSO-$d_6$. As shown above, analysis of this species in H$_2$O reveals that the carboxylic acid peak is fully deprotonated. Given that PEt$_4^+$ \textit{Ga}_{416}_6 is completely intact upon isolation by mild acidification and filtration, the abrupt change in host solubility was attributed to the protonation/deprotonation of the pendent carboxylates. This constitutes the first $M_4L_6$ tetrahedral host where variability in overall charge can be induced by adjusting the pD of the bulk solution. The acidified, low-charge state was assigned to be $12^-$, analogous to host 1. It is challenging to definitively assign a anionic charge value to the high-charge state due potential fast-exchange of protons between the carboxylates, which reduce the overall anionic charge. However, it has been demonstrated that the effective variation in charge is significant enough to impart new properties, such as a drastic change in solubility.

![Figure 2.12](image)

**Figure 2.12.** $^1$H NMR spectrum of PEt$_4^+$ \textit{Ga}_{416}_6 isolated from water at pH 7. The spectrum features the appearance of a new carboxylic acid peak, which is concomitant with an abrupt change in solubility.

Subsequent studies of PEt$_4^+$ \textit{Ga}_{416}_6 rates of guest self-exchange, as well as amide H–D exchange rates, unfortunately showed no significant deviations from those measured for host 1. Attempts at PSM via activation of the carboxylic acids for amidation also failed, presumably due to the high steric bulk around host vertices. Although no definitive insights regarding the overall anionic charge could be garnered from these experiments, PEt$_4^+$ \textit{Ga}_{416}_6 offers the potential for many interesting applications and lessons to be learned. Future efforts must achieve the synthesis of solvent-occupied Ga$_{416}_6$ under template free conditions or a method to force excretion of tetraethylphosphonium from Ga$_{416}_6$.

### 2.8 Conclusions

In conclusion, the development of a late stage ligand precursor as well as ligand diversification and PSM has attempted to take steps towards meeting the challenge of structure diversification in self-assembly $M_4L_6$ tetrahedra. The late stage ligand precursor can be made on gram scale under chromatography-free conditions and has been demonstrated to successfully be
developed into ligands which reliably self-assemble. Furthermore, modest examples of PSM of these hosts have been shown. While the work presented is somewhat preliminary, the development of amine-, azide-, and carboxylic acid-functionalized ligands may open doors for interesting opportunities to gain insights into supramolecular catalysis and host behavior.
2.9 Supporting Information

2.9.1 General Methods

Unless otherwise noted, all reactions were carried out in oven-dried glassware sealed with rubber septa under a nitrogen atmosphere with Teflon-coated magnetic stir bars, with the exception of those performed in NMR tubes. Reaction progress was monitored using thin layer chromatography on Silicycle Siliplate™ glass backed TLC plates (250 μm thickness, 60 Å porosity, F-254 indicator) and visualized with 254 nm UV light or stained by submersion in a basic potassium permanganate solution. Flash column chromatography was performed on MP Biomedicals SiliTech silica gel 32-63D. Dimethylformamide (DMF), tetrohydrofuran (THF), diethylether (Et₂O), methylene chloride (DCM), and triethylamine were dried by passed the previously degassed solvents through activated alumina columns under argon. Deuterated solvents were purchased from Cambridge Isotope Laboratories and used as received. All other reagents were purchased from Sigma Aldrich and Fischer Scientific and used as received without further purification. Proton nuclear magnetic resonance (¹H NMR) and carbon nuclear magnetic resonance (¹³C NMR) spectra were taken with AV-300, AVB-400, AVQ-400, AV-500, DRX-500, or AV-600 Bruker spectrometers operating at 300MHz, 400MHz, 500 MHz, or 600 MHz. Chemical shifts are reported in parts per million (ppm) with reference to the appropriate residual solvent signal. ¹H NMR: CDCl₃ (δ: 7.26 ppm), DMSO-d₆ (δ: 2.50 ppm), MeOD (δ: 3.31 ppm), D₂O (δ: 4.79 ppm). ¹³C NMR: CDCl₃ (δ: 77.16 ppm), DMSO-d₆ (δ: 39.52 ppm). ¹H NMR multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), sept (septet), m (multiplet). Electrospray ionization mass spectra (ES(−)-MS), (ES(+)-MS) were obtained on a Thermo LTQ-FT-ICE (7T, ESI) at the QB3 mass spectrometry facility of the University of California, Berkeley.
2.9.2 Synthetic Procedures for Previously Unreported Compounds and Experiments

Permethylated ligand precursor 8

The known lithium terephthalate salt 7 (3.5 g, 14.5 mmol, 1 eq.) and HATU (12.1 g, 31.9 mmol, 2.2 eq.) were dissolved in DMF (40 mL) with stirring. The resulting pale yellow solution was treated dropwise with triethylamine (5.7 mL, 40.6 mmol, 2.8 eq.) then stirred for 1 hour. To this dark brown solution, 1,5-diaminonaphthalene (1.1 g, 6.96 mmol, 0.5 eq.) was added in portions and the resulting mixture was stirred for 18 hours at ambient temperature. The reaction mixture was then filtered to isolate 8 (3.8 g) in 93% yield as a bright yellow solid, which was used with no further purification.

\[ \text{H NMR (500 MHz, Chloroform-}d) \delta 10.65 \text{ (s, 2H), 8.51 (d, } J = 7.7 \text{ Hz, 2H), 8.10 (d, } J = 8.4 \text{ Hz, 2H), 7.88 (d, } J = 8.6 \text{ Hz, 2H), 7.73 – 7.60 (m, 4H), 4.19 \text{ (s, 6H), 4.05 (s, 6H), 3.97 (s, 6H).} \]

\[ \text{C NMR (126 MHz, Chloroform-}d) \delta 165.75, 162.49, 153.43, 152.41, 133.95, 130.17, 129.79, 127.17, 126.80, 126.56, 126.40, 119.64, 117.11, 62.44, 62.26, 52.73. \]

\[ \text{HRMS (m/z): calculated for } [C_{32}H_{30}O_{10}N_2Na]^+ 625.1793; \text{ observed, 625.1787.} \]

Methyl ether-protected ligand precursor 6

Permethylated ligand precursor 8 (5.5 g, 9.15 mmol, 1 eq.) was suspended in anhydrous DCM (85 mL) with stirring. At ambient temperature, potassium trimethylsilanolate (2.8 g, 22 mmol, 2.4 eq.) was added in small portions. The resulting cloudy mixture was stirred for 12 hours, concentrated in vacuo, and then suspended in water (50 mL). This mixture is acidified with 1M HCl to an approximate pH of 3, sonicated for 15 minutes, then filtered to isolate 6 (3.88 mg) in 74% yield as a yellow solid.
\(^1\)H NMR (600 MHz, DMSO-\(d_6\)) \(\delta\) 10.53 (s, 2H), 8.04 (d, \(J = 8.4\) Hz, 2H), 7.81 (d, \(J = 7.2\) Hz, 2H), 7.65 (t, \(J = 8.0\) Hz, 2H), 7.50 (s, 4H), 3.98 (s, 6H), 3.90 (s, 6H).

\(^{13}\)C NMR (151 MHz, DMSO-\(d_6\)) \(\delta\) 167.03, 165.10, 152.18, 151.06, 134.36, 133.67, 129.60, 129.27, 125.99, 125.05, 123.76, 122.94, 120.96, 61.90, 61.73.

HRMS (m/z): calculated for \([\text{C}_{30}\text{H}_{25}\text{O}_{10}\text{N}_{2}]^–\), 573.1515; found, 573.1505.

The previously reported intermediate 10\(^{43}\) (2.1 g, 5.36 mmol, 1 eq.) was dissolved in thionyl chloride (30 mL), treated with one drop of DMF, and then the resulting mixture was stirred for 12 hours at ambient temperature. Excess thionyl chloride was removed under vacuum and the isolated yellow crystals were dissolved in anhydrous DCM (80 mL). To the resulting yellow solution, 1,5-diaminonaphthalene (406 mg, 0.257 mmol, 0.48 eq.) was added in several portions, followed by dropwise addition of triethylamine (0.8 mL). The dark reaction mixture was stirred for 12 hours, then quenched with water (10 mL). The organic layer was washed with 1N HCl (60 mL x 2), 1M NaOH (60 mL x 2), brine (60 mL x 2), then dried over MgSO\(_4\), filtered, then concentrated under vacuum. The residue was then dissolved in a minimal amount of DCM and the product isolated by precipitation with hexanes to yield 10 as a tan powder (2.25 g) in 96% yield.

\(^1\)H NMR (300 MHz, Chloroform-\(d\)) \(\delta\) 10.38 (s, 2H), 8.23 (d, \(J = 7.6\) Hz, 2H), 8.09 (d, \(J = 8.4\) Hz, 2H), 7.73 (d, \(J = 8.4\) Hz, 2H), 7.55 (d, \(J = 6.5\) Hz, 4H), 7.56 – 7.40 (m, 10H), 7.17 (t, \(J = 7.2\) Hz, 6H), 7.07 (t, \(J = 7.4\) Hz, 4H), 5.29 (d, \(J = 7.8\) Hz, 8H), 3.93 (s, 6H).

\(^{13}\)C NMR (126 MHz, Chloroform-\(d\)) \(\delta\) 165.76, 162.50, 152.13, 151.34, 136.60, 134.90, 133.36, 131.26, 130.27, 129.30, 129.13, 128.88, 128.79, 128.71, 128.64, 126.79, 126.74, 126.68, 126.14, 119.31, 117.62, 78.06, 52.72. Missing carbon resonances may be due to overlapped resonances.

HRMS (m/z): calculated for \([\text{C}_{56}\text{H}_{47}\text{O}_{10}\text{N}_{2}]^\cdot\), 907.3225; found, 907.3232.
Benzyl-ether protected ligand precursor 9

In a 250 mL round bottom flask, intermediate 11 (2.515 g, 2.77 mmol, 1 equiv.) was suspended in anhydrous DCM (75 mL). At ambient temperature, potassium trimethylsilanolate (1.4 g, 11.09 mmol, 4 equiv.) was added in small portions. The resulting cloudy mixture was stirred for 12 hours, concentrated under reduced pressure, and then suspended in deionized water. This suspension was acidified with 1M HCl to an approximate pH of 3 and filtered to isolate 9 (2.11 g, 2.41 mmol, 87% yield) as a grey powder.

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta = 13.33$ (b, 1H), 10.55 (s, 1H), 7.92 (d, $J = 8.8$ Hz, 1H), 7.70 (d, $J = 6.8$ Hz, 1H), 7.60 (dd, $J = 10.5$, 7.2 Hz, 2H), 7.49 (b, 2H), 7.38 (b, 5H), 7.30 (b, 4H), 5.20 (s, 2H), 5.16 (s, 2H).

$^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta = 166.8$, 165.0, 151.0, 150.0, 137.0, 136.6, 135.3, 133.4, 129.9, 128.9, 128.3–128.1 (overlapping peaks), 125.4, 124.0, 122.6, 121.1, 76.0, 75.7.

HRMS (FTMS-ESI) m/z: calculated for [C$_{54}$H$_{41}$O$_{10}$N$_2$]$^-$, 877.2767; found 877.2758.

**A general procedure for the synthesis of ligands 12, 13, and 14**

Precursor 6 (2.9 g, 5.08 mmol, 1 eq.) was dissolved in DMF (10 mL), and HATU (3.9 g, 10.15 mmol, 2 eq.) was added in several portions, followed by dropwise addition of triethylamine (2 mL) with stirring. The resulting yellow, heterogeneous reaction mixture was stirred for 1 hour at ambient temperature, followed by dropwise addition of the N-Boc-diamine of interest (11.2 mmol, 2 eq.) and stirring for 12 hours. Methanol (5 mL) was added to the resulting yellow suspension. The solution was filtered and washed with cold methanol to give the desired product as a pale solid, which was used without further purification.

Protected ligand (0.5 mmol) was suspended in dry DCM (10 mL) followed by dropwise addition of boron tribromide (3 mL) at -78 °C. The resulting orange solution was stirred for 12 hours at ambient temperature and quenched by slow addition of ice (200 mL). The mixture was allowed to melt and filtered to collect a yellow precipitate. This precipitate was then refluxed in water (25 mL) for 12 hours, then collected by filtration.
**Ethylamine-functionalized ligand 12**

$^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 12.78 (s, 2H), 12.04 (s, 2H), 11.01 (s, 2H), 9.08 (s, 2H), 7.97 (t, $J = 7.9$ Hz, 2H), 7.85 – 7.78 (m, 4H), 7.69 – 7.63 (m 2H), 7.48 (d, $J = 7.9$, 2H), 3.59 (t, $J = 5.7$ Hz, 4H), 3.06 (t, $J = 5.6$ Hz, 4H).*

*Amine protons are unaccounted for due to extreme broadening, presumably due to hydrogen bonding with high concentrations of residual water.

HRMS (FTMS-ESI) m/z: calculated for $[C_{30}H_{31}O_8N_6]^{+}$ 603.2198; found 603.2204.

**Butylamine-functionalized ligand 13**

$^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$ 13.20 (s, 2H), 11.97 (s, 2H), 11.00 (s, 2H), 9.08 (s, 2H), 7.97 (d, $J = 8.6$ Hz, 2H), 7.72 – 7.66 (br s, 2H), 7.65 (d, $J = 8.5$ Hz, 2H), 7.62 (d, $J = 9.0$ Hz, 2H), 7.49 (d, $J = 8.6$ Hz, 2H), 3.37 (t, $J = 6.0$ Hz, 4H), 2.84 (app q, $J = 6.5$ Hz, 4H), 1.61 (br. s, 4H).*

*Amine protons are unaccounted for due to extreme broadening, presumably due to hydrogen bonding with high concentrations of residual water. Another set of methylene peaks is obscured by the large signal from residual water.

HRMS (FTMS-ESI) m/z: calculated for $[C_{34}H_{39}O_8N_6]^{+}$ 659.2824; found 659.2822.
**Hexylamine-functionalized ligand 14**

$^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 13.24 (br. s, 2H), 11.96 (br. s, 2H), 10.99 (s, 2H), 9.04 (s, 2H), 7.96 (d, $J$ = 8.2 Hz, 2H), 7.72 – 7.57 (m, 6H), 7.50 (d, $J$ = 8.4 Hz, 2H), 2.83 – 2.75 (m, 4H), 1.67 – 1.52 (m, 8H), 1.34 (br. s, 8H). *

* Amine protons are unaccounted for due to extreme broadening, presumably due to hydrogen bonding with high concentrations of residual water. Another set of methylene peaks is obscured by the large signal from residual water.

HRMS (FTMS-ESI) m/z: calculated for [C$_{38}$H$_{46}$O$_8$N$_6$Na]$^+$ 737.3274; found 737.3269.

**A general procedure for the preparation of PEt$_4^+$ ⊂ Ga$_4$12$_6$ and PEt$_4^+$ ⊂ Ga$_4$13$_6$ hosts**

Ligand (0.116 mmol, 6 equiv.) and KOH (35 mg, 0.620 mmol, 32 eq.) were dissolved in degassed D$_2$O (1.2 mL), then treated with tetraethylphosphonium bromide (18 mg, 0.078 mmol, 4 eq.). The resulting yellow solution was then treated with gallium nitrate (20 mg, 0.078 mmol, 4 equiv.) and stirred 1 hour at 60 ºC, then transferred to an NMR tube for small-scale studies.

$^1$H NMR (500 MHz, D$_2$O) $\delta$ 8.13 (d, $J$ = 7.4 Hz, 12 H), 7.78 (d, $J$ = 7.4 Hz, 12 H), 7.29 (d, $J$ = 8.6 Hz, 12 H), 7.12 (app. t, $J$ = 8.8 Hz, 24 H), 3.41 – 3.31 (m, 12 H), 3.24 – 3.16 (m, 12 H), 2.57 – 2.42 (m, 24 H), -1.35 – -1.44 (m, 12 H), -1.65 – -1.79 (m, 8 H).
**PEt₄⁺ ⊂ Ga₄13₆**

1H NMR (500 MHz, D₂O) δ 8.10 (d, J = 5.1 Hz, 12 H), 7.77 (d, J = 5.1 Hz, 12 H), 7.25 (d, J = 6.5 Hz, 12 H), 7.12 (app. d, J = 7 Hz, 24 H), 3.39 – 3.28 (m, 12 H), 3.20 – 3.10 (m, 12 H), 2.70 – 2.59 (m, 24 H), 2.35 – 2.25 (m, 24H), 1.22 – 1.07 (m, 24H), -1.35 – -1.45 (m, 12 H), -1.65 – -1.79 (m, 8 H).

**A general procedure for PSM experiments with amine-functionalized hosts**

Solutions of PEt₄⁺ ⊂ Ga₄12₆ (0.005 mmol/0.4 mL) in deuterated solvents or H₂O were treated dropwise with pre-dissolved solutions of PSM reagents (0.06-0.6 mmol) at ambient temperature with stirring. These samples were then transferred to NMR tubes and the reaction was monitored over time. Samples that appeared unchanged after 6 hours were then heated to 60 °C with continued monitoring.

Azide-functionalized ligand 15

Ligand 12 (50 mg, 0.083 mmol, 1 eq.), potassium carbonate (46 mg, 0.33 mmol, 4 eq.), imidazole-1-sulfonyl azide salt* (0.2 mmol, 2.4 eq.), and copper sulfate pentahydrate (1 mg, 0.004 mmol, 0.05 eq.) were suspended in degassed methanol (0.5 mL) under nitrogen and stirred as a yellow-green suspension for 12 hours. The resulting pale pink reaction mixture was acidified dropwise with 1M HCl until pH < 4. Methanol was removed under vacuum, and the resulting aqueous suspension was diluted further in water (2 mL) and filtered to give the desired product 15 as a pale red powder (27 mg, 0.039 mmol, 48% yield).
1H NMR (500 MHz, DMSO-$d_6$) δ 12.82 (br. s, 2H), 12.08 (br. s, 2H), 11.08 (s, 2H), 9.17 (s, 2H), 7.99-7.90 (m, 6H), 7.64 (d, J = 8.5 Hz, 2H), 7.50 (s, 2H), 3.59 (br. s, 4H), 3.05 (br. s, 4H).

HRMS (FTMS-ESI) m/z: calculated for [C$_{30}$H$_{25}$N$_{10}$O$_8$Na]$^+$ 677.1827; found 677.1824.

*both the chloride and sulfate salts of imidazole-1-sulfonyl azides are competent at di-azo transfer. In addition, a slight excess of potassium carbonate appears to accelerate the reaction.

A general procedure for PSM experiments with azide-functionalized host PEt$_4^+$ ⊂ Ga$_4$15$_6$

Solutions of PEt$_4^+$ ⊂ Ga$_4$15$_6$ (0.01 mM – 0.001 mM) in deuterated solvents or H$_2$O were treated with the PSM reagents (4-20 eq.) at ambient temperature with stirring. These solutions were treated dropwise with prepared solutions of (Cu(I)-TBTA)$_X$ ($X$ = Cl, Br, I, NO$_3$) (0.1-0.6 eq) in acetonitrile, then diluted with t-butanol until the solution was homogeneous (about 20-40% by volume.) These samples were then stirred at ambient temperature, then transferred to NMR tubes, and the reaction was monitored over time. Samples that appeared unchanged after several hours were then heated to 60 °C with continued monitoring. Reactions were quenched by dropwise addition of 1M HCl until the reaction reached pH < 4, followed by filtration through a 0.2 µm syringe filter. The filtered solids were then dissolved in DMSO-$d_6$ for 1H NMR spectroscopy and analysis with MALDI-TOF.
**Modified ligand 15**

MALDI-ToF-MS (dithranol) m/z: Calculated for $[C_{36}H_{33}N_{10}O_{10}]^-$ 765.24; found 765.68.

**Carboxylic acid-functionalized ligand 16**

Ligand precursor 6 (482 mg, 0.80 mmol, 1 eq.) was suspended in anhydrous DCM (10 mL) and treated dropwise with neat boron tribromide (1.06 mL, 11.20 mmol, 14 eq.) at -78 °C. This orange mixture was stirred for 12 hours at ambient temperature. The resulting mixture was quenched by pouring into an ice bath and then allowed to warm to room temperature. The resulting yellow slurry was filtered to give a wet powder, which was then resuspended in water (15 mL) and heated to reflux overnight. The reaction mixture was cooled, filtered, and dried under vacuum overnight to give ligand 16 (401 mg, 0.77 mmol, 96% yield) as a yellow powder. This ligand was used without further purification.

$^1$H NMR (500 MHz, DMSO-$d_6$) δ 11.97 (s, 2H), 11.02 (s, 2H), 7.99 (d, $J = 8.5$ Hz, 2H), 7.96 (d, $J = 7.3$ Hz, 2H), 7.66 (d, $J = 8.0$ Hz, 2H), 7.62 (d, $J = 8.3$ Hz, 2H), 7.41 (d, $J = 8.6$ Hz, 2H). *

*Amide and carboxylic acid peaks are unaccounted for, presumably due to extensive hydrogen bonding with the high concentration of residual water in the sample.

HRMS (m/z): calculated for $[C_{26}H_{17}O_{10}N_2]^-$, 517.0889; found, 517.0886.
General considerations for synthesis and experiments with carboxylate-functionalized host $\text{PET}_4^+ \subset \text{Ga}_4\text{16}_6$

All studies were performed in D$_2$O (except for those were performed in H$_2$O to enable observation of exchangeable protons) in NMR tubes to enable convenient $^1$H NMR analysis. Solvents were typically degassed prior to use, but it appears that the host is not air sensitive enough to warrant this precaution. All samples were prepared with six equivalents of ligand 16, four equivalents of $\text{Ga(acac)}_3$, three equivalents of guest (when relevant), and 100 mM $\text{K}_3\text{PO}_4$ in D$_2$O as the source of base. Buffers are prepared from 100 mM $\text{K}_3\text{PO}_4$ in D$_2$O with the addition of DCl, and the reported pDs have been corrected ($\text{pD}_{\text{read}} + 0.4$) to account for the isotope effect at the glass electrode.

At basic pH in H$_2$O:

$^1$H NMR (500 MHz, H$_2$O) $\delta$ 13.13 (s, 12H), 7.91 (d, $J = 7.8$ Hz, 12 H), 7.60 (d, $J = 8.6$ Hz, 12 H), 7.05 (d, $J = 8.6$ Hz, 12 H), 6.96 (t, $J = 8.1$ Hz, 12 H), 6.66 (d, $J = 8.6$ Hz, 12 H), -1.49 – -1.59 (m, 12 H), -1.80 – -1.89 (m, 8 H).

Isolated from neutral pH, in DMSO-$d_6$:

$^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 14.94 (s, 12H), 12.71 (s, 12H), 8.61 (d, $J = 7.8$ Hz, 12 H), 7.52 (d, $J = 8.7$ Hz, 12 H), 7.28 (d, $J = 8.8$ Hz, 12 H), 6.98 (t, $J = 8.0$ Hz, 12 H), 6.88 (d, $J = 8.8$ Hz, 12 H), -1.54 – -1.65 (m, 12 H), -1.90 – -2.00 (m, 8 H).
2.10 References


Chapter 3

Isolating and Deconvoluting the Role of Supramolecular Host Charge in Microenvironment Catalysis
3.1 Preface

While chapters 2 and 4 describe efforts to expand the structural scope of supramolecular assemblies by ligand modification, there is also ample opportunity in diversifying the metals used to construct them. This chapter describes a new, octaanionic silicon(IV) analog to the reported dodecaanionic Ga(III) catalyst of $M_4L_6$ stoichiometry, which are revealed to be isostructural with the exception of a reduction in overall charge. These two catalysts, when examined in parallel transformations, provide a unique experimental point of entry into isolating the role of electrostatic and charge effects in microenvironment catalysis. Specifically, an aza-Cope rearrangement, in which the substrate experiences no change in charge, and a mechanistically distinct Nazarov cyclization, in which the neutral substrate undergoes an increase in charge to access cationic transition states, were examined. Though these effects have been highlighted as important in many enzymatic and synthetic catalysis systems, this work stands as the first time charge effects have been experimentally distinguished in a synthetic microenvironment. This work was done in collaboration with Mariko Morimoto, with crystallographic support from Dr. Eugene Kapustin.

3.2 Introduction to Charge and Electrostatic Effects in Microenvironment Catalysis

Starting from humble beginnings, some supramolecular catalysts now rival enzymes in accelerating rates of catalyzed reactions. Like enzymatic active sites, the microenvironments within these self-assembled catalysts encapsulate substrate molecules with specificity and utilize non-covalent host–guest interactions to induce significant rate accelerations and impart remarkable product selectivities. Thus, supramolecular hosts are important to study not only for the design of improved synthetic catalysts, but also to advance our understanding of the governing principles that underlie enzymatic catalysis.

The catalytic activity of microenvironments in supramolecular hosts and enzymes is dictated by multiple parameters, including overall charge, cavity size, and the degree of solvent exclusion. Among these, charge and electrostatic effects are of particular interest because they have been established as crucial to enzymatic and supramolecular reactivity. Despite the abundance of enzymes that are proposed to rely heavily on general charge and electrostatic effects for catalysis, cases where these effects can be experimentally isolated and quantified are very limited. One such rare example is chorismute mutase, a metabolic enzyme that catalyzes the rearrangement of chorismate to prephenate. Specifically, the significance of a cationically charged residue has been demonstrated without perturbing other enzyme-substrate interactions (Figure 3.1A). Replacement of a specific cationic arginine with neutral citrulline maintains the precise substrate orientation and geometry of the wildtype enzyme but incurs a severe penalty in catalytic effectiveness, underscoring that electrostatic effects play a crucial role in catalysis. Various synthetic systems also ascribe supramolecular phenomena to these effects (Figure 3.1B), but experimental studies that explicitly examine the role of charge in supramolecular catalysis are lacking. Evidently, it is quite challenging to deconvolute the consequences of charge/electrostatic effects from other specific influences on the reactivities of both natural and synthetic systems.
Figure 3.1. A: Mutation of cationic arginine to neutral citrulline maintains the precise substrate orientation and geometry of wildtype chorismate mutase but incurs a severe penalty in catalysis. This validates that electrostatic and charge effects play a crucial role in enzymatic catalysis. B: Examples of charged supramolecular systems; Rebek’s tetraammonium cavitand, Ward’s Co₈L₁₂⁶⁺ cube where orange lines represent ligands with only one is shown, and Fujita’s Pd₆L₄¹²⁺ octahedron.³⁵,¹⁸,²⁸ Supramolecular host Ga-1 (K₁₂Ga₄₂₆) developed by Raymond and co-workers is a suitable candidate for the development of an isostructural catalyst with varied charge. The unusually large anionic host charge (12⁻) is due to four trianionic homochiral Ga(III) triscatecholate vertices (Figure 3.2).²⁹ Host Ga-1 effectively catalyzes a variety of transformations in polar solvents such as water. Two notable examples are the Ga-1-catalyzed Nazarov cyclization of a dienol substrate as well as aza-Cope rearrangements of cationic enammoniums.³⁰,³¹ Subsequent kinetics and DFT-based investigations have probed the origins of these rate enhancements, proposing several factors such as anionic host charge and constrictive binding.³²,³³ Thus, we envisioned an
experimental investigation of the specific role of charge in these reactions by changing the metal vertices in Ga-1 to reduce the overall host charge while maintaining the chemical structure and geometry of the microenvironment.

**Figure 3.2.** The [Ga$_4$Z$_6$]$_{12}^-$ tetrahedron Ga-1 and the [Si$_4$Z$_6$]$_8^-$ tetrahedron Si-3. Only one ligand 2 is shown of six and counterions are omitted for clarity. Blue lines represent 2 and spheres represent metal or metalloid ions.

### 3.3 Synthesis and Characterization of the Octaanionic Silicon-Based Assembly

To this end, Si(IV) was chosen as an alternative vertex to access an isostructural M$_4$Z$_6$ host with overall reduced charge (Figure 3.2). Triscatecholate Si(IV) complexes are well reported in the literature, wherein the hypervalent metalloid coordination environment is pseudooctahedral, similar to that of Ga(III) in host Ga-1. 34,35 To test if an M$_4$Z$_6$ host with Si(IV) vertices could form, six equivalents of ligand 2 and four equivalents of tetramethyl orthosilicate were heated in DMF in the presence of tetraethylphosphonium iodide, a strongly binding guest that acts as a template. The appearance of six aromatic resonances in the $^1$H NMR spectrum in concomitance with a diagnostic upfield shift in the resonances of one equivalent of the tetraethylphosphonium cation confirmed that the host-guest inclusion complex PEt$_4^+\subset$ Si-3 had formed (Figure 3.3).

**Figure 3.3.** $^1$H NMR spectrum of host-guest inclusion complex PEt$_4^+\subset$ Si-3. The simplicity of the aromatic region points to the T-symmetry of the host, while the dramatic upfield shift of the guest resonances indicates strong encapsulation. This upfield shift, which results from the magnetic shielding effect of the naphthalene walls, is a useful diagnostic for internal association.
Conditions were then screened for the template-free synthesis of Si-3. This is often the most challenging step of accessing new cavity-bearing architectures, and has previously prohibited access to related octaanionic, catalytically active hosts. In addition to the lack of the thermodynamic driving force of strong guest binding, multiple iterations of self-assembly and self-correction are required for high conversion to the desired host. Ultimately, long reaction times and elevated temperatures were necessary for the clean assembly of template-free Si-3, likely due to the low lability of mechanically coupled silicon catecholate bonds. Electrospray mass spectrometry (ESI-MS) confirmed the Si$_4$2$_6$ stoichiometry and octaanionic charge of Si-3, primarily detected with various counterions in the 3$^-$ and 4$^-$ charge states (Figure 3.4).

Further support for the proposed structure of Si-3 was obtained by single crystal X-ray diffraction analysis. Single crystals suitable for X-ray diffraction were grown by vapor diffusion of benzene into a solution of NEt$_4^+$ ⊂ Si-3 in DMSO and measured with synchrotron radiation. The crystal structure of Si-3 was solved and refined in the space group P-1, with two hosts in the asymmetric unit, which are averaged for Si-3 structural parameters. Both hosts contain one guest molecule of NEt$_4^+$ fully occupying the intrinsic cavity of the host. In order to balance the overall 16$^-$ charge between the two octaanionic hosts, all NEt$_4^+$ and K$^+$ ions were initially located and refined. The crystal structure contains a few highly disordered solvent molecules such as DMSO and benzene. Due to this, the structure could not be converged even after 150 cycles of refinement. To determine whether the incomplete assignment of solvent molecules would be problematic for assigning the overall geometry of Si-3, all the solvent molecules were removed from the model, except for the two internal guests, and the crystal structure was then treated...
with the PLATON/SQUEEZE solvent masking procedure to account for the unassigned electron density. The deviations in geometry of hosts before and after solvent masking was applied were found to be negligible and the solvent masked structure was used for further interpretation.

In comparing the analogous structures of \( \text{NET}_4^+ \subset \text{Ga-1} \) and \( \text{NET}_4^+ \subset \text{Si-3} \), both structures are ideal tetrahedra (Figure 3.5).\(^{37}\) The angle between two vertices and the centroid generated based on the Si(IV) and Ga(III) centers is 109.1(1) and 109.5(1) °, respectively. The geometrical parameters of Si-3 and Ga-1 were also compared. The edge length of Si-3 is 12.655(2) Å, which is slightly shorter than that of in Ga-1 at 12.663(3) Å. The volumes of the hosts are 238.85(6) Å\(^3\) and 239.3(1) Å\(^3\), respectively, and the average metal-oxygen distance is 1.78(1) Å for Si-3 and 1.96(1) Å for Ga-1. The slight deviation in the geometrical parameters can be explained by the different temperatures at which the structures were measured (100 K for Si-3 and 168 K for Ga-1) and different crystallization conditions. In summary, despite minor perturbations in ligand conformation, both Si-3 and Ga-1 are ideal tetrahedra and the Si(IV) and Ga(III) coordination environments are remarkably well preserved. These data thus confirm the architecture of Si-3, and demonstrate the isostructural relationship between Si-3 and Ga-1.

![Figure 3.5](image)

*Figure 3.5. Left: \([\text{Ga}_4\text{L}_6]^{12-}\) Ga-1. Center: Overlay of Si-3 in green and Ga-1 in red. Right: \([\text{Si}_4\text{L}_6]^8\) Si-3. Counterions, solvents, and guests are removed for clarity.*

### 3.4 Silicon-Based and Gallium-Based \( \text{M}_4\text{L}_6 \) Assemblies as Mechanistic Probes in Catalysis

With isostructural catalysts Si-3 and Ga-1 in hand, the next step was to compare their catalytic activities in order to probe the effects of host charge. Because Si-3 is designed to mirror all the features of catalyst Ga-1 except for the overall anionic character, their catalytic profiles should be identical unless the reaction mechanism is sensitive to a change in substrate charge. Two mechanistically distinct reactions were selected to profile the catalytic abilities of Si-3 and Ga-1: the aza-Cope rearrangement and the Nazarov cyclization. In the aza-Cope rearrangement of enammonium substrates accelerated by host Ga-1, the cationic substrate charge is maintained throughout the reaction, resulting in no change in overall charge of the host–guest complex. In contrast, the Nazarov cyclization involves protonation of a neutral substrate, incrementally increasing the cationic charge in the cavity. Investigating the Ga-1- and Si-3-catalyzed reactions in parallel should thus enable the determination of the extent to which their rate accelerations depend on anionic host charge.
3.4.1 Evaluation of Constrictive Binding Effects via the Catalytic Aza-Cope Rearrangement

The aza-Cope rearrangement features a [3,3] sigmatropic shift of allyl- or propargyl-enammonium substrates to generate γ,δ-unsaturated iminium species via a cationic chair-like transition state (Figure 3.6). Under reaction conditions with catalyst Ga-1, an enammonium substrate such as 4 is initially encapsulated prior to the rate-limiting sigmatropic shift. It was postulated earlier that rate acceleration from Ga-1 derives from a reduction of the entropic barrier of cyclization due to constrictive binding within the host. Specifically, the proposed chair-like conformation of the encapsulated substrate resembles the sigmatropic rearrangement transition state more closely than the preferred extended conformations the substrate adopts in bulk solution. Note that the resulting iminium product 5 is also strongly encapsulated, but hydrolyzes upon rapidly reversible egress to the corresponding γ,δ-unsaturated aldehyde 6. This precludes product inhibition and enables catalyst turnover. Because iminium hydrolysis was previously shown to proceed outside the host cavity, the reaction proceeds with a true retention of cationic charge inside Ga-1.

Figure 3.6. Proposed mechanism for Si-3- or Ga-1-catalyzed aza-Cope rearrangement.

One consideration when comparing the kinetics of the Si-1- and Ga-3-catalyzed aza-Cope rearrangement is the difference in binding strength (association constant) of the cationic substrate due to a variation in anionic charge of the catalysts. It is quite likely that the association constant of the enammonium substrate is significantly smaller with octaanionic catalyst Si-3, compared to that of its dodecanionic counterpart Ga-1. However, treatment of 4 with catalytic
amounts of Si-3 in DMSO-\textit{d}_6 immediately generated the quantitatively encapsulated host-substrate complex 4 \subset Si-3, evidenced by upfield resonances in the \textit{1}H NMR spectrum corresponding to one equivalent of 4 (Figure 3.7A). The subsequent reaction was tracked, and it was noted that the concentration of 4 \subset Si-3 is maintained at early reaction times via catalyst turnover and further encapsulation of free 4 (Figure 3.7B). This has been previously observed with Ga-1, where it was also shown that the aza-Cope rearrangement proceeds with first-order dependence on the host-substrate complex [enammonium \subset Ga-1].\textsuperscript{39} We thus concluded that any differences in the association constant of 4 with Si-3 or Ga-1 were negligible at early reaction times in which the catalyst cavities are fully occupied. Therefore, the Ga-1- and Si-3-catalyzed aza-Cope rearrangements of 4 can both be analyzed by the method of initial rates.

\textbf{Figure 3.7.} \textit{A}: Quantitatively encapsulated 4 within host-substrate complex 4 \subset Si-3. \textit{B}: The concentration of 4 \subset Si-3 is maintained at early reaction times via catalyst turnover.

The catalytic aza-Cope rearrangement of 4 was monitored at 37% catalyst loading with Si-3 and Ga-1 (Figure 3.8). The reactions proceeded at similar rates, with an average rate constant (\(k_{obs}\)) of 1 \times 10^{-2} \text{ s}^{-1} and 6.5 \times 10^{-3} \text{ s}^{-1} for Si-3 and Ga-1, respectively (Table 3.1). The excellent agreement in rates for the Si-3- and Ga-1-catalyzed rearrangement suggests that the two microenvironments exert a near-identical influence on 4, despite the 33% difference in overall host charge. Furthermore, these data stand as a strong upholding of the conclusions of our crystallographic analysis that the hosts are isostructural (\textit{vide supra}). Consequently, these data confirm our previous analysis that the rate acceleration can be assigned as a consequence of constrictive host-substrate interactions in which host charge plays a negligible role.
Figure 3.8. *Upper:* Representative kinetics trace for the Ga-1- or Si-3-catalyzed aza-Cope rearrangement. *Lower:* Representative log plot for the Ga-1- or Si-3-catalyzed aza-Cope rearrangement (Si-3 shown).

<table>
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<tr>
<th>Catalyst</th>
<th>Trial</th>
<th>$k_{obs}$ (s$^{-1}$)</th>
<th>Substrate Conc. (mM)</th>
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</table>

Table 3.1. Rate constants ($k_{obs}$) for the catalyzed and uncatalyzed aza-Cope rearrangement.
3.4.2 Evaluation of Electrostatic and Charge Effects via the Catalytic Nazarov Cyclization

We proceeded to investigate the effect of altering host charge for our second model reaction, the Ga-1- and Si-3-catalyzed Nazarov cyclization. In bulk solution, the Nazarov cyclization of dienols such as 7 requires strong Lewis or Brønsted acids which promote ionization of the C–O bond to generate the key carbocationic intermediate, followed by cationic 4π-electrocyclic ring closure. In the presence of catalytic Ga-1, however, the Nazarov cyclization of 7 proceeds efficiently under basic aqueous conditions with no other additives. The proposed Ga-1-catalyzed mechanism invokes reversible binding of 7 driven by the hydrophobic effect, followed by reversible protonation and ionization to release water (Figure 3.9). It is noteworthy that encapsulated 7 is protonated efficiently despite the basic pH of the exterior medium. The resulting dienyl carbocation 8 then undergoes irreversible 4π-electrocyclization followed by proton loss to generate isomers of the cyclopentadiene product 9. Because 9 and substrate 7 are competitive guests, product inhibition was precluded by trapping 9 with an excess of maleimide as the corresponding Diels-Alder adduct, which is too bulky for encapsulation.

![Proposed mechanism for Si-3- or Ga-1-catalyzed Nazarov rearrangement.](image)

The overall course of the reaction for the Ga-1-catalyzed Nazarov cyclization could be monitored over several hours at room temperature. In contrast, our preliminary observations of the analogous Si-3-catalyzed reaction under identical conditions did not show significant progress,
even after twelve hours. This disparity in reaction rate motivated a quantitative rate comparison of Si-3 and Ga-1 as catalysts. Heating the reaction with catalyst Si-3 to 72 °C was sufficient to measure the reaction rate ($k_{obs}$) on a practicable experimental timescale (Figure 3.10). The Ga-1-catalyzed reaction, however proceeds very rapidly and suffers from catalyst decomposition at elevated temperatures, precluding reliable rate constant extraction. An Eyring analysis of the Ga-1-catalyzed reaction was thus used to extrapolate the corresponding rate constant at 72 °C. The two reactions proceed with a significant difference in rate, with $k_{obs} = 2.2 \times 10^{-4}$ s$^{-1}$ for Si-3 and $1.5 \times 10^{-1}$ s$^{-1}$ for Ga-1 (Table 3.2). This corresponds to a 680-fold difference in rate acceleration for dodecaanionic Ga-1 relative to octaanionic host Si-3.

**Figure 3.10.** *Upper:* Representative kinetics trace for the or Si-3-catalyzed Nazarov cyclization. *Lower:* Representative log plot for the Si-3-catalyzed Nazarov cyclization.
<table>
<thead>
<tr>
<th>Trial</th>
<th>$k_{\text{obs}}$ (s$^{-1}$)</th>
<th>Substrate Conc. (mM)</th>
<th>Catalyst loading (%)</th>
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Table 3.2. Rate constants ($k_{\text{obs}}$) for the Si-3-catalyzed Nazarov cyclization.

This dramatic change in rate acceleration is particularly striking in light of the near-identical reaction rates of the Ga-1- and Si-3-catalyzed aza-Cope rearrangement. As showcased in the aza-Cope rearrangement, the isostructural relationship between hosts Ga-1 and Si-3 exerts control over the degree of constrictive binding during the irreversible electrocyclization step. Thus, the decrease in catalytic efficacy of Si-3 for the Nazarov cyclization is interpreted as the identification of the effect of reducing anionic charge. DFT-studies have proposed that the significance of the anionic host charge stems not only from stabilization of the initial protonation step, but also of the subsequent carbocationic intermediates and transition state. The observed 680-fold reduction in rate not only demonstrates that the anionic charge stabilizes cations, but also puts into perspective the magnitude of this effect; an attenuation in charge from 12$^-$ to 8$^-$ was sufficient to shift the rate constant ($k_{\text{obs}}$) substantially. This experimental rate comparison thus serves as an important proof of concept, which aids us in understanding the extent to which host charge influences reactivity. Follow-up studies to quantify the relative thermodynamic stabilization invoked by incremental increases in anionic host charge are currently underway.

3.5 Conclusions

In conclusion, the development of octaanionic catalyst Si-3 as an isostructural analog of dodecaanionic catalyst Ga-1 has enabled a unique experimental investigation that allows the connection of supramolecular structural features with specific mechanisms of reactivity. A Ga-1- and Si-3-catalyzed aza-Cope rearrangement in which the substrate experiences no change in charge proceeded at comparable rates, demonstrating the dominant effect of constrictive binding over host charge on the observed rate acceleration. The catalytic abilities of hosts Ga-1 and Si-3 were then compared in a mechanistically distinct Nazarov cyclization, in which the neutral substrate undergoes a change in charge to access cationic intermediates and transition states. The significant consequences (680-fold difference in rate constants, $k_{\text{obs}}$) of reducing host charge from 12$^-$ in Ga-1 to 8$^-$ in Si-3 stand as an experimental validation of the significant stabilizing effect of the anionic host charge in reactions that feature a build-up of cationic charge. Though charge and electrostatic effects have been highlighted in catalysis, this is the first example in which these effects have been experimentally deconvoluted from constrictive binding effects in a synthetic microenvironment system.43
3.6 Supporting Information

3.6.1 General Methods

Unless otherwise noted, all reactions were carried out in oven-dried glassware sealed with rubber septa under a nitrogen atmosphere with Teflon-coated magnetic stir bars, with the exception of those performed in NMR tubes. Reaction progress was monitored using thin layer chromatography on Silicycle Siliaplate™ glass backed TLC plates (250 μm thickness, 60 Å porosity, F-254 indicator) and visualized with 254 nm UV light or stained by submersion in a basic potassium permanganate solution. Flash column chromatography was performed on a Teledyne Isco CombiFlash Rf instrument using pre-packed silica gel columns. Dimethylformamide (DMF), tetrahydrofuran (THF), diethylether (Et₂O), methylene chloride (DCM), and triethylamine were dried by passed the previously degassed solvents through activated alumina columns under argon. Deuterated solvents were purchased from Cambridge Isotope Laboratories. All other reagents purchased from Sigma Aldrich and Fischer Scientific and were used as directly received without further purification. Proton nuclear magnetic resonance (¹H NMR) and carbon nuclear magnetic resonance (¹³C NMR) spectra were taken with AV-300, AVB-400, AVQ-400, AV-500, DRX-500, or AV-600 Bruker spectrometers operating at 300MHz, 400MHz, 500 MHz, or 600 MHz. Chemical shifts are reported in parts per million (ppm) with reference to the appropriate residual solvent signal. ¹H NMR: CDCl₃ (δ: 7.26 ppm), DMSO-d₆ (δ: 2.50 ppm), MeOD (δ: 3.31 ppm), D₂O (δ: 4.79 ppm). ¹³C NMR: CDCl₃ (δ: 77.16 ppm), DMSO-d₆ (δ: 39.52 ppm). ¹H NMR multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), sept (septet), m (multiplet). The temperatures of kinetic experiments performed within the NMR probe were determined from the ¹H NMR chemical shifts of ethylene glycol and methanol samples, and varied ±0.1 °C. Electrospray ionization mass spectra (ES(−)-MS) were obtained on a Thermo LTQ-FT-ICE (7T, ESI) at the QB3 mass spectrometry facility of the University of California, Berkeley. Mass spectrometry data were processed and interpreted using MassLynx software. X-ray diffraction data for Si-3 were collected using synchrotron radiation at Endstation 11.3.1 at the Advanced Light Source at the Lawrence Berkeley National Laboratory.
3.6.2 Synthetic Procedures for Previously Unreported and Modified Compounds

K\textsubscript{12}[Ga\textsubscript{4}2\textsubscript{6}] Assembly Ga-1

Assembly Ga-1 was synthesized according to a modified procedure.\textsuperscript{44} In a 250 mL three-neck round bottom flask equipped with a 500 mL addition funnel, ligand 2 (0.50 g, 1.16 mmol, 6 equiv.) and Ga(acac\textsubscript{3}) (0.285 g, 0.77 mmol, 4 equiv.) were combined in degassed MeOH (25 mL). This solution was further sparged with N\textsubscript{2} for 20 minutes. In the meantime, acetone (200 mL) was added to the addition funnel and the resulting mixture was sparged with N\textsubscript{2} for 30 minutes. KOH (0.13 g, 2.32 mmol, 12 equiv.) was added dropwise as a 1M solution in degassed MeOH. The milky white solution became homogeneous upon addition of base, and the yellow solution was stirred under N\textsubscript{2} for thirty minutes. Acetone was then added dropwise via the addition funnel. Upon the first signs of precipitation, the addition was halted, and the solution was stirred for an additional hour to allow the slow precipitation of Ga-1. Assembly Ga-1 was isolated by filtration as a pale-yellow solid, dried briefly under vacuum, and immediately transferred to an air free glovebox.

\textsuperscript{1}H NMR (500 MHz, Methanol-d\textsubscript{4}) \(\delta\) 8.05 (d, \(J = 7.8\) Hz, 12H), 7.77 (d, \(J = 8.6\) Hz, 12H), 7.25 (d, \(J = 8.2\) Hz, 12H), 6.96 (t, \(J = 8.2\) Hz, 12H), 6.66 (dd, \(J = 7.3, 1.6\) Hz, 12H), 6.37 (t, \(J = 7.8\) Hz, 12H).

K\textsubscript{8}[Si\textsubscript{4}2\textsubscript{6}] Assembly Si-3

To a 150 mL three-necked round bottom flask equipped with a reflux condenser was added ligand 2 (300 mg, 0.70 mmol, 6 equiv.) and anhydrous DMF (24 mL) under N\textsubscript{2}. Tetramethylorthosilicate (69 \(\mu\)L, 0.47 mmol, 4 equiv.) was added to the reaction mixture. The resulting homogeneous solution was stirred at 150 °C for 60 h, then allowed to cool to ambient temperature and treated with excess potassium bicarbonate (600 mg) as an aqueous solution (3 mL). After stirring the resulting mixture for 15 minutes, the resulting precipitate was removed by filtration, and the filtrate was concentrated \textit{in vacuo}. The brown residue was dissolved in dry methanol (90 mL), then treated dropwise with dry ether (130 mL) to generate a tan precipitate, which was removed by filtration. The filtrate was concentrated \textit{in vacuo} to yield assembly Si-3 as a tan powder.

\textsuperscript{1}H NMR (500 MHz, DMSO-d\textsubscript{6}) \(\delta\) 11.27 (s, 12H), 8.02 (d, \(J = 7.8\) Hz, 12H), 7.33 – 7.17 (m, 24H), 6.90 (t, \(J = 8.3\) Hz, 12H), 6.52 (d, \(J = 5.7\) Hz, 24H).
To a flame dried 50 mL round bottom flask was added (Z)-pent-2-en-1-ol (1 mL, 9.83 mmol, 1 equiv.) as a solution in dry Et₂O (25 mL). Tosyl chloride (2.80 g, 14.7 mmol, 1.1 equiv.) was then added in one portion. The solution was cooled to 0 °C, and powdered KOH (4.6 g, 81.9 mmol, 6 equiv.) was added in several portions over a period of 15 minutes, and the heterogeneous mixture was stirred at room temperature for 2 hours. The reaction mixture was diluted with brine (20 mL) and extracted with Et₂O (20 mL x 2). The combined organic phases were dried over magnesium sulfate, filtered, and concentrated in vacuo to yield 11 as a colorless oil, which was used in the subsequent reaction without further purification.

Alcohol S2 was synthesized according to a modification of a literature procedure. To a flame dried 25 mL three-neck round bottom flask equipped with a reflux condenser was added (Z)-2-bromobut-2-ene (96%) (0.3 mL, 2.92 mmol, 2 equiv.) as a solution in dry Et₂O (10 mL). High sodium lithium granules (0.04 g, 5.84 mmol, 4 equiv.) were added, and the resulting heterogeneous mixture was stirred at 38 °C in the dark (to prevent alkene isomerization) for 2 hours. Unreacted lithium was removed by transferring the orange solution via cannula to a separate 25 mL round bottom flask. The solution was cooled to 0 °C in an ice bath and the reaction was quenched by the slow addition of ethyl formate (0.12 mL, 1.47 mmol, 1 equiv.) diluted to 50% with Et₂O. The mixture was stirred for an hour, poured into a saturated aqueous NH₄Cl solution, and extracted three times with Et₂O (5 mL x 3). The combined organic layers were dried over magnesium sulfate, filtered, and concentrated in vacuo to yield alcohol S2 as a red liquid. Due to its decomposition at room temperature, S2 was used immediately in the subsequent reaction without further purification.

Ketone S3 was obtained by automated column chromatography using a solvent gradient of 0-2% ethyl acetate in hexanes. The title compound was obtained as a yellow liquid in 18% yield (0.31 mg, 2.2 mmol). S3 was methylated to obtain 7 by a literature procedure.
3.6.3 Procedures for Kinetic Experiments

Kinetic data points represent the average of multiple runs. All solvents were degassed by sparging N₂ for 30 minutes prior to use. Sodium p-toluenesulfonate was used as an integration standard for the Nazarov cyclization, and the aromatic resonances of the tosylate counterions of substrate 4 were used as an integration standard for the aza-Cope rearrangement.

Aza-Cope rearrangement kinetics

\[
\begin{align*}
\text{4} & \quad \text{40%} \quad \text{1% D₂O in DMSO-d₆} \quad 323 \text{ K} \quad \text{5} \\
& \quad \text{H₂O} \quad \text{6} \quad \text{NH₂OTs}
\end{align*}
\]

In an N₂ atmosphere wet glove box, assembly Ga-1 or Si-3 (3.6 µmol, 0.4 equiv) was added from a stock solution in DMSO-d₆ to an NMR tube. The tube was fitted with a rubber septum before being removed from the glovebox, and electrical tape was applied to seal the septum. The sample was inserted into the prewarmed, tuned and shimmed NMR spectrometer and was allowed to equilibrate for two minutes. Substrate 4 (9 µmol, 1 equiv) was added via microsyringe as a concentrated solution in DMSO-d₆ to total a volume of 600 µL in the NMR tube. ¹H NMR spectra were acquired every 60 seconds (ns = 4, d₁ = 10 s) until 50% of the starting material was consumed.

Nazarov cyclization kinetics

\[
\begin{align*}
\text{1} & \quad 7\% \quad \text{1:1 DMSO-d₆/D₂O} \quad 345 \text{ K}, \text{pD} = 8.0
\end{align*}
\]

In an N₂ atmosphere wet glove box, assembly Si-3 (0.9 µmol, 0.07 equiv.), maleimide (40.0 mg, 260 µmol, 20 equiv.), and sodium p-toluenesulfonate (3.0 mg, 15.4 µmol) were added from stock solutions to total a volume of 0.5 mL of degassed 1:1 DMSO-d₆/D₂O (buffered with 100 mM phosphate buffer, adjusted to pD = 8.0) in an NMR tube. The tube was fitted with a rubber septum before being removed from the glovebox, and electrical tape was applied to seal the septum. The sample was inserted into the prewarmed, tuned and shimmed NMR spectrometer and was allowed to equilibrate for two minutes. Substrate 5 (2.0 mg, 13.0 µmol) was added as a concentrated solution in 1:1 DMSO-d₆/D₂O via microsyringe, and ¹H NMR spectra were acquired every 180 seconds (ns = 3, d₁ = 10 s) until 50% of the starting material was consumed.
3.6.4 Representative Spectra and Kinetic Traces for the Aza-Cope rearrangement

Representative spectra for the Ga-1 catalyzed aza-Cope rearrangement.

3 minutes

External starting material 4

Encapsulated 4

15 minutes

Rearranged product 6

Product 6
Representative kinetic trace for the Ga-1-catalyzed aza-Cope rearrangement

Representative log plot for the Ga-1 catalyzed aza-Cope rearrangement

\[ y = 0.0006x - 2.2871 \]
\[ R^2 = 0.9904 \]
Kinetic trace for the uncatalyzed aza-Cope rearrangement in DMSO-$d_6$

Log plot for the uncatalyzed aza-Cope rearrangement in DMSO-$d_6$
3.6.5 Representative Spectra and Kinetic Traces for the Nazarov cyclization

Representative spectra for the Si-3-catalyzed Nazarov cyclization

5 minutes

Maleimide
Starting material 7

45 minutes

Hydrolyzed maleimide
DA adduct (syn and anti) 10
Maleimide and the hydrolyzed maleimide were both tested for background reactivity with substrate 7. Neither were found to promote any reaction with 7.

Two points from the Eyring analysis of the Ga-1-catalyzed Nazarov cyclizations were reproduced to confirm that experimental error was sufficiently low to rely on an extrapolation to 72 °C.\textsuperscript{32}
3.6.6 Extended Electrospray Mass Spectrometry Data

ESI-MS data for Si-3 collected from a 50 µM solution in 9:1 MeOH/DMSO.

*General spectrum*

*Region of interest (4⁺ charge state)*
Region of interest (3⁻ charge state)

Detected (lower) and modeled (upper) isotope patterns for Si-3 as [K₄(Si₂₂₆)]⁴⁻.
Detected (lower) and modeled (upper) isotope patterns for Si-3 as $[\text{Na}_4\text{K}_4\text{Si}_4\text{O}_{10}]^{3-}$.
3.6.7 Crystallographic Analysis of Assembly Si-3

**NEt₄⁺ ⊂ K₈[Si₄L₆] Assembly Si-3.** Crystals suitable for single crystal X-ray diffraction were grown from a solution of NEt₄⁺ ⊂ Si-3 in DMSO by vapor diffusion of benzene. A slightly modified procedure was used to synthesize host Si-3 for X-ray diffraction, in which KHCO₃ was replaced with KOH as the base. The crystals shatter upon desolvation and necessitate rapid handling, and Krytox was required to manipulate the crystals without de-solvation.

*The asymmetric unit in structure NEt₄⁺ ⊂ Si-3 with probability ellipsoids. Counterions and solvent molecules are omitted for clarity.*
<table>
<thead>
<tr>
<th>Name</th>
<th>Si-3</th>
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<tbody>
<tr>
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<td>$c$, Å</td>
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<td>$\gamma$, °</td>
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<tr>
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<tr>
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<tr>
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</tbody>
</table>
3.6.8 $^1$H NMR Spectra

$^1$H NMR spectrum of solvent-occupied or “empty” Si-3
$^{1}H$ NMR spectrum of host Si-3 with encapsulated PEt$_4^+$
$^{1}H$ NMR spectrum of solvent-occupied or “empty” host Ga-1

MM-cluster-MeOD, 1:1
CC: 12180212 AV-S00 TBIP prods
$^{1}H$ DD NMR

= Ga(III)
3.7 References


(43) Portions of this chapter will appear in a publication: Hong, C. M.*; Morimoto, M.*; Kapustin, E. A.; Alzakhem, N. A.; Bergman, R. G.; Raymond, K. N.; Toste, F. D. *J. Am. Chem. Soc.*, manuscript currently under revision.

Chapter 4
An Unusual Enzyme-Like Mechanism of Guest Recognition in a Flexible Supramolecular Host
4.1 Preface

While previous chapters have focused on deliberate endeavors to expand the chemical scope and mechanistic insights of supramolecular catalysis, this study was born of a serendipitously discovered phenomenon and is a detailed exercise in deconvoluting dynamic molecular recognition events. Specifically, this chapter describes a host of Ga$_4$L$_4$ stoichiometry that self-assembles in an $S_4$-symmetric conformation. This subsequently isomerizes to a $T$-symmetric conformation upon binding of sufficiently large guests. The transformation is shown to proceed through a conformational selection mechanism, an unusual pathway which has thus far only been demonstrated in a select range of enzymatic systems. This comprehensive study demonstrates that a simple chemical system can stand as a model for analogous behavior in biological systems that are often challenging to experimentally deconvolute, and speaks to the symbiotic relationship between the fields of enzymology and supramolecular chemistry. This work was done in collaboration with Dr. David M. Kaphan.

4.2 Introduction to Configurationally Adaptive Binding

The understanding of protein–ligand molecular recognition is of significance to the study of enzymatic catalysis and allosteric regulation of cell signaling, as well as to the design of improved and efficient drugs.$^1$ The high levels of specificity observed in enzyme catalysis and protein–ligand binding have been classically accepted to proceed predominantly by the “induced fit” hypothesis (Koshland–Nemethy–Filmer model) introduced in 1958, rather than a concerted binding model.$^2,^3$ The induced fit model stipulates that a protein encounters its ligand in an inactive state, and an optimum fit is achieved only after structural rearrangement is induced by the interaction with the ligand (Figure 4.1). Seven years later, an alternative mechanism for ligand recognition was proposed, referred to as “conformational selection” or the Monod–Wyman–Changeux model.$^4$ This mechanistic hypothesis postulates that the enzyme exists in solution as a dynamic distributions of Boltzmann-populated conformations, and the ligand selectively interacts with the active conformation of the protein to form the ligated complex. This subsequently shifts the equilibrium distribution of protein conformations.

![Figure 4.1. Induced fit and conformational selection mechanisms of substrate binding.](image-url)
Until recently, conformational selection was considered by some to be an uncommon or rare mechanism, with few definitive examples reported in the literature. However, technical advances in single molecule fluorescence and NMR relaxation experiments in the past decade have resulted in the accumulation of significant evidence in support of conformational selection as a relevant mechanism for protein–ligand interactions. While indirect evidence for conformational selection can be garnered by techniques including crystallographic analysis, selective mutation of receptors, and in silico molecular dynamics, the most compelling evidence to distinguish the induced fit and conformational selection mechanisms arises from the kinetic profile of the binding event. Specifically, the rate of approach to the binding equilibrium shows saturation kinetics in ligand concentration for induced fit and inverse dependence on ligand concentration for the conformational selection mechanism (Figure 4.2). Deciphering the mechanism of protein–ligand recognition in the context of these two general mechanisms has since become a very active topic of study in biophysics and enzymology.

![Figure 4.2. Qualitative trends of rate constants as a function of ligand concentration for induced fit (blue dots •), conformational selection (orange dots •), and concerted binding (red squares □) mechanisms.](image)

The mechanisms for induced fit and conformational selection each consist of two consecutive reversible reactions, where guest binding precedes isomerization in induced fit binding and isomerization precedes guest binding in conformational selection (Figure 4.1). A complete description of their respective rate laws is beyond the scope of this work and can be found in the literature. However, under the “rapid equilibrium approximation”, a kinetic scenario that often prevails, expressions for the rate constant of approach to binding equilibrium ($k_{obs}$) can be derived (Table 4.1, equations 1–3). Specifically, the rapid equilibrium approximation makes the simplifying assumption that the ligand-binding step is fast and reversible on the time scale of the conformational change, allowing the consecutive equilibria to be treated independently (see the Supporting Information).

For an induced fit mechanism, the rate law for relaxation of inactive enzyme ($E_i$) to the active enzyme ($E_a$) bound to substrate ($L$) can be expressed by applying a pseudoequilibrium approximation to the guest binding step. This enables an expression for the concentration of intermediate $E_iL$ in terms of $[E_i\text{total}]$ and $[L]$. The concentration of $[E_iL]$ can then be used to express the overall rate constant ($k_{obs}$) as the sum of the forward and the backward rate constants for conformational change. For a simple reversible reaction, the first-order rate constant that describes approach to equilibrium is equal to the sum of the forward and reverse rate constants.
Reversible bimolecular reactions simplify to this form when the concentration of one of the components is sufficiently large, which applies due to the presumed excess of substrate relative to the enzyme. The rate law for an induced fit mechanism shows saturation kinetics in ligand concentration in the forward direction, while the reverse reaction is independent of ligand. This results in overall saturation kinetics with respect to ligand concentration.

Table 4.1. Observed rate constants \( (k_{\text{obs}}) \) for approach to substrate binding equilibrium under concerted, induced fit, and conformational selection mechanisms.

In contrast, a similar analysis applied to the mechanism for conformational selection reveals that the forward reaction is independent of ligand concentration, while the reverse reaction is inhibited by the ligand. The resulting rate constant \( (k_{\text{obs}}) \) describing the approach to equilibrium under the conformational selection mechanism shows overall partial inverse order in ligand concentration. While counterintuitive, this prediction has been experimentally validated in the molecular biology literature.\(^{12-14}\) It is important to note that not all binding events that proceed via conformational selection show ligand inhibition; when the rapid equilibrium approximation does not hold, approximately first order or saturation behavior can be observed. However, if ligand inhibition is observed, the induced fit mechanism can be definitively ruled out in favor of conformational selection. Finally, it is important to note that many biological systems follow a much more complicated mechanism that may incorporate elements of both conformational selection and induced fit. This contributes to the difficulty in quantitatively analyzing many of these systems.

4.3 Ligand Design Principles in Synthetic Self-Assembled Systems

The symbiotic relationship between molecular biology and supramolecular chemistry suggests that similar kinetic scenarios might arise in dynamic synthetic systems. Simple biomimetic supramolecular assemblies have served as model systems to provide insight into the underlying stereoelectronic interactions that drive molecular recognition and enzymatic reactivity in biological systems, and in turn, lessons from biology have contributed to the design of synthetic receptors and catalysts. Self-assembled nanovessels, including hydrogen bond and metal–ligand based assemblies, have been particularly fruitful as models for the study of molecular recognition and microenvironment catalysis.\(^{15-19}\)
The Raymond, Toste, and Bergman collaboration has extensively studied the host–guest chemistry and catalytic behavior of $K_{12}Ga_4L_6$ tetrahedral assembly 1, which is composed of six biscatecholate ligands bridging four homochiral ($\Delta\Delta\Delta\Delta$ or $\Lambda\Lambda\Lambda\Lambda$) pseudo-octahedral gallium(III) atoms (Figure 4.3A). Assembly 1 features a range of enzyme-mimetic behaviors including hydrophobic encapsulation of neutral and anionic guests, Michaelis–Menten-type kinetics for a range of catalytic applications with rate accelerations ($k_{\text{cat}}/k_{\text{uncat}}$) of up to $1.9 \times 10^7$ fold, unusual product selectivities reminiscent of enzymatic catalysis, and even protein-like amide H–D exchange behavior. While assembly 1 has proven a fruitful supramolecular enzyme mimic, it is inherently limited with respect to the steric and electronic properties of the catalyst active site. While structural analogues of 1 have been reported, the rapid diversification of novel $M_4L_6$ assemblies is significantly impaired by the fact that tetrahedra of $M_4L_6$ stoichiometry are entropically disfavored relative to the $M_2L_3$ helicate (Figure 4.3B). Only the carefully tuned geometry of the internal spacer in the bis-bidentate ligand of 1 prevents fragmentation to the helicate, and subtle variations from this structure often lead to the incomplete self-assembly of the tetrahedron or selective formation of the $M_2L_3$ helicate.

**Figure 4.3.** A: $K_{12}Ga_4L_6$ assembly 1. B: The helicate to tetrahedron equilibrium.

In contrast, $M_4L_4$ tetrahedra assembled from $C_3$-symmetric tris-bidentate ligands are less likely to suffer from this entropic complication, given that the ligands are sufficiently rigid. The Raymond group has previously reported the synthesis of $Ga_4L_4$ assembly 2 (Figure 4.4); however,
the scope of host–guest chemistry observed with 2 was prohibitively limited in comparison with assembly 1. It was hypothesized that the disparity in encapsulation between these hosts may be attributed to a difference in the innate flexibility or “breathability” of their cavity structures. In much the same way that a highly specific protein−ligand interaction requires that the protein undergo a configurational change to accommodate and conform to the structure of its ligand (vide supra), so too should supramolecular hosts conform to complement their guest molecules for optimal binding.

In assembly 1, the amide substitution on the central naphthalene linker is offset from the center of the ligand, which allows the walls of the cavity to expand and contract through rotation about the amide−aryl nitrogen−carbon bond. This is evidenced by the crystallographic observation that the encapsulated guest inside 1 can influence the host volume from at least 253 to 435 Å³. In contrast, the amide−aryl bonds within the ligand of assembly 2 are oriented radially from the origin of C₃-symmetry, and as a result, any structural accommodation of guest molecules must occur predominantly by enthalpically costly bond distension. It was therefore hypothesized that the introduction of rotational flexibility to the M₄L₄ structural manifold might reintroduce the “breathability” required for efficient guest encapsulation while opening the opportunity for host diversification by maintaining the entropic benefits of assembly.

In accordance with this hypothesis, tetrahedral assembly 3 was selected as a synthetic target by self-assembly of ligand 4 (Figure 4.4). Host 3 is isomeric to assembly 2, differing only in the meta-substitution pattern of the trianiline-linker that generates the C₃-symmetry of ligand 4 (as opposed to para-substitution in the ligands of host 2). This meta-substitution pattern introduces 60° of curvature in each arm of the ligand and offsets the metal binding moiety from the center of the ligand. This was expected to grant assembly 3 the necessary flexibility to conform to guest molecules without incurring a significant enthalpic penalty. I discuss here the dynamic structural consequences of this simple isomeric substitution, including the observation of a guest-induced structural reorganization upon encapsulation of an appropriately large guest molecule. Mechanistic investigation of the guest-induced host reorganization supports the conclusion that a conformational selection mechanism of molecular recognition is operative, the first such observation in the context of synthetic supramolecular enzyme mimics.

Figure 4.4. Previously reported Ga₄L₄ assembly 2 and the topic of this work, isomeric assembly 3.
4.4 Synthesis of Assembly 3

Synthetic access to ligand 4, the precursor for self-assembly of host 3, was achieved in a manner similar to the ligand precursor to host 2 (Scheme 4.1).\textsuperscript{33} 3-nitroacetophenone 5 was subjected to dehydrative trimerization by the addition of dry potassium pyrosulfate to molten 5 at 100 °C, followed by the addition of catalytic sulfuric acid to afford 1,3,5-tris(3'-nitrophenyl)benzene 6. Known trianiline 7\textsuperscript{35} could be accessed by tin/HCl reduction of 6, which was then acylated with 3.2 equivalents of 2,3-dimethoxybenzoyl chloride (freshly prepared from the corresponding carboxylic acid) and excess triethylamine, yielding the hexamethyl-protected ligand 8. Finally, the deprotected ligand 4 was accessed by treatment of 8 with excess boron tribromide at -78 °C, followed by suspension of the crude product in water at reflux for twelve hours to hydrolyze any boronic esters formed in the deprotection. Ligand 4 was accessed in overall 47% yield from known trianiline 7 and 2,3-dimethoxybenzoyl chloride.

With ligand 4 in hand, the synthesis of host 3 was attempted under the established self-assembly conditions. Ligand 4 and Ga(acac)\textsubscript{3} in equimolar quantities were suspended in degassed methanol-$d_4$ followed by the addition of three equivalents of potassium hydroxide as a methanolic solution. This gave rise to spontaneous self-assembly to form a single species, assigned as host 3, which could be isolated by precipitation with acetone. However, the $^1$H NMR spectrum of the resulting assembly revealed the presence of 24 unique resonances, which is inconsistent with the eight unique chemical environments of a M$_4$L$_4$ host of T-symmetry (Figure 4.5, upper spectrum). This low symmetry species was persistent, even upon heating in methanol for several days (60 °C). Furthermore, the addition of potential guest cation
tetraethylphosphonium iodide resulted in a rapid reduction in the number of proton resonances from 24 to eight, along with the appearance of two new resonances around -0.5 and -1.5 ppm (Figure 4.5, lower spectrum). These resonances and their integrations were indicative of a molecule of tetraethylphosphonium encapsulated within host 3, with $^1$H NMR resonances shifted upfield due to close contact with the aromatic ring currents of the assembly walls. These observations are consistent with formation of $T$-symmetric $M_4L_4$ inclusion complex $\text{PET}_4^+ \subset 3$; however, the structure of the initially formed low symmetry species remained unclear.

![Diagram of assembly 3 and its conformations](image)

Figure 4.5. $^1$H NMR spectrum of assembly 3 immediately after assembly (upper spectrum) and after the addition of approximately 1.5 equivalents of tetraethylphosphonium iodide (lower spectrum).

4.5 Characterization of Assembly 3 and its Conformations

Three possible structures can explain the species observed from host 3 in the absence of a guest. First, the increased flexibility of ligand 4 might alleviate the mechanical coupling that enforces homochirality on the Ga(III) centers in assemblies 1 and 2; if this is the case, then assembly 3 might adopt the $S_4$-symmetric, mixed metal-chirality isomer ($\Lambda\Lambda\Delta\Delta$) in the absence of guest. Second, it is also plausible that a reduction in overall symmetry of host 3 may arise from a collapse of the flexible ligand walls while maintaining homochirality of the Ga(III) metal centers. This conformational variation would reduce the host to $D_2$ symmetry and might arise from pinching two pairs of vertices together. Lastly, it is also conceivable that if the ligand 4 is
sufficiently flexible, an \( \text{M}_2\text{L}_2 \) structure of \( D_2 \) symmetry could initially form under self-assembly conditions. This would require that the addition of a guest initiates a rapid rupture and dimerization of two complexes to form the observed inclusion complex \( \text{PEt}_4^+ \subset T-3 \).

Diffusion-ordered NMR spectroscopy (DOSY) and electrospray mass spectrometry (ESI-MS) were employed to differentiate the predicted \( \text{M}_4\text{L}_4 \) host 3 from this potential \( \text{M}_2\text{L}_2 \) structure. It was anticipated that a low symmetry \( \text{M}_4\text{L}_4 \) host would have a similar hydrodynamic radius and therefore rate of diffusion compared to those of the inclusion complex \( \text{PEt}_4^+ \subset T-3 \). In contrast, an \( \text{M}_2\text{L}_2 \) complex would have a significantly smaller hydrodynamic radius and thus diffuse much more quickly. DOSY NMR revealed a diffusion coefficient of \( 1.52 \times 10^{-5} \text{ cm}^2/\text{s} \) for low symmetry host 3, while the complex \( \text{PEt}_4^+ \subset T-3 \) had a diffusion coefficient of \( 1.96 \times 10^{-5} \text{ cm}^2/\text{s} \) (Figure 4.6). The proximity of these values was a strong indication that the \( \text{M}_4\text{L}_4 \) stoichiometry is preserved between two configurations of 3. ESI-MS measurements of the low symmetry host 3 corroborated this conclusion, as signals consistent with an \( \text{M}_4\text{L}_4 \) host were observed (Figure 4.7).\(^{36}\) These data together supported the conclusion that the host 3 adopts a low symmetry conformation in the absence of guest.

**Figure 4.6.** Left: The diffusion of \( S_4 \)-symmetric assembly 3 (\( D = 1.52 \times 10^{-5} \text{ cm}^2/\text{s} \)) by DOSY NMR. Right: The diffusion of \( T \)-symmetric complex \( \text{PEt}_4^+ \subset T-3 \) (\( D = 1.96 \times 10^{-5} \text{ cm}^2/\text{s} \)) by DOSY NMR.

An \( S_4 \)-symmetric \( \Lambda\Lambda\Delta\Delta \) conformation of host 3 may form a “collapsed” assembly, reducing the internal cavity volume and, by extension, reducing the entropic penalty incurred by trapping solvent molecules inside. Desymmetrization of the assembly from \( T \) to \( S_4 \) symmetry would be consistent with the three-fold increase in unique proton resonances upon isomerization. While the majority of \( \text{M}_4\text{L}_4 \) and \( \text{M}_4\text{L}_6 \) tetrahedra exhibit \( T \) symmetry due to ligand-enforced mechanical coupling, examples of \( \text{M}_4\text{L}_6 \) assemblies have been reported to exist as near-statistical mixtures of \( T-(\Delta\Delta\Delta\Delta/\Lambda\Lambda\Lambda\Lambda) \), \( C_3-(\Lambda\Delta\Delta\Delta/\Delta\Lambda\Lambda\Lambda) \), and \( S_4 \)-symmetric \( \Lambda\Lambda\Delta\Delta \) structures.\(^{37-41}\)
Similarly, a $D_2$-symmetric conformation of host 3 arising from a pair of pinched vertices and collapsed ligands would also reduce the internal cavity volume. If indeed the $D_2$-symmetric structure was an energetic minimum for host 3, the discrimination of these structures with $^1$H NMR would require that their barrier of interconversion be significantly higher than would be expected for a process invoking simple conformational exchange of ligands 4 within host 3.

![Figure 4.7](image)

**Figure 4.7.** Detected (upper spectrum) and modeled (lower spectrum) isotope patterns consistent for $S_4$-symmetric assembly 3 in the $3^-$ charge state: $[K_6H_3(Ga_44_4)]^3$.

### 4.6 Conformational Fluxionality of Host 3

To interrogate the configurational changes that give rise to the reduction of overall symmetry in 3, the dynamics of host isomerization were studied. Because the $C_3$-symmetry of ligand 4 is broken upon self-assembly into host 3, the unique chemical environments created by this desymmetrization were expected to be related by conformational exchange of each unique ligand resonance in the cases of both $S_4$- and $D_2$-symmetric proposed structures of host 3. If the low symmetry of host 3 is a consequence of mixed Ga(III) chirality ($\Delta\Delta\Lambda\Lambda$), then the exchange process that interconverts the desymmetrized protons was anticipated to proceed via stepwise Bailar twists by analogy to studies in the literature on the mechanisms of Ga(III) triscatecholate and helicate isomerizations.\(^{42,43}\) The first Bailar twist from $S_4$-symmetric 3 would generate a $C_3$-symmetric intermediate, and a second Bailar twist would then provide either the $T$-symmetric 3 or a second isomeric $S_4$-symmetric host 3 (Figure 4.8).

It should be noted that the plausibility of an empty $T$-symmetric host 3 as an intermediate in the self-exchange of $S_4$-3 depends on the relative energetic barriers for the $S_4$-$C_3$ Bailar twist.
and the $C_3$-$T$ Bailar twist. Because these relative barriers could not be probed, analysis of the kinetics parameters of self-exchange do not necessarily directly inform the barrier of $S_4$- to $T$-symmetric host isomerization involved in guest binding. However, as these processes are mechanistically related, these self-exchange parameters are taken to shed a qualitative light on this process.

![Figure 4.8. The proposed stepwise Bailar twist mechanism for $\Delta\Delta\Lambda\Lambda$-$3$ degenerate isomerization.](image)

The degenerate isomerization of 3 was initially established by the observation of $^1$H NMR peak coalescence at elevated temperatures. In D$_2$O, the 24 observed resonances of 3 coalesced into eight broad peaks at approximately 90 °C, implying the observation of the time-averaged $T$-symmetric conformation from rapid $S_4$-$3$ isomerization (Figure 4.9). However, line-broadening effects and complications with the temperature dependence of isotropic chemical shifts prevented an accurate determination of kinetic activation parameters from this method.

![Figure 4.9. Variable temperature $^1$H NMR spectra demonstrating the coalescence of host 3 resonances at elevated temperatures in D$_2$O.](image)
To avoid these issues, the technique of selective inversion recovery (SIR) \(^1\)H NMR was employed to measure the isomerization kinetics of the low symmetry host 3 (Figure 4.10). SIR experiments provide an excellent method of measuring rates of slow-exchange (rates of 0.01 s\(^{-1}\) to 100 s\(^{-1}\)) in resolved systems at equilibrium.\(^{43}\) A typical experiment proceeds by selective inversion of the spin population for a unique resonance, effectively labeling those nuclei via magnetization. This is followed by a variable delay time, during which the chemical-exchange process causes a measurable magnetization transfer of the spin-labeled nuclei between the exchange-related chemical environments, followed by the acquisition of a 1D spectrum. This process is repeated for a series of increasing delay times to generate a profile of magnetization transfer over time. The chemical exchange between the inverted signal and the exchanging signal results in signal attenuation in the exchanging resonance and accelerated relaxation in the inverted signal. By modeling these rates in conjunction with independently measured T\(_1\) relaxation times, the rate of chemical exchange can be extracted.

**Figure 4.10.**

A: Total inversion magnetization (the difference in integral at equilibrium and at increasing mixing times) for selectively inverted proton resonance (7.50 ppm) and two resonances related by chemical exchange (8.38 ppm and 6.97 ppm, respectively) for a representative SIR experiment.  
B: Normalized integral value as a function of mixing time for the three resonances related by exchange. 
C: \(^1\)H NMR spectrum of 3 indicating the resonance selectively inverted (blue circles •) and the resonances attenuated as a result of chemical exchange (red • and green • circles).
Although SIR NMR experiments are typically performed on exchange reactions between two chemically distinct environments, the data collection and analysis were extended to evaluate the three-fold symmetric exchange of ligand resonances upon isomerization of assembly 3. Inversion of the singlet appearing in the $^1$H NMR spectrum of 3 at 7.50 ppm resulted in the attenuation of signal intensity for the resonances at 8.38 and 6.97 ppm in a manner characteristic of symmetric three-fold exchange. The rates of this process were measured at temperatures from 33 to 53 °C to extract the kinetic parameters of activation by Eyring analysis (Figure 4.11). This analysis afforded an enthalpy of activation ($\Delta H^*$) of 12.7(3) kcal/mol and an entropy of activation ($\Delta S^*$) of -17.4(5) cal/mol*K, corresponding to a free energy of activation ($\Delta G^*$) of 17.8(3) kcal/mol at 298 K.

These data show that the host isomerization event is significantly entropically disfavored, indicating an ordered transition state. These observations are consistent with the expected parameters for a consecutive Bailar twist mechanism for the degenerate isomerization of $S_4$-symmetric 3 ($\Delta\Delta\Lambda\Lambda$). In comparison, the isomerization of a mononuclear Ga(III) triscatecholate proceeds with an enthalpy of activation ($\Delta H^*$) of 11.0 kcal/mol, an entropy of activation ($\Delta S^*$) of -11.4 cal/mol*K, and a free energy of activation ($\Delta G^*$) of 14.4 kcal/mol at 298 K. The activation parameters for these two processes are very similar, and the slight increase of both the entropy and enthalpy of activation for isomerization of 3 is attributed to the effect of minor mechanical coupling by the polymacrocyclic framework of the host. I note here that while the data presented do not explicitly rule out the collapsed structure of $D_2$-symmetry, they are much more consistent with the expected observations for a double Bailar twist. In conjunction with literature precedents for self-assembled tetrahedra, as well as the behavior of the host in the presence of guest (vide infra), this hypothesis is greatly favored. Having established this species as the $S_4$-symmetric $M_4L_4$ 3 of mixed chirality, the mechanism of this configurational fluxionality was investigated.
4.7 Evaluation of the Conformational Selection Mechanism for Guest Binding

To understand the mechanism by which the addition of a guest affects the isomerization of assembly 3 from the $S_4$-symmetric conformation to the $T$-symmetric isomer, the rate dependence of the approach to the encapsulation equilibrium with respect to guest concentration was studied. A number of supramolecular systems have been shown to undergo configurational changes to accommodate and conform to their guest molecules.44–53 This phenomenon is often referred to imprecisely as “induced fit” binding in reference to the overall shift in host conformation, rather than the mechanism by which this conformational change takes place. To the best of our knowledge, no mechanistic studies have been performed on synthetic hosts to differentiate between an induced fit and conformational selection binding mechanism. By analogy to enzymatic substrate binding, supramolecular assembly 3 might display either induced fit or conformational selection behavior upon binding of ammonium guests (Figure 4.12).

![Figure 4.12. Induced fit and conformational selection mechanisms for encapsulation of ammonium guests by assembly 3.](image)

The approach to encapsulation equilibrium was observed by cooling a methanolic solution of empty host 3 in an NMR tube to -78 °C, followed by addition of the guest at low temperature and then allowing the resulting mixture to warm in a precooled NMR spectrometer. At 8 °C, the approach to equilibrium occurred on an appropriate time scale for kinetic studies, and this relaxation was observed to follow pseudo-first order kinetics (Figure 4.13A). The rate of approach to encapsulation equilibrium ($k_{obs}$) for both tetraethylammonium and tetrapropylammonium guests upon encapsulation within assembly 3 were found to be inhibited by the concentration of guest (Figure 4.13B). Guest inhibition of the encapsulation relaxation rate is the classic kinetic signature of a conformational selection mechanism of molecular recognition (vide supra). If the rapid equilibrium approximation – guest exchange is rapid compared to host isomerization – holds true, then the rate constant for approach to equilibrium is shown in equation 4 on the basis of the mechanism depicted in Figure 4.12 by analogy to the established analysis of enzymatic conformational selection. The observation of inhibition with respect to the concentration of ammonium is consistent with equation 4 (Figure 4.13C). This initially unexpected observation stood as the first piece of evidence supporting the conformational selection mechanism of guest binding for assembly 3.
Figure 4.13. A: (Left) Kinetic profile for approach to equilibrium of tetraethylammonium encapsulation in methanol at 8 °C (blue circles ● are NEt₄⁺ ⊂ T-3, red squares ■ are S₂-3). (Right) Natural log plot displaying first-order kinetics for approach to equilibrium. B: Rate dependence of the approach to encapsulation equilibrium on the concentration of tetraethylammonium ion concentration (left) and tetrapropylammonium ion concentration (bottom) for cluster 3 (right). C: Equation 4, which defines the rate of approach to equilibrium in a conformational selection mechanism of guest binding.

To evaluate the applicability of the rapid equilibrium approximation for this system, the rate of guest self-exchange for tetraethylammonium with the inclusion complex NEt₄⁺ ⊂ 3 was evaluated by SIR NMR spectroscopy. Eyring analysis of this system revealed that the barrier of guest self-exchange (ΔG⧧ = 16.2(8) kcal/mol at 298 K) is dominated by entropic contributions (ΔS⧧ = -46.3(30) cal/mol*K) with only a modest enthalpic component (ΔH⧧ = 2.3(1) kcal/mol) (Figure 4.14). These observations are consistent with the previous analysis of guest self-exchange in host 1, where a significant entropic contribution to the barrier was observed.⁵⁴ The measured kinetic parameters for guest self-exchange and degenerate host isomerization were extrapolated to 8 °C as an indicator of whether the rapid equilibrium approximation was valid. At this temperature, guest self-exchange occurs with a barrier of 15.3(8) kcal/mol, as compared to 17.6(3) kcal/mol.
for degenerate host isomerization, implying that guest self-exchange occurs at a rate greater than 60-fold faster than that of host isomerization. As noted, the degenerate host isomerization is only an approximation for the isomerization process for $T$-symmetric guest binding; however, these processes are likely mechanistically related, and their activation barriers should be similar. This analysis supports the application of the rapid equilibrium approximation in this system.

Figure 14.4. Eyring plot and activation parameters for the self-exchange reaction of tetraethylammonium from NEt$_4^+$ ↔ 3, generated by SIR experiments.

However, while guest-inhibited relaxation rates are strong support for a conformational selection mechanism of guest binding, this does not preclude the additional possibility of fast, reversible guest association to the $S_d$-3 before equilibration. Indeed, there is support for the encapsulation of guest by the $S_4$-symmetric host, as evidenced by the significant change in the $^1$H NMR chemical shift of the ammonium ion in the presence of $S_4$-symmetric host (Figure 14.5). As the low symmetry host is consumed, a concomitant increase in the magnitude of the ammonium ion chemical shift is observed. This is consistent with the ammonium undergoing encapsulation or external association by $S_4$-3 that is fast and reversible relative to the NMR measurements.

In light of this evidence, the first guest association step of the induced fit pathway is viable, while the second isomerization step is prohibited by internal guest. Because the isomerization proceeds via stepwise Bailar twists, we hypothesize that the $C_2$-symmetric prismatic transition state of the Bailar twist constricts the cavity volume sufficiently that guest encapsulation inhibits the overall process. This drives the unique conformational selection mechanism (Figure 14.16).

Further evidence for guest association to $S_4$-3 is gathered from the interaction between 3 and tetramethylammonium. Tetramethylammonium, presumably due to decreased steric demand compared to its ethyl and propyl-congeners, does not induce an increase in symmetry in 3. However, shifts in the $^1$H NMR spectrum indicate association of the guest to the low symmetry host. It was hypothesized that if the ethyl- and propylammonium guests were acting as inhibitors to the Bailar twist isomerization mechanism, thus preventing the induced fit mechanism of guest binding, then the addition of tetramethylammonium to $S_4$-3 would also inhibit the rate at which degenerate isomerization occurred in the low symmetry host.
Figure 4.15. Spectroscopic evidence for the association of \( \text{NEt}_4^+ \) to the \( S_4 \)-symmetric host 3 (left), indicated by an upfield shift of free \( \text{NEt}_4^+ \) that disappears concomitantly with \( S_4 \)-3 (right).

\[
\begin{align*}
\text{A} & \quad \text{Conformational Selection} \\
\text{N} & + \quad S_4 \text{-3} \\
\text{N} & \quad \text{Induced Fit} \\
\text{T} & + \quad T \text{-3} \\
\end{align*}
\]

Figure 4.16. A: The conformational selection mechanism of guest association to 3, with rapid, reversible association of \( \text{NEt}_4^+ \) to \( S_4 \)-3. B: The Bailar twist mechanism depicting the isomerization of one Ga(III) catecholate vertex, which proceeds through a rigid, \( C_3 \)-symmetric prismatic transition state.
To evaluate this possibility, I again turned to SIR NMR to study the rates of degenerate $S_4$-$3$ isomerization in the presence of the tetramethylammonium guest. Indeed, it was observed that increasing concentrations of ammonium ion had a marked inhibitory effect on the rate of degenerate self-exchange for $S_4$-$3$, further supporting the hypothesis that a guest molecule disfavors the induced fit pathway by increasing the barrier to the Bailar twist isomerization mechanism (Figure 4.17). Furthermore, this observation provides further evidence to discount the possibility that the low symmetry host represents the collapsed, homochiral $D_2$-symmetric structure proposed earlier (vide supra). The degenerate self-exchange reaction from this structure would be expected to be unaffected or increased in rate in the presence of an internal guest molecule. Notably, even a guest too small to shift the overall population of $S_4$-$3$ to $T$-$3$ is still a significant deterrent to the stepwise Bailar twist isomerization.

![Figure 4.17](image)

**Figure 4.17.** Rates of degenerate self-exchange of $S_4$-$3$ in the presence of varying concentrations of tetramethylammonium guest.

Further evidence for reversible preassociation of tetraethylammonium to $S_4$-$3$ prior to isomerization can also be gathered from evaluating the guest concentration dependence on the pseudo-zero order initial rate of approach to encapsulation equilibrium, as opposed to the first-order rate constant, which describes the extended kinetic profile. As previously described, the observed rate constant ($k_{obs}$) for a reversible reaction is the sum of the forward and backward rate constants. Inspection of equation 4 (reproduced below, Figure 4.18) reveals that the forward rate constant contribution (equation 4, first term) is independent of guest concentration, while the reverse rate constant contributes the guest inhibition to the overall rate constant (equation 4, second term). If the conformational selection mechanism is amended (Figure 4.16A) to include reversible guest binding for the low symmetry host ($k_3/k_3$), a new expression for $k_{obs}$ is derived, as shown in equation 5.

In this alternative expression of the observed rate constant ($k_{obs}$), both the forward and reverse reaction display inhibition with respect to the concentration of the guest. The initial rate of the reaction is dominated by contributions from the forward direction (equations 4 and 5, first term) due to the lack of appreciable product accumulation. It is therefore possible to discern these mechanisms by assessing the effect of guest concentration in the initial rates regime of the
approach to encapsulation equilibrium. By isolating the contribution from the forward reaction, an absence of inhibition in the initial rates regime would indicate a simple conformational selection mechanism, while guest-dependent inhibition of initial rates would provide evidence for fast and reversible association to $S_4$-3 in addition to the conformational selection pathway.

$$k_{obs} = k_1 + \frac{k_2}{k_2 - k_1} \frac{1}{k_2} [NR_4^+]$$

$$k_{obs} = k_1 \frac{1}{1 + \frac{k_2}{k_2 - k_1} [NR_4^+]} + k_1 \frac{1}{1 + \frac{k_2}{k_2 - k_1} [NR_4^+]}$$

**Figure 4.18.** Equation 4 describing the rate of approach to equilibrium in a conformational selection mechanism of guest binding, and equation 5 describing the amended conformational selection mechanism with a preequilibrium association to the initial conformation of host.

Repetition of the encapsulation kinetics experiments at decreased temperature allowed accurate determination of the pseudo-zero-order initial rate as a function of ammonium concentration, which displayed clear inhibition by the ammonium guest (Figure 4.19). This observation underscores the conclusion that a complete mechanistic picture for the relaxation to guest binding equilibrium for assembly 3 involves guest dissociation from $S_4$-symmetric $\Delta\Delta\Lambda\Lambda$-3, followed by reversible host isomerization, and then guest binding to $T$-symmetric homochiral 3. Taken together, this constitutes the first observation of a conformational selection mechanism in a synthetic system. Furthermore, identification of the mechanism for host rearrangement as stepwise Bailar twists provides a satisfying rationale for why conformational selection is active over the more commonly observed induced fit binding mechanism.

**Figure 4.19.** Initial rates for approach to encapsulation equilibrium as a function of the concentration of tetraethylammonium ion concentration for host 3.
4.8 Preliminary Scope of Neutral Guest Binding in Host 3

With the configurationally adaptive binding behavior of assembly 3 with tightly bound ammonium guests characterized, I next sought to probe the phenomenon of hydrophobic guest encapsulation within assembly 3. Two reasons motivated this brief substrate scope of neutral guest binding. First, neutral guests are encapsulated within anionic metal–ligand assemblies very weakly in comparison to that of their cationic ammonium counterparts, due to the lack of a Coulombic driving force for their association. Instead, the encapsulation of neutral guests has been shown to be driven mainly by the hydrophobic effect, where the entropically costly penalty of solvating the interior of the host and the hydrophobic guest is relieved upon association.\textsuperscript{55} Given the hypothesis that the $S_4$-3 is likely collapsed and features a smaller cavity than 2 or $T$-3 with fewer trapped solvent molecules (hence, lower affinity to sufficiently large cationic guests), the question was raised whether neutral guest encapsulation would occur to a lesser degree.

Second, the meta-substitution pattern in ligand 4 and subsequently assembly 3 was originally incorporated from the previously synthesized assembly 2 with the intention of addressing poor host–guest properties (\textit{vide supra}). Briefly, increased host flexibility as a consequence of additional degrees of rotational freedom and smaller solvent-exposed apertures in 4 were hypothesized to amend the poor host–guest properties of assembly 2. To test whether this design feature did indeed, improve the encapsulation properties of M$_4$L$_4$ hosts, a preliminary range of hydrophobic guests was tested for their encapsulation in assembly 3 and 2 (Figure 4.20). Gratifyingly, a significant improvement was observed for the encapsulation of small hydrophobic guests in the $T$-symmetric conformation of assembly 3.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure420.png}
\caption{The scope of encapsulation of neutral guests in assemblies 2 and 3.}
\end{figure}
4.9 Generality of Guest-Dependent Configurational Flexibility

The generality of this conformational phenomenon was further interrogated and established by the synthesis of a new assembly 9 bearing similar design features, namely the meta-substituted phenyl linker. Ligand 10, the precursor to assembly 9, was accessed by construction of the trianiline core 11, followed by the acylation/deprotection strategy used to access ligand 4 (Scheme 4.2). The ligand core was accessed through a triple S_N_Ar reaction between cyanuric chloride 12 and excess 3-nitroaniline, affording the previously reported tris(3-nitroaryl)melamine complex 13. Triple nitro group reduction of 13 with tin metal and hydrochloric acid afforded trianiline 11, which was followed by acylation with 2,3-dimethoxybenzoyl chloride, providing hexamethyl protected ligand 14. Complete demethylation with boron tribromide yielded ligand 10. Ligand 10 was accessed in overall 52% yield from known compound 13 and 2,3-dimethoxybenzoyl chloride.

As expected, a low symmetry species assigned as ΔΔΛΛ-9 could be generated by the subjecting ligand 10 to self-assembly conditions. The addition of tetraethylphosphonium iodide to the initially observed assembly 9 induced the conversion of ΔΔΛΛ-9 to PEt₄⁺⊂T-9 (Figure 4.21). These conclusions were supported by DOSY NMR spectroscopy (Figure 4.22) and ESI-MS measurements (Figure 4.23). Despite the significant architectural changes between ligands 4 and 10, including new modes of rotational flexibility and potentially strong internal hydrogen bonds, it appeared that the observed self-assembly behavior of assembly 3 is conserved for this bent C₃-symmetric motif in assembly 9.
Figure 4.21. $^1$H NMR spectrum of assembly 9 immediately after assembly (upper spectrum) and after the addition of approximately 1.5 equivalents of tetraethylphosphonium iodide (lower spectrum).

Figure 4.22. Left: The diffusion of $S_d$-symmetric assembly 9 ($D = 5.62 \times 10^{-7} \text{ cm}^2/\text{s}$) by DOSY NMR. Right: The diffusion of $T$-symmetric complex PEt$_4^+$ ⊂ T-9 ($D = 7.94 \times 10^{-7} \text{ cm}^2/\text{s}$) by DOSY NMR.
Figure 4.23. Detected (upper spectrum) and modeled (lower spectrum) isotope patterns consistent for $S_4$-symmetric assembly 9 in the $3^-$ charge state: $[K_3H_4(Ga_{10})]^{3-}$.

4.10 Conclusions

Understanding the mechanism of binding for molecular recognition processes is essential for the rational design of novel host–guest systems, whether in the biological context of drug design or in the context of synthetic supramolecular sensors and catalysts. In this chapter, a previously unknown motif in supramolecular metal–ligand self-assembly was identified which gave rise to a configurationally dynamic phenomenon upon recognition of a tetraalkylammonium guest. This feature was taken advantage of in to study the mechanism by which encapsulation of guests takes place. Specifically, the introduction of appropriate flexibility into the ligand structure of a self-assembled tetrahedral $M_4L_4$ host leads to a thermodynamic preference for the $\Delta\Delta\Lambda\Lambda$-mixed metal center chirality state of $S_4$ symmetry. The addition of guest molecules of sufficient steric bulk induces a structural rearrangement to afford the homochiral host of overall $T$ symmetry. It was found that the rate at which this increase in symmetry occurs is inversely correlated to the concentration of the guest molecule, which is singularly consistent with a conformational selection mechanism of molecular recognition. This is the first defined recapitulation of such a mechanism in an entirely synthetic system. This behavior is known to be the operant mechanism of substrate binding for many biological systems and reinforces the intimate relationship between synthetic and biological supramolecular systems. In particular, this work highlights the useful role of synthetic supramolecular analogs to biological systems and their application as model systems for biology.\textsuperscript{56}
4.11 Supporting Information

4.11.1 General Methods

Unless otherwise stated, all reactions were performed in flame-dried or oven-dried glassware sealed with rubber septa under a nitrogen atmosphere. Reaction solutions were stirred by Teflon-coated magnetic stir bars with the exception of those performed in NMR tubes. Dry dichloromethane (DCM) and triethylamine (TEA) were obtained by passing these previously degassed solvents through activated alumina columns. All other solvents were degassed by thoroughly sparging with nitrogen. All other reagents were used as received from Acros, Sigma Aldrich, or Fisher. Deuterated solvents were purchased from Cambridge Isotope Laboratories and used without further purification. Reactions were monitored by thin layer chromatography (TLC) on Silicycle Siliaplate™ glass backed TLC plates (250 µm thickness, 60 Å porosity, F-254 indicator) and visualized by UV irradiation and p-anisaldehyde stain. Volatile solvents were removed under reduced pressure with a rotary evaporator and dried on high vacuum on a Schlenk line. $^1$H NMR and $^{13}$C NMR spectra were taken with Bruker spectrometers operating at 300, 400, 500, or 600 MHz for $^1$H (75, 100, 125, and 150 MHz for $^{13}$C). Chemical shifts are reported in parts per million (ppm) relative to the residual solvent signal. $^1$H NMR: CDCl$_3$ ($\delta$: 7.26 ppm), DMSO-d$_6$ ($\delta$: 2.50 ppm), MeOD ($\delta$: 3.31 ppm), D$_2$O ($\delta$: 4.79 ppm). $^{13}$C NMR: CDCl$_3$ ($\delta$: 77.16 ppm), DMSO-d$_6$ ($\delta$: 39.52 ppm). NMR data are reported as follows: chemical shift (multiplicity, coupling constants where applicable, number of hydrogens). Coupling is reported with the following symbols: s = singlet, bs = broad singlet, d = doublet, t = triplet, dd = doublet of doublets, dt = doublet of triplets, m = multiplet. All variable temperature NMR experiments were calibrated using an ethylene glycol and methanol standard. High-resolution mass spectrometry (HRMS) was performed on a Thermo LTQ-FT-ICE (7T, ESI) by the QB3/Chemistry Mass Spectrometry Facility at the University of California, Berkeley and on a PerkinElmer AxION 2 (ESI, positive or negative mode) at the Catalysis Center at the University of California, Berkeley.
2,3-dimethoxybenzoic acid (2.33 g, 12.8 mmol, 3.2 equiv.) was dissolved in dry DCM (96 mL) in a 250 mL Schlenk flask. Thionyl chloride (1.88 mL, 25.6 mmol, 6.4 equiv.) was added dropwise, followed by 6 drops of DMF. The reaction mixture was stirred overnight, and then volatiles were removed in vacuo. The resulting residue was redissolved in dry DCM (96 mL), and previously reported trianiline 7 (1.41 g, 3.4.00 mmol, 1 equiv.) was added in one portion, followed by the dropwise addition of triethylamine (3.6 mL). The resulting reaction mixture was stirred overnight. DCM (100 mL) was added, and then the solution was washed with 1M NaOH (50 mL), and 1M HCl (50 mL). The organic layer was dried over magnesium sulfate and concentrated under reduced pressure. The residue was purified by silica gel chromatography (30-50% ethyl acetate in hexanes) to afford 8 (1.94 g, 57% yield) as an off-white solid.

**1H NMR (600 MHz, Chloroform-d)** δ 10.15 (s, 1H), 8.02 (s, 1H), 7.87 (s, 1H), 7.81 (t, J = 8.4 Hz, 2H), 7.52 (app. s, 2H), 7.22 (t, J = 7.9 Hz, 1H), 7.11 (d, J = 7.9 Hz, 1H), 4.04 (s, 3H), 3.93 (s, 3H).

**13C NMR (151 MHz, Chloroform-d)** δ 163.24, 152.77, 147.49, 142.19, 139.04, 129.70, 127.03, 125.65, 124.82, 123.53, 123.15, 119.50, 119.23, 116.00, 61.91, 56.31.

HRMS (m/z): calculated for [C51H45N3O9Na]+, 866.3054; observed, 866.3482.

To a 100 mL round bottom flask was added 8 (1.94 g, 2.3.0 mmol, 1.0 equiv.) and dry DCM (50 mL). The resulting solution was cooled to -78 °C, and boron tribromide (5.18 g, 20.7 mmol, 9 equiv.) was added slowly. The reaction mixture was allowed to warm to room temperature overnight, and then poured over ice (~50 g). Upon reaching room temperature, the resulting biphasic mixture was filtered. The solid was suspended in water (50 mL) and then the resulting suspension was heated to reflux for 12 hours. The reaction mixture was allowed to cool to room temperature, and the solid was filtered and washed with copious amounts of water, to afford ligand 4 (1.53 g, 83%) as a beige solid.
H NMR (600 MHz, DMSO-$d_6$) $\delta$ 11.66 (s, 1H), 10.46 (s, 1H), 9.42 (s, 1H), 8.14 (s, 1H), 7.93 (s, 1H), 7.85 (d, $J = 7.4$ Hz, 1H), 7.67 (d, $J = 7.1$ Hz, 1H), 7.55 (t, $J = 7.5$ Hz, 1H), 7.48 (d, $J = 7.6$ Hz, 1H), 7.00 (d, $J = 7.3$ Hz, 1H), 6.80 (t, $J = 7.6$ Hz, 1H).

$^{13}$C NMR (151 MHz, DMSO-$d_6$) $\delta$ 167.91, 148.40, 146.20, 141.54, 140.49, 138.66, 129.40, 124.48, 123.09, 120.68, 119.92, 119.06, 118.43, 118.31, 117.02.

HRMS (m/z): calculated for [C$_{45}$H$_{32}$N$_3$O$_9$]$^-$, 758.2139; observed, 758.2120.

Self-assembly of Host 3

To a 1 L 3-neck flask was added ligand 4 (942 mg, 1.24 mmol, 1 eq.), Ga(acac)$_3$ (455 mg, 1.24 mmol, 1 eq.), and degassed methanol (40 mL) under a nitrogen atmosphere. The resulting suspension was stirred for 10 minutes, then treated dropwise with potassium hydroxide (209 mg, 3.72 mmol, 3 eq.) predissolved in degassed methanol (3 mL). The reaction mixture became homogeneous upon stirring for 30 minutes, after which degassed acetone (400 mL) was added dropwise over 4 h to precipitate out $S_4$-symmetric host 3 (1.05 g, approx. 88% yield) collected as a beige powder upon filtration.

$^1$H NMR (500 MHz, Methanol-$d_4$) $\delta$ 8.45 – 8.34 (m, 8H), 8.25 (d, $J = 7.8$ Hz, 4H), 7.50 (s, 4H), 7.38 (s, 4H), 7.35 – 7.18 (m, 24H), 7.08 (d, $J = 8.5$ Hz, 4H), 7.03 – 6.96 (m, 8H), 6.93 – 6.87 (m, 8H), 6.75 (d, $J = 7.3$ Hz, 4H), 6.63 (d, $J = 7.3$ Hz, 4H), 6.53 (d, $J = 6.9$ Hz, 4H), 6.44 (t, $J = 7.9$ Hz, 4H), 6.35 – 6.28 (m, 8H), 6.22 (t, $J = 7.5$ Hz, 4H), 6.22 (t, $J = 7.5$ Hz, 4H), 6.02 (t, $J = 7.6$ Hz, 4H).

Hexamethyl protected ligand 14

In a 50 mL round bottom flask, previously reported trinitroaryl compound 13$^{56}$ (1.9 g, 3.88 mmol, 1 eq.) was suspended in ethanol (15 mL) and subsequently treated dropwise with a solution of
SnCl₂ dihydrate (8.0 g, 35.5 mmol, 9.15 eq.), 11.65 M HCl (18 mL), and ethanol (18 mL) via an addition funnel. The resulting reaction mixture was heated to reflux for 18 h, allowed to cool to ambient temperature, then filtered. The resulting solids were dissolved in boiling water then filtered. The aqueous filtrate was neutralized with 1 M KOH until pH > 11, and the resulting precipitate was filtered, washed with water, and washed with cold methanol to afford trianiline 11 as an off-white solid (1.4 g, 3.51 mmol) in 90.3% yield, which was then carried on without further purification.

A 100 mL flask was charged with 2,3-dimethoxybenzoic acid (1.52 g, 8.34 mmol, 3.3 equiv.) under a nitrogen atmosphere. Thionyl chloride (4 mL, 32.90 mmol, 13 equiv.) was added dropwise, followed by the addition of 3 drops of DMF. The reaction mixture was stirred overnight, and then the volatiles removed in vacuo. The resulting residue was redissolved in dry DCM (25 mL) and treated with the freshly prepared trianiline 11 (1.01 g, 2.53 mmol, 1 equiv.) in one portion. The resulting orange suspension was treated dropwise with triethylamine (4 mL) and the reaction was stirred overnight. The reaction mixture was then diluted with DCM (25 mL), washed with 1M HCl (30 mL) followed by 1M NaOH (30 mL), and brine (40 mL). The organic layer was dried over Na₂SO₄ and concentrated in vacuo. The resulting off-white material was dissolved in hot methylene chloride (~50 mL) and precipitated by layering with ether (~75 mL), and the resulting solid was collected by filtration, affording protected ligand 14 (1.47 g, 65%) as an off-white solid.

1H NMR (500 MHz, Chloroform-d) δ 9.86 (s, 1H), 7.94 (s, 1H), 7.68 (d, J = 8.4 Hz, 1H), 7.59 (s, 1H), 7.39 (d, J = 7.9 Hz, 1H), 7.23 (d, J = 6.6 Hz, 1H), 7.17 (t, J = 7.9 Hz, 1H), 7.12 (t, J = 8.0 Hz, 1H), 7.01 (dd, J = 8.0, 1.5 Hz, 1H), 3.87 (s, 3H), 3.84 (s, 3H).

13C NMR (126 MHz, Chloroform-d) δ 163.17, 152.60, 147.23, 138.81, 138.68, 129.22, 126.96, 124.67, 122.92, 116.99, 115.69, 115.44, 112.82, 61.69, 56.14.

HRMS (m/z): calculated for [C₄₈H₄₅N₉O₉Na]+, 914.3248; observed, 914.3295.

In a 25 mL flask, 10 (115 mg, 0.14 mmol, 1 equiv.) was dissolved in dry DCM (5 mL). The solution was cooled to -78 °C and treated dropwise with boron tribromide (0.3 mL, 2.84 mmol, 20 equiv.). The resulting yellow mixture was stirred for 1 hour at -78 °C, and then slowly warmed to ambient temperature and stirred for 8 additional hours. The reaction mixture was then poured slowly into a large beaker of ice (~25 g). Upon reaching ambient temperature, the biphasic slurry was then filtered and washed with water (3 x 100 mL) to give ligand 6 (100 mg, 88%) as an off-white powder.
$^1$H NMR (500 MHz, Chloroform-$d$) $\delta$ 11.70 (br. s, 1H), 10.35 (s, 1H), 9.71 (s, 1H), 7.90 (s, 1H), 7.73 (s, 1H), 7.45 (d, $J = 8.5$ Hz, 1H), 7.28 (br. s, 2H), 6.99 (d, $J = 7.5$ Hz, 1H), 6.78 (t, $J = 7.8$ Hz, 1H).

$^{13}$C NMR (126 MHz, DMSO-$d_6$) $\delta$ 167.65, 162.79, 148.38, 146.19, 139.49, 138.00, 128.59, 119.07, 118.51, 118.47, 117.41, 117.02, 116.27, 114.24.

HRMS (m/z): calculated [C$_{42}$H$_{34}$N$_9$O$_9$]$^+$, 808.2474; found, 808.2464.
4.11.3 Procedures for Non-Preparative Reactions

Kinetics for relaxation to encapsulation equilibrium

In a wet N\textsubscript{2} atmosphere glove box, to an NMR tube was added host 3 (0.25 µmol, 1.0 equiv) and 2-TMS-ethanol (5.0 µmol) as a stock solution in methanol-\textit{d}\textsubscript{4} (400 µL). The NMR tube was sealed with a septum-topped cap, removed from the glove box, and the cap was sealed with parafilm. The NMR tubes were stored at -78 °C in a dry ice / acetone bath. Immediately prior to use, the appropriate ammonium salt was added as a stock solution in methanol-\textit{d}\textsubscript{4} (100 µL) while submerged in the dry ice / acetone bath. The reaction mixture was then placed into the precoced, tuned and shimmed NMR spectrometer (on an identical dummy sample), and an automatic kinetics program was used to collect regular time points.

Neutral guest binding in host 3

In a wet N\textsubscript{2} atmosphere glove box, an NMR tube was charged with host 3 (approx. 10 mg) and degassed D\textsubscript{2}O (0.7 mL). Neutral, hydrophobic guests were then injected or added as a solid (10 mg or 10 µL), then sonicated for 10 minutes.
4.11.4 Derivations of Observed Rate Constants ($k_{obs}$) in Equations 2, 3

The first order rate constant for approach to equilibrium in a reversible reaction

\[
\begin{align*}
A & \xrightleftharpoons[k_{-1}]{k_1} B \\
k_{obs} &= k_1 + k_{-1}
\end{align*}
\]

\[
\frac{\partial [A]}{\partial t} = k_1[A]_r - k_1[B]_r
\]

\[
[A]_o + [B]_o = [A]_o + [B]_o = [A]_r + [B]_r
\]

\[
k_1[A]_o = k_1[B]_o
\]

\[
[B]_o = [A]_o + \frac{k_1}{k_{-1}}[A]_r - [A]_r
\]

\[
\frac{\partial [A]}{\partial t} = k_1[A]_r - k_1([A]_o + \frac{k_1}{k_{-1}}[A]_o - [A]_r)
\]

\[
\frac{\partial [A]}{\partial t} = k_1[A]_r - k_1[A]_o - k_1[A]_o + k_3[A]_o
\]

\[
\frac{\partial [A]}{\partial t} = (k_1 + k_{-1}) \times ([A]_r - [A]_o)
\]

For a bimolecular reaction that is reversible, if one component is significantly greater in concentrations (i.e. [L] >> [E]), then the forward reaction becomes pseudo-first order, with the reagent of larger concentration absorbed into the rate constant, as in the one step binding event:

\[
E \xrightarrow{k_{on}[L]} E[L] \\
k_{obs} = k_{on}[L] + k_{off}
\]

For the analyses of the conformational selection and the induced fit mechanisms, two assumptions are made: (1) ligand exchange is fast compared to host isomerization, and (2) the ligand concentration is large compared to host concentration. Since the isomerization step is significantly slower than guest binding, the two reactions can be treated independently.
**Induced fit mechanism:**

\[
E_i \xrightleftharpoons[k_{\text{off}}][k_r] E_iL \xrightleftharpoons[k_r][k_{\text{off}}] E_aL \quad k_{\text{obs}} = k_r \frac{[L]}{k_{\text{off}} + [L]} + k_r
\]

\[
\frac{\partial [E_iL]}{\partial t} = k_r [E_iL] - k_r [E_aL]
\]

\[
k_{\text{on}}[L][E_i] = k_{\text{off}} [E_iL]
\]

\[
[E_{i,\text{total}}] = [E_i] + [E_iL]
\]

\[
k_{\text{on}}[L] ([E_{i,\text{total}}] - [E_iL]) = k_{\text{off}} [E_iL]
\]

\[
k_{\text{on}}[L][E_{i,\text{total}}] = k_{\text{off}} [E_iL] + k_{\text{on}}[L][E_iL]
\]

\[
[E_iL] = \frac{k_{\text{on}}[L][E_{i,\text{total}}]}{k_{\text{off}} + k_{\text{on}}[L]} = \frac{[L][E_{i,\text{total}}]}{k_{\text{off}} + k_{\text{on}}[L]} = \frac{[E_{i,\text{total}}]}{k_{\text{on}}[L]}
\]

\[
\frac{\partial [E_aL]}{\partial t} = k_r \frac{[L]}{k_{\text{off}} + [L]} [E_{i,\text{total}}] - k_r [E_aL]
\]

\[
k_{\text{obs}} = k_r \frac{[L]}{k_{\text{off}} + [L]} + k_r
\]

**Conformational selection mechanism:**

\[
E_i \xrightarrow[k_{\text{on}}][k_r] E_a \xrightarrow[k_{\text{off}}][k_{\text{on}}[L]] E_aL \quad k_{\text{obs}} = k_r + k_r \frac{1}{1 + k_{\text{on}}[L]}
\]

\[
- \frac{\partial [E_i]}{\partial t} = k_r [E_i] - k_r [E_a]
\]

\[
k_{\text{on}}[L][E_a] = k_{\text{off}} [E_aL]
\]

\[
[E_{a,\text{total}}] = [E_a] + [E_aL]
\]

\[
k_{\text{on}}[L][E_a] = k_{\text{off}} ([E_{a,\text{total}}] - [E_a])
\]

\[
k_{\text{on}}[L][E_a] + k_{\text{off}} [E_a] = k_{\text{off}} [E_{a,\text{total}}]
\]

\[
[E_a] = \frac{k_{\text{off}} [E_{a,\text{total}}]}{k_{\text{off}} + k_{\text{on}}[L]} = \frac{[E_{a,\text{total}}]}{1 + \frac{k_{\text{on}}[L]}{k_{\text{off}}}}
\]

\[
\frac{\partial [E_aL]}{\partial t} = k_r [E_i] - k_r \frac{1}{1 + \frac{k_{\text{on}}[L]}{k_{\text{off}}}} [E_{a,\text{total}}]
\]

\[
k_{\text{obs}} = k_r + k_r \frac{1}{1 + \frac{k_{\text{on}}[L]}{k_{\text{off}}}}
\]
4.11.5 Extended Electrospray Mass Spectrometry Data

ESI-MS data for host 3 collected from a 200 µM solution in 1:1 MeOH/H₂O.

**General spectrum**

![General spectrum](image1)

**Region of interest**

![Region of interest](image2)
Detected (upper) and modeled (lower) isotope patterns for host 3 as \([H_4K_5(Ga_4xi)]^{3-}\).
ESI-MS data for host 9 collected from a 500 µM solution in H₂O.

*General spectrum*

*Region of interest*
Detected (upper) and modeled (lower) isotope patterns for host 9 as $[\text{H}_4\text{K}_5\text{Ga}_6\text{O}_{16}]^{3-}$.
4.11.6 Measured Rates for Kinetic Studies

Rates for the degenerate isomerization of host $S_4\cdot3$ generated by SIR at various temperatures

<table>
<thead>
<tr>
<th>Temp (K)</th>
<th>$k_{obs}$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>310.40</td>
<td>1.26</td>
</tr>
<tr>
<td>315.65</td>
<td>1.71</td>
</tr>
<tr>
<td>320.90</td>
<td>2.50</td>
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<td>326.15</td>
<td>3.58</td>
</tr>
<tr>
<td>331.40</td>
<td>4.81</td>
</tr>
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</table>

Rate dependence of the approach to encapsulation equilibrium of host 3 on tetraethylammonium concentration

<table>
<thead>
<tr>
<th>NEt$_4^+$ (mM)</th>
<th>$k_{obs}$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.8</td>
<td>1.03E-3</td>
</tr>
<tr>
<td>8.6</td>
<td>1.46E-3</td>
</tr>
<tr>
<td>7.5</td>
<td>1.78E-3</td>
</tr>
<tr>
<td>6.0</td>
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</tr>
<tr>
<td>3.6</td>
<td>4.97E-3</td>
</tr>
</tbody>
</table>

Rate dependence of the approach to encapsulation equilibrium of host 3 on tetrapropylammonium concentration

<table>
<thead>
<tr>
<th>NPr$_4^+$ (mM)</th>
<th>$k_{obs}$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>3.65E-3</td>
</tr>
<tr>
<td>7.6</td>
<td>3.95E-4</td>
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<tr>
<td>3.1</td>
<td>6.49E-3</td>
</tr>
</tbody>
</table>

Rates for the self-exchange reaction of tetraethylammonium from the inclusion complex NEt$_4^+ \subset T\cdot3$ generated by SIR at various temperatures

<table>
<thead>
<tr>
<th>Temp (K)</th>
<th>$k_{obs}$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>257.91</td>
<td>4.37</td>
</tr>
<tr>
<td>286.41</td>
<td>5.30</td>
</tr>
<tr>
<td>278.90</td>
<td>6.82</td>
</tr>
<tr>
<td>299.90</td>
<td>9.34</td>
</tr>
<tr>
<td>320.90</td>
<td>13.31</td>
</tr>
</tbody>
</table>
Rate dependence of the degenerate isomerization of host $S_4\cdot3$ on tetramethylammonium concentration relative to one equivalent of host

<table>
<thead>
<tr>
<th>$\text{NMe}_4^+$ (equiv.)</th>
<th>$k_{\text{obs}}$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.97</td>
</tr>
<tr>
<td>1.10</td>
<td>0.69</td>
</tr>
<tr>
<td>1.94</td>
<td>0.63</td>
</tr>
<tr>
<td>3.57</td>
<td>0.54</td>
</tr>
<tr>
<td>7.55</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Rate dependence of the approach to encapsulation equilibrium of host 3 on tetraethylammonium in the initial rates regime

<table>
<thead>
<tr>
<th>$\text{NEt}_4^+$ (mM)</th>
<th>$k_{\text{obs}}$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2</td>
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</tr>
<tr>
<td>4.7</td>
<td>1.85E-7</td>
</tr>
<tr>
<td>5.9</td>
<td>9.53E-8</td>
</tr>
</tbody>
</table>
4.11.7 $^1$H and $^{13}$C NMR Spectra of Previously Unreported Compounds

*Hexamethyl protected ligand 8*
Ligand 4

DMK.V.0哈尔 CS3MLH6 copy.1.6d
AV-600 750 proton starting parameters 11/16/08 RN
Hexamethyl protected ligand 14
4.12 References


(10) Vogt, A. D.; Di Cera, E. Conformational Selection Is a Dominant Mechanism of Ligand Binding. *Biochemistry (Mosc.)* **2013**, *52* (34), 5723–5729.


(33) Yeh, R. M.; Xu, J.; Seeber, G.; Raymond, K. N. Large M4L4 (M = Al(III), Ga(III), In(III), Ti(IV)) Tetrahedral Coordination Cages: An Extension of Symmetry-Based Design. Inorg. Chem. 2005, 44 (18), 6228–6239.


