Studies of Organic Molecule Recognition by Synthetic Cyclophane Receptors in Aqueous Media

Thesis by

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Acknowledgments

My graduate career at Caltech has been a lengthy one. During my time here, Ronald Reagan, George Bush, and Bill Clinton have occupied the White House; the Whittier Narrows, Landers, and Northridge earthquakes have jolted Southern California; and over one hundred episodes of *Star Trek: The Next Generation* have gone into production. During this time, I've also interacted with some of the most diverse and interesting people that I have ever encountered. These people have had a greater impact on me than three presidents, three major earthquakes, and one hundred episodes of *Star Trek* ever could, and I would like to acknowledge some of them here.

I would like to thank Dennis Dougherty, my research advisor, for the opportunity to work on some really interesting research projects. His input into my scientific efforts here was first-rate, and his enthusiasm for the projects and for science in general was truly remarkable. I'm also grateful that his idea of professional development includes more than lab work. The ample time I spent teaching at Caltech was some of the more rewarding, and I'm glad to have had it. I'm also thankful for the time and the freedom that I was allowed for deciding the future of my scientific career. Quite honestly, not every advisor would let a sixth-year graduate student in chemistry take a lab course in neurobiology.

All of the cyclophane work presented here is based on the efforts of Mike Petti and Tim Shepodd, who developed the exceptional host P. Rich E. Barrans Jr. developed the fine programs for determining binding constants from all of the NMR and CD binding data that I could generate. Jonathan Forman deserves credit for developing circular dichroism into a useful method for generating binding constants, and I'm glad to have made use of it. I also want to recognize

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Many people deserve thanks for their friendship during my time at Caltech. I'm indebted to Donnie Cotter for introducing me to the joys of backpacking and the pecan rolls at Pie 'N Burger, Nancy Shreve for all of the wonderful conversations disguised as tennis matches, Laura Mizoue for keeping 249 Crellin a bastion of calm during my stay here, and Elizabeth Burns for her general wit and wisdom. Andy Fyfe deserves special thanks for always knowing how to make a bad situation better, and for his continual encouragements.

Finally, I would like to thank my family, especially my mom, for their love and support throughout this long endeavor.

Abstract

The behaviors of six new cyclophane receptors for organic guest molecules in aqueous media are reported. These new hosts are modifications of more basic parent structures, and the main goal of their examination has been to determine how the modifications affect host selectivity for cationic guests. In particular, we have been interested in determining how additional non-covalent binding interactions can complement the cation-π interactions active in the parent systems. Three types of modifications were made to these systems. Firstly, neutral methoxy and bromine substituents were added to produce four of the six new macrocycles. Secondly, two additional aromatic rings (relative to the parent host) capable of making cation-π interactions with charged guest species were appended. Thirdly, a negatively charged carboxyl group was attached to produce a cavity in which electrostatic interactions should enhance cationic guest binding. ^{1}H-NMR and circular dichroic techniques were employed to determine the binding affinities of a wide variety of organic guests for the parent and modified structures in aqueous media.

Bromination of the parent host greatly enhances its binding in a general fashion, primarily as the result of hydrophobic interactions. The addition of methoxy groups does not enhance binding, apparently as a result of a collapse of the hosts into a conformation that is not suitable for binding. The appendage of extra aromatic rings enhances the binding of positively charged guests, most likely in response to more complete encapsulation of guest species. The addition of a negatively charged carboxylate enhances the binding to only selective groups of cationic guests. AM1 calculations of the electrostatic potentials of several guests molecules suggests that the enhancements seen with the modified

receptor compared to the parent are most likely the result of close contact between regions of highest potential on the guest and the appended carboxylate.

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Chapter 1: Introduction and Background Information on Cyclophane Receptors.

Introduction

Since its birth in studies of alkali metal ions complexed by crown ethers carried out by Pedersen, Lehn, and Cram in the late 1960s,[1] the field of supramolecular chemistry has grown considerably into a broad discipline which incorporates numerous perspectives from chemistry, physics, and biology.[2] Throughout its history, a major goal of the discipline has been to achieve an understanding and a mastery of the wide array of intermolecular forces that are active in molecular complexation. Many elegant binding studies of organic and inorganic species have now documented the roles played by hydrogen bonding,[3] donor-acceptor interactions,[4] hydrophobic effects,[5] solvent effects,[6] and electrostatic interactions,[7-10] as well as other factors required for successful binding such as preorganization and cooperativity. Originally, the field perhaps could be most commonly described as the study of bimolecular complexation events with the ultimate aim of building artificial enzymes. Today, however, any description of supramolecular chemistry must also include the studies of polymeric complexations and their goals: mainly, crystal engineering, control of micellular and bilayer species, as well as the construction of nanoscale devices.[2] Clearly, much has changed since the potassium ions were first observed to be complexed by 18-crown-6.

In the Dougherty group at Caltech, our efforts[11] in the field have centered around investigations of what have come to be known as cation-π interactions, the stabilization that results from interactions between a positive charge and the electron-rich face of an aromatic ring (figure 1.1). While conceptually simple to understand, the interaction presumably involves a number of fundamental

forces, such as charge-dipole, charge-quadrupole, charge-induced dipole, and London dispersion terms.[11h] This interaction had been observed in the gas phase ion studies of Kerbarle[12] and Meot-Ner (Mautner),[13] and had been postulated to be a fundamental force for the stabilization of protein quaternary structure.[14]

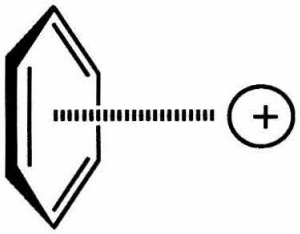
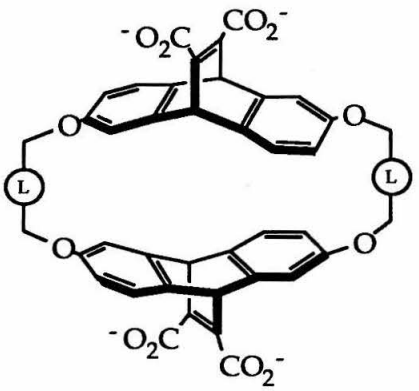


Figure 1.1. The Cation-π Interaction

Our investigations of cation-π interactions have centered around the use of macrocyclic cyclophane hosts, such as P (shown in figure 1.2). This host contains two rigid ethenoanthracene units, which provide a large concave hydrophobic surface for binding, and is denoted as P for the *p*-xylyl groups that serve as "linkers" for the two units. Using cation-π interactions, host P serves as a general receptor for positively charged guests such as alkyliminium,† quaternary ammonium, guanidinium, and sulfonium compounds in both aqueous[11a,b,c,j] and organic[11d] media. Elegant studies[11f,h] have shown that hosts P and C (figure 1.2) catalyze both alkylation and dealkylation reactions through the stabilization of positively charged transitions states (presumably through cation-π interactions and polarization effects). The work with P and related structures led us to propose[11g] that the aromatic rings of tyrosine, phenylalanine, and tryptophan bind quaternary ammonium groups in a wide variety of protein structures, in particular in receptors that bind the neurotransmitter acetylcholine (ACh). The

† This term is used throughout this thesis to define the class of molecules that includes alkylated quinolines, isoquinolines, pyridines, and related structures.



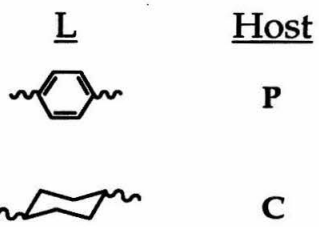


Figure 1.2. Classic Host Structures

proposal has received considerable recent support. Based on more recent computational modeling studies, we have postulated[11i] that through cation-π interactions, side chain aromatics may serve as the selectivity filters for potassium ions in voltage-gated and other potassium channels.

Having observed the remarkable binding properties of the host P receptor, we became interested in trying to modify its basic structure. Six new cyclophanes (Figure 1.3) were examined, and are presented in this thesis. The main focus of the work was to modify the electronic structure of P to enhance host selectivity for cationic guest molecules. Three different types of modifications were made. Firstly, neutral methoxy and bromine substitiuents were appended (in various combinations) to the host P structure to produce the OMP, TMP, TBP, and TMTBP macrocycles (Figure 1.3). It was hoped that these π-donating substituents would result in more-electron rich and/or more polarizable cavities capable of making improved cation-π interactions between host and guest species. Secondly, a negatively charged carboxyl group was attached to P to produce the CP cavity (Figure 1.3). In this study, we wished to improve cationic guest binding through enhanced electrostatic interactions. Thirdly, another

ethenoanthracene subunit was added to produce the cyclophane D3 (Figure 1.3). The D3 structure presents two additional aromatic rings capable of making cation-π interactions with charged guest species.

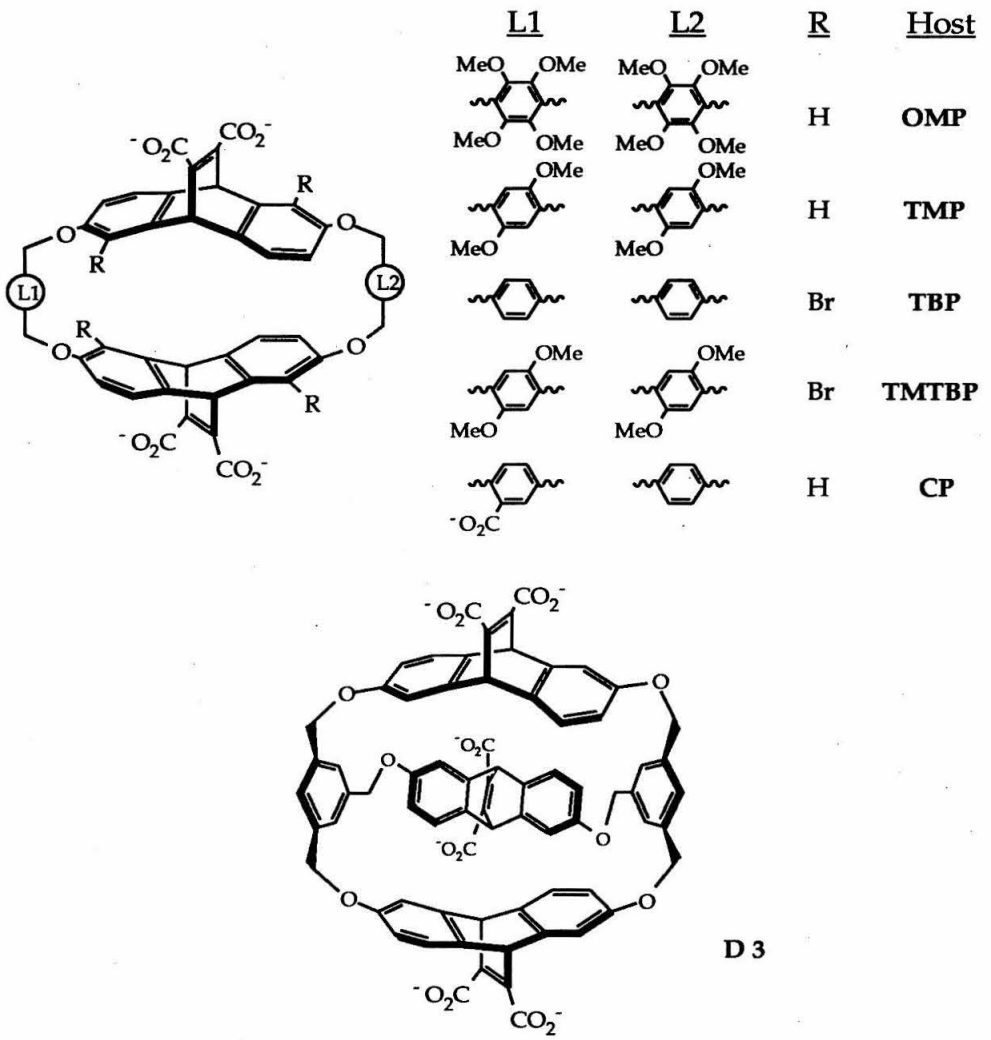


Figure 1.3. Definition of "New" Host Structures

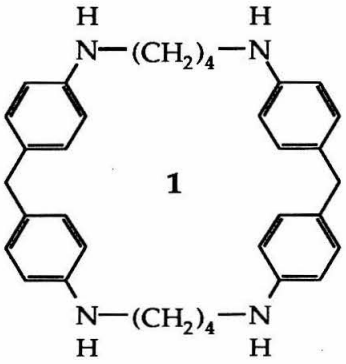
As mentioned above, our work with host P and related structures has led us to postulate that nature uses the aromatic rings of tyrosine, phenylalanine, and tryptophan side chains to bind quaternary ammonium groups in a wide variety of protein structures.[11g] Of particular interest to us have been acetylcholine ligand gated ion channels (AChRs).[15] The binding sites in these receptors for

acetylcholine, a quaternary ammonium compound, have been found to contain a large number of highly conserved aromatic residues.[16] These membrane-bound proteins (and the class of ion channels to which they belong) are presently the objects of intense scientific study. In collaboration with the Lester/Davidson and Abelson groups here at Caltech, we have begun a project to express receptors (AChRs) containing artificial amino acids located at or near the binding site for acetylcholine in *Xenopus* oocytes. The goal of the project is to use a modified version of the procedures described by P. G. Schultz for artificial amino acid incorporation[17] in conjunction with modern neurophysiological techniques (such as functional expression, as well as voltage and patch clamping)[18] to study these fascinating receptors. The project is ongoing.

The syntheses of the host species studied are discussed in chapter 2 of this work. Methods used to study the host structures (NMR and CD titration methods, solvent selection, guest selection and synthesis, complexation induced chemical shift changes, etc.) are covered in chapter 3. The results of neutral modifications made to the host are discussed in chapter 4. Discussions of the properties of the CP and D3 hosts are found in chapters 5 and 6, respectively. Synthetic procedures which might prove useful in the synthesis of future host structures are presented in chapter 7. Chapter 8 describes how this work might serve as a foundation for further studies. A description of the project aimed at expression in *Xenopus* oocytes of AChRs containing artificial amino acids and details of some of the synthetic procedures utilized therein are desribed in chapter 9. Before proceeding to this material however, it is necessary to provide a more detailed description of some previous studies done in the Dougherty group involving cation-π interactions, and an outline of the context in which they belong.

Background Information

The study of cyclophanes[4,5,6a,7-11,19-26] in aqueous and oganic media has proved helpful in understanding the intricate operation of intermolecular forces in biological systems. The first cyclophane shown to include an apolar guest in both aqueous solution and in the solid state was reported by Koga and coworkers in 1980.[20] The tetraprotonated macrocycle **1** complexed the apolar guest compound 2,6-naphthalenediol with reasonably high affinity in aqueous solution at a pH less than 2. This work firmly established ^{1}H NMR spectroscopy as one of the primary methods for studying complexation events between organic species, and inspired numerous subsequent investigations, including our own efforts. Studies in several groups, most notably those of Lehn,[24] Koga,[7] Vögtle,[10,22] and Diederich,[4,5a,6,9] centered on the creation of new receptors based on this first Koga macrocycle. The results of these early efforts helped establish the importance of hydrophobic and close-contacting electrostatic interactions in the binding of small organic guest molecules in aqueous solutions.



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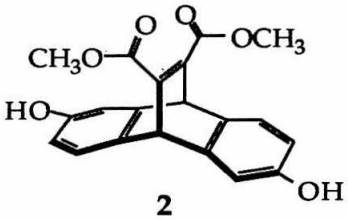


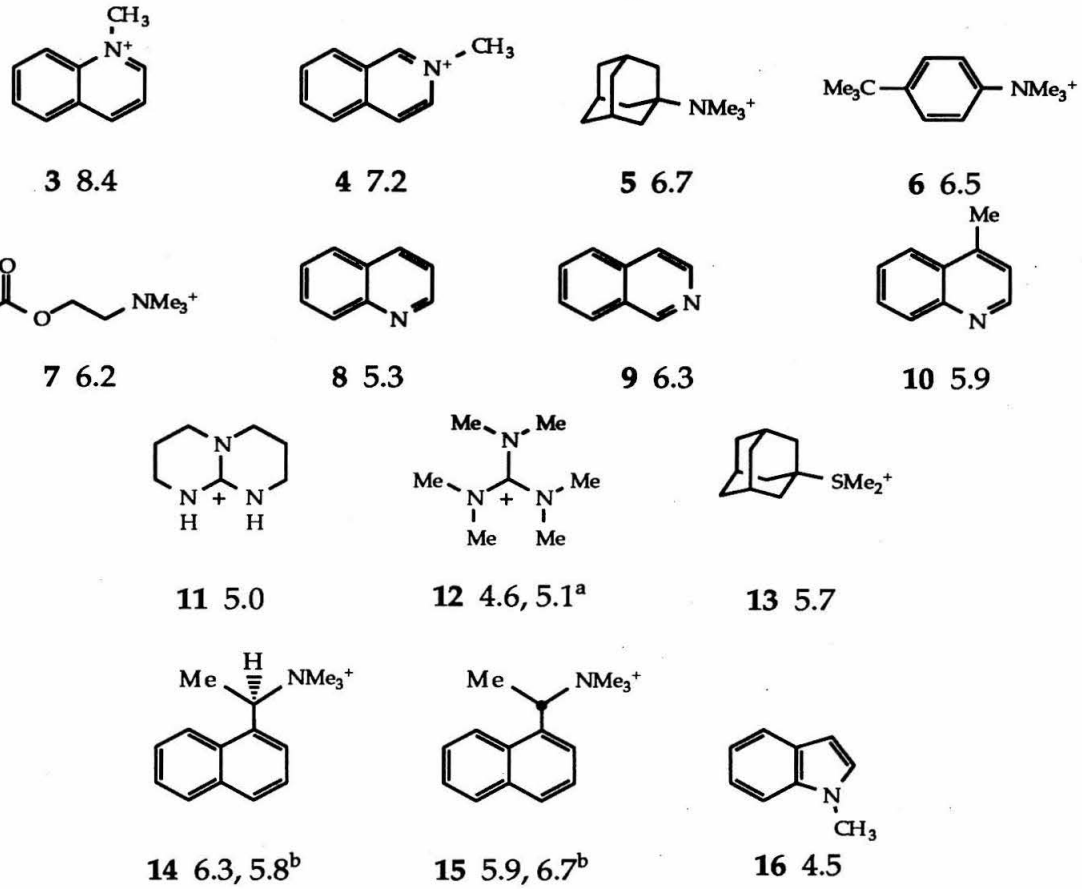
2

Using the Koga host as a starting point, previous members of the Dougherty group (Mike Petti and Tim Shepodd) developed the ethenoanthracene compound **2** as a building block for a new class of cyclophane receptors.[11a-c] The coupling of this unit with various aromatic or aliphatic linkers produced the

tetramethylesters of cyclophane hosts, such as P and C, shown in figure 1.2. These hosts differ from the Koga macrocycle (and those of other researchers) in several respects. Firstly, the ethenoanthracene units of the P and C hosts provide a concave hydrophobic surface for binding, which, unlike the diphenylmethane moieties of the Koga host, is absolutely rigid. As such, the P and C structures maintain a higher degree of preorganization. Secondly, the ethenoanthracene moieties enforce a rigorous separation of the water-solubilizing carboxylate groups from the binding site. It was assumed that such a separation would increase the overall hydrophobicity of the binding site, and hence improve guest binding in aqueous media. Thirdly, by incorporating carboxylates into the design, hosts P and C achieve solubilities much closer to neutral pH than does the original Koga host. Finally, the ethenoanthracene subunits are chiral, with C_2 symmetry, and can be used to create chiral host molecules. It was hoped that the 2-fold symmetry in these chiral receptors would provide the host structures with some degree of enantioselectivity.

Of the host structures created in our lab, host P has been the most thoroughly examined. The free energies of binding ($-\Delta G°$) of a sampling of guest molecules with P in aqueous borate buffer (pH ~ 9) are shown in figure 1.4. The guests presented highlight some of our most important findings. Before discussing these findings however, a few comments about the guests and the host conformations to which they bind should be made. All of the guest molecules presented in the figure are sterically well suited to bind with one of two host conformations. CPK and computer modeling suggest that flat aromatic guests, such as **3** and **8**, bind well with the rhomboid conformation of the host (shown in figure 1.5). This conformation is C_2 symmetric, and as such maintains inequivalent cavity openings. A recent X-ray structure[27] of the tetramethylester

Figure 1.4. -ΔG° (kcal/mol) in Borate for Binding to Host P



3 8.4 **4** 7.2 **5** 6.7 **6** 6.5

7 6.2 **8** 5.3 **9** 6.3 **10** 5.9

11 5.0 **12** 4.6, 5.1[a] **13** 5.7

14 6.3, 5.8[b] **15** 5.9, 6.7[b] **16** 4.5

[a] Two values are for two enantiomers of guest. [b] Two values are for the (R,R,R,R) and (S,S,S,S) absolute configurations of the host respectively.

of P revealed a rhomboid conformation in near perfect agreement with this predicted structure. Evidence supporting guest binding with this host conformation is presented in a discussion of complexation induced changes in ^1H NMR chemical shift values in chapter 3. Larger guests molecules, such as **5**, are better suited to bind with the toroid conformation of the host (figure 1.5). This D_2 symmetric form of the receptor presents equivalent cavity openings for guest complexation. ^1H NMR difference NOE data[11c] on the tetramethylester in CD_2Cl_2 support the conformations of the aryl OCH_2 groups shown in the figure for the host in both toroid and rhomboid conformations. Circular dichroism

(CD) studies of guest complexation provide further evidence of this two-state model, as changes in the CD spectrum of P are noticeably different when binding flat iminium vs. the larger tetraalkylammonium compounds.[28]

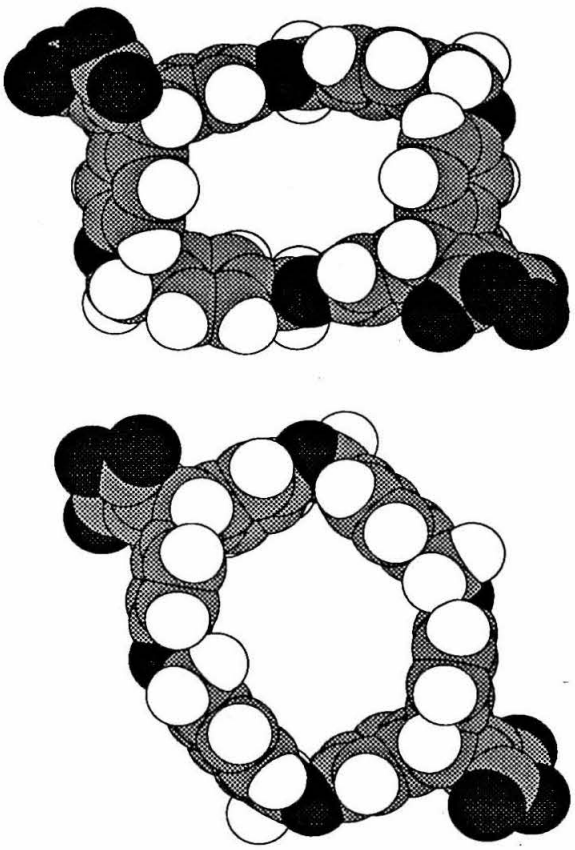


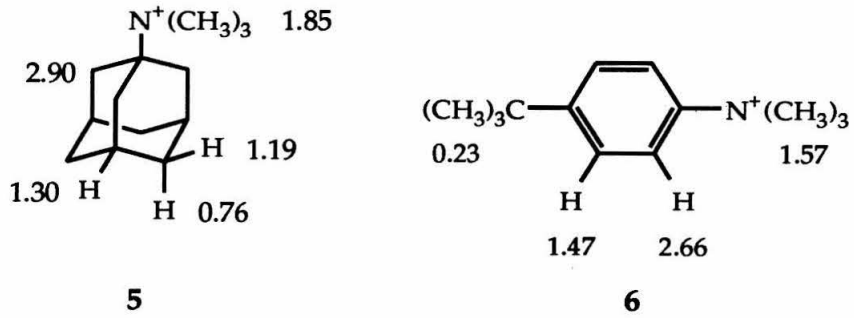
Figure 1.5. CPK Representations of Host P [(S,S,S,S) absolute configuration] in the Rhomboid (top) and Toroid (bottom) Binding Conformations

Returning to the binding data in figure 1.4, several observations are readily apparent. In aqueous borate, host P shows a high affinity for freely water soluble guests. As such, the significant stabilizations are more reflective of true attractions between host and guest species, rather than of repulsions between the guests and water. Electron-deficient neutral compounds such as quinoline **8** and isolquinoline **9** are more tightly bound to the cavity of P than is the electron rich, more hydrophobic indole compound **16**, suggestive of strong π-stacking donor-

acceptor interactions. The host displays a reasonable degree of enantioselectivity, evidenced not only by the differential binding of the enantiomers **14** and **15,** but also by the differential binding of the enantiomeric conformations of hexamethylguanidinium **12.** Most importantly, however, host P binds a wide variety of positively charged guests with high affinity. Iminium (**3, 4**), quaternary ammonium (**5, 6, 7, 14,** and **15**), sulfonium (**13**), and guanidinium compounds (**11, 12**) are all observed to undergo complexation. Perhaps most surprising is the observation that iminium compounds bind with higher affinity to P than do more hydrophobic, neutral quinoline and isoquinoline guests. For example, compound **3** is more stably bound to P than is compound **10** by 2.5 kcal/mol, despite the fact that calculations indicate that **3** is more stably solvated in water by 46.5 kcal/mol![11j] This stabilization of charged guests suggests a special attraction between these compounds and the host structure.

This special attraction is further evidenced by chemical shift changes in the ^{1}H NMR spectra of compounds **5** and **6** complexed with host P. Guest protons buried in the host cavity are extensively shielded by the aromatic rings of the host, and resonances that move upfield to a greater extent are considered to be in more direct contact with the host cavity. The calculated maximum upfield shifts of the guest protons of **5** and **6** determined from NMR binding studies (figure 1.6) show that the signals of the quaternary ammonium groups move to a greater extent than do signals from more hydrophobic portions of these molecules. The results clearly indicate a preferential interaction between the quaternary ammonium groups of these guests and the aromatic binding cavity.

We proposed that these observations were the results of cation-π interactions, in which the positively charged guest species were stabilized through direct



5 **6**

Figure 1.6. Calculated Maximum Upfield Shifts (in ppm) for Guest Protons upon Binding to Host P

contact with the electron-rich π clouds of the aromatic rings of the host.[11c] Experiments comparing the binding affinities of hosts P and C for charged compounds support this hypothesis. As seen in table 1.1, host C, with its aliphatic cyclohexyl linkers, displays a lower affinity than P for cationic guests **3**, **4**, and **5** , but a similar affinity for neutral compounds **8**, **9**, **10**, and **16**. The results imply that the aromatic rings rather than the carboxylates of P are critical for cationic binding. The special function of the aromatic rings is further supported by binding experiments of the tetramethylester of P in chloroform.[11d] In this solvent, guests **3**, **4**, and **5** complexed with appreciable affinities ($-\Delta G°$ of 3.5, 2.5, and 2.1 kcal/mole respectively) to the uncharged receptor. Quinoline and isoquinoline were not bound by the receptor in this medium, suggesting that charge is a critical component of the interaction.

The initial reports of these findings were the first to document the importance of cation-π interactions in synthetic systems.† Subsequently, we began to hypothesize that these interactions might also play a role in the binding of quaternary ammonium compounds in biological systems. Encouragingly, the

† However, such interactions had been reported at that time to occur in the gas phase and had been suggested to stabilize protein tertiary and quaternary structures (references 12-14). Subsequent reports by other investigators verified the operation of cation-π interactions in other cyclophane systems (reference 8d).

Table 1.1. Comparative Binding Affinites of Hosts P and C[a]

Guest	Host P	Host C
3	8.4	6.3
4	7.2	6.0
5	6.7	5.4
8	5.3	5.9
9	6.3	6.3
10	5.9	6.0
16	4.5	4.8

[a] $-\Delta G°$ (kcal/mol)

neurotransmitter acetylcholine **7** binds well to the hydrophobic cavity of P, with a dissociation constant of 28 μM - comparable to its affinity in many biological receptors. The results of our binding studies on charged guests in conjunction with a rigorous search of the literature, led in 1990 to a publication[11g] from our group providing ample evidence that the aromatic side chains of phenylalanine, tyrosine and tryptophan residues actively stabilize quaternary ammonium compounds through cation-π interactions. This hypothesis differed from popular opinion, which held that cation binding was largely the domain of the negatively charged carboxylate residues. In natural systems that were examined, aromatic residues were found consistently at the binding sites for quaternary ammonium compounds, even when carboxylates were not. Modeling studies of phosphocholine binding to the crystallographic structure of the immunoglobin Fab McPC603 as well as NMR solution studies indicated that tryptophan and tyrosine side chains of the antibody make close contacts with the positively charged N-methyl groups of the hapten.[29] Labeling studies of the α-subunit of the AChR isolated from *Torpedo* indicated a large number of aromatic residues at the agonist binding sites for this class of ligand-gated ion channels.[16] The cloning of numerous AChRs underscored the importance of these residues, as

they were found to be highly conserved in all of the receptors.[15] Similar studies indicated the presence of aromatic residues at the "anionic" binding site of acetylcholine esterase (AChE).[30] Shortly after the publication of our hypothesis, an X-ray crystallographic structure of the *Torpedo* esterase was reported by Sussman and coworkers.[31] The structure was spectacular. It showed an active site lying at the bottom of a deep gorge; both were lined with a large number of aromatic residues. Since that time, our proposal has been bolstered by modeling studies of G-protein coupled receptors (GPCRs), which suggest that aromatic residues partially comprise the binding sites for ammonium compounds in all sequenced receptors.[32] The identification of numerous aromatic residues in the pore regions of voltage-gated potassium channels,[33] a region blocked by tetraethylammonium, presents further evidence supportive of our hypothesis.

The studies of host P and related compounds and the subsequent proposal that nature uses aromatic π systems in the binding of cationic molecules exemplify the role that relatively simple chemical models can play in understanding the complex interactions of natural systems. With this in mind, the work presented in this thesis was undertaken. It is hoped by the author that the six new host structures presented here ultimately will be considered to have increased that understanding.

Chapter 2: Syntheses of Host Structures.

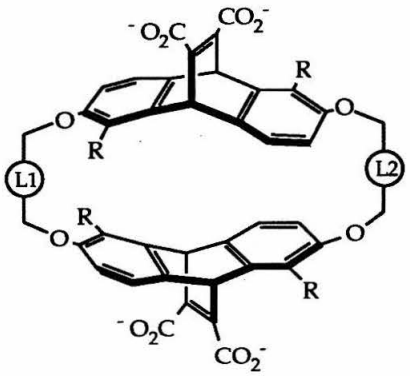
Introduction

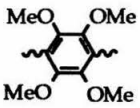
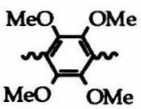
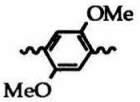
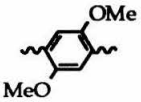
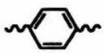
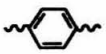
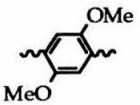
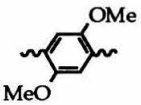
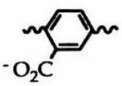
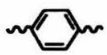
Eight different macrocycles (figure 2.1) were used during the investigations presented in this thesis. Each macrocycle consists of a number of ethenoanthracene units tethered through benzylic ether linkages. The similarity is of course intentional, as the macrocycles are all the end products of similar convergent synthetic schemes (figure 2.2). The appropriate 2,6-dihydroxyethenoanthracene compound **1** or **2** is synthesized. These compounds are then condensed with the appropriate bromomethylarenes **3-8** to generate the macrocycles as their methyl esters. Hydrolysis of the esters yields the macrocycles in their acid forms, ready for study in aqueous media. This chapter describes the details of these host assembly processes.

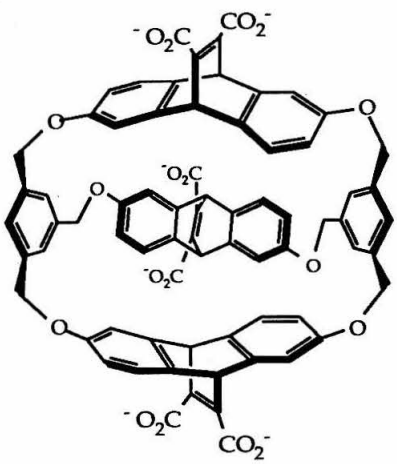
Discussion

Syntheses of the Ethenoanthracene Subunits. The first goal in the synthesis of a host structure is the proper assembly of the ethenoanthracene unit **1** or **2**. As mentioned in chapter 1, these building blocks are inherently chiral. In order to avoid generating diasteriomeric structures during the macrocyclization steps of host synthesis, only enantiomerically pure forms of compounds **1** and **2** were used.

The syntheses of the enantiomerically pure forms of compound **1** have been previously reported.[11c] These procedures are diagrammed in figure 2.3. Anthraflavic acid **9** is reduced with aluminum amalgam to produce 2,6-dihydroxyanthracene **10**.[11c,34] The hydroxyl groups of **10** are then protected using *tert*-butyldimethylsilyl chloride to produce **11**. The diethylaluminum chloride catalyzed Diels-Alder reaction of (+) dimenthyl fumarate **12** with **11**



L1	L2	R	Host
		H	**P**
		H	**M**
		H	**OMP**
		H	**TMP**
		Br	**TBP**
		Br	**TMTBP**
		H	**CP**



D 3

Figure 2.1. Host Structures Studied in This Thesis

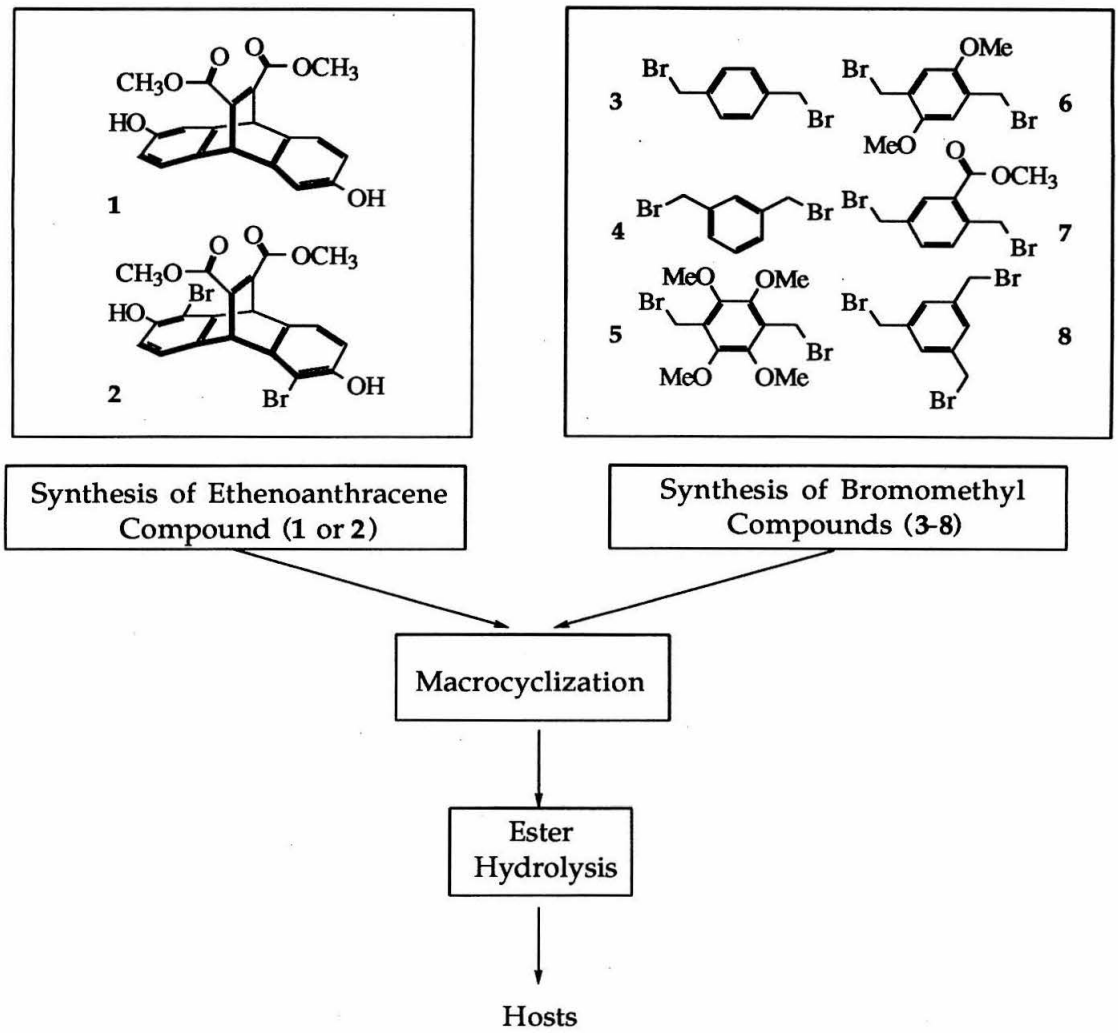


Figure 2.2. Precursors to Host Structures and General Synthetic Scheme

produces the ethanoanthracene compounds **13** and **14**.† This reaction is critical to the overall synthesis, as the stereocenters of the final ethenoanthracene products are set during this step. This reaction proceeds with complete facial selectivity of the dienophile[35] and yields only two (one *syn* **13** and one *anti* **14**) of four possible adducts. The two diastereomeric products are separated using a combination of chromatographic and recrystallization techniques. Reaction of the separated products with diphenyl diselenide and potassium *tert*-butoxide, followed by deprotection with hydrochloric acid, yields the ethenoanthracenes **15** and **16**. These compounds are easily transesterified with methanesulfonic acid to produce the enatiomerically pure forms of **1** (denoted as (-)-**1** and (+)-**1** in the figure).

The enatiomerically pure forms of the brominated ethenoanthracene material **2** [(-)-**2** and (+)-**2**] are produced in an analogous fashion[11j] from 1,5-dibromo-2,6-dihydroxyanthracene **17**,[36] as outlined in figure 2.4. A few aspects of the synthesis deserve comment. The Diels-Alder reaction of **18** with dimenthyl fumarate proceeds at a much slower rate than does the reaction with **11** and produces only a single adduct **19**. The subsequent oxidation-deprotection and transesterification reactions, which generate **20** and (-)-**2** respectively, also proceed at slower rates than their counterparts described above.

The assignments of the absolute stereochemistries of the ethenoanthracene compounds **1** and **2** are critical, and can be done through the analysis of both the products themselves and the Diels-Alder adducts from which they were produced. As reported elsewhere,[11c] the *syn/anti* assignment was made for **13** and **14** based on ^{1}H-^{1}H, NOE-correlated NMR (NOESY) studies of the

† The use of (-)-dimenthlyfumarate produces the enantiomers of compounds **13** and **14**.

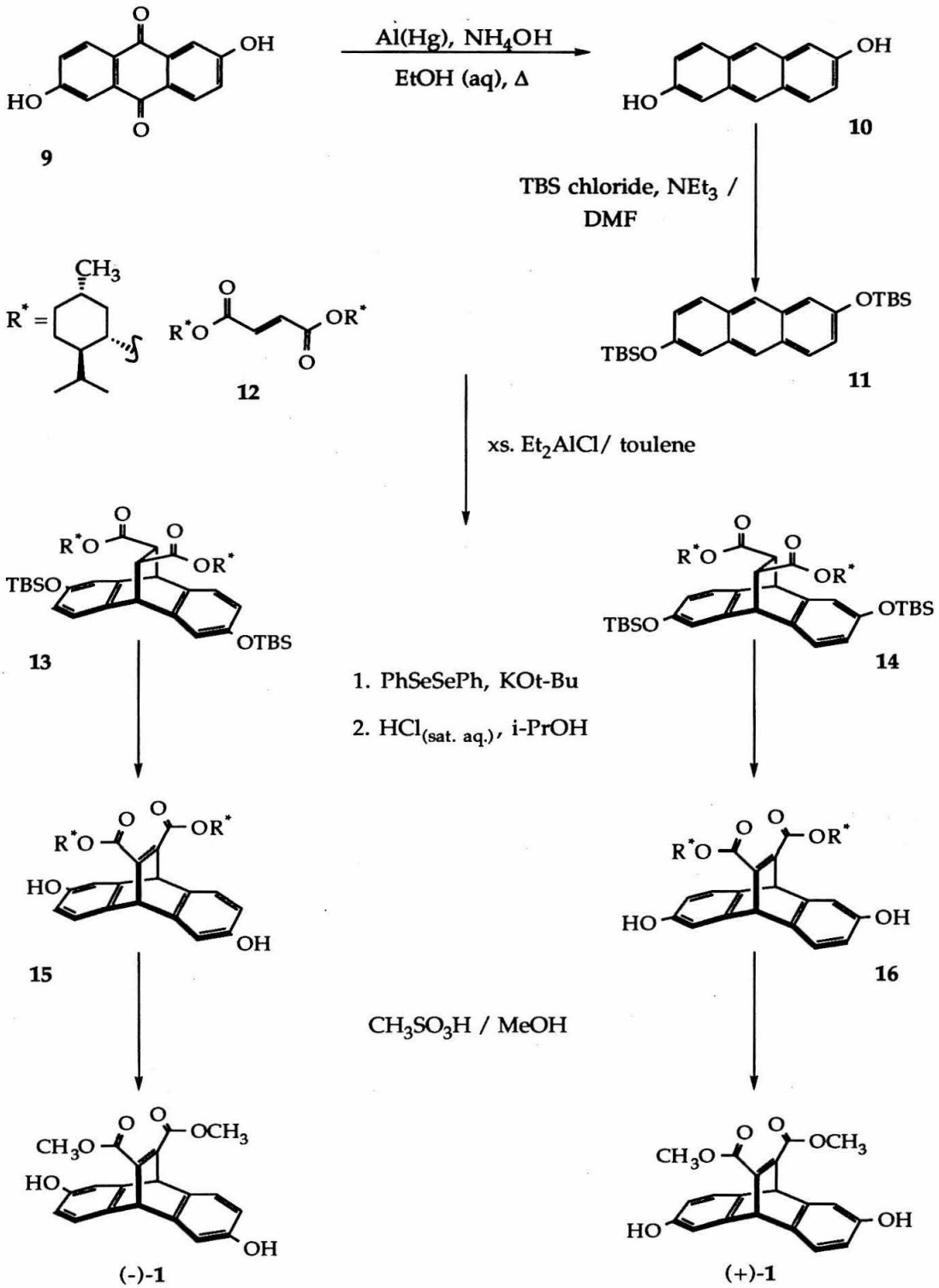


Figure 2.3. Synthesis of Ethenoanthracene **1**

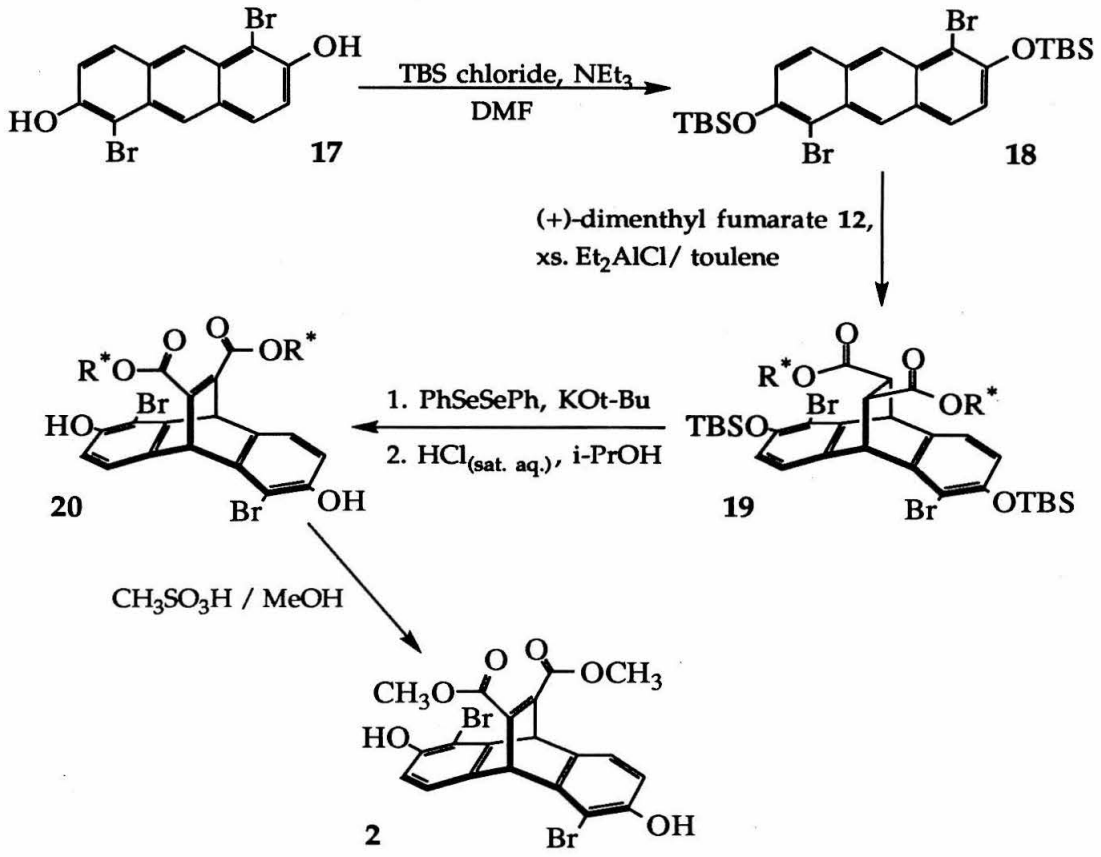


Figure 2.4. Synthesis of Dibromoethenoanthracene **2**. The (-)-**2** enantiomer is shown.

compounds as well as the qualitative matching of ^{1}H NMR chemical shift patterns of **13** and **14** with those of similar *syn/anti* pairs reported in the literature. The specific rotations of the two enantiomers of **1** provided further support for the **13/14** assignments, as studies of C_2-symmetric bridged anthracenes have shown a consistent relationship between the sign of $[\alpha_D]$ and absolute configuration.[37]

The assignment of the absolute configuration of **2** was made in a similar fashion. The ^{1}H NMR spectrum of **19** bears a close resemblance to that of compound **13**, suggesting a *syn* product. Furthermore, the specific rotation of **2**,

when produced from (+)-dimenthyl fumarate, is consistent in sign and magnitude with that of compound **1** with an (*S,S*) configuration. This also suggests an assignment of **19** as a *syn* product. The stereochemical assignment of **2** was also made using the excitonic chirality method of Nakanishi.[38] A CD spectrum of the bis(dimethylaminobenzoate) derivative of bisphenol **2**, prepared from (-)-dimenthyl fumarate, was taken. The compound displayed a strong positive Cotton effect at ca. 315 nm, followed by a negative Cotton effect at shorter wavelengths that was somewhat weaker due to overlap with signals of the ethenoanthracene. This is in agreement with the positive chirality expected for **2** with an (*S,S*) configuration. Again the evidence is consistent with the assignment of **19** as a *syn* product.

Syntheses of Linker Units. Contrary to the complexity of the ethenoanthracene syntheses, those of the linking groups have always been relatively simple. (Compounds **3** and **4** are available from commercial sources.) This not only reflects the ease of generating such compounds, but also a wariness on our part of building too extensively on the most conformationally flexible region of the host structure.

The syntheses of the remaining linkers **5-8** are outlined in figure 2.5. The synthesis of compound **5** (figure 2.5a) was accomplished using procedures reported by Syper et al.[39] Benzoquinone **21** is hydroxymethylated to produce 2,5-dimethoxybenzoquinone **22**. This compound is then reduced to produce the hydroquinone **23**, which is subsequently methylated to produce 1,2,4,5-tetramethoxybenzene **24**. Reaction of this compound with bromomethyl methyl ether in trifluoroacetic acid is used to produce **5** in good yield. This last step can also be used to generate compound **6** from 1,4-dimethoxybenzene **25** (figure 2.5b). Compound **7** was produced from the conversion of 2,5-dimethylbenzoic

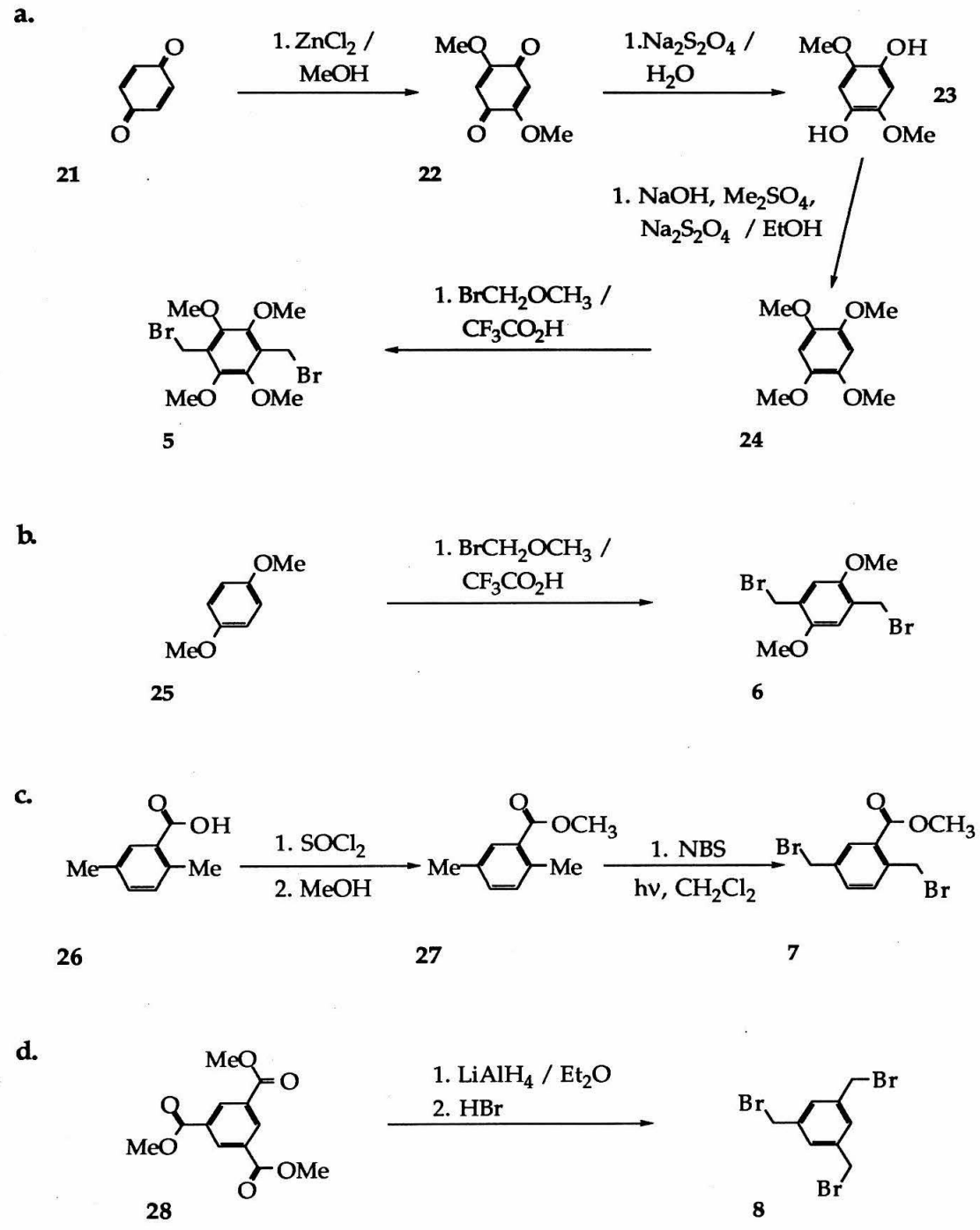


Figure 2.5. Syntheses of Linking Groups **5-8**

acid **26** to its methyl ester **27**, and the subsequent reaction of **27** with N-bromosuccinimide (figure 2.5c). Finally, trimethyl-1,3,5-benzene tricarboxylate **28** is converted in a one-pot synthesis to compound **8**, according to methods reported by Bilger et al. (figure 2.5d).[40]

Generation of the Host Structures. All host structures were constructed through condensations of the ethenoanthracene units with the appropriate di- or tribromides in anhydrous DMF using cesium carbonate as a base. The condensations were carried out in one of two fashions. Macrocycles P, M, OMP, TMP, TMTBP, and D3 (methyl ester forms) were generated in a single step. In this step, syringe pump techniques were used to deliver a solution of the ethenoanthracene unit and the linking agent (in the appropriate ratio) to a suspension of Cs_2CO_3 in DMF. The CP macrocycle was produced in a sequential fashion, as outlined in figure 2.6. In this procedure, *p*-xylene dibromide **3** is first condensed in the presence of an excess of **1** to produce compound **29**. This compound is then further reacted with **7** via syringe pump techniques to create the pentamethyl ester of CP.

The methyl esters of all macrocycles were cleaved through the use of cesium hydroxide in aqueous solutions of DMSO (5-10% v/v). The purified host products were then dissolved in deuterated aqueous borate buffer (pD ~ 9), in preparation for binding studies.

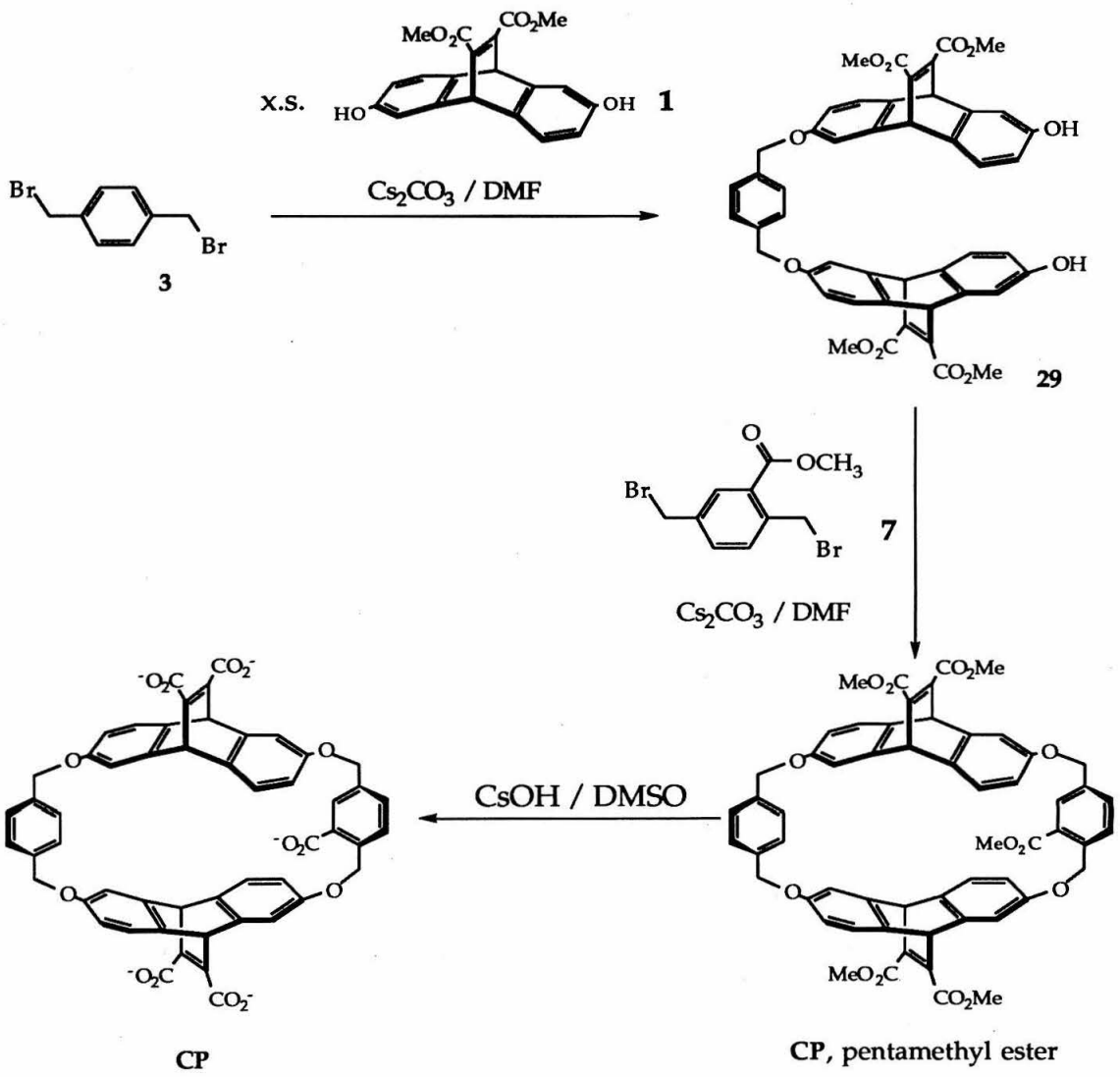


Figure 2.6. Stepwise Synthesis of the CP Macrocycle

Experimental Section

General. NMR spectra were recorded on JEOL JNM GX-400, Bruker AM-500 or General Electric QE-300 spectrometers. Routine spectra were referenced to the residual proton signals of the solvents and are reported in ppm downfield of 0.0 as δ values. All coupling constants, *J*, are in Hz. All aqueous host solutions for study were prepared in borate-d buffer. Spectra of aqueous solutions were referenced to an internal standard of 3,3-dimethylglutarate (DMG, δ 1.09). Optical rotations were recorded on a Jasco DIP-181 digital polarimeter at 298 K. All circular dichroism (CD) experiments were carried out using a JASCO J-600 spectropolarimeter with either 1.0 or 0.5 cm pathlength quartz cells. Preparative centrifugal chromatography was performed on a Harrison Research Chromatotron model 7924T using silica plates. Melting points were determined on a Thomas Hoover melting point apparatus, and are corrected. HPLC was performed on a Waters dual 510 pump liquid chromatograph system equipped with a Waters 490E wavelength detector. Mass spectral analyses were performed at the University of California, Riverside.

Compounds **3, 4, 9, 21, 26,** and **28** were procured from commercial sources. Compounds **1, 5, 8, 10, 11, 12, 13, 14, 15, 16, 17, 22, 23,** and **24** were prepared as described in the literature.[41]

1,4-Bis(bromomethyl)-2,5-dimethoxybenzene (6). 1,4-Dimethoxybenzene (2.76 g, 20 mmol, 1 equiv.) was placed in a flask and dissolved in glacial acetic acid (13 mL). Bromomethyl methyl ether (3.6 mL, 44 mmol, 2.2 equiv.) was added and the solution allowed to sit under nitrogen at room temperature for twelve hours. An off-white precipitate was filtered off and washed with CCl_4. The material was then recrystallized from chloroform to yield **6** (2.54 g, 7.8 mmol,

39%); ^{1}H NMR ($CDCl_3$) δ 6.85 (s, 2H), 4.51 (s, 4H), 3.85 (s, 6H); mp 203-204 °C, literature value 206 °C.

Methyl 2,5-dimethylbenzoate (27). 2,5-Dimethylbenzoic acid **26** (3.95 g, 26.3 mmol, 1 equiv.) was combined with thionyl chloride (4.0 mL, 55 mmol, 2.1 equiv.) in a 50 mL round-bottomed flask fitted with a reflux condenser. A calcium chloride drying tube was attached to the condenser, and the thionyl chloride brought to reflux. After six hours the resulting clear solution was cooled, and the reflux condenser replaced with a distillation apparatus. The excess thionyl chloride was removed under reduced pressure. The residue was then diluted with 20 mL of methanol and allowed to stir at room temperature overnight. The methanol was then removed via rotary evaporation. The resulting oil was dissolved in methylene chloride (50 mL) and extracted twice with a saturated solution of sodium bicarbonate (2 X 100 mL), followed by two further extractions with distilled water (2 X 100 mL). The organic layer was then dried over magnesium sulfate, filtered and concentrated to yield **27** (4.05 g, 24.6 mmol, 93%): ^{1}H NMR ($CDCl_3$) δ 7.71 (s, 1H), 7.16 (d, J = 3, 1H), 7.10 (d, J = 3, 1H),3.86 (s, 3H), 2.53 (s, 3H), 2.32 (s, 3H).

Methyl 2,5-bis(bromomethyl)benzoate (7). Methyl 2,5-dimethylbenzoate **27** (2.46 g, 15.0 mmol, 1 equiv.) was dissolved in 50 mL of methylene chloride in a 100 mL round-bottomed flask. N-Bromosuccinimide (5.61 g, 3.15 mmol, 2.1 equiv.) was added to the flask. The reaction vessel was fitted with a reflux condenser, and its contents heated to refluxing. The flask was irradiated for seven minutes using a General-Electric model RSKB sunlamp with a 275W, 110-125V, A.C. bulb. The solution was allowed to reflux overnight. It was then cooled and filtered, to remove precipitated succinimide, and concentrated. The residue was chromatographed over silica gel, using CH_2Cl_2 as an eluent to

separate the product from residual succinimide. The resulting material was twice recrystallized from cyclohexane to yield the desired dibromide in purified form (980 mg, 3.04 mmol, 20% yield): ^{1}H NMR ($CDCl_3$) δ 7.97 (d, J = 2, 1H), 7.50 (dd, J = 6, 2, 1H), 7.43 (d, J = 6, 1H), 4.92 (s, 2H), 4.46 (s, 2H), 3.93 (s, 3H).

1,5-Dibromo-2,6-bis(*tert*-butyldimethylsiloxy)anthracene (18). 2,6-dihydroxyanthracene (4.20 g, 20.0 mmol, 1 equiv.) was added to a 500 mL flask fitted with an addition funnel, an argon adapter, and a septum. The addition funnel was also sealed with a septum. The flask was purged with argon and the anthracene was dissolved in 80 mL dioxane. Bromine (6.08 g, 38.0 mmol, 1.9 equiv.) was dissolved in 80 mL dioxane and the resulting solution transferred to the addition funnel. The bromine solution was added to the anthracene solution over 10 minutes, and the resulting solution was stirred for two hours. The reaction mixture was then filtered, and the precipitate was washed with dioxane. The solid was dissolved in 80 mL DMF. *Tert*-butyldimethylsilyl chloride (9.045 g, 60.0 mmol, 3 equiv.) and triethylamine (8.40 mL, 60.3 mmol, 3 equiv.) were added. Golden crystals formed immediately. These were filtered off, washed with methanol, and recrystallized from isooctane to yield the title compound (4.710 g, 7.9 mmol, 40% yield); ^{1}H NMR ($CDCl_3$) δ 8.65 (s, 2H), 7.89 (d, J = 9, 2H), 7.14 (d, J = 9, 2H), 1.08 (s, 18H), 0.30 (s, 12H).

(9*S*, 10*S*, 11*R*, 12*R*)-1,5-Dibromo-2,6-bis(*tert*-butyldimethylsiloxy)-9,10-dihydro-11,12-dicarboxyethenoanthracene Bis[(+)-menthyl ester] (19). An oven-dried round bottomed flask was fitted with a stopper, a septum, and an argon inlet. Di-(+)-menthyl fumarate (2.75 g, 7.0 mmol, 1 equiv., 7.0 mL of a 1 M solution in toluene) was added to the flask and cooled to 0 °C. Diethylaluminum chloride (5.063 g, 42.0 mmol, 6 equiv, 23.3 mL of a 1.8 M solution in toluene) was slowly added to the fumarate solution, which turned orange. The ice bath was

removed and the solution was allowed to warm to room temperature. Protected anthracene compound **18** (4.17 g, 7.0 mmol, 1 equiv.) dissolved in 40 mL of toluene was then added to the flask. After 8 hours the solution was pale yellow in color, and thin layer chromatography of the solution (silica gel plates, 3% ether in hexane v/v) indicated complete consumption of the dimenthyl fumarate (R_f 0.35), but incomplete consumption of anthracene. Another equivalent of dimenthyl fumarate was then added. Another 0.5 equivalents were added after sixteen hours. After 24 hours the reaction mixture was poured over a chilled biphasic solution of 50 mL of toluene and 150 mL of saturated sodium potassium tartrate. (*Caution! Gas evolution!*) The biphasic solution was then filtered through a Celite pad. The organic layer and a further toluene extract of the aqueous phase were combined, dried with magnesium sulfate, filtered, and concentrated.

A single Diels-Alder adduct (R_f 0.25) was visible by thin layer chromatography (silica gel plates, 3% ether in hexane). Flash column chromatography (silica gel, 2-5% ether in hexane) was used to isolate the product **19** (5.73 g, 5.79 mmol, 83% yield based on the anthracene starting material); ^{1}H NMR ($CDCl_3$) δ 7.12 (d, J = 9, 2H), 6.58 (d, J = 9, 2H), 5.08 (s, 2H), 4.56 (td, 2H), 3.17 (s, 2H), 1.80-0.50 (m, 36H), 0.98 (s, 18H), 0.20 (s, 6H), 0.15 (s, 6H). **(9*R*, 10*R*, 11*S*, 12*S*)-1,5-Dibromo-2,6-bis(*tert*-butyldimethylsiloxy)-9,10-dihydro-11,12-dicarboxy-ethenoanthracene Bis[(-)-menthyl ester]** can be prepared in an analogous fashion using di-(-)-menthyl fumarate.

(9*R*,10*R*)-1,5-Dibromo-2,6-dihydroxy-11,12-dicarboxyethenoanthracene Bis[(+)-menthyl ester] (20). Compound **19** (9*S*, 10*S*, 11*R*, 12*R*) (2.196 g, 2.20 mmol, 1.0 equiv.) and diphenyl diselenide (1.107 g, 3.55 mmol, 1.6 equiv.) were dissolved in 50 mL of dry toluene. Potassium *tert*-butoxide (698 mg, 6.22 mmol, 2.8 equiv., 6.2 mL of a 1.0 M solution in THF) was added, and the reaction

allowed to stir for 45 minutes at room temperature. Isopropyl alcohol (260 mL) and HCl (37% aqueous, 16 mL) were then added and the solution heated to 50 °C. After two days the solution was cooled to room temperature and poured onto a biphasic solution of ethyl acetate (500 mL), $NaHCO_3$ (600 mL, saturated aqueous) and 1 M potassium phosphate buffer (200 mL, pH = 7). After gas evolution had ceased, the organic layer was separated, dried with $MgSO_4$, filtered, and concentrated. The residue was then chromatographed over flash-grade silica (35-50% ethyl acetate in isooctane) to yield the desired product (1.366 g, 1.80 mmol, 81% yield); ^1H NMR (CD_3CN) δ 7.21 (d, *J* = 8, 2H), 6.64 (d, *J* = 8, 2H), 5.83 (s, 2H), 4.79 (td, 2H), 2.15 - 1.70 (m, 38H). **(9*S*, 10*S*)-1,5-Dibromo-2,6-dihydroxy-11,12-dicarboxyethenoanthracene Bis[(-)-menthyl ester]** can be prepared from the (9*R*, 10*R*, 11*S*, 12*S*) enantiomer of compound **19**.

(9*R*, 10*R*)-1,5-Dibromo-2,6-dihydroxy-11,12-dicarbomethoxyethenoanthracene (2). The (9*S*, 10*S*, 11*R*, 12*R*) enantiomer of ethenoanthacene **20** (858 mg, 1.13 mmol) was dissolved in methanol (30 mL). Methanesulfonic acid (1.8 mL) was added and the solution brought to reflux. After 5 days the reaction appeared complete by thin layer chromatography (silica gel plates, pet. ether: ether 1:1). The reaction mixture was then cooled to room temperature and mixed with ethyl acetate (80 mL) and 1 M potassium phosphate buffer (80 mL pH = 7). The organic layer and another extraction of the aqueous layer were combined, dried with $MgSO_4$, filtered, and concentrated. The residue was then chromatographed over flash-grade silica (50 - 0% petroleum ether in ether) to yield the purified product **2** (466 mg, 0.91 mmol, 80%); ^1H NMR (CD_3CN) δ 7.30 (bs, 2H), 7.25 (d, *J* = 8, 2H), 6.62 (d, *J* = 8, 2H), 5.91 (s, 2H), 3.77 (s, 6H); [a_D] -103° (*c* 0.13, CH_3CN); EI-MS, *m/e* 508 (M^+), 510 (M^++2), 449 (M-CO_2Me), 451 (M+2-CO_2Me); HRMS 507.91400, calc. for $C_{20}H_{14}{}^{79}Br_2O_6$: 507.91571. **(9*S*, 10*S*)-1,5-Dibromo-2,6-**

dihydroxy-11,12-dicarbomethoxyethenoanthracene] can be made from the (9*S*, 10*S*, 11*R*, 12*R*) enantiomer of ethanoanthacene **20**.

Compound 29. Into a 100 mL three-necked round-bottomed flask fitted with a stopper, a septum, and an Ar gas adapter were placed 1.46 g of cesium carbonate (4.50 mmol, 15 equiv.) and 528 mg of (9R,10R)-2,6-dihydroxy-11,12-dicarbomethoxyethenoanthracene **1** (1.50 mmol, 5 equiv.). The flask was then purged with argon, and 40 mL of anhydrous dimethyformamide was injected into the vessel. A solution of *p*-xylene dibromide **3** (79 mg, 0.30 mmol, 1.0 equiv.) in 10 mL anhydrous DMF was then delivered via syringe pump into the ethenoanthracene solution over four hours. The reaction mixture was then allowed to stir at room temperature for two days. (Note: Both the reaction vessel and the gas-tight syringe used in the reaction were covered with foil to shield the reactants and products from light.) The solution was then acidified using a few drops of concentrated aqueous HCl until the yellow color of the solution dissipated. The solution was then filtered to remove residual solids and the solvent stripped off in vacuo. Preparative centrifugal thin layer chromatography was employed for further purification. Using 2 mm thick silica gel plates and a 3:1 (v/v) eluent solution of ether: petroleum ether, the excess ethenoanthracene (250 mg, 0.71 mmol) was first recovered. Altering the eluent solution to a 1:1 mixture of diethylether: chloroform yielded the desired compound **29** (147 mg, 0.18 mmol, 61%): ^{1}H NMR (acetone-d_6) δ 8.26 (s, 2H), 7.41 (s, 4H), 7.28 (d, J = 8, 2H), 7.20 (d, J = 8, 2H), 7.13 (d, J = 2, 2H), 6.96 (d, J = 2, 2H), 6.60 (dd, J = 8, 2, 2H), 6.44 (dd, J = 8, 2, 2H), 5.43 (s, 2H), 5.41 (s, 2H), 5.04 (s, 4H), 3.71 (s, 12H).

Macrocyclizations. As described previously,[11c] the tetramethyl ester of hosts P and M were prepared by a condensation of **1** with *p*- or *m*-bis(bromomethyl)benzene **3** or **4** in a suspension of cesium carbonate in

anhydrous DMF. The tetramethyl esters of the new host compounds (OMP, TMP, TBP, and TMTBP) were prepared similarly, using the ethenoanthracenes **1** and **2** and the appropriate bis(halomethyl) compounds **3**, **5**, and **6**. In all macrocyclizations, enantiomerically pure ethenoanthracenes were coupled, although both *R,R* and *S,S* forms were used. Workup of the macrocyclic products differed slightly from that previously reported for host P, however. After the macrocyclizations were complete, the reactions were filtered and the DMF evaporated. The residues were then chromatographed over flash-grade silica, using either 5% ether or 5% ethyl acetate in methylene chloride, in order to separate the macrocyclic compounds from baseline impurities. The macrocycles were then isolated from higher order macrocycles using preparative centrifugal thin layer chromatography (silica plates, 0-5% ether in CH_2Cl_2 gradient, or, 5% ethyl acetate in CH_2Cl_2).

OMP, Tetramethyl Ester. Yield 6%; ^{1}H NMR ($CDCl_3$) δ 7.23 (d, J = 2, 4H), 6.93 (d, J = 8, 4H), 6.32 (dd, J = 8, 2, 4H), 5.18 (s, 4H), 5.02 (s, 8H), 3.75 (s, 12H), 3.64 (s, 24H); FAB-MS *m/e* 1149 (M^+).

TMP, Tetramethyl Ester. Yield 10%; ^{1}H NMR ($CDCl_3$) δ 7.05 (d, J = 8, 4H), 6.94 (d, J = 2, 4H), 6.69 (s, 4H), 6.42 (dd, J = 8, 2, 4H), 5.21 (s, 4H), 4.99 (AB, J = 13, Δν = 93 Hz, 8H), 3.75 (s, 12H), 3.48 (s, 12H); FAB-MS *m/e* 1028 (M^+); HRMS 1028.3312, calc. for $C_{60}H_{52}O_{16}$: 1028.3255.

TBP, Tetramethyl Ester. Yield 10%; ^{1}H NMR ($CDCl_3$) δ 7.23 (s, 8H), 7.08 (d, J = 8, 4H), 6.41 (d, J = 8, 4H), 5.80 (s, 4H), 5.07 (AB, J = 13, Δν = 116 Hz, 8H), 3.78 (s, 12H); FAB-MS *m/e* MH^+ cluster 1220-1230 (1225 100 integral % within cluster); HRMS 1220.9320, calc. for $C_{56}H_{41}O_{12}{}^{79}Br_4$: 1220.9331.

TMTBP, Tetramethyl Ester. Yield 31%; ^{1}H NMR ($CDCl_3$) δ 7.07 (d, J = 8, 4H), 7.05 (s, 4H), 6.56 (d, J = 8, 4H), 5.80 (s, 4H), 5.06 (AB, J = 13, Δν = 88 Hz, 8H), 3.79 (s, 12H), 3.67 (s, 12H); FAB-MS, *m/e* 1344 (M^+); HRMS 1343.9673, calc. for $C_{60}H_{48}{}^{79}Br_2{}^{81}Br_2O_{16}$: 1343.9635.

CP, Pentamethyl Ester. Cesium carbonate (276 mg, 0.84 mmol, 4 equiv.) was placed in a 250 mL three-necked round-bottomed flask fitted with a stopper, a septum and an Ar gas adapter. The system was then purged with argon, and charged with 50 mL of anhydrous DMF. In a separate flask, compound **29** (171 mg, 0.21 mmol, 1 equiv.) and methyl 2,5-bis(bromomethyl)benzoate (69 mg, 0.21 mmol, 1 equiv.) were dissolved into 20 mL of anhydrous DMF. The contents of this flask were then drawn into a 25 mL gas-tight syringe. The solution was then injected into the cesium carbonate suspension over 48 hours via a syringe pump. After the injection was complete, the solution was stirred for an additional 24 hours. The syringe and the main reaction vessel were shielded from light throughout the course of the reaction. The DMF solution was then filtered to remove cesium salts. The collected solids were washed twice with approximately 10 mL of DMF. The filtrate and washings were then combined and the DMF removed in vacuo. The residue was then mixed with $CHCl_3$, filtered and concentrated in vacuo. The crude material was then purified via flash chromatography over silica gel with an eluent mixture of 5% Et_2O in CH_2Cl_2 (v/v). Further purification using preparative centrifugal thin layer chromatography (1 mm thick silica gel plate using an eluent gradient of CH_2Cl_2 to 5% Et_2O in CH_2Cl_2 (v/v)) afforded the pentamethyl ester of the CP host (53 mg, 0.05 mmol, 26% yield): ^{1}H NMR ($CDCl_3$) δ 7.95 (d, J = 2, 1H), 7.37 (d, J = 8, 1H), 7.37 (dd, J = 8,2, 1H), 7.20 (s, 4H), 7.09 (d, J = 8, 1H), 7.07 (d, J = 8, 2H), 7.05 (d, J = 8, 1H), 6.90 (d, J = 2, 1H), 6.89 (d, J = 2, 1H), 6.88 (d, J = 2, 1H), 6.84 (d, J = 2,

1H), 6.40 (dd, J = 8, 2, 1H), 6.39 (dd, J = 8, 2, 1H), 6.36 (dd, J = 8, 2, 1H), 6.35 (dd, J = 8, 2, 1H), 5.47 (s, 2H), 5.22 (s, 1H), 5.21 (s, 2H), 5.20 (s, 1H), 5.12-4.99 (m, 6H), 3.91 (s, 3H), 3.75 (s, 12H); FAB-MS m/e 967 (MH^+); HRMS 967.2986, calculated for $C_{58}H_{47}O_{14}$ (MH^+) 967.2965.

D3, Hexamethyl Ester. The compound was prepared using the standard macrocyclization procedure previously described, except that the ethenoanthracene compound **1** was reacted with mesitylene tribromide **8** in a 3:2 mole ratio. Yield 7%; ^1H NMR ($CDCl_3$) δ 6.99 (s, 6H), 6.75 (d, J = 8, 6H), 6.65 (d, J = 2, 6H), 6.04 (dd, J = 8, 2, 6H), 5.22 (s, 6H), 4.93 (AB, J = 15, Δν = 36 Hz, 12H), 3.87 (s, 18H); FAB-MS m /e 1285 (MH^+); HRMS 1285.3870, calculated for $C_{78}H_{61}O_{18}$ (MH^+) 1285.3857.

Ester Hydrolysis: All tetraacid macrocycles were prepared from the corresponding tetramethyl esters, using the following hydrolytic procedure. The tetraesters were dissolved in 1-2 mL of DMSO. Ten equivalents of cesium hydroxide in a 1M aqueous solution (2.5 equiv. for each methyl ester) were added and the solution allowed to stir overnight at room temperature. Water (1-2 mL) was then added and the solution allowed to stir for 24 hours. The solution was then frozen solid and lyophilized. The resulting residue was dissolved in a minimum amount of water and passed over a cation exchange column (neutral pH, Dowex, 50X4, NH_4^+ form) using doubly distilled water as the eluent. Fractions containing host were determined by UV activity on TLC silica gel plates. The approriate fractions were then combined and lyophilized to give the acid compounds. Aqueous solutions of these host compounds were prepared by adding an appropriate amount of aqueous CsOD (1.0 M in D_2O) to the residue and diluting the resulting solution with deuterated borate buffer to the desired volume. (60-80% yields)

OMP, Tetraacid. ^{1}H NMR (borate, referenced to internal DMG δ 1.09) δ 7.28 (d, *J* = 8, 4H), 6.82 (dd, *J* = 8, 2, 4H), 6.76 (d, *J* = 2, 4H), 5.12 (s, 4H), 5.03 (AB, *J* = 10, Δν = 81, 8H), 3.49 (s, 24H).

TMP, Tetraacid. ^{1}H NMR (borate, referenced to internal DMG δ 1.09) δ 7.21 (d, *J* = 9, 4H), 6.93 (d, *J* = 2, 4H), 6.56 (s, 4H), 6.54 (dd, *J* = 9, 2, 4H), 5.17 (s, 4H), 4.96 (AB, *J* = 10, Δν = 45 Hz, 8H), 3.17 (s, 12H).

TBP, Tetraacid. ^{1}H NMR (10% CD_3CN/90% borate, referenced to internal DMG δ 1.09) δ 7.45 (s, 8H), 7.28 (d, *J* = 8, 4H), 6.76 (d, *J* = 8, 4H), 5.74 (s, 4H), 5.16 (AB, *J* = 13, Δν = 102 Hz, 8H).

TMTBP, Tetraacid. ^{1}H NMR (10% CD_3CN/90% borate, referenced to internal DMG δ 1.09) δ 7.28 (d, *J* = 8, 4H), 7.24 (s, 4H), 6.76 (d, *J* = 8, 4H), 5.75 (s, 4H), 5.14 (AB, *J* = 13, Δν = 95 Hz, 8H), 3.76 (s, 12H).

CP, Pentaacid. The pentaacid of the CP macrocycle was prepared from its pentamethyl ester, using the general ester hydrolysis conditions listed above with the following modifications. After stirring overnight in the basic DMSO/water mixture, the solution was frozen and lyophilized. The residue was dissolved in 25 mM aqueous ammonium acetate solution (1-2 mL) with acetic acid being added as necessary to bring the solution to pH ≈ 7. The pentaacid was then isolated by preparative HPLC. Using a Whatman Magnum 9 column (50 cm, Partisil 10, ODS-3), we found the following HPLC conditions suitable: flow rate of 4.5 mL/min.; observation at 260 nm; eluent gradient of 25 mM aqueous ammonium acetate (t = 0 through t = 10 min.), followed by a gradient eluent of 0 to 30% acetonitrile in 25 mM ammonium acetate over 50 min. The desired product eluted after approximately 42 minutes. The appropriate collected fractions were combined, frozen, and lyophilized. The material was then purified

by ion-exchange chromatography as described above: ^{1}H NMR (10% CD_3CN / 90% borate, referenced to internal DMG δ 1.09) δ 7.53 (s, 1H), 7.37 (d, *J* = 8, 2H), 7.36 (s, 4H), 7.28 (d, *J* = 8, 1H), 7.23 (d, *J* = 8, 1H), 7.21 (d, *J* = 8, 1H), 7.19 (d, *J* = 8, 1H), 7.17 (d, *J* = 8, 1H), 7.03 (bs, 4H), 6.62 (dd, *J* = 8, 2, 1H), 6.60 (dd, *J* = 8, 2, 1H), 6.59 (dd, *J* = 8, 2, 1H), 6.54 (dd, *J* = 8, 2, 1H), 5.32 (s, 2H), 5.19 (s, 3H), 5.18 (s, 1H), 5.13 (d, *J* = 4, 2H), 5.10 (bs, 4H).

D3, Hexaacid. The hexaacid of the D3 bicycle was prepared directly from the hexamethyl ester compound, using the procedure described above for the CP pentaacid. However, a flow rate of 6.75 mL/min. and a gradient eluent of 0 through 50% acetonitrile in 25 mM aqueous ammonium acetate over 50 minutes, starting at t =0, was used to isolate the compound by HPLC. The compound eluted after approximately 40 minutes. ^{1}H NMR (10% CD3CN / 90% borate, referenced to internal DMG δ 1.09) δ 7.10 (s, 6H), 6.95 (d, *J* = 8, 6H), 6.91 (d, *J* = 2, 6H), 6.14 (dd, *J* = 8, 2, 6H), 5.24 (s, 6H), 4.98 (AB, *J* = 15, Δν = 25 Hz, 12H).

Chapter 3: Methods for Studying Host-Guest Complexations.

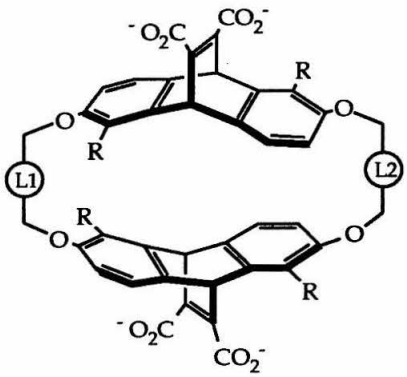
Introduction

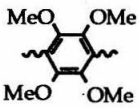
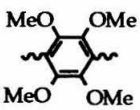
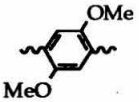
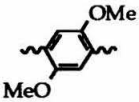
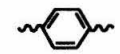
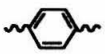
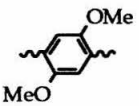
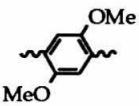
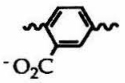
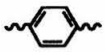
The primary goal of the cyclophane work presented in this thesis is to determine how selective functionalization of the P and M host structures (figure 3.1) affects guest binding. In the first two chapters of this thesis, six new host structures (figure 3.1), the rationale behind their creation, and the procedures used to synthesize them were introduced. In this chapter, the guest molecules (figure 3.2), and methods and techniques used to study the host structures are presented. This information will come in handy in chapters 4, 5, and 6, as we describe the results of our investigations of the new host structures.

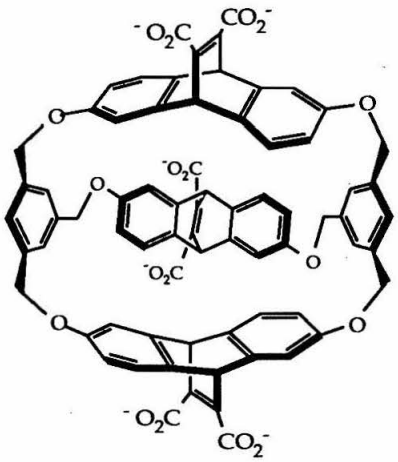
The ^{1}H NMR and circular dichroism (CD) methods for obtaining the free energies of guest binding ($-\Delta G^\circ$) are outlined. This is followed by a description of the aggregation properties of each host. The aggregation behavior of a host largely dictates how each of these two methods for obtaining binding energies can be employed. The role of solvation upon guest binding is discussed in terms of the binding affinities of host P in three different solvents (0%, 10%, and 15% acetonitrile by volume in aqueous borate). The guest molecules used in the studies of the new receptors are described along with some general information about their binding properties with host P. The chapter concludes with a description of how changes in the NMR chemical shifts of both guests and hosts can be used to extract structural information about the host-guest complexes.

Discussion

Methods for the Determination of Binding Affinities. As mentioned in chapter 1, Koga's early work[20] established ^{1}H NMR as the preeminent method for the determination of association constants in the study of cyclophanes. Our



L1	L2	R	Host
		H	**P**
		H	**M**
		H	**OMP**
		H	**TMP**
		Br	**TBP**
		Br	**TMTBP**
		H	**CP**



D 3

Figure 3.1. Definition of Host Structures

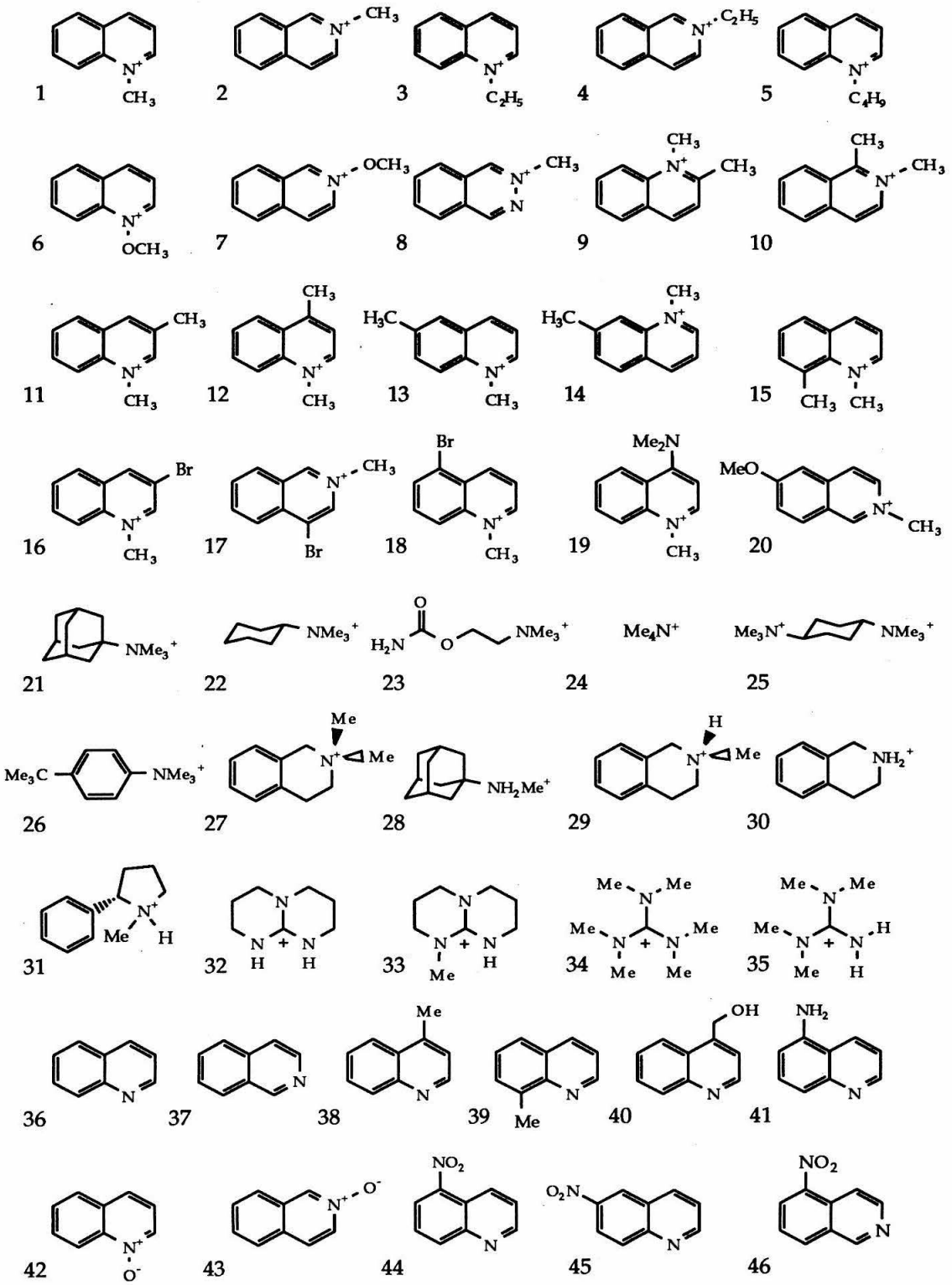


Figure 3.2. Guest Structures

work has been no exception, and most of the binding affinities determined in this thesis are the products of NMR titration experiments. All spectra of solutions containing both host and guest compounds displayed only time averaged signals of complexed and uncomplexed species. Under these fast exchange conditions, the association constants are determined by the best fits between the observed and calculated positions of the guest and host chemical shifts at various concentrations of these molecular species. There are several unknowns that must be solved in these fitting procedures: the association constant (K_a) and the differences between the chemical shifts of the free and bound host and guest protons (denoted as D values here). Given that the model is nonlinear with respect to the association constant, iterative means for optimizing solutions for the unknown parameters must be employed.

This was accomplished in a least-squares sense here, using the sophisticated EMUL/MULTIFIT package developed by Rich Barrans Jr. in the group.[42] In these programs all chemical shift data (and hence the D values) are fit to a single association constant, and the methods provide superior data fits to other methods under most circumstances. Most of the data has also been subjected to the LUCIUS/PORTIA statistical analysis package[42] in order to obtain an estimate of the error bars for the ΔG° values reported. In general error bars were on the order of $\pm$ 0.2 kcal/mol. Naturally, the programs will only be effective if the percentage of host and guest bound covers a broad range over the course of a titration experiment. Host and guest concentrations were varied accordingly in all experiments, in order to satisfy this requirement.

During the course of the work in this thesis, another method for the determination of guest binding affinities using circular dichroic techniques was

developed by group members Jonathan Forman and Rich Barrans Jr.[28] As mentioned in chapters 1 and 2, each of the host species studied here is chiral and has a characteristic CD spectrum. Upon guest complexation, marked changes in the CD spectrum of a host may occur. The ellipticites ($\theta_{obs\ \lambda}$) at a large number of wavelengths are recorded for solutions of various host and guest concentrations. Using a fitting procedure based on the MULTIFIT program, the differences between the observed and calculated changes in the ellipticity ($\theta_{obs\ \lambda}$) are used iteratively to determine the association constant (K_a) and the differences in the values of molar ellipticity ($\Delta\varepsilon_\lambda$) between the host and the host-guest complex. As with NMR titration methods, the procedure is useful only if a broad range of host and guest percent bound values is covered during a titration experiment.

Different conditions limit the effectiveness of both methods. Under typical experimental conditions, NMR methods become unreliable in our hands if $-\Delta G°$ exceeds 8.0 or drops below 3.5 kcal/mol. CD methods are best suited to conditions in which $-\Delta G°$ exceeds 4.0 but is less than 10.5 kcal/mol. As such, CD methods are preferred for the study of guests with high affinity for the host molecules, while NMR methods are preferred for the complexations of lesser stability. The CD method also operates in a lower concentration range, which is useful in the study of poorly water-soluble hosts. NMR methods, however, are more easily executed and facilitate access to structural information about the host guest complex. As such, NMR methods were generally preferred in the work presented here.

Aggregation Properties of the Host Structures. All of the host structures discussed here contain hydrophilic and hydrophobic portions and, like surfactants, will significantly aggregate near or above a certain concentration, the

critical aggregation concentration or (CAC).[5a, 19] Aggregate structures provide additional nonspecific binding sites for guest molecules and as a result distort the chemical shift data obtained during ^{1}H NMR binding experiments. Such distortions cannot accomodated in the 1:1 host-guest binding models which form the basis of the EMUL/MULTIFIT programs and must be rigorously avoided. Consequently, an investigation of the aggregation properties of any new host structure is necessary before binding studies are conducted.

As mentioned in chapter 2, eight host structures (Figure 3.1) were used throughout the course of the investigations presented in this thesis. After the synthesis of a host compound is completed, it is dissolved into an aqueous borate buffer (pH $\approx$ 9).[11c] Aqueous borate is the standard medium in which binding studies are performed. An investigation of the aggregation properties of the host by ^{1}H NMR ensues. In these studies the spectra of host solutions of various concentrations are recorded. A comparison of these spectra then determines a concentration under which the chemical shifts of the host species are invariant. Host solutions below this concentration are considered to be relatively free of aggregates.

As reported previously, aggregation problems for the basic hosts P and M (Figure 3.1) can largely be avoided at concentrations below 300 μM.[11c] The added methoxy groups of the new OMP and TMP structures were shown to increase greatly the critical aggregation concentration. For OMP, no significant aggregation was found to occur up to the highest concentration sampled, 3.2 mM. Solutions of the TMP host were relatively free of aggregates below 1 mM. The additional carboxylate of the CP macrocycle relative to P was also found to retard aggregation. The chemical shifts of the host were invariant up to concentrations of 1.5 mM, the highest concentration examined. All other

structures, TBP, TMTBP, and D3, were found to aggregate significantly in aqueous borate solutions. Solutions of these hosts are significantly aggregated at concentrations of 99, 107, and 22 μM respectively, the lowest concentrations examined for each of these by ^{1}H NMR.

A host concentration of at least 200-300 μM is generally necessary in order to carry out a binding study by NMR. For successful CD experiments, host concentrations of 1-2 μM are suitable. While borate solutions of TBP, TMTBP, and D3 in borate are presumably amenable to study by the latter method, they are clearly ill-suited for study by NMR techniques. The addition of 10% to 15% acetonitrile by volume to solutions of host in aqueous borate was found to suppress aggregation for each of these compounds, allowing NMR titrations to be performed using host concentrations of at least 300μM.

Solvent Effects. A change in the solvent system from aqueous borate to 10% or 15% acetonitrile (v/v) in borate should have significant effects on the binding properties of the host. In general, the hydrophobic component to binding should be attenuated upon addition of the organic cosolvent and the free energy of binding ($-\Delta G°$) should drop accordingly. We have been able to assess the impact of these solvent changes through extensive studies of host P. While we have used numerous guests (figure 3.2) in the study of host compounds, a distinctive subset of these guests, consisting of mostly charged iminium compounds, was used to assess the impact of the acetonitrile addition. The results of binding studies of these guests in borate solutions containing 0%, 10%, and 15% acetonitrile by volume are shown in table 3.1.

For the eight comparisons of cationic guests in both borate and 10% solutions, $-\Delta G°$ was found to be larger in borate by an average of 2.49 ± 0.19 kcal/mol. The

Table 3.1. -ΔG° (kcal/mol) for binding to host P in borate solutions containing 0%, 10%, and 15% acetonitrile (v/v).

	Solvent		
Guest	borate	10% acetonitrile	15% acetonitrile
1	8.4	5.8	4.8
2 (iodide salt)	7.3	4.8	
2 (chloride salt)	7.2	4.9	4.3
6	7.4	5.1	
7	6.8	4.4	
8	6.5	4.1	
9		5.7	5.0
10		5.5	4.8
11		5.9	5.3
12		5.9	5.4
15		6.3	5.6
17		5.7	4.9
19	8.5	6.1	5.6
21	6.7	4.1	

correlation is good, and suggests that we can compare borate and 10% acetonitrile numbers by simply adding 2.5 kcal/mol to -ΔG° for the acetonitrile system. A decrease in -ΔG° was also observed on moving from 10% to 15% acetonitrile solutions. For the nine comparisons made, the 10% numbers were on average larger by 0.68 ± 0.16 kcal/mol. Again, the effect is consistent and allows for meaningful comparisons of binding values determined in the different solvents.

The observed decreases in binding on moving from aqueous solutions to mixed aqueous/organic solvents are in the expected general direction as described above. Diederich has established an excellent linear correlation between hydrophobic binding and solvent $E_T(30)$ values.[6a] Given the reported solvent polarity parameters for mixed water/acetonitrile systems,[43] our system appears to be more sensitive to solvent polarity than Diederich's over the limited range of $E_T(30)$ values probed here.

Guest Selection and Counterions. Figure 3.2 shows a comprehensive list of the guests used in the binding studies reported in this thesis. The guests are simple compounds, readily available from commercial sources or easily synthesized from such. Some of the compounds, such as **1, 2, 21,** and **36,** are standard probes for testing the binding affinities of the host structures for various classes of compounds. Others have more subtle purposes as steric probes of the host structure or as indicators of their electronic properties. For instance, the series of N,X-dimethylquinolinium compounds (**9, 11, 12, 13, 14,** and **15**), in which a methyl group is moved systematically around the quinolinium structure, were used to probe how steric substitution affects guest binding. Compounds **9** and **10** were used to determine how different electronic properties of isostructural guests affect their binding.

Most of the guests are cations. As a result, there is a general concern about the role played by counterions in guest binding. We have always assumed that the anions of our aqueous buffer, 10 mM in borate, would overwhelm any counterion originally provided by the guest. This assumption was specifically tested in binding studies of N-methylisoquinolinium **2** with host P. In these studies both the chloride and iodide salts of the cationic guest were used. The

binding energies, reported in table 3.1 differ by only 0.1 kcal/mol, well within the error bars.

While the binding affinities of various guests can provide information about the structure/function relationships of a system, a great deal of information is also afforded by close examination of the complexation behavior of the individual guest compounds. Upon binding, the guest experiences a change in its molecular environment. In host P, the guests move from an aqueous environment into contact with six aromatic rings. These changes are evidenced by the upfield shifts in the ^1H NMR spectra of the guests, which are used to obtain the association constants. It is important to remember that in an NMR binding experiment, one obtains two types of fitted parameters, an association constant (K_a) and the differences in the shifts between free and bound species (D values) . In compiling the D values of the guest resonances, one can assemble a picture (or shift pattern) of the guest in its complexed state. This was demonstrated in chapter 1 for the compounds **21** and **26**. For these compounds, the calculated D values of the trimethylammonium groups of the molecules are greater than those observed for the hydrophobic portions of the molecules. This observation was used to conclude that the more soluble charged portions of these molecules lie preferentially in the host P binding site.[11c]

Information of this type becomes invaluable in comparison studies of host structures, like those presented here. By comparing the shift patterns for a guest complexed to different hosts, we can determine if our modified host structures have altered the guest's binding orientation. This information can then be used to ferret out the root causes of any increases or decreases in the binding energies. A strong correlation between altered binding orientations and altered binding

energies may indicate the presence of new intermolecular contacts. No correlation may indicate the presence of more subtle factors.

While such information is inherently useful, in many cases calculating D values for all protons within a guest in order to generate a shift pattern isn't easy. Complexation-induced line broadening, the shifting of a signal through other resonances, etc. can make obtaining the necessary shift information impossible. However, since we are mainly interested in a relative comparison of the D values (i.e., which of two proton resonances shifts to a greater extent), and not the values themselves, the equivalent information can be obtained in a simpler fashion.

The observed shifts we seen in our NMR titrations are weighted averages of the signals of the free and bound states of the host. The observed signals can be described by equation (1), in which δ_{free} and δ_{obs} are the unbound and time averaged shifts of the guest proton, respectively, and χ equals the mole fraction of guest bound.

$$\delta_{obs} = \delta_{free} + \chi D \quad (1)$$

Simple rearrangement gives equation (2).

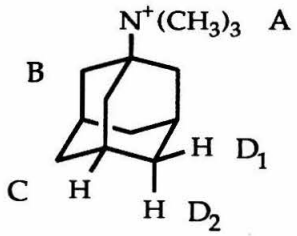
$$\delta_{obs} - \delta_{free} = \chi D \quad (2)$$

In practical terms, for any given spectrum containing both host and guest species we can determine the quantity χD for each guest proton by simply subtracting its observed chemical shift from its uncomplexed chemical shift. Each spectrum shows all guest signals at a particular mole fraction of guest bound; i.e., χ is constant in any given spectrum. As such, by comparing the values of χD we can obtain the same structural information available from rigorous determinations of

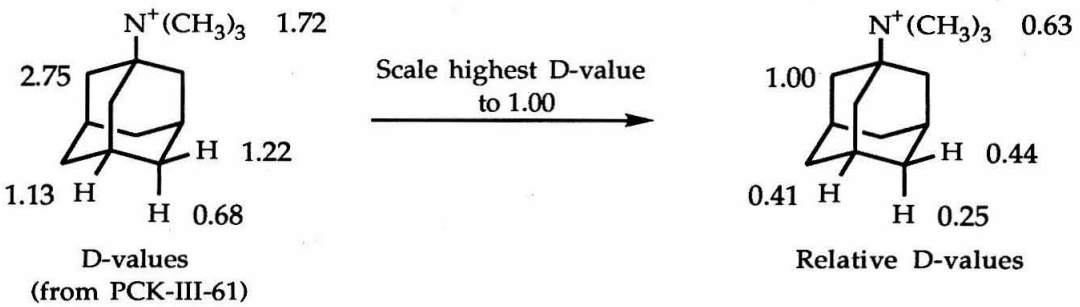
all guest D values. To determine these relative shift patterns, only two spectra are needed: a spectrum of the free guest and an appropriate spectrum of the guest complexed to the host species. An example of the method is shown in figure 3.3 for the binding of guest **21** to host CP. It should be noted that in the figure, the agreement is excellent between the relative shift values calculated from both D-values and directly from the two spectra.

Structural information is also available from following changes in the host signals upon guest complexation. This data is typically examined on a less sophisticated level here, with note being taken only as to the direction of the complexation-induced changes. For instance, in the binding of host P with a series of flat aromatic naphthalene-sized guests (both charged and uncharged), general trends in the shift patterns of the host are observed. The 1,5-, 3,7-, OCH_2, and linker protons (see figure 3.4) all experience shielding upon binding. The 4,8-protons shift downfield.[11c] The trends observed are consistent with the binding of these guests in the C_2-symmetric rhomboid conformation of the host shown in figure 3.5, with the guest displaced toward the front of the host.

a) Proton labels for compound **21**.



b) Calculation of relative shift value of each proton from D-values of experiment.



c) Calculation of relative shift values from the spectrum of the unbound guest and a single spectrum from the binding titration.

		Proton				
spectrum		A	B	C	D_1	D_2
21 in borate	δ_{free}	2.99	2.07	2.31	1.66	1.74
PCK-III-161.D	δ_{obs}	1.50	-0.31	1.34	0.60	1.14
	$\delta_{free} - \delta_{obs}$	1.49	2.38	0.97	1.06	0.60
	$\Delta\delta_{rel}$	0.63	1.00	0.41	0.45	0.26

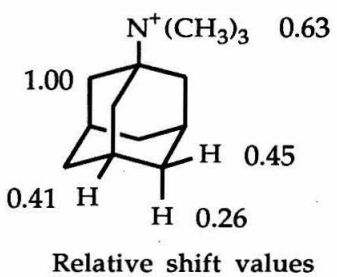


Figure 3.3. Example: determination of relative shift values for **21** complexed to host CP in borate. Data taken from PCK-III-61.

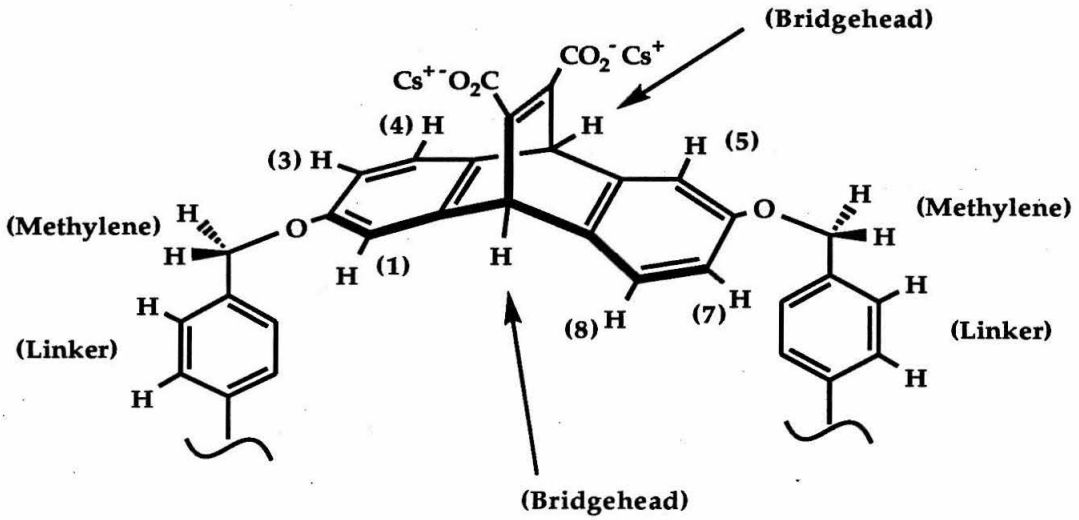


Figure 3.4. General labelling scheme for host protons. The name of each proton is placed next to its position.

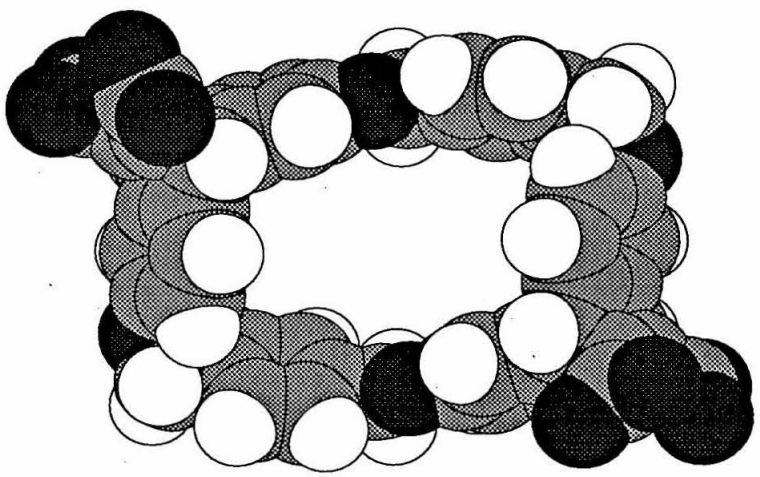


Figure 3.5. CPK Representation of the Rhomboid Conformation of Host P

Experimental Section

NMR spectra were recorded on JEOL JNM GX-400, Bruker AM-500 or General Electric QE-300 spectrometers. Routine spectra were referenced to the residual proton signals of the solvents and are reported in ppm downfield of 0.0 as δ values. All coupling constants, *J*, are in Hz. Spectra from aqueous binding studies were referenced to an internal standard of 3,3-dimethylglutarate (DMG, δ 1.09). Optical rotations were recorded on a Jasco DIP-181 digital polarimeter at 298 K. All circular dichroism (CD) experiments were carried out using a JASCO J-600 spectropolarimeter with either 1.0 or 0.5 cm pathlength quartz cells.

All host solutions for NMR binding studies were prepared in borate-d buffer as described in chapter 2. The preparation of the borate buffer itself is described below. The host solutions were quantified by NMR integrations against a primary standard solution of DMG in borate-d. For mixed-solvent binding studies, acetonitrile (10 or 15% v/v) was added as needed to aliquots of these host solutions. Guest solutions for NMR binding studies were prepared by dissolution of the compounds in the appropriate volumes of 0, 10 or 15% v/v acetonitrile in borate-d buffer. Guest solution concentrations were determined gravimetrically or through NMR integrations against DMG. All binding studies were performed by subsequent addition of aliquots of guest solutions to an NMR tube containing a solution of host compound that was initially approximately 300 μM. Binding data were fit to an appropriate association constant using the MULTIFIT or EMUL programs.

All chloroform binding studies were performed using the tetramethyl esters of host molecules. All host and guest solutions involved were quantified by NMR

integrations against a primary standard of *p*-dimethoxybenzene. All data were fit using the EMUL binding program.

Solutions for CD binding studies were prepared in borate buffer (pH = 9) prepared from water passed through a Milli-Q purification system. Host concentrations varied between 1-3 μM. In a typical study, CD spectra of 5-6 solutions of equivalent host but varying guest concentrations were used. The spectra and the Δε values of the host were fit to an association constant using the CDFIT program. Guest iodide and bromide salts were exchanged for chloride using Dowex® 1X8–400 ion exchange resin.

Guests **1**, **23**, **24**, **28**, **30**, **31**, **32**, **33**, **35**, **36**, **37**, **38**, **39**, **41**, **42**, **43**, **44**, **45**, and **46** were available from commercial sources. Compounds **6**, **7**, **19**, **20**, **27**, **29** and **34** were prepared as described in the literature.[44] Guests **2**, **3**, **4**, **5**, **8**, **9**, **10**, **11**, **12**, **13**, **14**, **15**, **16**, **17**, **18**, **21**, **22**, **25**, and **26** were prepared through alkylation of the appropriate amines, quinolines, isoquinolines and pyridines with the appropriate bromo- or iodoalkanes. Compound **40** was prepared by reduction of 4-quinolinecarboxaldehyde with sodium borohydride.

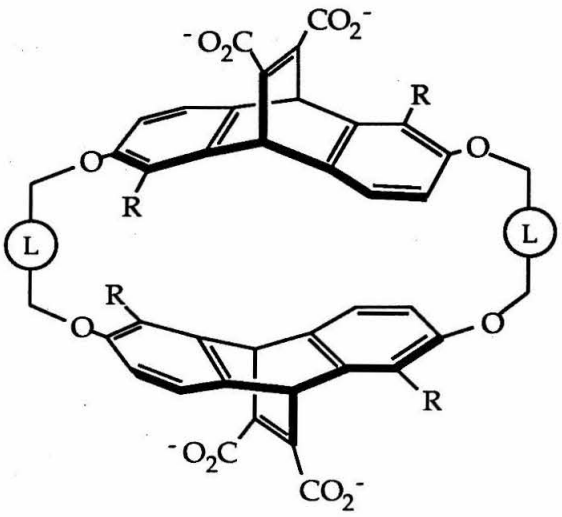
The 10 mM deuterated cesium borate buffer at pH ≈ 9 (borate-d) was prepared as follows. High purity boric oxide (31.3 mg) was dissolved in 100 g of D_2O. Cesium deuteroxide (467 μL of a 1M solution dissolved in D_2O) was then added and the solution mixed thoroughly. Borate solutions for CD studies were prepared in an analogous fashion.

Chapter 4: The Effects of Neutral Substituents on the Binding Properties of a Macrocyclic Host Compound.

Introduction

Perhaps some of the most convincing evidence documenting the importance of cation-π interactions as a force for molecular recognition was obtained through comparisons of the binding properties of the hosts P and C (shown in figure 4.1).[11c] In these studies it was shown that the replacement of the aromatic linkers of P with the aliphatic linkers of C significantly reduced the affinity of the host for hydrophilic cationic guests such as N-methylquinolinium **1**, N-methylisoquinolinium **2**, and adamantyltrimethylammonium **3** (figure 4.2). The host's affinity for neutral hydrophobic molecules such as quinoline **23** and isoquinoline **24** was unaltered by the modification. The results clearly suggest a strong role played by aromatic rings in the binding of cationic guests. In light of this and other data,[11d] we became interested in trying to increase the magnitude of these cation-π interactions through modifications of the host P structure. This chapter describes some of our efforts toward achieving this goal through the addition of neutral substituents to the basic host structure of P. Four new host structures were constructed (figure 4.1) with modifications being made to both the ethenoanthracene and linker regions of the host P structure. The binding affinities of these new structures for a wide variety of organic guest molecules were determined. A comparison of these results with each other and with those obtained for host P was performed to evaluate the effects of the modifications.

Methoxy groups were used to alter the linker regions of the macrocycle. We have studied three host structures modified in this fashion, denoted as OMP, TMP, and TMTBP in figure 4.1. Through π-donation to the aromatic



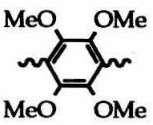
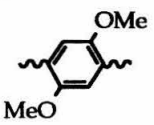
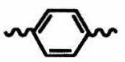
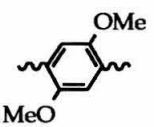
L	R	Host
	H	**P**
	H	**C**
	H	**OMP**
	H	**TMP**
	Br	**TBP**
	Br	**TMTBP**

Figure 4.1. Definition of Host Structures

rings of the linkers, these functional groups could enable the host to present more electron-rich aromatic faces to interact with cationic guest molecules. The methoxy groups should also increase the overall cavity depth of these structures, allowing for more extensive contact to occur between host and guest species. As reported for other similar host molecules, this can lead to large enhancements in guest binding. Finally, these relatively hydrophilic groups should make the host structures more resistant to aggregation[†] , which would be of general utility.

[†] The addition of methoxy groups to aromatic compounds has been found to drastically reduce the partition coefficients of such compounds between water and octanol. (reference 45) Furthermore, the addition of methoxy groups to other cyclophane hosts has been reported to drastically reduce their abilities to aggregate in aqueous media (reference 21a).

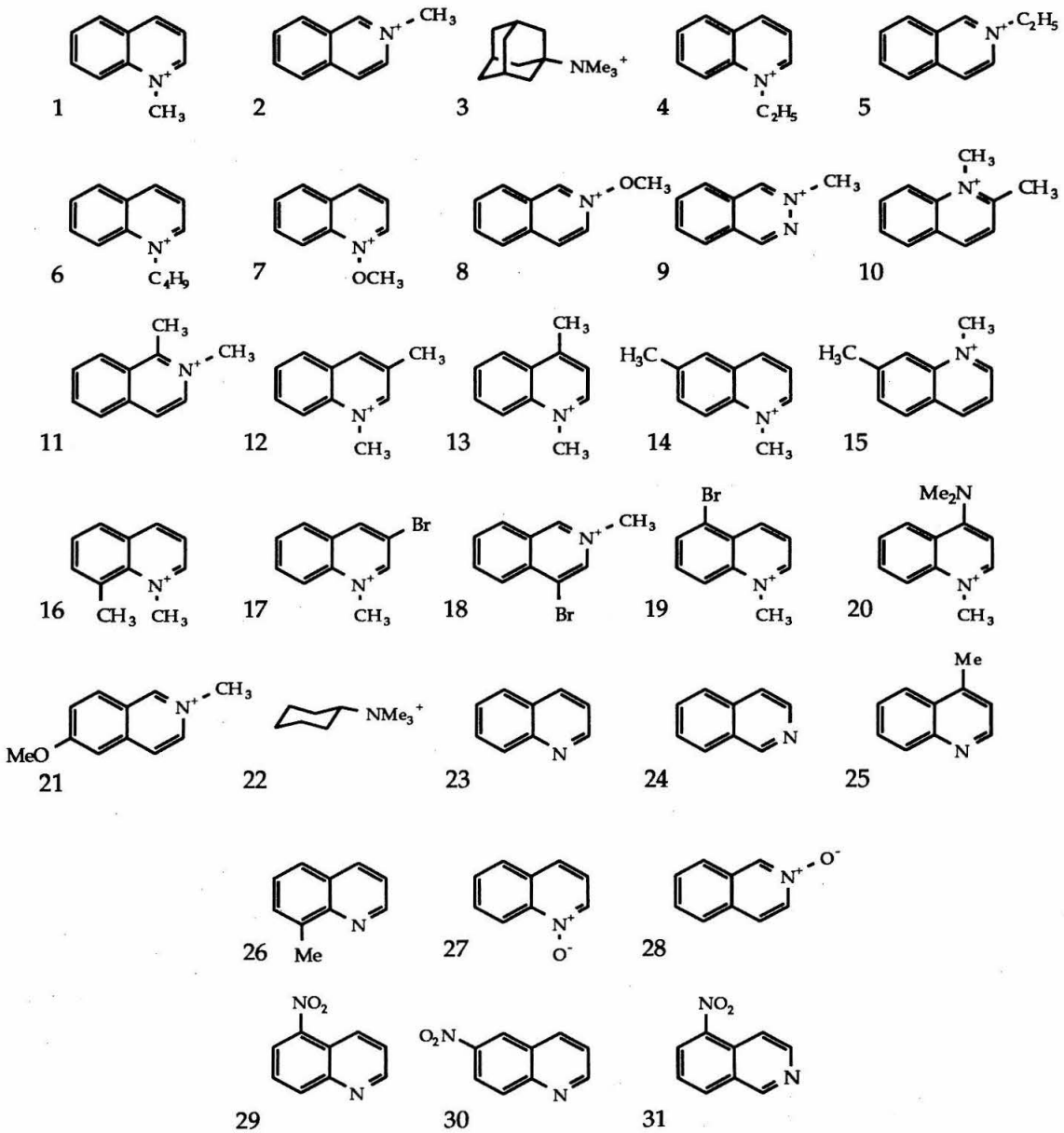


Figure 4.2. Guest Structures

Modifications to the ethenoanthracene portions of the basic host structure were made through the addition of halogen atoms. We've examined two macrocycles here, denoted TBP and TMTBP (figure 4.1), in which four bromine substituents have been positioned around the periphery of the binding cavity. These bromine substituents are highly polarizable, and it was hoped that they might supplement the aromatic recognition elements of the host and lead to altered guest specificities. The increase in the stability of stacked halogenated nucleotide sequences in aqueous solution and the close contacts made between halogen atoms and the purine and pyrimidine rings in molecular crystals of nucleotides and nucleosides suggested that the halogens could exert a strong influence on the binding properties of the receptors.[46] As with the methoxy substituents, the bromine atoms increase the overall cavity depth, and should allow for more extensive contact between host and guest. The positioning of the bromine atoms on the ethenoanthracene subunits should also limit the rotational mobility of the aryl-OCH_2 linkages of the host. As a result, hosts TBP and TMTBP should be conformationally less flexible than either the P, OMP, or TMP structures.

Results and Discussion

Octamethoxy Host (OMP). The first structure examined was the OMP host in which eight methoxy groups have been appended onto the basic structure of host P. Satisfyingly, the host was highly resistant to aggregation (chapter 3), displaying no appreciable aggregation at concentrations as high as 3.2 mM. However, as seen in table 4.1 the eight methoxy groups of OMP had a disastrous effect upon guest binding. In aqueous borate (pH~9), the binding affinities of the OMP host for guests **1**, **3**, and quinoline **23** were all

Table 4.1. The Effect of Methoxy Groups on Binding[a]

	OMP	TMP		P	
guest	borate	borate	10% MeCN	borate	10% MeCN
1	3.6	6.5	5.3	8.4	5.8
2		5.7	4.5	7.2	4.9
3	2.9	6.0	4.0	6.7	4.1
6		6.6			5.7
10			5.0		5.7
11			5.3		5.5
23	~0	4.2	<3.5	5.3	<3.5

[a] $-\Delta G°$ (kcal/mol)

significantly lower than observed with host P. A similar decrease in binding energy was seen for the binding of **1** with the tetramethyl ester of OMP in chloroform (1.5 kcal/mole versus 3.5 kcal/mol with the tetramethyl ester of P).

The results highlight a naïveté in the design of the OMP host structure. In total, OMP contains four pairs of methoxy groups in positions *ortho* to each other. Numerous experimental and theoretical studies have documented the tendency of *o*-dimethoxybenzene compounds to adopt nonplanar conformations.[47] The steric influence of the methylene linkers of the host should rigorously enforce such a tendency here. It is reasonable to assume that OMP directs several of its substituents into the cavity at any given time. CPK modeling indicates that while this doesn't prohibit guest binding, it does impose a sizable steric impediment. In essence the cavity is filled by the additional substituents. Guests which bind to the cavity are faced with the energetic burden of moving these groups out of the way and into less stable conformations.

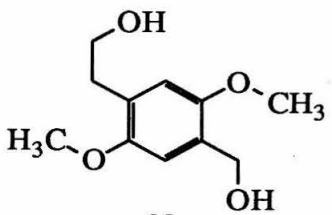
Tetramethoxy Host (TMP). In a second attempt at using methoxy substituents to modify the binding properties of host P, the number of methoxy groups was scaled back to four to produce the TMP host structure (figure 4.1). Steric factors, which may contribute to the nonplanarity of the methoxy groups in the OMP structure, have been greatly reduced in TMP. In addition, there should also be a greater intrinsic preference of the methoxy groups for planarity in the linkers of TMP, as compared to OMP, based on the available theoretical and experimental data of *o*- and *p*-dimethoxybenzenes.[47a]

The TMP host proved to be more highly resistant to aggregation than P (chapter 3). However, like OMP, TMP was a universally poorer host than P. For several comparisons in borate, the difference in binding affinities between P and TMP is in the 1.0 - 1.5 kcal/mol range. The difference is substantially reduced with the addition of 10% acetonitrile (v/v) to the solvent. This solvent effect can be stated another way. For three available comparisons (guests **1**, **2**, and **3**) the differences in $-\Delta G°$ between borate and 10% acetonitrile average less than 1.5 kcal/mole with TMP. This is roughly 60 percent of the 2.5 kcal/mol increment seen with P (chapter 3), suggesting a special solvent effect with TMP.

To rationalize the poor performance of TMP, a collapsed conformation of the unbound host is invoked, in which the linker groups are rotated so as to position two methoxy groups in the binding cavity. Essentially, the molecule binds itself. Binding a guest then requires the unfavorable removal of the methoxy groups from the cavity, and $-\Delta G°$ is diminished accordingly. Support for this analysis is provided by the ^1H NMR data. The linker O-CH_3 and aryl-H resonances are significantly shifted upfield in water (to 3.15 and

6.52 ppm respectively) versus chloroform (3.50 and 6.71 ppm, TMP-tetramethyl ester). Upon the addition of 10% acetonitrile (v/v) to a solution of TMP in borate, the resonances are shifted downfield to 3.56 and 6.90 ppm respectively. No other resonances in TMP or P show a similar effect. This is consistent with the model, in that the methoxy groups should shift upfield like typical guest protons if they are placed inside the cavity. Also, the host collapse should be less pronounced in the more organic solvents, and so the effect is only seen in a fully aqueous environment.

Additional support for this model comes from the downfield movement of the O-CH_3 and aryl-H resonances upon binding flat aromatic guests. This downfield movement stands in contrast to the upfield movement displayed by the linker proton signals of host P upon the binding of similar guests. In all solvents, upon binding flat aromatic guests, the TMP signals shift downfield to positions at approximately 3.8 and 6.9 ppm respectively, remarkably close to those recorded for the corresponding protons of compound **32** in chloroform (3.84 and 6.88 ppm respectively).[48] The resonances of **32** in chloroform may model those for the linker protons in an uncollapsed host state, and the downfield movement of the TMP linker signals to these positions in all solvents are consistent with the notion of a collapsed unbound host structure. The resonances of the ethenoanthracene subunits of TMP display a similar behavior to those of host P. Upon complexation of flat aromatic guests, the 1,5-, 3,7-, and methylene protons experience shielding, the 4,8-protons are deshielded (see figure 4.3 for numbering scheme of ethenoanthracene subunits). These data are



32

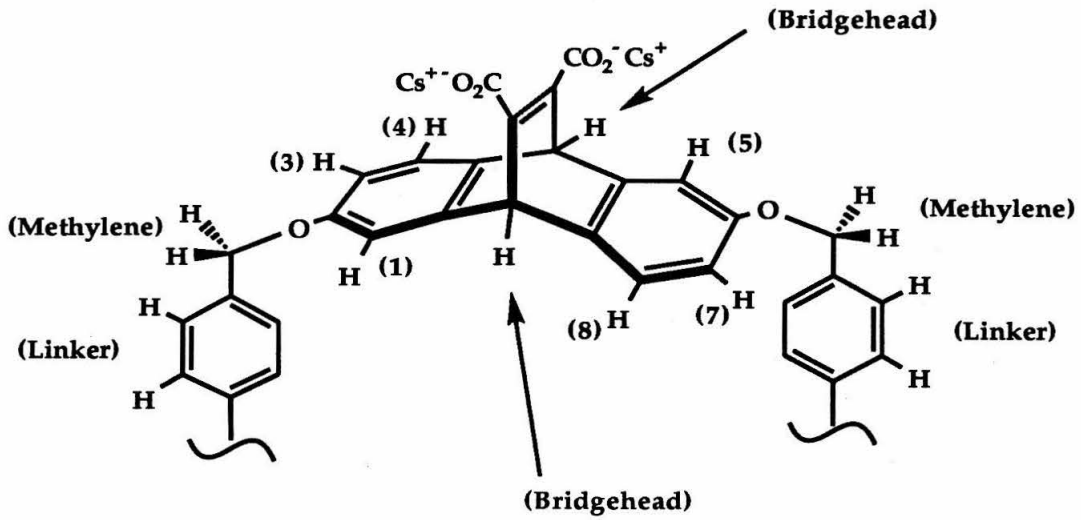
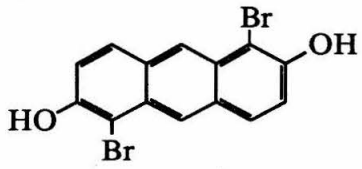


Figure 4.3. General labelling scheme for host protons. The name of each proton is placed next to its position.

consistent with the complexation of guests by TMP in the rhomboid conformation and with the overall locations of these protons in the collapsed and bound conformations of the TMP structure. More support for this model is obtained by comparing TMP to its brominated analogue (TMTBP), discussed below.

Tetrabromo Host (TBP). The results of our investigation of the OMP and TMP host had led us to believe that if enhanced binding affinities were to be seen from the addition of methoxy groups, these groups would need to be appended to the ethenoanthracene units themselves. A synthetic effort to do so was initiated and is described in chapter 7. During this period, however, we learned of procedures for the synthesis of 1,5-dibromo-2,6-dihydroxyanthracene **33**. As described in chapter 2, by building upon this molecule, we were able to create the dibromoethenoanthracene unit **34**, and by coupling **34** with the *p*-xylene dibromide linker groups, the TBP host structure.



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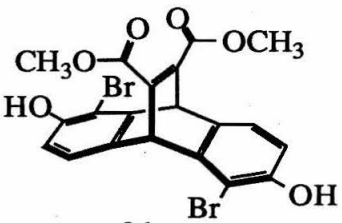
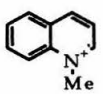
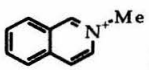
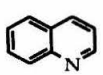


34

Host TBP showed a greater tendency to aggregate than host P in our aqueous buffer system. This was not unexpected, given the apolar nature of the halogen substituents. As a result, the binding affinities of guest molecules for TBP in borate buffer could be obtained only via CD measurements, using 1-2 μM concentrations of host. The free energies of binding ($-\Delta G°$) of four molecules were determined via this method as shown in table 4.2. The free energies obtained for these guests with P are listed for comparison. These are very strong interactions, with K_d for the TBP/**1** pair being 130 nM. All binding affinities have increased relative to host P, but the selectivity of the cavity for charged compounds **1**, **2**, and **3** versus the neutral **23** has been reduced significantly.

Table 4.2. - $\Delta G°_{298}$ (kcal/mol) for Binding to TBP and P in Borate

Guest	Host TBP	Host P
 **1**	9.3	8.4
 **2**	8.8	7.2
 **3**	7.8	6.7
 **23**	8.1	5.3

The properties of the TBP receptor were further elucidated via NMR titration studies in mixed acetonitrile/borate solutions. Most compounds were studied in 10% acetonitrile solutions. In this medium, however, studies of TBP with N-methylquinolinium compounds produced poorly fitting data.† Satisfactory results were obtained when these compounds were studied in 15% acetonitrile solutions. A number of isoquinolinium compounds were also studied in this medium, partially to serve as references to allow comparison with the 10% acetonitrile data. The results of these studies are reported in table 4.3.

Upon moving from borate to 10% acetonitrile solutions, the stabilities of all host-guest complexes decrease. It is noteworthy, however, that the more hydrophobic guests **3** and **23** experience a greater decrease in binding energy (4.0 and 4.3 kcal/mol, respectively) upon complexation with TBP than does the more hydrophilic isoquinolinium guest **2** (3.1 kcal/mole). These same guest molecules, when complexed with host P, undergo a more modest and uniform decrease in binding affinity of about 2.5 kcal/mol on moving from borate to 10% acetonitrile (as discussed above in our study of the TMP host and in chapter 3).

Remarkably, the enhanced affinity of the brominated receptor for neutral compounds is still clearly demonstrated in 10% acetonitrile. Some structures that are not measurably bound by P in this solvent system are substantially bound by TBP. The effect seems quite pronounced with the nitroaromatic structures (**29-31**), where $\Delta\Delta G$ values (P vs. TBP) as high as 1.8 kcal/mol are seen.

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† As a result there are a few guests for which only a binding affinity for P was reliably determined. These have been reported in table 4.3 for completeness

Table 4.3. The Effect of Bromination on Binding[a]

	TBP		P	
Guest	10% MeCN	15% MeCN	10% MeCN	15% MeCN
1		5.7	5.8	4.8
2	5.7	5.0	4.9	4.3
3	3.8		4.1	
4	5.8		5.4	
5	5.2		4.6	
6			5.7	
7	5.8		5.1	
8	5.1		4.4	
9	4.7		4.1	
10		5.9	5.7	5.0
11	6.5	5.9	5.5	4.8
12			5.7	
13		6.5	5.9	5.4
14			5.8	
15			5.7	
16		6.7	6.3	5.6
17		6.0	5.9	5.3
18	6.0	5.9	5.7	4.9
19			6.1	
20		6.9	6.1	5.6
21	4.8		5.0	
22	<3.5		< 3.5	
23	3.8		< 3.5	
24	3.9		<3.5	
25	5.0		3.5	
26	4.6		<3.5	
27	4.4		<3.5	
28	4.0		3.5	
29	6.1	5.8	4.3	4.1
30	5.4		4.6	
31	5.9		4.5	

[a] $-\Delta G°$ (kcal/mol)

These observations, the decrease in selectivity for cationic guests seen in borate, the greater drop in binding affinity seen for hydrophobic guests upon moving to 10% acetonitrile, and the greater affinity of TBP vs. P for neutral compounds in 10% acetonitrile, implicate nonspecific hydrophobic interactions with the bromine atoms as largely responsible for the increased binding affinities of host TBP relative to host P. Additional support for this argument is provided by the results of our studies using 15% acetonitrile (table 4.3). Across the series of quinolinium and isoquinolinium compounds, the increase in binding affinity upon brominating the host is fairly uniform, suggestive of nonspecific interactions. Also noteworthy within this series are the data obtained for the isostructural guest molecules **10** and **11**. These guests have the same binding constants, despite very different charge distributions. Similar results are obtained for the structurally similar but electronically variant guest pairs (**4** and **7**) and (**5** and **8**).

Given the large binding constants we have seen for TBP, we considered the possibility that it might produce strong binding in a purely organic solvent. As such we performed a quick survey of several guests in $CDCl_3$, binding to the tetramethyl ester of TBP. The results were [guest ($-\Delta G°$, kcal/mol)]: **1** (4.1), **2** (4.3), **23** (~0), **10** (3.8), **11** (3.7), **12** (3.6), and **18** (4.4). As discussed elsewhere, such substantial binding constants obtained in the absence of hydrophobic effects and any possible electrostatic contribution from the host carboxylates provide some of the most compelling evidence for the operation of cation-π interactions.[11d]

Tetramethoxytetrabromo Host (TMTBP). The modifications of TMP and TBP were combined to produce TMTBP. Given the relatively high critical aggregation concentrations of the OMP and TMP hosts, it was hoped that the methoxy groups of the TMTBP host structure would alleviate the aggregation problems experienced by TBP in borate. It was also hoped that the bromine substituents of the TMTBP would prevent the collapse seen with TMP, by limiting the conformational flexibility of the host. It was seen by CPK modeling of the TMTBP structure that the collapse could also be prevented through the formation of close contacts of the bromine atoms with the methyl portions of the methoxy substituents. In effect, the halogen atoms could provide an alternative to the hydrophobic interior of the cavity, thereby retarding collapse.

The TMTBP host proved more water soluble than TBP but not so much so that pure borate was a viable solvent for NMR binding studies. As a result, this molecule was studied exclusively in 10% acetonitrile/borate solutions. The results of binding studies with several guest molecules are located in table 4.4. Some of the binding data for hosts P, TMP, and TMTBP are reproduced here to allow for easier comparison of data. Compared to the methoxy host of TMP, the addition of bromines greatly enhanced binding. The binding of the TMTBP host is largely comparable to TBP, and so significantly superior to P. Adding methoxy groups to TBP produced no observable change in the complexation of isoquinolinium compounds **2** and **11**, and only a small drop in affinity for the tetraalkylammonium compound **3**. Interestingly, for the neutral compounds **23** and **25**, TMTBP was a significantly better host than TBP. In this system lepidine **25** has a binding

Table 4.4. Combined Effects of Bromine and Methoxy Substituents[a]

Guest	TMTBP	TBP	TMP	P
1	6.5		5.3	5.8
2	5.7	5.7	4.5	4.9
3	<3.5	3.8	4.0	4.1
10	6.6		5.0	5.7
11	6.3	6.5	5.3	5.5
23	5.0	3.8	<3.5	<3.5
25	6.4	5.0		3.5

[a] $-\Delta G°$ (kcal/mol), solvent 10% acetonitrile.

affinity comparable to that of N-methylisoquinolinium **1** ($-\Delta G°$ 6.5 kcal/mol), a phenomenon not observed previously with any of our host structures.

The neutral compounds may benefit from the greater cavity depth of the TMTBP structure. Increasing the cavity depth provides additional surface area capable of forming nonspecific van der Waals interactions with the guest molecules. The results with host TBP show that **25** and **23** have a lower affinity for the aromatic structure of the cavity than do quinolinium and isoquinolinium compounds. As such, these more "free-ranging" neutral guests might be expected to benefit more by the increased surface area of TMTBP, than would charged guests, which might be held in more specific conformations. Further experimentation is needed to examine this hypothesis.

As evidenced by the binding data, the addition of bromines to TMP appears to have fully compensated for the adverse effects of the methoxys. ^{1}H NMR chemical shift data support this analysis. For the uncomplexed host, the linker O-CH_3 and aryl-H resonances are positioned at 3.77 and 7.24 ppm respectively in 10% acetonitrile, well downfield of the positions recorded for the TMP linkers in this solvent. Upon the binding of flat aromatic guests, the

O-CH_3 resonance moves slightly downfield to around 3.9 ppm and the aryl-H resonance moves upfield to approximately 6.9 ppm. These complexed shift positions are quite similar to those of TMP in the bound state. Moreover, the upfield movement of the aryl-H resonance is now in the same general direction as that for similar protons of host P, as expected if the host were no longer in the collapsed state. It should be noted, however, that the effects seen here could also be the result of the proximal location of the protons to a deshielding halogen atom.[49]

Conclusions

We have examined four new host structures in which we have added neutral substituents to the basic host structure of P. Methoxy groups were added to the linker regions, and bromine atoms to the ethenoanthracene subunits. While the modifications led to facile control of phenomena such as aggregation, they had a more complicated impact upon the binding properties of the host structures. Left unchecked, the methoxy groups tended to decrease binding by filling in the host cavity with their hydrophobic methyl portions. In general, the addition of bromine atoms led to an increase in binding, mainly through an increase in nonspecific hydrophobic interactions. The primary purpose of these modifications was to improve stability of the host-guest complexes, formed between the host species and charged organic guests, through enhanced cation-π interactions. Our modifications were unsuccessful in this respect , and highlight the difficulty in trying to enhance a single aspect of binding. However, as evidenced by the systematic study of the TMP, TBP and TMTBP structures, the complex behaviors induced by the substituents can be understood and controlled to some degree.

Chapter 5: The Effects of Additional Negative Charge on the Binding Affinitiy of a Macrocyclic Receptor for Cationic Guests.

Introduction

Our studies of molecular recognition in aqueous media have shown that the cyclophane host P (**1**) serves as a general receptor for organic cations, including quaternary ammonium, iminium, guanidinium and sulfonium compounds.[11c,j] A primary binding force between these compounds and **1** is thought to be a cation-π interaction, in which the positively charged groups are stabilized by the π-clouds of the electron-rich aromatic rings of the host. Our results with host P have led us to propose that cation-π interactions are manifest in a wide variety of biological binding sites for quaternary ammonium compounds such as acetylcholine (ACh).[11g]

As mentioned in Chapter 1, much evidence supports the position that aromatic residues (instead of negatively charged carboxylates or phenolates) may be largely responsible for binding at the traditionally termed "anionic" sites for quaternary ammonium compounds. The crystal structure of phosphocholine bound to the immunoglobin Fab McPC603 indicates that aromatic tryptophan and tyrosine side chains of the antibody make close contacts with the positively charged N-methyl groups of the hapten.[29] Labeling studies of the α-subunit of the nicotinic acetylcholine receptor (AChR) isolated from *Torpedo* place a large number of highly conserved aromatic residues at the agonist binding site for this class of ligand-gated ion channels.[16] Similar studies indicate the presence of aromatic residues at the "anionic" binding sites of acetylcholine esterases (AChE).[30] X-ray crystallographic studies of the esterase isolated from *Torpedo* later identified the site at the bottom of a deep gorge, which was lined with a large number of aromatic residues.[31] More recently, the proposition has gained further support from the large number of aromatic residues located at the pore

regions of voltage-gated potassium channels,[33] a region susceptible to block by tetraethylammonium. Modeling studies of G-protein coupled receptors (GPCRs) also suggest the presence of conserved aromatic residues at aminergic ligand binding sites.[32]

The presence of aromatic residues at the binding sites for cholinergic ligands does not preclude, however, the involvement of anionic carboxylates in these regions. Indeed, there is substantial evidence for their participation. Close contacts can be seen between an aspartate residue and an N-methyl group in the crystal structure of phosphocholine bound to McPC603. Modeling suggests that similar contacts can be made between acetylcholine and Glu^{199} of the AChE.[29a] Furthermore, recent labeling studies indicate that negatively charged acidic residues from the γ and δ subunits contribute to the ligand binding sites of AChRs.[15a,50] Finally, in the modeling studies of aminergic GPCRs,[32] the aromatic moieties are proposed to work in conjunction with a highly conserved aspartate residue in the complexation of primary, secondary, tertiary and quaternary amines. As stated by the authors of those studies, "...this aromatic cluster contributes to the neutralization of the ammonium positive charge (through cation-π interactions), and tremendously reinforces the energy of interaction of the ion pair, compared with that possible in an aqueous environment."

In light of this information, a general picture of these binding sites is beginning to emerge in which negatively charged aspartate and glutamate residues act in synergy with aromatic tyrosine, tryptophan and phenylalanine residues to enable the binding of cationic guests. Such a juxtaposition of acidic and aromatic residues creates a relatively complex molecular environment, and discerning the roles played by the individual residues in the non-covalent

binding of aminergic guests can be difficult. Given the success of synthetic systems at analyses of this type, it was hoped that a structured host-guest system, containing both neutral aromatic and negatively charged functionalities, could provide additional insights into the nature of such environments.

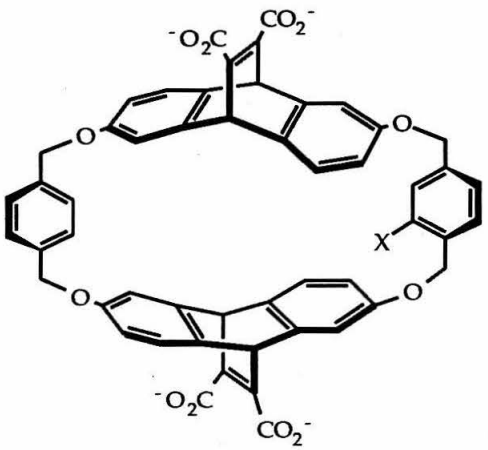
Our initial attempt in creating such a system, the CP host **2** (figure 5.1a), has a single carboxylate group added to the basic structure of host P. As shown in figure 5.1b, this carboxylate, located adjacent to the cavity, should be well solvated in an aqueous environment, but is positioned such that it can form close contacts with guest molecules upon binding. By placing a carboxylate group near the binding site, this structure intentionally deviates from our traditional host design in which solubilizing groups are held rigidly away from the binding cavity. Through comparison of the complexation strengths of the P and CP hosts for charged and uncharged guests, we hoped to gain an understanding of the role played by charged species near to and distant from the binding cavity. Such a study would compliment those carried out by Diederich[9] and by Schneider[8c,e] in which only the role of close contact between charges was evaluated.

The CP host was also created without drastic redesign of the system: a carboxylate replaced a hydrogen atom. Forces active in the host P system should thus be similarly active in the CP system. Hydrophobic and cation-π interactions should thus largely dominate the binding processes in both systems. In this respect the study differs considerably from those of other workers, most notably those of Lehn,[51] Schneider,[8f] and Vögtle,[10] in which electrostatics were shown to be the dominant forces of interaction.

The perturbations to the host P system were deliberately kept at a minimum, in hopes of better evaluating the direct interactions between the guest and the

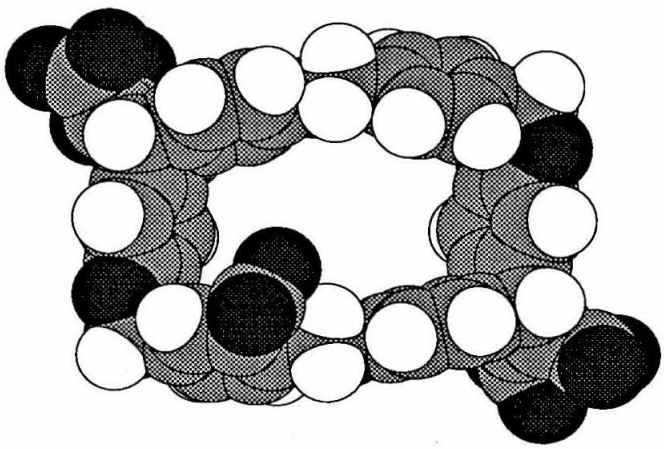
Figure 5.1. Host Structures

a) Definition of hosts P and CP.



Substituent	Host
X = H	P
$X = CO_2^-$	CP

b) CPK representation of the CP host structure in one of several possible rhomboid conformations of the molecule. The additional carboxylate is shown in the lower left-hand side of the diagram.



added carboxylate of CP. As mentioned previously, a variety of positively charged guests bind to host P in well defined conformations. By keeping structural changes to a minimum, we desired to capitalize on the structural control of the host-guest complex afforded by the host P system. Using this control, we hoped to illuminate how subtle differences in the electrostatic properties of these guests influence electrostatic binding. Massive changes to the host structure would preclude an evaluation of these factors.

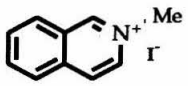
In addition to surveying the role of electrostatics in guest binding, we were interested in examining host CP for its potential to show enhanced guest affinities via the formation of a hydrogen bond. Given the strongly solvated nature of this group and geometrical constraints limiting interactions of potential guests to its apparently the less basic anti lone pairs,[52] stabilized hydrogen bond formation may seem like a remote possibility. However, we were encouraged by recent reports of synthetic systems in which hydrogen bonding in aqueous media played a significant role in guest binding.[53] We were encouraged further by recent calculations, which suggest that in aqueous solution the basicity of the syn and anti lone pairs of a carboxylate differ by only 1.25 pKa units.[54] A recent survey of the crystallographic data base also indicated no statistical preference for syn- over anti-hydrogen bond formation.[55]

Results and Discussion

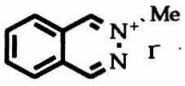
Binding Energies. The binding affinities of a wide variety of neutral and cationic guests for hosts P and CP in our aqueous borate system were determined by NMR titration experiments. The results of these studies can be seen in figure 5.2. Neutral quinoline compounds were examined to determine how the carboxylate affected cavity solvation, as well as for their potential to hydrogen-

Figure 5.2. $-\Delta G°_{298}$ (kcal/mol) in Borate for Binding to Hosts CP / P [a]

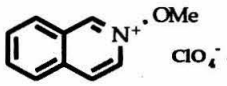
Iminium Compounds:



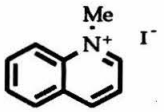
3 7.8 / 7.2



4 6.9 / 6.5



5 7.3 / 6.8

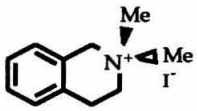


6 8.7 [(a)] / 8.4

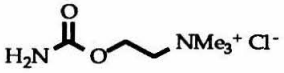
Quaternary Ammonium Compounds:

7 6.6 / 6.7

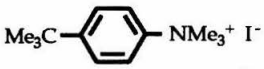
8 4.7 / 4.8



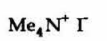
9 6.7 / 6.4



10 4.6 / 4.5



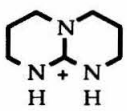
11 6.7 / 6.5



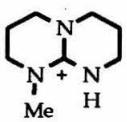
12 3.9 / 3.8

13 6.2 / 5.9

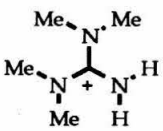
Guanidinium Compounds:



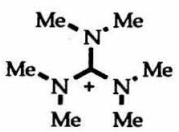
14 5.8 / 5.0



15 6.7 / 6.2



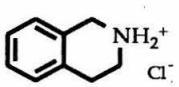
16 5.1 / 4.7



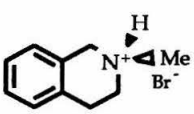
17 5.0 [(b)] / 4.9

Secondary and Tertiary Ammonium Compounds:

18 3.9 / 3.9

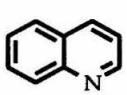


19 5.3 / 5.0

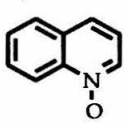


20 5.1 / 5.1

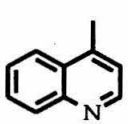
Neutral Aromatic Compounds:



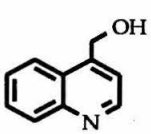
21 5.1 / 5.3



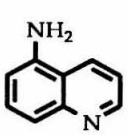
22 6.1 / 6.1



23 5.8 / 5.9



24 5.8 / 5.9



25 5.6 / 5.7

[a] (a) Value with CP determined from measurements of upfield shifts of N-Me protons for three samples with differing 1:1 ratios of host and guest, and a measured value of the maximum upfield shift for that resonance. See footnote (††, page X). (b) Values reported are weighted averages of rapidly interconverting enantiomers. Binding affinites of the enantiomers are (5.1, 4.8) and (5.1, 4.6) kcal/ mol with hosts CP and P respectively.

bond with the cavity. Iminium guests, such as N-methylisoquinolinium **3**, and tetraalkylammonium compounds, such as adamantyltrimethylammonium **7**, were studied for their abilities to interact electrostatically with the carboxylate group. Guanidinium compounds as well as secondary and tertiary amines were also examined for this purpose, in addition for their abilities to H-bond with the cavity.

Two observations are readily apparent from the data. Firstly, in aqueous media the neutral compounds studied (**21-25**) displayed neither an increase nor a decrease in their affinities for host CP relative to host P.† Secondly, not all of the positively charged compounds displayed an enhanced affinity for the CP cavity. In general, all of the iminium compounds (**3-6**) and most of the guanidinium compounds (**14-16**), except for hexamethylguanidinium **17**, displayed an enhanced affinity for the CP cavity on the order of 0.4 to 0.8 kcal/mol.†† The

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† Differences in $-\Delta G^{\circ}$ less than 0.2 kcal /mol are considered insignificant here.

†† A small improvement has been recorded here for the binding of **6** to the CP host. The 8.7 kcal/mol value for **6**/CP, however, lies outside of the range that can be directly determined using our standard NMR titration procedures. The number reported is the average of three values obtained by calculation of the association constant (Ka) as follows. Spectra were taken of three solutions of known host and guest concentrations. The ratio of host to guest was 1:1 in each sample. Direct measurements of the upfield shifts of the N-methyl protons ($\Delta\delta_{obs}$) were made in each case. A measured value for the maximum upfield shift of those protons ($\Delta\delta_{max}$) was obtained from a 10:1 host to guest solution. An estimate of the percentage of guest bound (G%) was made for each sample using the relationship (1).

(1) $\quad G\% = \Delta\delta_{obs} / \Delta\delta_{max}$.

This percentage can then be used to determine the concentrations of host, guest, and host guest complex ([H], [G], [H·G] respectively) in each of the solutions. The association constant can be determined from the calculated values of [H], [G], and [H.G] according to the equation (2).

(2) $\quad Ka = [H{\cdot}G]/([H]{\cdot}[G])$.

The binding energy reported may be less reliable than those obtained from standard binding experiments. However, increased line-broadening of the signal for the N-methyl protons observed in solutions of **6** with CP (at known concentrations) as compared to solutions of **6** with P (at identical concentrations) suggests a definite increase in binding affinity. As such the

tetraalkylammonium compounds (**7-13**), as well as the secondary and tertiary amines (**18-20**), in general were bound equally well by the P and CP macrocycles, with a few guests possibly displaying slight enhancements.

The observation that none of the neutral compounds displayed any preference for host CP has several implications. In terms of hydrogen bonding, since neither the 4-hydroxymethyl-, nor the 5-aminoquinoline compounds (**24** and **25** respectively) displayed any enhancement in binding, we are forced to conclude that these hydrogen donating functionalities were unable to increase complex stability. More powerful donating groups may be required. The results with the neutral guest compounds **21-25** do tell us something, however, about the overall environment of the binding cavity.

Since the addition of a carboxylate group to our basic host structure **1** greatly increased the water solubility of the CP cavity[†], it might have been expected that it would have also decreased the overall hydrophobicity of the binding site. This in turn would have reduced the binding affinity of CP for guest molecules. The neutral guests' equal affinities for the P and CP macrocycles suggests that the environment of the cavity has remained largely unperturbed. The carboxylate moiety has neither decreased the hydrophobicity of the cyclophane cavity nor, like other modifications on related host structures, stabilized a collapsed conformation of the host. This implies that the enhanced affinities seen of many of the positively charged compounds can be viewed more directly as the result of increased (presumably electrostatic) interactions with the CP macrocycle.

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enhanced affinity of 0.3 kcal/mole may be underestimated and is generally ignored when discussing the free energies of binding.

[†] No significant aggregation was observed throughout the concentration range examined (up to 1.5 mM).

It seemed strange that three different families of positively charged compounds, the iminium, tetraalkylammonium, and guanidinium groups, would behave so differently in their responses to the CP host. While perhaps it isn't surprising that all of the iminium compounds, as typified by **3**, displayed an enhanced affinity for CP, it is interesting that a majority of the tetraalkylammonium compounds, as typified by **7**, show equal preferences for the P and CP hosts. Of the tetraalkylammonium compounds only **9** and **13**, molecules designed either to resemble iminium compounds in structure or to carry an additional positive charge, displayed marginal improvements in binding affinity. Perhaps equally intriguing is the data obtained from studies with the guanidinium compounds **14-17**. Within this class of molecules, molecule **14** exhibited the highest increase in binding affinity observed in our studies, while molecule **17** exhibited no enhancement in binding. Given the strength of hydrogen bonds formed between carboxylates and guanidinium compounds,[56] it is tempting to attribute the increased affinities seen here to the formation of a hydrogen bonds. However, since the iminium and guanidinium compounds studied here display similar increases in binding affinities for CP, it was necessary to look for factors common to both types of compounds that may be responsible.

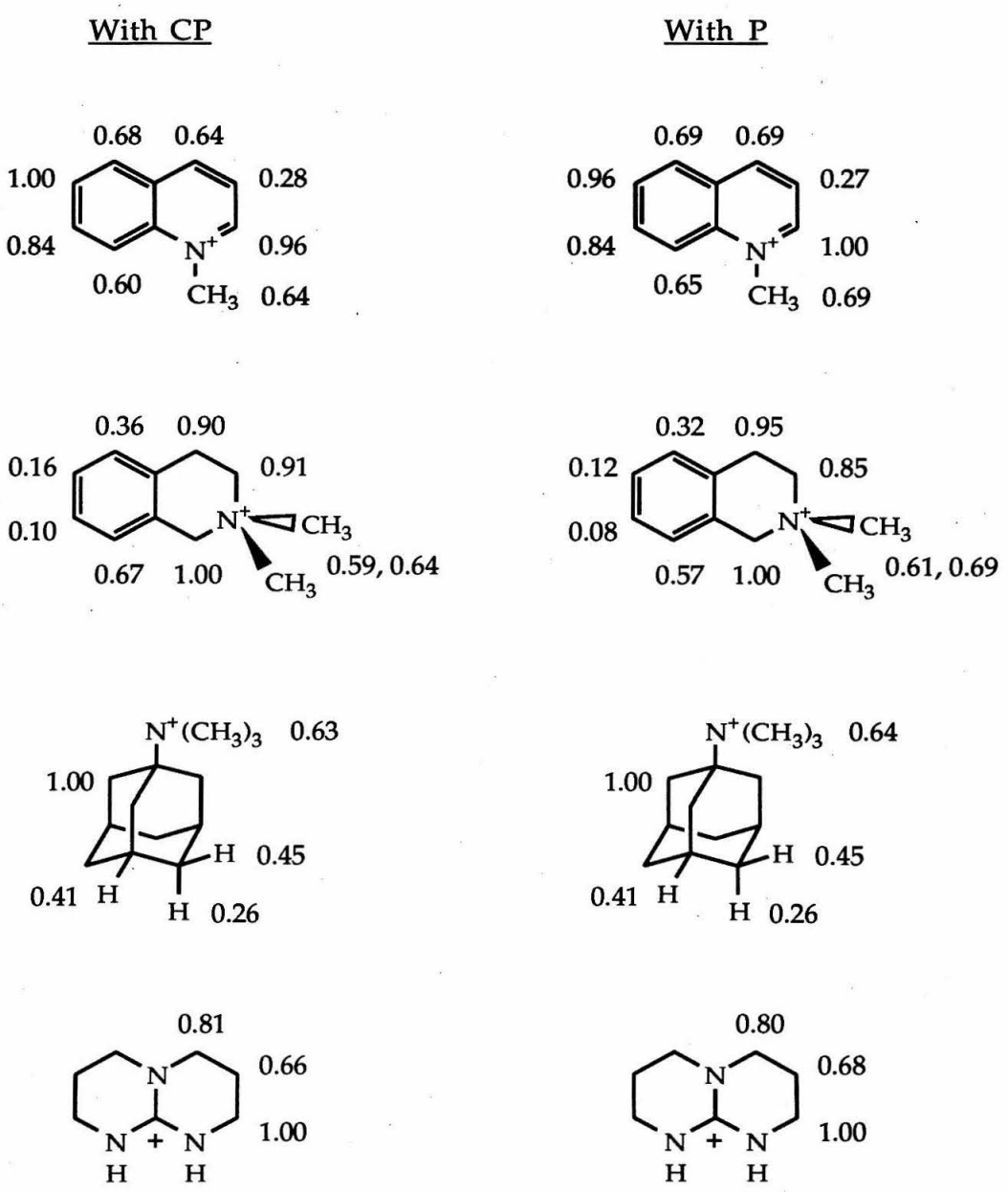
Before proceeding, it should be noted that the binding data in figure 5.2 have definite implications for the role played by the remote carboxylates of host P. The additional carboxylate of the CP host lies much closer to the cavity than the carboxylates on the ethenoanthracene subunits. If these remote groups played a significant role in the binding of guest molecules, then the additional carboxylate of CP should have enhanced the affinity of all positively charged guests for the cavity. Since many of the positively charged guests displayed no increased

affinity for the CP host, it can be inferred that the remote carboxylates play a negligible role in binding. Increased binding clearly requires more than the indiscriminate placement of an additional charge. Closer contact between the two oppositely charged species may be required for enhanced stabilization in aqueous media. A similar conclusion was reached by Schneider et al. in a recent study.[8e]

Chemical Shift Patterns. As mentioned in Chapter 3, an examination of the changes in the ^{1}H-NMR chemical shifts of both host and guest protons can be used to provide structural information about the host-guest complex. We determined the relative upfield shift patterns for a variety of guests complexed with both the P and CP cavities. These patterns are displayed in figure 5.3. In each case, the shift patterns of a given guest with both host structures were found to be nearly identical. No correlation between changes in chemical shift pattern and enhancement of binding affinity for the CP host could be drawn. As mentioned previously, relative shift patterns suggest an average of the environments sampled by the protons, relative to each other, upon complexation with a host molecule. The similarity between the shift patterns of the examined guests with both the P and CP structures, implies that the binding orientations of the guest molecules are largely unaffected by the addition of the carboxylate. Therefore, it would seem that in both systems the guest binding orientations are determined primarily by interactions of the guest molecules with the aromatic rings of the cyclophane cavities.

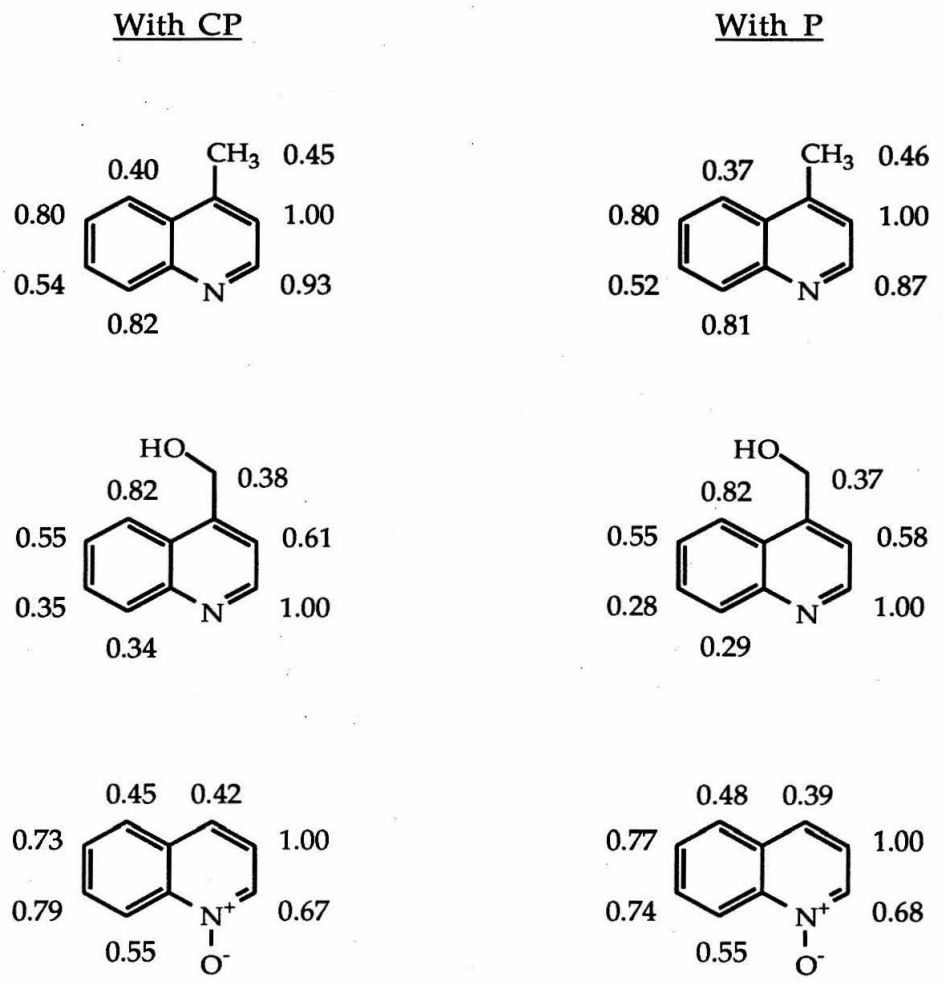
The determination of the guests' ^{1}H-NMR shift patterns was made easier by the large changes in chemical shift upon complexation with both the P and CP host systems. The changes are largely the result of the shielding influences of the hosts' aromatic rings. The chemical shifts of the host protons also change upon

Figure 5.3. Relative ^1H NMR Chemical Shift Patterns of Selected Guest Molecules complexed with Hosts CP and P in Borate.[a]



[a] Hydrogen atoms of the individual guests are not shown, unless needed to provide clarity.

Figure 5.3. Relative ^{1}H NMR Chemical Shift Patterns of Selected Guest Molecules complexed with Hosts CP and P in Borate (continued).



guest complexation. Study of these host complexation-induced shifts is also facilitated if the guest molecules contain aromatic rings.

When the complexation-induced changes in the chemical shifts of the host hydrogen atoms were examined using both the neutral **3-6** and charged aromatic guests **21-25**, two differing types of behavior were observed. In figures 5.4 and

5.5 the host aromatic regions of the 1H-NMR spectra of quinoline **21** (figure 5.4) and N-methylisoquinolinium **3** (figure 5.5) complexed to both the P and CP hosts are shown.† The results typify the shift behaviors seen for each class of guests. The spectra of the uncomplexed hosts are included in each figure for reference. The spectra of the CP molecules are more complicated than those of P, since the added carboxylate destroys the time-averaged four-fold symmetry observed in the spectra of P.[11c] However, as can be seen by comparison of the spectra of the uncomplexed P and CP cyclophanes, it is a simple matter to relate signals of CP to the analogous signals of P (see figure 5.6 for numbering).

On binding either neutral or cationic aromatic guests, upfield shifts of the 1,5-, 3,7,- and linker protons, and downfield shifts of the 4,8-protons of P are observed. These observations, in conjunction with the guest shift data, provide strong evidence that naphthalene-sized aromatic guests bind with the rhomboid conformation of P. Similar observations are made for CP. However, while the signals for each of the proton groups remain largely clustered together in the spectra of CP alone and complexed with the neutral aromatic **21**, a large splitting is observed for such protons when the CP host is complexed with the charged iminium **3**. These observed splittings are roughly centered around the chemical shift values observed for the time-averaged symmetrical protons of P complexed to **3**. This distinction between neutral and cationic aromatic guests is seen for all of the guests shown in figure 5.2.

The chemical shift of a proton is a good indicator of its time-averaged molecular environment. The clustering of signals observed for the proton groups

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† The concentrations of host and guest used to generate each spectrum were 250 mM and 2.5 mM respectively, and were chosen such that the percentage bound of the each host was greater than 99%.

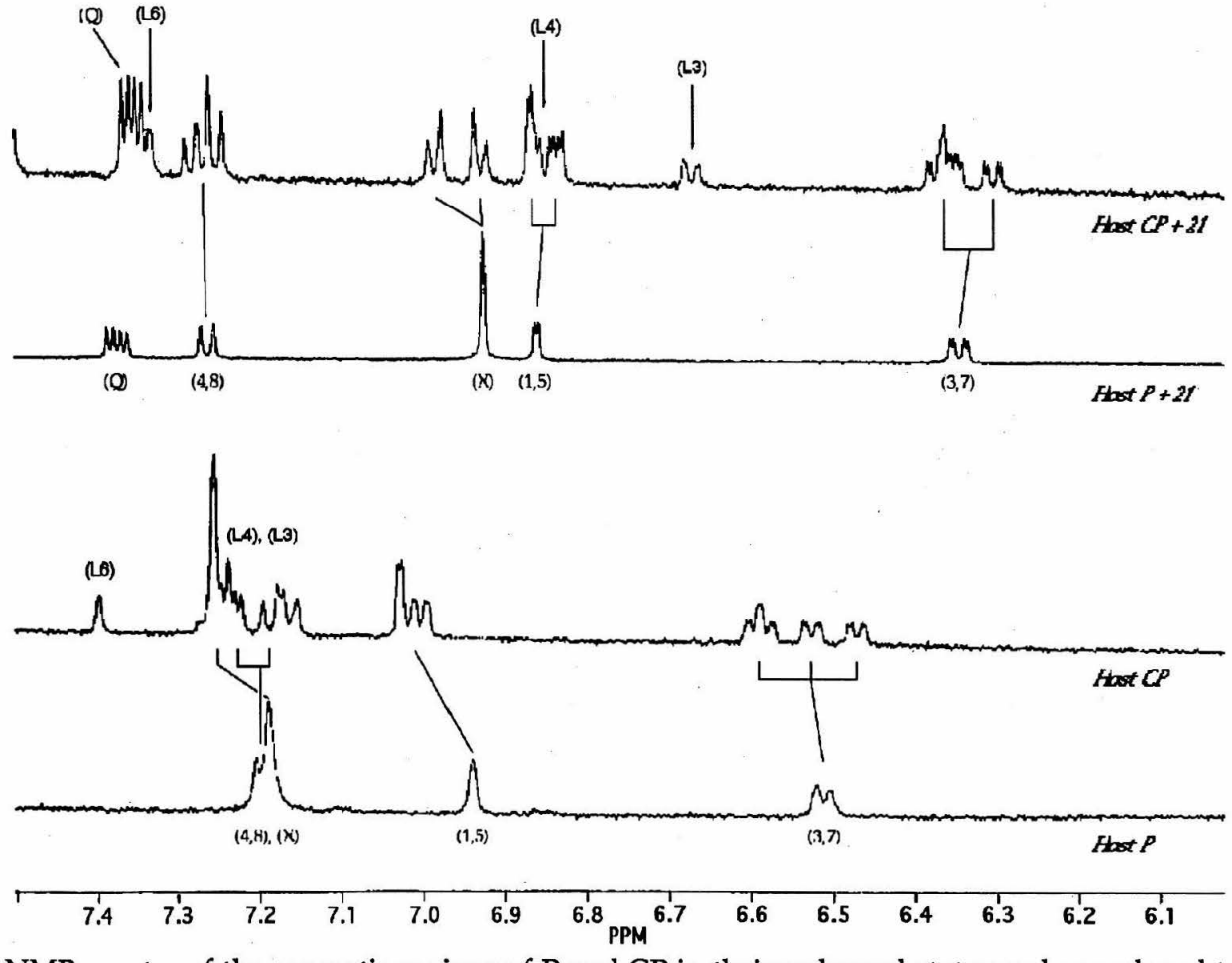


Figure 5.4. ^{1}H NMR spectra of the aromatic regions of P and CP in their unbound states and complexed to quinoline **21**. Branches between spectra of hosts identify resonances of similar host protons. Those protons are identified within the brackets ( ) above and below the spectra according to the labeling scheme outlined in figure 5.6. (Q) indicates a quinoline resonance.

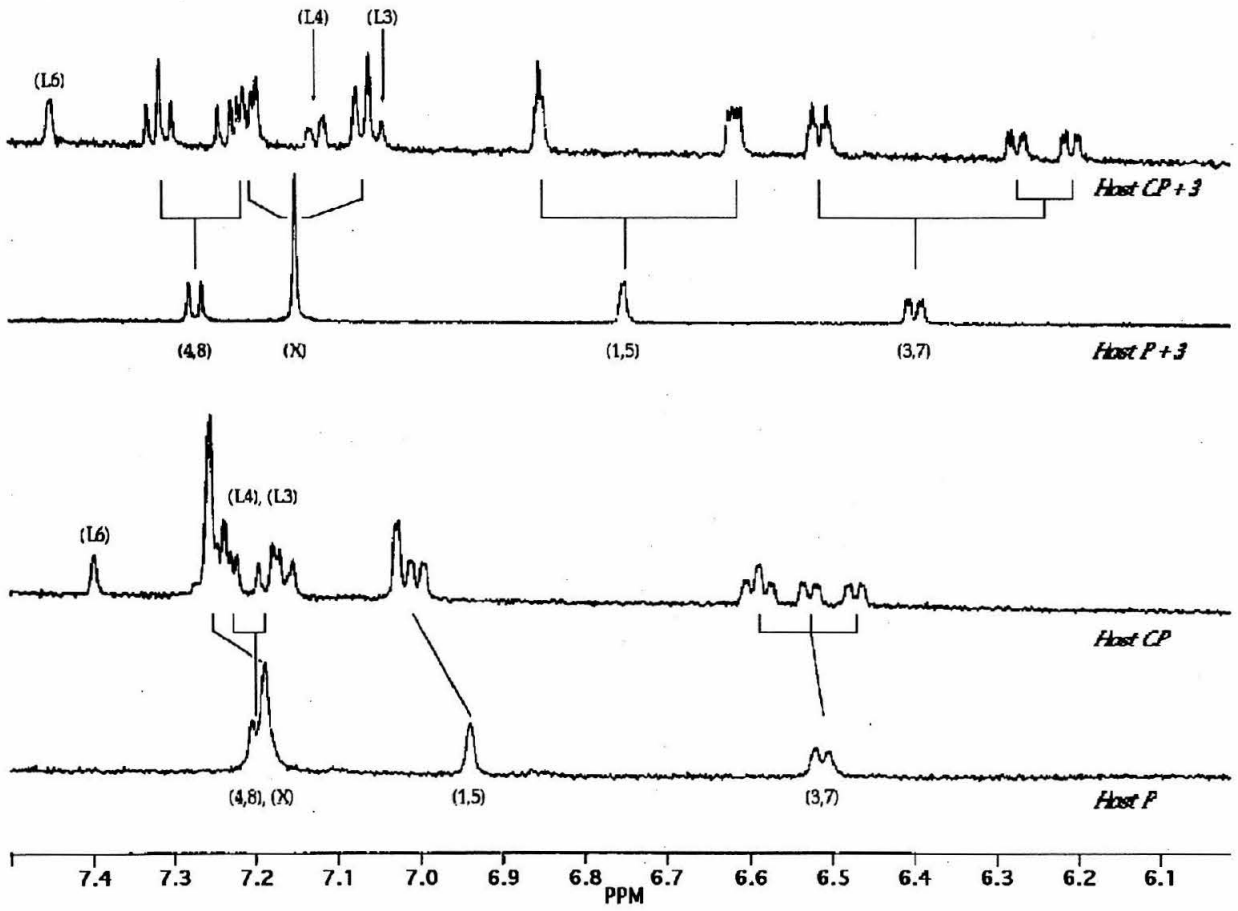


Figure 5.5. ^{1}H NMR spectra of the aromatic regions of P and CP in their unbound states and complexed to N-methylisoquinolinium **3**. Branches between spectra of hosts identify resonances of similar host protons. These protons are identified within the brackets ( ) above and below the spectra according to the labeling scheme outlined in figure 5.6.

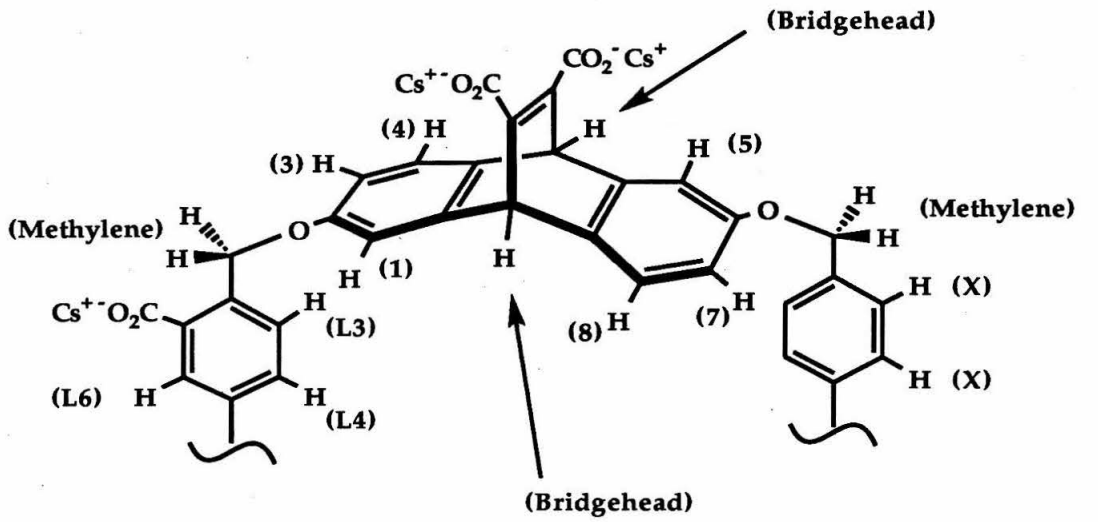


Figure 5.6. Numbering scheme for P and CP macrocycles. The name of each proton is placed next to its position.

of the unbound CP host and for the CP host complexed with the neutral quinoline compounds suggests that they see similar time-averaged chemical environments. In addition, given the similarities in the chemical shifts of these protons to those recorded for the analogous protons of host P, it seems reasonable to conclude that the signals observed in these circumstances are averaged signals of the four possible conformations of the CP host in the rhomboid conformation (figure 5.7). The observed splitting in the signals when iminium compounds such as **3** are complexed, and the centering of such splittings roughly around the positions seen for the time averaged signals of host P suggest that for these guests a subset of the four possible conformations is preferred. In other words, guests that show a definite enhanced affinity for the CP host, also show a definite conformational preference for the CP structure. It should be noted that no such splitting was observed in the spectra of the CP host complexed with the aromatic ring-containing, tetraalkylammonium compounds

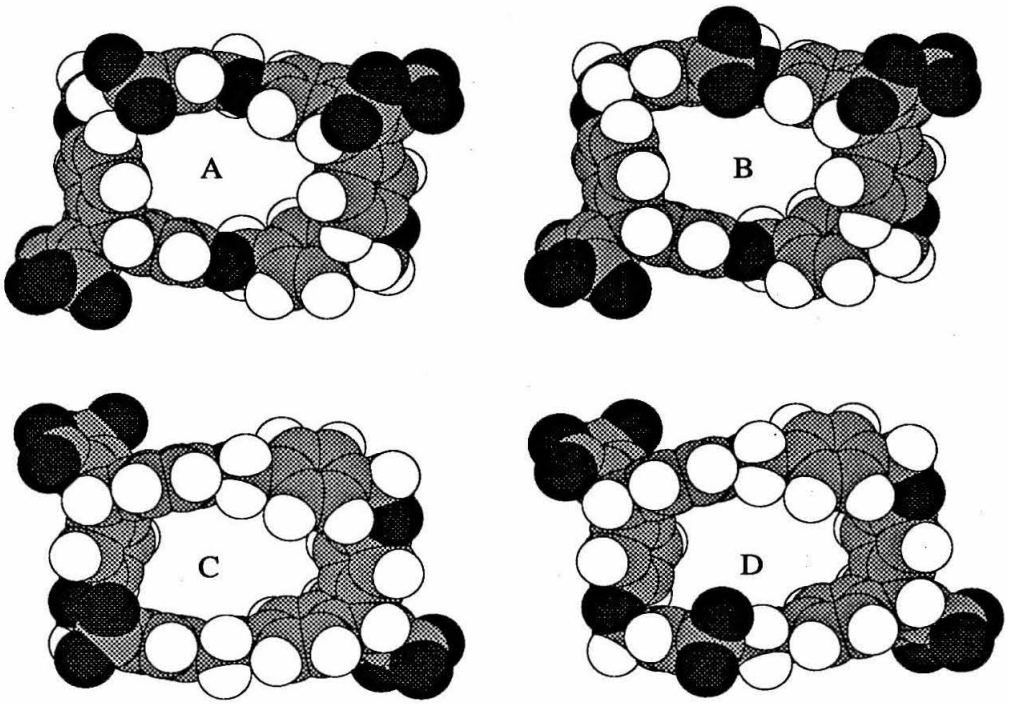


Figure 5.7. CPK representations of the four possible rhomboid conformations of the CP macrocycle. The additional carboxylate of the CP cavity is located in the front of each structure as follows (structure, position): A, left corner on the top half of structure; B, left of center on the top half of the molecule; C left corner of the lower half of the structure; D, left of center lower half of the molecule. Conformations A and B can be converted into conformations C and D respectively through a simple rotation of the aromatic linker. More complicated torsions convert A and B into conformations D and C respectively.

9 and **11**, suggesting that these guests do not impose a conformational preference upon the host.

Electrostatic Potentials. In addition to studies of the host and guest chemical shifts, an investigation of the electrostatic potentials of our guests proved insightful. Using the program Spartan, the AM1 electrostatic potentials were calculated and mapped onto surfaces of total molecular electron density for the guest molecules **3**, **4**, **7**, **14**, and **17**. The AM1 optimized geometries of all guest molecules were used in these calculations. The results of these calculations are shown graphically in Figure 5.8. All values of the electrostatic potential shown in

figure 5.8 are graphed according to the color spectrum determined for N-methylisoquinolinum **3**, with potentials greater than or equal to the maximum value of **3** (122.4 kcal/mol) shown in blue and potentials less than or equal to the minimum value of **3** (73.66) shown in red. This scale was chosen for several reasons. Since we were looking for a correlation between electrostatic potential and enhanced binding, the highest end of the color scale was set to the smallest calculated maximum electrostatic potential value for guests displaying clear enhancements in binding (compound **3**). In this way, the figure is more likely to highlight features common to all such guests. The lower end of the color scale was set somewhat more arbitrarily. The minimum electrostatic potential value of **3** is the third lowest of those shown. The lowest value observed is seen with compound **4**, and is located on the non-methylated nitrogen of that compound with its free lone pair of electrons. Setting the minimum to this value essentially blurs the more typical changes in potential seen for the other guests; as such, it was not selected. Setting the lower limits of the scale to values of either compound **3** or **7**, produced images of similar quality, and it seemed somewhat more prudent to select scale values from a single compound.

As expected, positive values of electrostatic potential were calculated for the all regions of the guest surfaces. However, the guests display significant variation in these potentials both within themselves and in comparison to each other. As can be seen in the figure, the most positive electrostatic potentials are observed on exposed regions of the surfaces of the iminiums **3** and **4** and the guanidinium **14**, guests which displayed substantial enhancements in their binding affinities for the CP host. Guests such as the tetraalkylammonium **7** and guanidinium **17** maintain localized regions of high positive electrostatic potential, comparable to those of **3** and **4**, but position these potentials in less

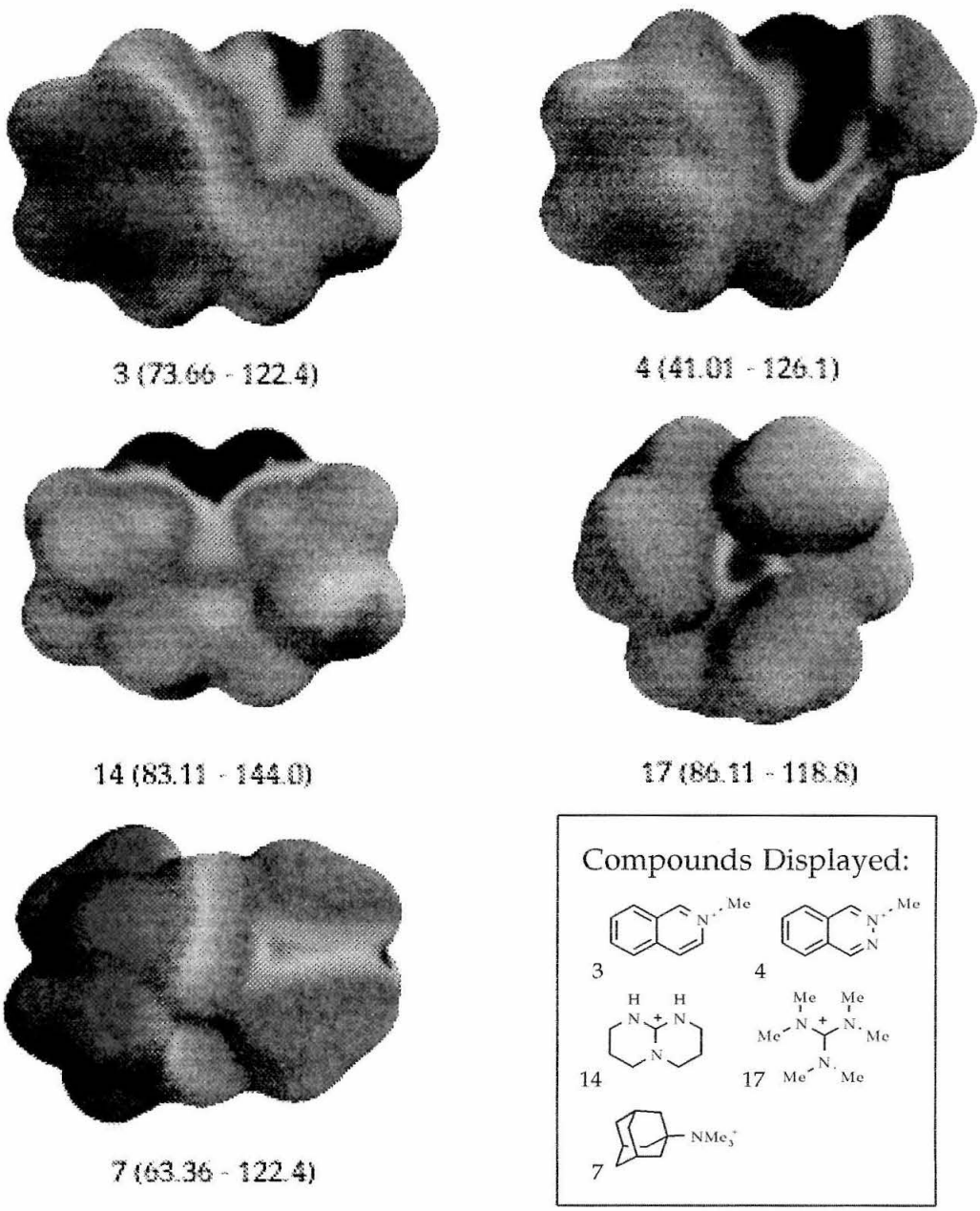


Figure 5.8. Calculated AM1 electrostatic potential surfaces for selected guest molecules. The electrostatic potential surface value spectrum (shown colorimetrically here) has been set for each guest to that determined for compound **3**, ranging from 73.66 (red) to 122.4 (blue) kcal/mol. The range of electrostatic potential values calculated for each guest is reported beneath its individual surface.

accessible regions of their surfaces. Consequently, cations such as **7** and **17** appear more diffuse in nature. The results of these calculations imply that exposed regions of electrostatic potential above a certain large positive value are required to produce an observably enhanced affinity to the CP host. CPK modeling suggests that for guests **3**, **4**, and **14** these regions of high electrostatic potential are ideally oriented, pointing out of the binding cavity up towards the anti lone pairs of the carboxyl group of the CP host. This is illustrated in figure 5.9, in which compound **3** is shown complexed with the CP cavity.

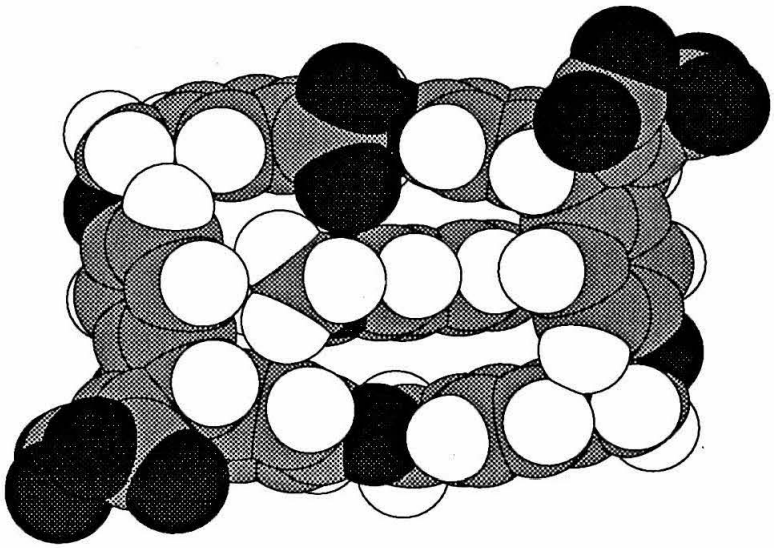


Figure 5.9. CPK representation of the CP host complexed to N-methylisoquinolinium **3**. The additional carboxylate of the host (left of center in upper half of the cavity) is shown here in close contact with the region of the guest calculated to contain the highest electrostatic potential.

Proposed Model. Taken together, the binding data, shift patterns, and AM1 calculations suggest a composite model for enhanced binding to the CP host in aqueous borate. The aromatic rings of the CP host determine the binding orientations of all guests. Once bound, guests with regions of high electrostatic potential pointing up out of the cavity interact favorably with the host

carboxylate, forming a contact ion pair . These additional favorable electrostatic interactions bias the conformations of the CP host. This model implies a specific criterion for enhanced binding in aqueous media through electrostatic interactions between organic, well solvated, charged guests and carboxylate functionalities.

This model does not explain why all positively charged guests do not display enhanced binding. Charged compounds are considerably solvated in this medium, and given the high dielectric of the aqueous borate buffer, any electrostatic interaction between charged compounds will be greatly attenuated. It is quite possible that in this medium, two diffuse charges (such as the guests and the carboxylate moiety of the CP host) will interact significantly only when forced into close proximity to one another. This hypothesis is supported by the inability of the carboxylate to influence the binding conformation of the guest molecules in our system. In addition, such interactions will only be favorable if they compete well against the energetic price of desolvation of the charged moieties. The regions of less positive electrostatic potential available to the carboxylate with tetraalkyammonium compounds **7-13** and guanidinium **17** should result in less favorable electrostatic interactions than observed with guests **3** or **14**. Such guests may not compete well against the desolvation of the carboxylate, and prefer to surround their charged functionalities with the aromatic rings of the host structure, forming solvent-separated ion pairs instead. No enhancement in binding is seen since one anionic counterion (borate) has simply exchanged for another (the carboxylate of CP).

Such an explanation is consistent with the binding data recorded for the trimethylammonium guests in figure 5.1. Of these guests studied, only **9** and **13** appear to show slight enhancement for the CP cavity. Guest **13** contains a second

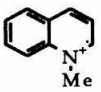
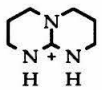
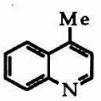
trimethylammonium group, which by necessity must protrude out of the binding cavity, and make close contact with the carboxylate of CP. Guest **9** was designed to appear more like the iminium **3**, forcing part of its tetraalkylammonium functionality to lift up and out of the cavity and into contact with the carboxylate. For both guests, the increases in binding affinity for CP were less than those observed with the iminium and guanidinium compounds, as might be expected from the electrostatic potential calculations.

Alternatively, one might suggest that close contacts are formed between the carboxylate and the tetraalkylammonium guests, but that increased stabilization is offset by a decrease in the entropy of the host system due to conformational preferences of the guest. However, as mentioned previously, no evidence for conformational preference was found for the aromatic tetraalkylammoniums examined. In addition no increase in binding with the CP host was seen for guest **7**. This compound binds with our host systems in the toroidal form. While the rhomboid conformation of the CP host has four diasteriomeric conformations, the torroidal form has only two. The achiral **7** should thus have to pay a smaller penalty upon binding (of about 0.4 kcal/mol at 298 K) since only one of two diasteriomeric conformations will be preferred.

Binding Studies in 10% Acetonitrile Solutions. To better interpret our aqueous results, a series of binding studies were performed in a solvent mixture of 10% acetonitrile / 90% borate-d buffer (v/v). This solvent mixture is approximately 4M in acetonitrile, and as such, the solvent environment is presumably substantially altered. Effects due to the solvation of the ionic species could be considerably different here. Furthermore, the lower dielectric of acetonitrile should slightly decrease the attenuation of electrostatic interactions

seen in purely aqueous solutions. The results of these studies can be seen in table 5.1.

Table 5.1. - $\Delta G°_{298}$ (kcal/mol) for Binding to Hosts CP and P in 10% MeCN / Borate

Guest	Host CP	Host P
 **3**	5.4	4.9
 **6**	6.2	5.8
 **7**	4.6	4.1
 **14**	3.8	< 3.5
 **25**	3.6	3.5

The neutral compound lepidine (**23**) again shows an equal affinity for both cavities. However, all of the positively charged guests examined displayed an enhanced affinity for the CP host in this mixed solvent. Iminium compounds **3** and **6** display similar enhancements to those seen in water (0.5 vs. 0.6 kcal/mol for **3**, and 0.4 vs. 0.3 kcal/mol for guest **4** in 10% acetonitrile and borate solutions respectively). In contrast to its binding properties in water, adamantyltrimethylammonium **7** exhibits a 0.5 kcal/mol preference for the CP cavity in 10 % acetonitrile. The relative upfield chemical shift patterns of **7** with both P and CP were virtually identical to those observed in water, implying that no changes in the structure of its host-guest complex had occurred. When

complexed to iminium compounds, the CP host again displayed large splittings in the chemical shifts of its aromatic protons.

The failure to observe enhancements greater than those seen in borate for all of the charged guests supports the hypothesis that alkyliminium and non-peralkylated guanidinium compounds interact with the CP host in a different fashion than tetraalkylammonium compounds. The greater organic content of the medium could have been expected to increase the stabilization of solvent-separated ion pairs. The enforced contact between the carboxylate of the CP host and the iminium or guanidinium guests, might be expected to benefit to a lesser extent, as the interactions between the two units remain largely unchanged.

Hydrogen Bonding. We had wanted to examine the role that hydrogen bonding might play in binding of guests to the CP macrocycle. To this end, the binding properties of the neutral guests **24** and **25**, ammonium guests **9, 18, 19,** and **20,** and the guanidinium guests **14-17** were determined in borate with the P and CP hosts. As mentioned previously, the weak hydrogen atom donors on the guests **24** and **25** appear to make a negligible impact upon complexation.

The guest series **9, 20** and **19,** and **14, 15, 16,** and **17** were chosen in hopes of seeing a consistent increase in binding enhancement for CP as the number of hydrogen binding sites increased. Binding studies with the ammonium compounds showed mixed results. A slight enhancement was seen for both tetrahydroisoquinoline hydrochloride **19** and the dimethyl compound **9,** whereas no improvement was seen with guest **20**. The results most likely reflect the protonation states of the guest molecules. The pKa of **19** has been measured at 9.4,[57] and thus our results probably reflect the effects of charged and uncharged guest competing for the binding site of CP. Recall that the pH of our buffer is 9.0.

Given that primary and secondary amines enjoy greater basicities in water than tertiary amines, one would expect the pKa of compound **20** to be somewhat lower. As such, its affinity for the CP cavity possibly represents the binding of an essentially neutral compound. A similar argument can describe the results with compound **18**. No evidence of a host-induced change in the pKa values of the ammonium compounds studied was found. Overall, these results are unsatisfying, and studies at a lower pH are warranted.

The studies of the guanidinium compounds **14-17** should avoid the basicity problems seen with the ammonium compounds. The pKa values of each of the partially alkylated compounds reported should be greater than 11.[57] As described previously, only the compound **17**, hexamethylguanidinium, displayed no increase in binding to CP. The compound **14**, which places two hydrogen bonding elements what would appear to be an ideal orientation for hydrogen bonding to the carboxylate. Smaller enhancements were seen for compounds **15** and **16**, which present only a single hydrogen to the carboxylate of CP upon binding.

While it may be tempting to attribute these results to hydrogen bond formation, it should be noted that these results are well described by the general model in which compounds presenting regions of greater electrostatic potential are bound to a more appreciable extent. The results of AM1 electrostatic potential calculations (not shown in figure 5.8) [†] suggest that compounds **15** and **16** should present regions of electrostatic potential to the carboxylate roughly equivalent to that presented by compound **3**. These compounds, **3**, **15** and **16**,

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[†] The ranges in electrostatic potential calculated for these guests were (77.64 - 123.7 kcal/mol) for compound **15** and (84.07 - 128.6 kcal/mol) for compound **16**. Like compound **14** the regions of highest electrostatic potential were centered on the N-H hydrogen atoms.

exhibit similar enhancements in binding energy. The results suggest a general electrostatic effect. However, if the guest regions of highest electrostatic potential (located on the N-H hydrogen atoms of the guanidinium compounds **14, 15,** and **16**) do form close contacts with the accessible CP carboxylate, this may in essence be considered a hydrogen bond, a primarily electrostatic interaction in nature. Further experimentation is required.

Implications for Natural Receptors. The implications of our studies with the P and CP hosts for the binding of charged molecules by natural receptors are perhaps best considered in terms of the binding data for the guests **3** and **7** collected in various solvents, shown in Table 5.2. Data for binding in chloroform were collected using the tetramethyl ester of host P.† Several insights can be discerned from the table. Firstly, the addition of an additional negative charge isn't necessarily beneficial for the binding of charged guests. Compound **7** and similar compounds displayed no improvement in binding in purely aqueous media. Secondly, given that some guests, such as **3**, do show increased affinity for the CP host in this solvent, it can be concluded that the specific properties of the individual guests matter. The guests need to be considered as more than point charges. Thirdly, the affinity of **7** in 10% acetonitrile solution for the CP host demonstrates that the solvation of the species involved must be considered. In this system, the increase in binding for **7** was displayed only as the solvent became more disordered. Finally, it should be noted that for P and CP, the binding affinities of all guests dropped as the solvent became more organic in composition. In other words, guest complexation with the aromatic rings of our host systems becomes less favorable as the hydrophobic components to binding are removed.

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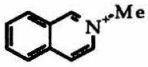
† Taken from reference 11d.

Table 5.2. $-\Delta G°_{298}$ (kcal/mol) of Guests **3** and **7** to Hosts P and CP in Various Solvents

Host	Solvent	 3	 7
P	Borate	7.2	6.7
P	10% CD_3CN in Borate (v/v)	4.9	4.1
P (tetramethylester)	$CDCl_3$	2.5	2.1
CP	Borate	7.8	6.6
CP	10% CD_3CN in Borate (v/v)	5.4	4.6

The last point is perhaps the most significant in discussing the biological binding sites for ammonium compounds. Many of these binding sites, as demonstrated for AChE[31] and suggested by modeling studies for the aminergic GPCRs,[32] lie at the bottom of deep clefts. As such, the guest compounds for these structures may visit several apolar sites along the way to their final destinations within these molecules. Consequently, the aromatic residues of the binding sites must compete for the guests not only with the solvent but with these alternative sites within the protein structure. As suggested by the decreased binding affinites of our hosts as the polarity of the solvent decreases, the aromatic residues of the natural sites may not compete effectively on their own against alternative environments.

The positioning of a carboxylate group may help the aromatic binding sites compete more effectively for substrates against sites composed of apolar residues, oriented dipoles, etc. The introduction of a poorly solvated carboxylate may introduce ion pairing as a means of effectively delivering the cationic

species to the proper location quickly upon its removal from aqueous medium. Such an arrangement for GPCRs and acetylcholine ligand gated ion channels would insure that the agonists reached their appropriate locations rapidly, and were held there until the changes in the conformational structure of the proteins relayed their appropriate signals. It is interesting to note that of the two carboxylates on the δ subunit thought to be located at the binding site of AChR, one of these is conserved in the many of the reported sequences of subunits for GABA gated ion channels,[58] and neither are conserved in several reported sequences of glycine receptors.[59] One can speculate that ion pairing may be necessary for the binding of GABA as well, but that glycine, which may serve as its own ion-pair, does not require pairing with the side chains of its receptor's subunits.

It is not being argued here that the presence of carboxylate groups does not aid in the complexation of guests pulled directly from aqueous media. However, our work demonstrates that high guest specificities can be achieved solely through contacts made with aromatic compounds. In addition, given the observation that oriented dipoles are usually preferred over countercharges in the stabilization of Arg, Lys, Glu, and Asp side chains buried at the intermolecular interfaces of oligomeric systems,[60] the necessity of a counter balancing carboxylate for this purpose is somewhat questionable.

In fact, a carboxylate at the binding AChE binding site could actually hinder that protein's overall function. It was mentioned at the beginning of this chapter that modeling implies contacts can be made between acetylcholine and Glu^{199} of the AChE. However, the crystal structure of the esterase hints that this side chain is most probably hydrogen bonded to the Glu^{443} side chain.[32] Glu^{199} is located in a hydrophobic environment, and mutation to glutamine showed little effect on

the kinetic parameters of the system.[61] All of these factors suggest that this residue may be uncharged. If the acetylcholine substrate were to experience an increase in stabilization from the carboxylate, our results with guest **7** in 10% acetonitrile suggest that upon hydrolysis, Glu^{199} may hinder the removal of the choline product.

Conclusions

Our studies with CP by no means provide definitive answers about cationic binding by natural receptors. However, it is hoped that these results might provide new ways of looking at such systems. We have described here the results of studies in which we examined the effects of a carboxylate group placed on the periphery of an aromatic cavity. Our results show that this addition had a minimal impact on the solvation of the cavity, and that complexation of this host by cationic guests was dominated largely by hydrophobic and cation-π interactions. Not all of the charged guest molecules displayed an enhanced affinity for the host in aqueous media, and enhancements seen were small compared to the overall binding affinities. The results suggest that the remote carboxylates of the host play a limited role in binding and that specific contact between the introduced carboxylate and the guests is a requirement for binding enhancement. Furthermore, AM1 calculations of guest electrostatic potentials indicate that increased binding for charged guests is dependent upon the presence of an exposed region of high electrostatic potential. The results suggest the formation of contact ion-pairs between the guest and the additional host carboxylate may be responsible for the increases in binding observed.

Chapter 6: More is Better. The Effects of Additional Aromatic Rings on the Binding of Cationic Guests by Cyclophane Receptors.

Introduction

Our work with host P (figure 6.1) has demonstrated the effectiveness of aromatic rings in the stabilization of positively charged compounds in aqueous and organic media.[11] As suggested by its structure, host P surrounds guest molecules with a necklace of aromatic rings, whose negatively charged faces interact favorably with relatively hydrophobic cations (tetraalkylammonium, alkyliminium, and guanidinium compounds, etc.). However, upon complexation with the monocyclic host P, a significant percentage of the surfaces of these cationic guests remains exposed to solvent. Many of these exposed regions could possibly experience significant stabilization through cation-π , aryl-aryl T-shaped stacking, and hydrophobic interactions, etc., if additional aromatic rings were to be placed above and below the plane of the binding cavity. In this chapter we discuss the results of our initial effort at providing "expanded coverage" of the complexed guest molecules, using the D3 macrocycle[†] (figure 6.1). The structure of D3 differs from that of our basic host P in two respects. Firstly, another ethenoanthracene unit has been added to provide two additional aromatic recognition elements. Secondly, the three ethenoanthracene units of D3 are joined to the aryl linking groups at positions *meta*, as opposed to *para* to each other as in host P. As such, the D3 host is more accurately described as a modification of the host M (figure 6.1).

We were interested in the deeper, larger cavity of D3 because it seemed well suited for the binding of guests such as adamantyltrimethylammonium **1** and N-methylquinolinium iodide **2** (see figure 6.2), guests well bound by hosts P and M. CPK modeling suggested that the chiral structure of D3 might also

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[†] The D3 structure is so named after its D_3 conformations of highest symmetry.

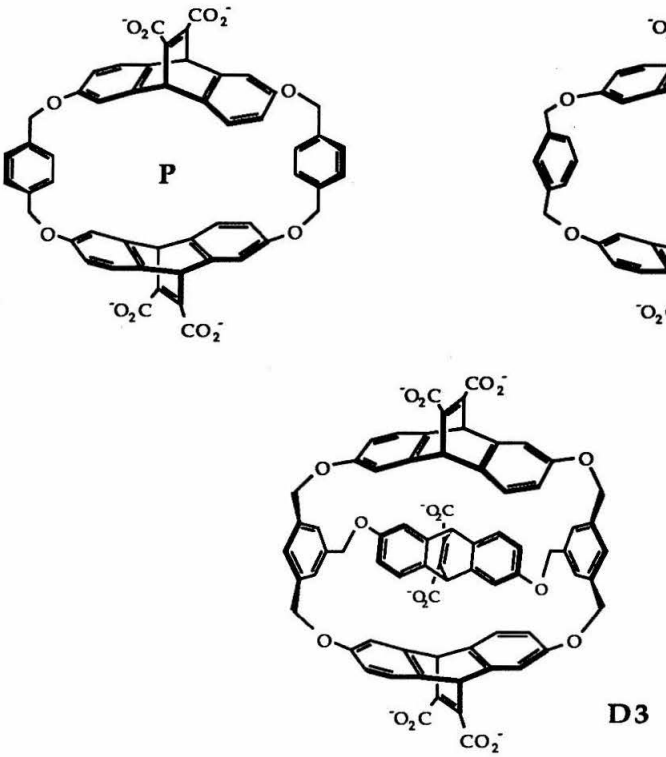
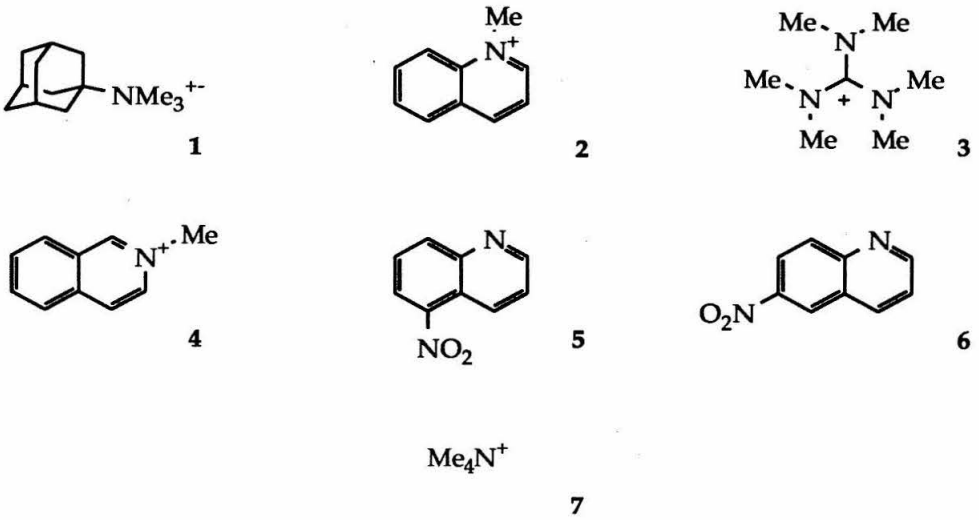


Figure 6.1. Host Structures

display a greater enantioselectivity than host P in the binding of the chiral D_3 conformations of hexamethylguanidinium **3**. Furthermore, the additional ethenoanthracene unit of D3 forms a bridge across the binding cavity of M, blocking access and egress of guest molecules to and from one side of the binding cavity. As such, it was suspected that the D3 bicycle would display altered kinetics of binding, possibly moving from the fast to the slow exchange regime on the ^{1}H NMR timescale.†

† Overall the D3 cyclophane bears a strong resemblance to fascinating structures recently reported by Collet et al. in reference 62. Collet's molecules form well defined, relatively rigid cavities that bind strongly to tetramethylammonium compounds (through cation-π interactions) in both chloroform and aqueous solutions. The binding is observed to be slow on the ^{1}H NMR timescale.

Figure 6.2. Guest Molecules



Results and Discussion

As mentioned in chapter 3, the D3 macrocycle aggregated at concentrations below 80 μM in borate buffer, too low to be effectively used in ^{1}H NMR binding titrations. This observation is somewhat surprising, given the six water-solubilizing groups of the structure. It was thought that the D3 host would be more freely soluble in our aqueous borate medium than the M and P cavities, since it contains a higher ratio of water-solubilizing ethenoanthracene units to hydrophobic linkers (3:2 as opposed to 1:1). The increased size of the hydrophobic surfaces of the molecule or its slightly more rigid structure as compared the M or P hosts may be responsible; we can only speculate. In any case, the aggregates dispersed upon the addition of 10% acetonitrile (v/v) to the D3 solution† so a number of binding studies were performed in this medium.

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† This solvent system of 10% acetonitrile, 90% borate-d (pD ~ 9) was chosen because of the large number of binding constants determined for host P in this mixture. See chapter 4.

Tetraalkylammonium compounds (**1** and **7**), alkyliminium compounds (**2** and **4**) and neutral aromatic compounds (**5** and **6**) were used as substrates. The binding affinities for the selected guest molecules with hosts D3, P and M[†] in 10% acetonitrile / 90% borate solutions (v/v) were determined by ^{1}H NMR. The results are shown in table 6.1. Extreme line broadening of the guest resonances in solutions of D3 made measurements with this host difficult.[††] In many of the spectra taken, this broadening precluded the observation of guest signals altogether.[†††] Fortunately, changes in the chemical shifts of the D3 host protons proved suitable for study. For all guests the 3,7 and the 4,8 host protons moved substantially downfield upon binding[††††]. (See figure 6.3 for labeling scheme.) Negligible shifting, both up- and downfield, was observed for the 1,5 and bridgehead protons. The linker protons moved substantially downfield with the tetraalkylammonium guests, but negligibly with guests **2**, **4**, **5**, and **6**. No line broadening problems were encountered with either the P or M cavities.

A comparison of the D3 and M binding data clearly indicates a strong preference for the D3 cavity by all guest compounds examined. For guests **2** and **4** the enhancements in binding are substantial (1.7 and 2.1 kcal/mol respectively), and represent some of the largest enhancements we've observed

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[†] Binding data for host M was generously provided by fellow group member Laura S. Mizoue.

[††] This general line broadening phenomenon as well as the use of the mixed solvent system precluded any meaningful study of compound **3**.

[†††] Host concentrations in these experiments varied from approximately 200 μM to 150 μM through dilution with guest stock solutions. Guest concentrations varied from 20 μM to approximately 750 μM over the course of the experiments.

[††††] This downfield shifting may be indicative of a partially collapsed conformation of the D3 host in its unbound state, in which the aryl linker groups of the molecule have rotated so as to partially fill its interior cavity.

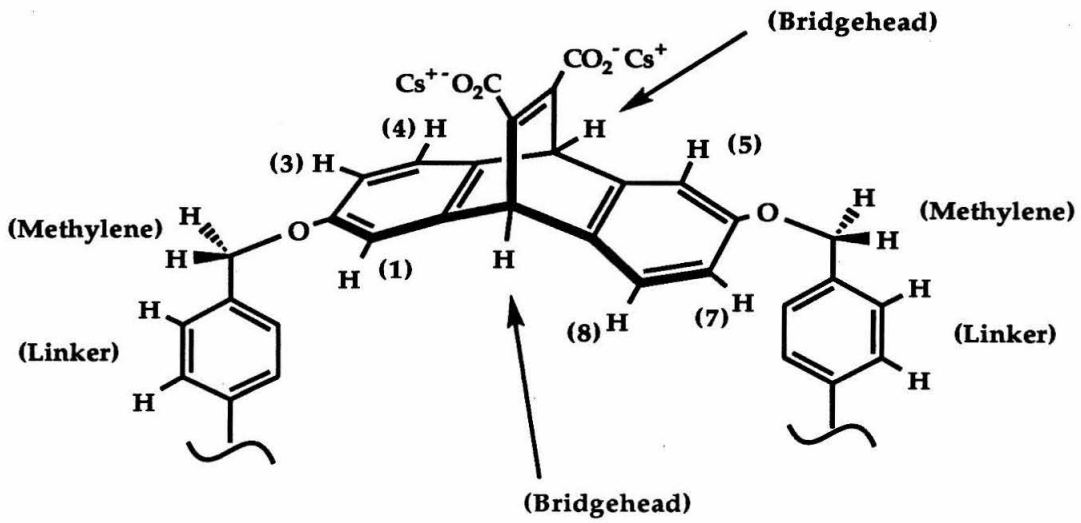
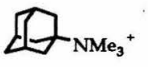
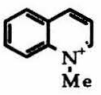
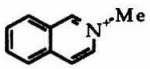
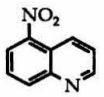
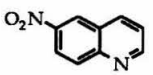


Figure 6.3. Numbering scheme for D3, P, and M. The name of each proton is placed next to its position.

with any of the new host structures presented (see chapters 1, 4, and 5). In this respect, the modification of the host structure has been highly successful, as additional recognition elements have clearly enhanced binding. Comparing host P with D3 is more difficult. A greater affinity for alkyliminium compounds is again seen for host D3. However, as evidenced by the binding data for the neutral aromatic compounds, the enhancement does not appear to extend to all naphthalene-sized guests. Of the tetraalkylammonium compounds, **7** displays a preference for the D3 cyclophane; **1** does not. It is tempting to attribute the enhanced binding of alkyliminium compounds with D3 to additional cation-π interactions between host and guest. It is also tempting to suggest that a greater penetration of the charged portion of **7** into the interior of the D3 cavity may be responsible for its enhancement as compared to **1**. However, the limited sampling of guests and the lack of guest shift data, which provide structural insight, do not allow for definite conclusions to be reached at this time.

Table 6.1. - $\Delta G°_{298}$ (kcal/mol) for Binding to Hosts D3, P, and M in 10% MeCN / Borate

Guest		Host D3	Host P	Host M
	**1**	4.0	4.1	< 3.5
	**2**	6.9	5.8	5.2
	**4**	6.9	4.9	4.8
	**5**	4.2	4.3	---
	**6**	4.8	4.6	---
Me_4N^+ I^-	**7**	3.8	< 3.5	---

The binding data and the dramatic line broadening observed for guests complexed with D3 do suggest that the on/off rates in this system have been considerably slowed by the additional ethenoanthracene unit. In systems like hosts M and P, guest exchange between the free and bound states is rapid, and the chemical shift values observed throughout the course of a binding experiment are weighted time-averaged signals of both free and bound states. In these systems, increased signal broadening is often observed for guest protons as the binding affinity of the guest increases. Assuming the on rate to be diffusion controlled[5] for all guests complexed to P, the line broadening is a direct result of a decreasing off rate that approaches the frequency difference between free and complexed species ($\Delta\nu$).[63] A decrease in the off rate may account for the guest-proton line broadening seen with the D3 system as well. However, if we assume similar changes in $\Delta\nu$ are observed for the guest

protons with both hosts, then given that several guests show equal affinities for the P and D3 cavities but only line-broaden with D3, any decrease in the off rate must be accompanied by a decrease in the on rate as well.

Conclusions

We have appended additional aromatic rings to our basic host structure and shown that significant enhancements in binding can occur by creating more extensive and favorable contact between host and guest species. In addition, we have shown that this modification has a definite impact on the kinetics of binding, moving the modified host structure closer to the ^{1}H NMR slow exchange regime. The poor water solubility of the D3 host and the added difficulty of extracting guest structural information pose drawbacks to the system. The system should be viewed positively, however, perhaps as a starting point for future designs.

Chapter 7: Synthetic Leads for New Host Structures.

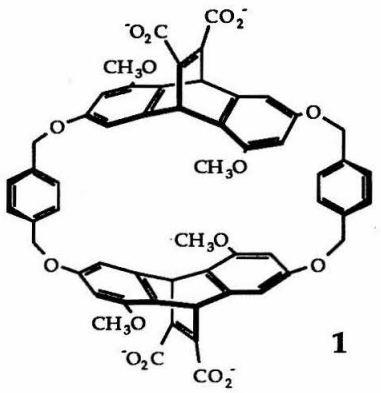
Introduction

In addition to the OMP, TMP, TBP, TMTBP, CP, and D3 hosts presented in this thesis, attempts were made at building several other host structures. Synthetic difficulties often halted the production of these molecules. In several instances methods were discovered around these difficulties. However, by the time these solutions were discovered, either different hosts were already in later stages of development or the reasons for building the structures had evaporated. This chapter documents some of these synthetic methods, in hopes that they may be useful to other members of the group in the future.

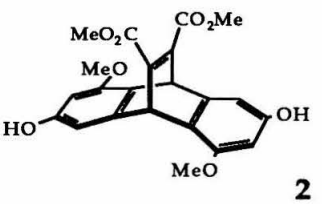
Discussion

Development of a Second TMP Host. In chapter 4, the results of our efforts to enhance cationic guest binding through the addition of methoxy groups to the structure of host P were discussed. The OMP and TMP structures examined did not enhance the binding of any guests relative to P, due the stabilization of collapsed states in these molecules One possible way to prevent this collapse would be to place the methoxy groups on the ethenoanthracene subunits of the host. These subunits are less flexible than the linker regions of our hosts. As such, the appropriate positioning of methoxy groups on these regions of the host might have a less dramatic impact on the conformational flexibility of the structure. The host structure **1** was designed. The methoxy groups of **1** replace the 4,8-protons of the parent host P, largely to avoid the conformational problems that might arise from a substitution *ortho* to the benzyl ether. (See chapter 4.) In keeping with our general synthetic scheme for the production of host molecules (chapter 2), the synthesis of **1** requires the construction of the ethenoanthracene

unit **2**. This molecule in turn depends upon the successful synthesis of 1,5-dimethoxy-3,7-dihydroxyanthracene **3** (figure 7.1).



1



2

1,3,5,7-Tetrahydroxyanthraquinone[64] **4** (figure 7.1) was chosen as the starting point for this synthesis. This compound is readily available in multi-gram quantities from the condensation of 3,5-dihydroxybenzoic acid in concentrated sulfuric acid. The procedure for making this compound is found in the experimental section. By taking advantage of steric influences and the differing acidities of the two types of hydroxyl groups in the structure of **4**, we hoped to selectively protect the 3,7 positions in this molecule. This protection would be followed by methylation at the 1,5-positions, deprotection of the 3,7-positions, and reduction to the anthracene **3**.

Selective protection of the at the 3,7-positions of **4** was easily accomplished using *tert*-butyldimethylsilyl chloride in the presence of triethylamine in DMF to produce **5** (figure 7.1). The procedure used to generate this species is recorded in the experimental section. Sadly, however, all attempts at the methylation of **5** failed. Removal of the silyl protecting groups and methylation at all positions was the general result of these efforts. Initial protection of the 3,7 positions was also accomplished with benzyl bromide, producing the compound **6** (figure 7.1).

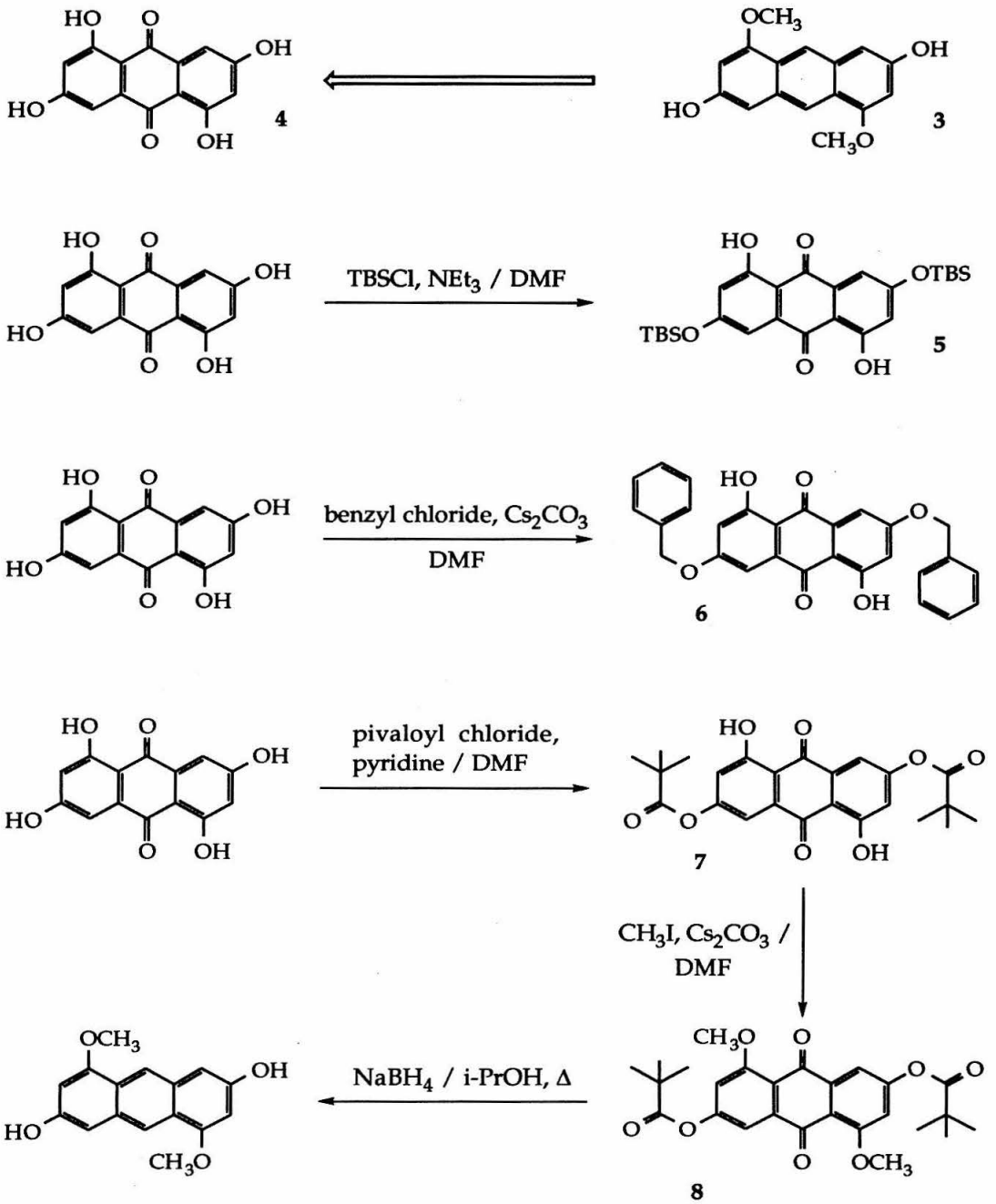
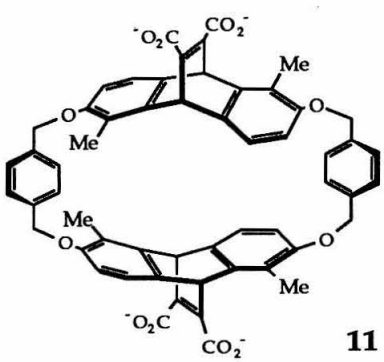


Figure 7.1. Attempted Methods for Producing Compound **3** from **4**

However this compound was not generated cleanly, and this approach was abandoned.

Much later it was discovered that compound **4** could be selectively protected using pivaloyl chloride to produce compound **7** (figure 7.1). Methylation of this compound with iodomethane in DMF using cesium carbonate as a base produced the compound **8**. It was found that **8** could be reduced directly to the anthracene **3** with sodium borohydride in refluxing isopropanol. The procedures to produce **7**, **8**, and **3** are recorded in the experimental section of this chapter. Further work on this project was halted by the advanced stage of the author's graduate career and by the development of the CP host.

General Reactions with 2,6-Dihydroxyanthracene. The synthesis of the TBP host was made possible by the synthesis of 1,5-dibromo-2,6-dihydroxyanthracene **9** from 2,6-dihydroxyanthracene **10** (figure 7.2). Electrophilic substitution reactions on anthracene usually favor the 9,10-positions. However, the 1,5-positions of **10** are competitive with its 9,10-positions because of the strong electron-donating abilities of the hydroxy groups.



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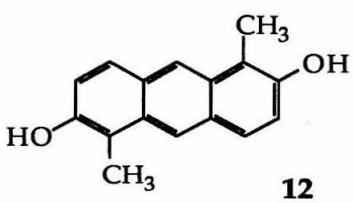


12

Our initial investigation of the TBP host generated some erroneous binding data (not presented here!) that suggested a unique role for bromine atoms of that molecule. The poor quality of the data was later revealed, but not before it had

launched an effort at producing the tetramethyl-substituted host structure **11**, by way of the anthracene **12**. The synthesis of this molecule was to proceed by using the unique substitutions afforded dihydroxyanthracene.

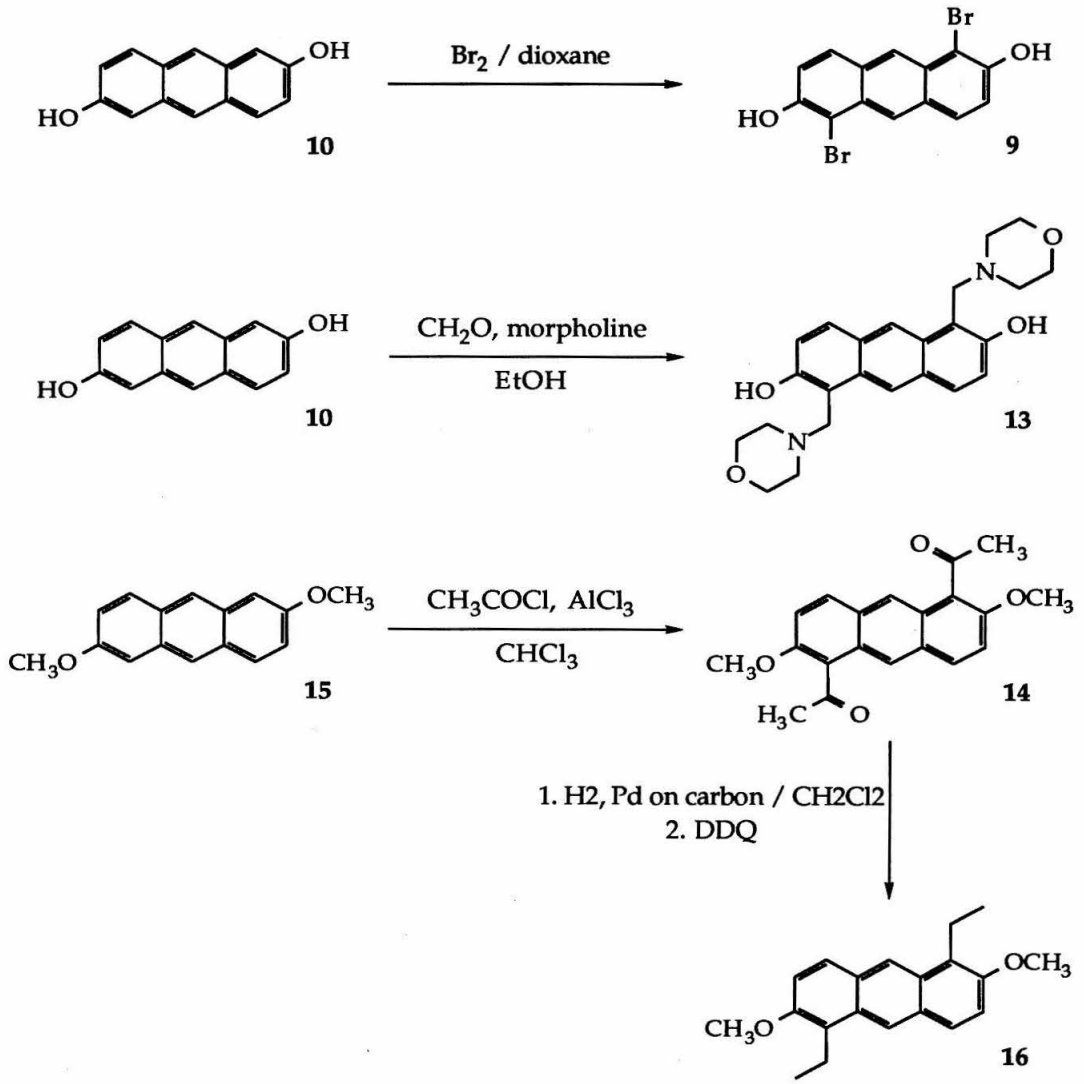
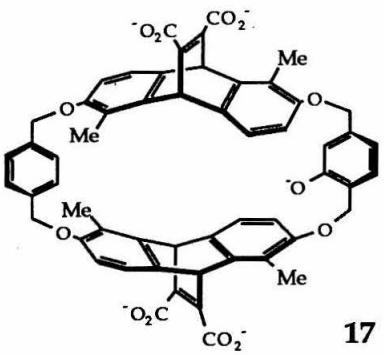


Figure 7.2. Some Miscellaneous Reactions of Anthracene Compounds

One reaction that proceeded well was the reaction of **10** with formaldehyde and morpholine in ethanol. This procedure cleanly generated the bis-substituted compound **13** (figure 7.2). The subsequent routes to producing the desired **12**

using this compound didn't pan out. However, the generation of **13** itself suggests the viability of facile substitution at the 1,5-positions of **10**. The procedure for its generation is located in the experimental section of this chapter.

Acylation can also be performed at this position. Compound **14** was generated from 2,6-dimethoxyanthracene **15**, using acetylchloride and aluminum trichloride (figure 7.2).[65] Compound **14** was then reduced to the diethyl compound **16**. A procedure for this reduction is recorded in the experimental section. The deprotection of this compound to afford the dihydroxy compound was halted after the poor quality of our original data was exposed.



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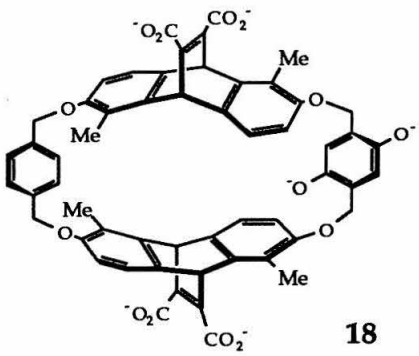


18

Phenolic Host Structures. Before the CP host was synthesized, efforts to place additional charge on the basic structure of P centered on the construction of the hosts **17** and **18**. Several dihalomethyl linkers **19-23** (figure 7.3) were reacted with the ethenoanthracene unit **24** in DMF, using cesium carbonate as a base. None of these efforts were successful. In general, either the linkers fell apart under the conditions of the macrocyclization, or, as in the case of the quinone linkers, were susceptible to Michael additions.

Nitroveratryl ethers[66] might be used to successfully generate these host structures. As benzyl ethers these groups should be stable under typical macrocyclization conditions. These groups can also be easily removed by

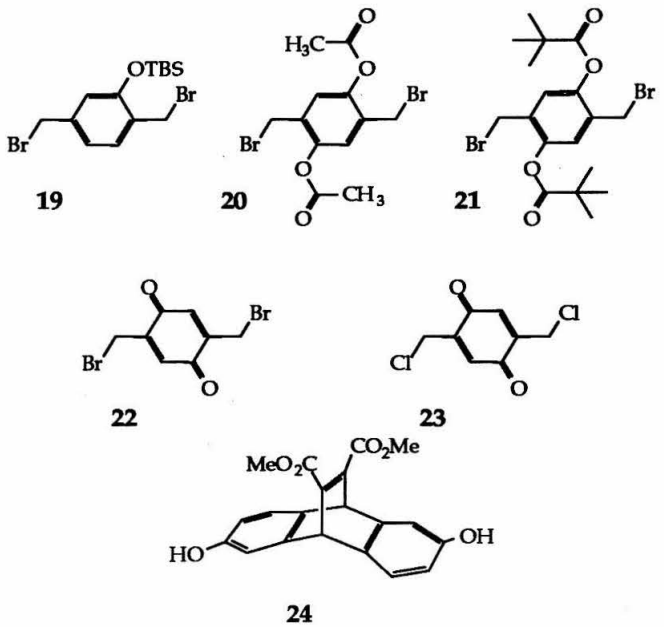


Figure 7.3. Some molecules **19-23** that do not macrocyclize with compound **24** using Cs_2CO_3 in DMF.

photolysis[66] after workup of the macrocyclization. The linker **25** (figure 7.4) was designed to produce the host **17**. It is thought that **25** can be constructed from 2,5-bis-(hydroxymethyl)phenol **26**. Reaction of **26** with nitroveratryl chloride[67] **27** could produce the intermediate **28**. Reaction of **28** with mesityl chloride should produce the desired compound **25**. A test reaction of 2-(hydroxymethyl)phenol **29** with **27** in DMF using cesium carbonate as a base proceeds cleanly to produce **30** (figure 7.4) . The success of this test reaction bodes well for the synthesis of the linker **25**. Again however, the advanced stage of the author's graduate career and the development of the CP host prevented the further pursuit of this approach.

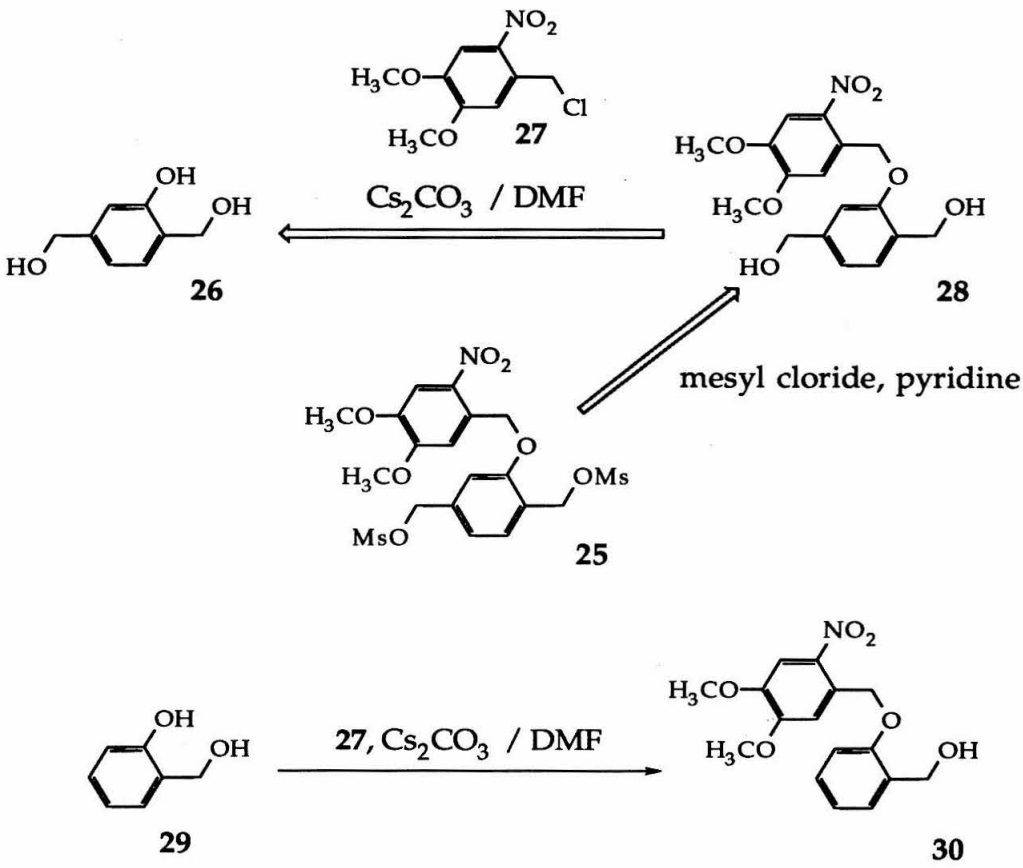


Figure 7.4. Toward Building Compound **25**

Select Experimental Procedures

1,3,5,7-Tetrahydroxy-9,10-anthraquinone (4). The compound was synthesized using a modified version of the procedure reported by Barth and Senhofer in reference 64. 3,5-Dihydroxybenzoic acid (20.0g, 0.13 mol) was placed in a three-necked round-bottomed flask fitted with a stopper, a reflux condenser and a thermometer. Concentrated aqueous sulfuric acid (45 mL, 0.82 mol) was added, and the reaction mixture was heated to 120 °C. The reaction mixture was held at this temperature for one hour with vigorous stirring. The reaction solution was poured over 500 mL of an ice/water mixture. After the resulting

precipitate had settled, the acidic liquid was decanted away as best possible. The resulting thick slurry was neutralized with sodium bicarbonate. After neutralization, the precipitate was again allowed to settle and as much of the liquid phase decanted off as possible. The solid was isolated by filtration (a slow process given the fine particulate nature of the precipitate), washed with water, collected and dried in an 80 °C oven. The blackish-green solid was ground with a mortar and pestle and recrystallized from a 1:1 mixture of MeOH:DMF at 63 °C to yield a yellowish-green powder (7.04 g, 0.03 mol, 51%): ^{1}H NMR (DMSO-d_6) δ 12.63 (s, 2H), 11.30 (s, 2H), 7.06 (d, J = 2, 2H), 6.48 (d, J = 2, 2H).

3,7-Bis(*tert*-butyldimethylsiloxy)-1,5-dihydroxy-9,10-anthraquinone (5). 1,3,5,7-Tetrahydroxy-9,10-anthraquinone (1.00 g, 3.68 mmol, 1.0 equiv.) was added to a 250 mL three-necked round-bottomed flask fitted with an addition funnel, a septum and an argon gas adapter. The flask was purged with argon, and the anthraquinone was dissolved in 50 mL of anhydrous DMF. Triethylamine (1.04 mL, 7.47 mmol, 2.02 equiv.) was added to the flask, which was subsequently chilled to -20 °C. *tert*-Butyldimethylsilyl chloride (1.13 g, 7.47 mmol, 2.02 equiv.) in 20 mL of anhydrous DMF was placed in the addition funnel and added to the anthraquinone solution dropwise over one hour. The solution was warmed to room temperature and stirred for another eight hours. The resulting precipitate was then filtered off. Thin-layer chromatography of the precipitate (silica gel plates, eluent solution of 4:1 petroleum ether: methylene chloride) indicated the almost-exclusive formation of a single product. Purification of the precipitate by flash chromatography (silica gel, eluent solution of 4:1 petroleum ether: methylene chloride) yielded 1.44 g (2.89 mmol, 78%) of the desired product: ^{1}H NMR ($CDCl_3$) δ 12.63 (s, 2H), 7.23 (d, J = 2, 2H), 6.67 (d, J = 2, 2H), 0.98 (s, 18H), 0.26 (s, 12H).

3,7-Bispivaloyl)1,5-dihydroxy-9,10-anthraquinone (7). Into a three-necked round-bottomed flask fitted with a stopper, a septum, and an argon gas adapter was placed 2.72 g (10 mmol, 1.0 equiv.) of 1,3,5,7-tetrahydroxy-9,10-anthraquinone. The flask was purged with argon and the solid dissolved in 100 mL of anhydrous DMF. Pyridine (2.0 mL, 25 mmol, 2.5 equiv.) was added. Pivaloyl chloride (2.6 mL, 21 mmol, 2.1 equiv.) was drawn up into a 5 mL gas-tight syringe and injected into the anthraquinone solution overnight via a syringe pump. The reaction mixture was poured onto 100 mL of water. The resulting yellow precipitate was filtered off, dissolved in methylene chloride, and passed through a pad of silica gel. The organic solvent was then stripped off, and the residue was recrystallized from isopropanol to yield the product as a yellow-orange solid (2.92 g, 6.6 mmol, 66%): ^{1}H NMR ($CDCl_3$) δ 12.58 (s, 2H), 7.56 (d, J = 2, 2H), 7.04 (d, J = 2, 2H), 1.38 (s, 18H).

3,7-Bispivaloyl-1,5-dimethoxy-9,10-anthraquinone (8). A three-necked round-bottomed flask was outfitted with a stopper, a septum, and a reflux condenser capped with an argon gas adapter. 3,7-Bis(pivaloyl)-1,5-dihydroxy-9,10-anthraquinone (2.00 g, 4.55 mmol, 1.0 equiv.) and cesium carbonate (7.50 g, 23.0 mmol, 5.0 equiv.) were added to the reaction vessel, which was subsequently purged with argon. DMF (50 mL) and iodomethane (2.8 mL, 45 mmol, 10 equiv.) were then added. The solution was heated to 40 °C and allowed to stir overnight. Water was added to the reaction mixture, causing a yellow precipitate to crash out of the solution. This material was filtered off, dissolved in methylene chloride, dried with magnesium sulfate, filtered and concentrated. Flash chromatography over silica gel, using an eluent solution of 1:1 ethyl acetate: isooctane, followed by recrystallization from ethyl acetate yielded 1.04 g (2.22

mmol, 49%) of the desired compound: ^{1}H NMR ($CDCl_3$) δ 7.54 (d, J = 2, 2H), 6.98 (d, J = 2, 2H), 4.00 (s, 6H), 1.37 (s, 18H).

3,7-Dihydroxy-1,5-dimethoxyanthracene (3). 3,7-Bispivaloyl-1,5-dimethoxy-9,10-anthraquinone (234 mg, 0.5 mmol, 1.0 equiv.) and sodium borohydride (189 mg, 5.0 mmol, 10 equiv.) were placed in a three-necked round-bottomed flask fitted with a stopper, a septum, and a reflux condenser. The reflux condenser was capped with an argon gas adapter, and the system purged with argon. Isopropanol (25 mL, degassed) was then injected into the reaction chamber and brought to refluxing. After six hours the reaction was cooled and poured onto 1 mL of concentrated aqueous hydrochloric acid in 40 mL of water. Aqueous phosphate buffer (pH ≈ 7) was then added. This aqueous solution was extracted several times with methylene chloride. All organic extractions were combined, dried over magnesium sulfate, filtered and concentrated. The residue was chromatographed over silica gel with an eluent solution of 1:1 ethyl acetate: isooctane to yield 50 mg of a yellow powder (0.19 mmol, 37%) as the desired product. The material had a dark blue fluorescence in THF, which turned light green upon the addition of base: ^{1}H NMR (CD_3OD) δ 8.29 (s, 2H) 6.74 (d, J = 2, 2H), 6.44 (d, J = 2, 2H), 4.00 (s, 6H).

1,5-Bis(morpholinylmethyl)-2,6-dihydroxyanthracene (13). 2,6-Dihydroxyanthracene (5.00 g, 23.8 mmol, 1 equiv.) and morpholine (4.35 g, 49.9 mmol, 2.1 equiv.) were placed in a 1000 mL flask fitted with a thermometer, a stopper, and a nitrogen gas adapter. Ethanol was added until the anthracene dissolved (approximately 500 mL), and the solution was heated to 50 °C. Formaldehyde (37% aqueous w/w, 4.05 g, 49.9 mmol, 2.1 equiv.) was added and the reaction allowed to proceed for 9 hours. (A precipitate began to form after about 5 minutes.) The solution was then cooled in an ice bath, and the solid

product was filtered off. The solid was dried in vacuo, affording 8.04 g (19.8 mmol, 83%) of the desired material: ^{1}H NMR ($CDCl_3$) δ 8.20 (s, 2H), 7.78 (d, J = 8, 2H), 7.08 (d, J = 8, 2H), 4.23 (s, 4H), 3.76 (bs, 8H), 2.69 (bs, 8H).

1,5-Diethyl-2,6-dimethoxyanthracene (16). A solution of 1,5-diacetyl-2,6-dimethoxyanthracene (500 mg, 1.55 mmol) in methylene chloride (20 mL) was reacted overnight at room temperature under one atmosphere of hydrogen with 200 mg of palladium on carbon. After this time the solution was filtered to remove the catalyst. P-chloranil (750 mg, 3.00 mmol) was added to the solution, which was then refluxed for several hours. The solution was cooled, the excess chloranil filtered off, and the solution concentrated. Chromatography over silica gel, using an eluent gradient of 25 to 33% methylene chloride in petroleum ether (v/v), afforded 126 mg of the product as a yellow crystalline material: ^{1}H NMR ($CDCl_3$) δ 8.41 (s, 2H), 7.86 (d, 2H), 7.30 (d, 2H), 3.95 (s, 6H), 3.18 (q, 4H), 1.17 (t, 6H).

Chapter 8: Closing Remarks about the Cyclophane Studies and Suggestions for Future Studies.

Summary Perspective

The cyclophane work reported in this thesis represents one particular approach to the study of intermolecular forces, mainly the use of synthetic systems. This approach is valuable, in that it affords some degree of control to be engineered into the study of interacting host and guest species. The host and guest molecules involved, in general, have relatively simple structures, stripped of much of the complexity characteristic of biological systems. The solvent environment is carefully chosen. Through such measures, it is hoped that one can extract detailed information about a specific binding or catalytic property of interest.

The study of intermolecular forces using synthetic systems enjoyed a great deal of popularity throughout much of the 1980s. In many ways, the conditions were ideal for studies of this type. A vast repertoire of synthetic techniques was available. Access to high field ^{1}H NMR instruments became more routine, and the effectiveness of ^{1}H NMR as a technique for extracting information about the complexation event was neatly demonstrated. The increased speed and availability of computers facilitated modeling studies. In consequence, numerous studies detailing the roles played by hydrogen bonding, π-stacking, electrostatic, hydrophobic, cation-π and other interactions in the binding process were published.[2-11, 19-26]

The cyclophane work presented here was performed between the fall of 1988 and the fall of 1993.† This was a period of time after many of the initial studies documenting the effectiveness of various forces in synthetic systems had been

† The author has been at Caltech somewhat longer (since the fall of 1987). However the efforts of that first year are not presented here.

completed. As such, the work is perhaps best thought of as a second generation effort. Host P and its well defined complexation abilities in aqueous and organic media form the foundation for all of the new structures presented here. The goal of the investigations was to evaluate the effects of added structural complexity on the well-defined binding properties of host P. In the case of the OMP, TMP, TBP, and TMTBP hosts, neutral methoxy and bromine substituents were added. In the case of the CP receptor, a carboxylate was added. In the case of the D3 receptor, an additional ethenoanthracene unit was added.

As discussed in chapters 4, 5, and 6, certain aspects of each host structure were surprising. The nonplanarity of its linkers fills in the binding cavity of OMP. In the TMP structure, the addition of hydrophilic methoxy groups causes the host cavity to collapse for hydrophobic reasons. The TBP host binds neutral compounds with high affinity in aqueous-organic solvent mixtures. The bromine atoms of the TMTBP host seem to prevent the collapse seen with TMP. The CP cavity displayed unforeseen enhancements relative to P for selected types of charged compounds in aqueous media. The D3 structure with its high affinities for charged compounds inexplicably aggregates in aqueous media. The surprises of this work highlight how much we still have to learn about the complex intermolecular behaviors of molecules in solution. This conclusion is a general one and can be heard throughout the field of molecular recognition today. We may be able to methodically alter the composition of a molecule through chemical synthesis, but we still cannot routinely tailor its properties to exact specifications.

If the work presented here highlights how much we still have to learn about the interactions between molecules, it also demonstrates what we can learn by the systematic study of artificial receptors. This was perhaps best demonstrated

by the study of the CP host system. Through comparison of its binding properties with those of P, we were able to learn that not all positively charged guests show enhanced binding with the CP structure in aqueous borate. The guest molecules were scrutinized in detail. Their binding affinities, their electronic composition, their chemical shift patterns, and how they affected the shift patterns of the host were examined. From all of this, we were able to piece together a plausible explanation for the selective enhancements in the binding of cationic guests: mainly, that enhanced binding would only be seen if the guest could place a region of high positive electrostatic potential in close contact with the added carboxylate of the CP host. We may not have been able to predict this from the onset, but this information will certainly come in handy in the design of future receptors, and hopefully in understanding the complex behaviors of biological systems.

Future Possibilities

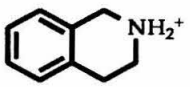
The cyclophane work discussed in this thesis poses additional questions that might be readily answered. A few of these are loose fragments from the work presented here, and can be answered with the existing host structures. Others require the synthesis of new structures. A few of these questions and structures (of particular interest to the author) are presented in the following sections.

Possibilities based on the CP Host. While host P binds quaternary ammonium compounds particularly well, it does not bind primary and secondary amines with any great affinity. This is presumably due the greater solvation energies of these compounds in water. In light of this information, the results of the modeling studies of the G-protein coupled receptors (GPCRs)[32] are somewhat surprising. In these studies three conserved aromatic residues were

present not only at the binding site for the quaternary ammonium compound acetylcholine, but also at the binding sites for the primary ammonium compounds such as histamine and serotonin. One might suspect that the tyrosine side chains stabilize the ammonium group through hydrogen bond formation, as suggested by crystallographic studies of other biomolecules. However, in many of the GPCRs for primary amines, tyrosine is not one of the three conserved residues, which suggests that the aromatic residues stabilize the aminergic agonists through other (i.e., cation-π) interactions. The authors of those modeling studies proposed that the aromatic residues work in just such a fashion in conjunction with a conserved carboxylate residue. The aromatic residues aid in neutralizing the positive charge of the ammonium group through cation-π interactions while reinforcing the energy of interaction of the ion pair by providing a lower dielectric medium than water.

The work on GPCRs inspired our studies of the CP host. This molecule places a negative charge near its aromatic binding site, and as such, may serve as a rough model for the binding motif of these proteins. It was hoped that the added carboxylate would give CP the ability to bind secondary and tertiary amines with high affinity. Comparisons of the binding properties of the P and CP hosts for guests of this type were made, but as stated in chapter 5, the results were clouded by the high pH (~ 9) of the aqueous borate medium. Such studies should be redone at a lower pH (~7) to evaluate more properly the binding affinities of CP receptor for these types of compounds. At this pH the previously examined guest compounds **1-3**, should be protonated. The additional carboxylate of CP (as compared to P) should remain deprotonated at this pH.

1



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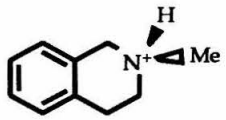

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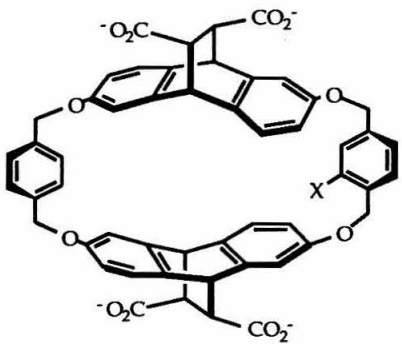
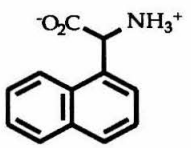
The solubility of the P and CP hosts might pose a problem at this pH. Should that be the case, the ethanoanthracene based compounds **4** and **5** (figure 8.1) might suffice. In these compounds, the conjugation between the carboxylate solubilizing groups has been disrupted. This should lower the second pKa of the ethanoanthracene unit, and make the hosts soluble at lower pH.



Substituent	Host
X = H	4
X = CO_2^-	5

Figure 8.1. Ethanoanthracene Versions of the P and CP Hosts

If the binding studies of the CP host at lower pH show enhanced binding affinites as compared to P, it might be interesting to look at guests such as **6** and **7**. In the zwitterionic form of compound **6**, the negatively charged carboxylate might reduce the cost of desolvating the positively charged ammonium group sufficiently to allow for meaningful interactions with host P. Compound **7**, incapable of doing such, may not. If this is the case, we might want to think of the operation of the GPCRs in another way. One could argue that the carboxylate contributes to the



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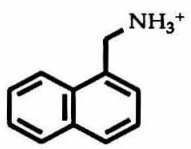


7

neutralization of the ammonium positive charge, enough to allow favorable interactions with the aromatic rings of the receptors. This may be a better way of thinking about the binding event anyway, especially if distortions in the positions of the conserved aromatic residues play a central part in the signaling mechanism.

Besides determining how well the CP system binds secondary and tertiary amines, there are a few more points of the CP system that need to be resolved. Firstly, the true nature of the interaction between guanidinium compounds and the CP host needs to be exposed. It remains possible that these compounds are stabilized through hydrogen bond formation with the CP carboxylate; our study was inconclusive on this point. An effort at obtaining crystals of this complex should be undertaken to provide more concrete evidence.

Secondly, the conformational preference of the host for iminium compounds and other charged guests should be more thoroughly examined. Specifically, it is of interest to determine which of the rhomboid conformations is preferred by these guests, and to see if this preference extends to the guanidinium type guests. This could most likely be accomplished through a series of ROESY experiments on solutions of CP alone and complexed to various guests. The proton labeled L6 in figure 5.6 has a distinctive resonance well separated from other aromatic resonances in spectra of CP. This proton occupies a different environment in each of the four rhomboid conformations of the CP host. Thus, the formation of intramolecular cross peaks should give a good indication of the host's molecular conformation

The CP project suggests several "new " host structures as well. As mentioned in chapter 7, the CP project was originally the outgrowth of an attempt to make

the phenolic host **8** (Figure 8.2). Given the unique binding properties of the CP cavity, it is of interest to see how the different charge distribution of **8** will affect cationic guest binding. One might expect that the more diffuse nature of the additional negative charge in **8** relative to CP might provide a more blanket enhancement in cationic guest binding relative to P. It is also of interest to see how additional carboxylates might further affect guest specificity. Along these lines, the host structures **9-11** (figure 8.2) might prove informative. It would be interesting to know whether binding continues to increase or whether it levels off after the addition of a given number of carboxylates.

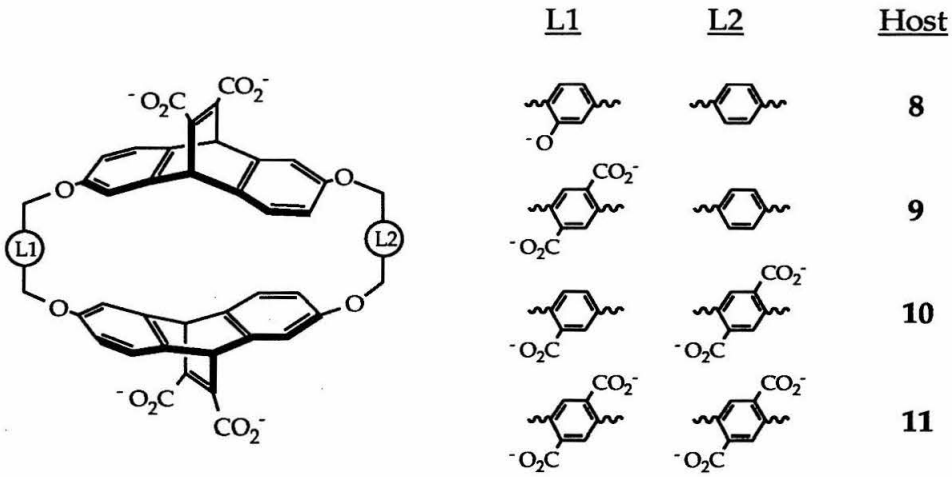


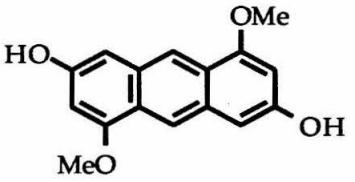
Figure 8.2. Possible Structures Based on the CP Host

Possibilities Based on the D3 and TMP Cavities. The enhanced binding affinities seen with the D3 cavity warrant further investigation. This structure was designed to more completely surround the cationic guest species. Significant enhancements in cationic guest binding were seen for this structure as compared to the M and P cavities. This suggests that guests bound to the M and P structures have not been saturated in terms of the number of cation-π interactions that they can form.

The D3 structure's low aggregation concentration did prevent us from examining its properties in our aqueous borate medium. As such, we were unable to obtain a measurement of the upper limits to the enhancements in binding seen with this strucutre. The slow on/off rates of the system in 10% acetonitrile/90% aqueous borate also hindered the investigation of the host compound by ^{1}H NMR. The extreme line broadening of the guest signals under these conditions prevented us from obtaining information about the guests' orientation in the host-guest complex. While CD methods for binding constant determination would prove helpful in providing answers to the first problem, it would not easily provide answers to this second problem.

Increasing the solubility of the host structure in aqueous media may solve both of these problems. ^{1}H NMR studies could then be performed. In aqueous media the D3 structure should display higher affinities for charged guest compounds, which should manifest themselves as slower guest off rates. If so, then in aqueous media, guest binding may drop into the slow exchange limit. This would facilitate binding constant determination, and provide direct structural information about the binding orientation of the guest species

One way to increase the hydrophilicity of the D3 structure is through the addition of methoxy groups. As seen in the studies of OMP and TMP these groups vigorously suppress aggregation in aqueous media. Appending these groups to the linker units of the D3 structure, however, is not a feasible option. Such a linker would be quite sterically hindered, and it is doubtful that significant quantities of macrocycle could be synthesized. The addition of methoxy groups to the ethenoanthracene groups provides a feasible alternative. As mentioned in chapter 7, the anthracene **12** could be used to produce the



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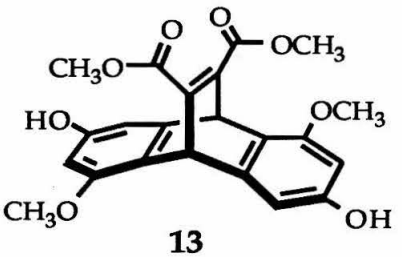

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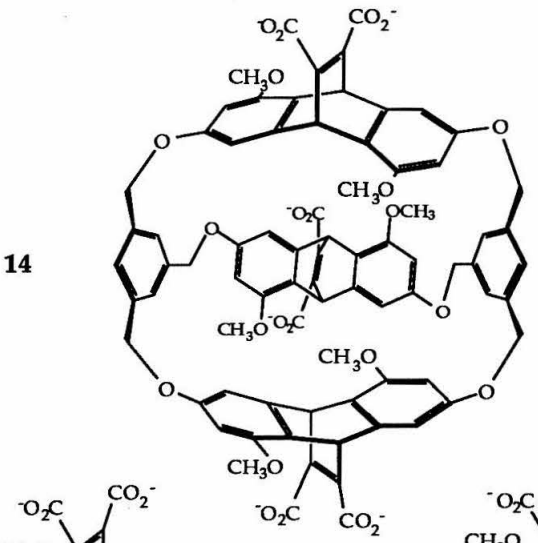
ethenoanthracene **13**. This unit could then be cyclized with mesitylene tribromide to produce the cavity **14** (figure 8.3).



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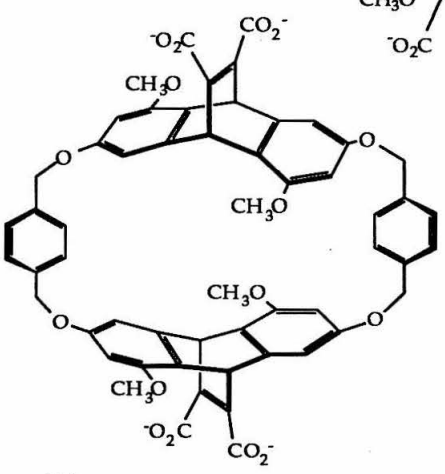

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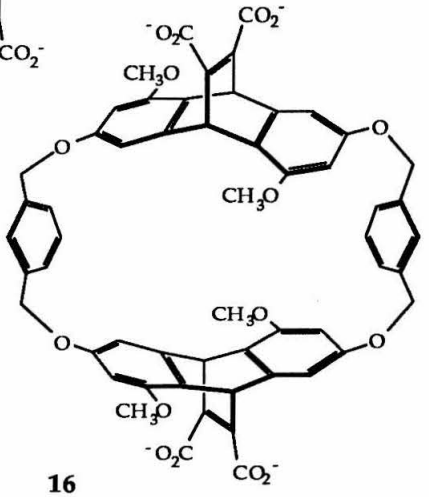

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Figure 8.3. Possible Methoxy-Substituted Host Structures.

The binding properties of **14** could then be contrasted to those of host structures **15** and **16** (figure 8.3). These structures are worthy of study in themselves. By comparison with P and M they could provide answers to how methoxy groups appended to the ethenoanthracene units of the host affect binding overall. It would be interesting to see if these structures do not collapse like TMP, due to the more judicious placement of the methoxy groups on the rigid ethenoanthracene units. If the host structures do not collapse, perhaps we could finally determine if the aryl methoxy groups can electronically enhance interactions between the host and guest species.

Chapter 9: Toward the Functional Expression in *Xenopus* Oocytes of Acetylcholine Ligand-Gated Ion Channels Incorporating Artificial Amino Acids .

Introduction

Fast chemical synaptic transmission is mediated by the family of ligand-gated ion channels. The family contains both anionic and cationic selective members which generate electric signals in response to specific neurotransmitters, such as acetylcholine, γ-aminobutyric acid, glycine, serotonin, and glutamate.[15,18,59b] These ion channels are currently the objects of intense scientific study, in hopes of better understanding neural processes.

The study of ion channels has been greatly facilitated by the advent of heterologous expression techniques. Oocytes from the African toad, *Xenopus laevis*, are commonly used vehicles for expression.[68] The oocytes are easily manipulated, are amenable to a wide variety of electrophysiological and electrochemical techniques , and are capable of translating exogenous RNA and DNA messages into membrane-bound proteins. Numerous studies on acetylcholine and other ligand-gated ion channels attest to the success of this expression system.[15]

A collaborative effort between the Dougherty, Lester/Davidson, and Abelson groups at Caltech and the Schultz group at Berkeley was begun to enable the *Xenopus* system to express proteins which incorporate artificial amino acids at specific sites in their primary sequences. This capability would add a new dimension to the mutagenesis studies of ion channels performed with the *Xenopus* system, by enabling the incorporation of more sophisticated probes of protein structure and function.

The Schultz group has already reported *in vitro* methods for unnatural amino acid incorporation into proteins.[17] In these methods, the codon in the DNA for

the natural amino acid at the position interest is replaced with the *amber* nonsense codon, using standard oligonucleotide directed mutagenesis techniques. The *amber* codon is one of the natural "blanks" in the genetic code, and is not recognized by any of the typical tRNAs involved in protein synthesis. A tRNA is then constructed to recognize the *amber* codon. This tRNA is then chemically aminoacylated with an artificial amino acid of interest. The modified DNA and the tRNA are then injected into an *in vitro* transcription-translation system to produce microgram quantities of the protein of interest.

It was hoped that key components of those techniques could be adapted for *in vivo* use with *Xenopus* oocytes. As mentioned above, *Xenopus* oocytes are routinely used to translate exogenous RNAs and DNAs into membrane-bound proteins. In theory, one need only co-inject the modified message into the cell along with the appropriate aminoacylated tRNA. The tRNA must be designed, however, to resist recharge by the oocyte's own aminoacylation machinery.

The confidence of success was high at the beginning of the project, since it was realized that minute amounts of the artificial protein (approximately 10^6 channels per oocyte) are needed to generate the electric signals necessary for detailed study. The low frequency of usage of the *amber* codon by the oocyte further bolstered this optimism. As such, the injection of a tRNA capable of recognizing this codon was less likely to disrupt the overall health of the oocyte than would tRNAs capable of recognizing the other stop codons.

The initial protein chosen for study was the nicotinic acetylcholine ligand-gated ion channel (AChR) from the mouse neuromuscular junction. AChRs of this type have been the most widely examined members of the ligand-gated ion channel family.[15] Like other neuromusclar AChRs, this oligomeric protein

contains five subunits: two α, one β, one γ and one δ. Four messenger RNAs must be injected into the oocyte to allow for the functional expression of this channel. The mRNA encoding for the α subunit was chosen for modification with the amber codon. The codon for a tyrosine residue at position 190 (*Torpedo californica* sequencing) was chosen for replacement with the UAG sequence of the *amber* codon. This residue is thought to form part of the acetylcholine binding site, and its sequence position has been the target of several site-directed mutagenesis studies.

Direct oversight of the project is provided by Drs. Dougherty, Lester, Davidson, and Abelson. Design of a new tRNA used in the project was carried out by Dr. Jeff Sampson and Dr. Peggy Saks (Caltech) . Generation of the t-and mRNAs, injections into oocytes, and electrophysiological recordings (2-electrode voltage clamp) were made by Dr. Mark Nowak (Caltech). Samples of aminoacylated and non-aminoacylated yeast phenylalanine tRNAs were provided by Dr. Schultz. The author of this thesis was responsible for the synthesis of several amino acids and related products. The project is currently ongoing. Only the synthetic efforts of the author are reported in detail here, in hopes that they may prove valuable when the time for their proper use arrives.

Generation of the Aminoacyl-dCA Fragments

A direct transfer of the methods[17c,h] used by Schultz for the generation of aminoacyl-tRNAs was desired for our studies with *Xenopus*. In general, a tRNA transcipt is run off in which the last two nucleotide bases (CA) at the 3'-end of the molecule have been deleted. The tRNA is then enzymatically ligated to an aminoacylated fragment of 5'-phospho-2'-deoxycytidylyl(3',5')adenosine (dCA) **1**.

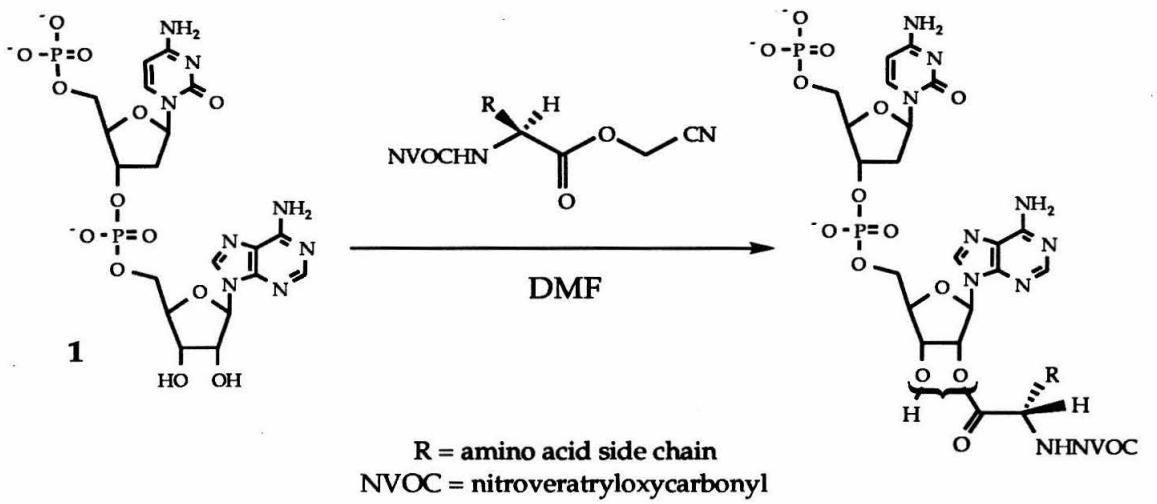


Figure 9.1. Aminoacylation of Dinucleotide Fragment **1**

The aminoacylation of **1** is carried out as shown in figure 9.1. Compound **1** in its basic form is reacted with the cyanomethylester of the desired amino acid in DMF. The fragment dCA is used instead of CA in order to carry out this reaction more effectively.† The amino termini of the amino acids involved are protected with the photolabile nitroveratryloxycarbonyl[69] (NVOC) group to prevent their reaction with the cyanomethylesters. After the ligation of the aminoacylated fragment to the tRNA, this group can be easily removed by intense irradiation at 350 nm for several minutes.

Compound **1** was synthesized as shown in figure 9.2. 6-N,6-N, 2'-O,3'-O-tetrabenzoyladenosine **2** is coupled to the phosphoramidite **3** in dry acetonitrile solution, using tetrazole as a catalyst. Treatment with iodine in THF/H_2O solution followed by reaction with *p*-toluenesulfonic acid yielded the phosphotriester **4**. The coupling of **4** with the phosphoramidite[70] **5** in acetonitrile

† Schultz has found that the incorporation of dCA fragments into the tRNA does not effect its ability to synthesize protein *in vitro*.

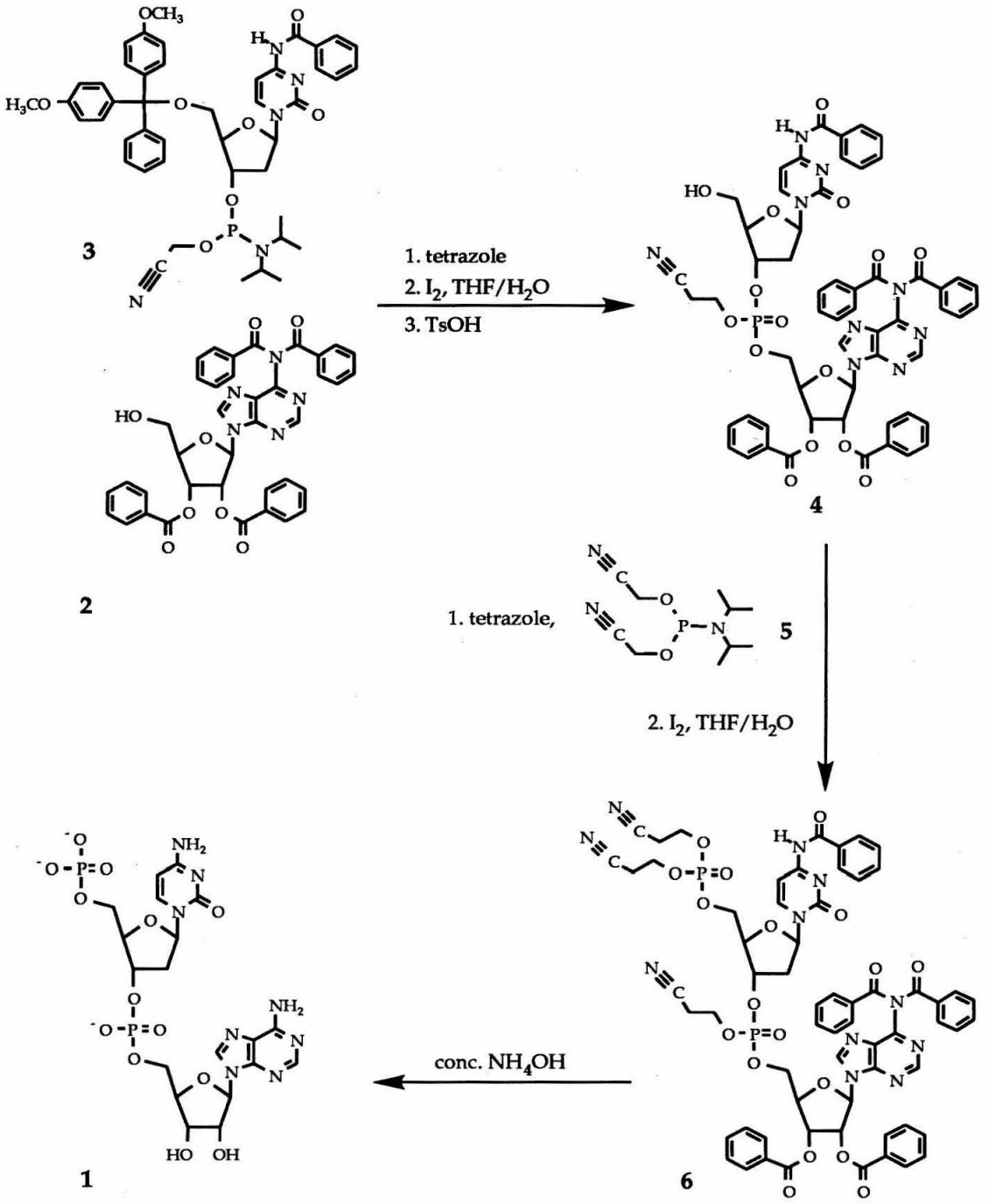


Figure 9.2. Synthesis of Compound **1** (dCA)

using tetrazole as a catalyst followed by oxidation in THF/H_2O solution yielded **6**. Treatment of **6** with concentrated ammonium hydroxide produced the desired dCA compound **1**.

Three protected amino acids **7-9** (figure 9.3) were synthesized for coupling with the dCA fragment. Compounds **7** and **8** are, of course, derivatives of the two naturally occurring amino acids phenylalanine **10** and tyrosine **11**. Compound **9** is the NVOC-cyanomethylester of trimethyllysine. While trimethyllysine **12** is a naturally occurring amino acid, it is not directly translated into protein chains. As such, its successful use in this system would be something of a novelty.

As mentioned previously, a tyrosine site in the α subunit was selected for replacement with unnatural amino acids. The use of compounds **7** and **8** would create (if successful) a known mutant[71] and the native proteins. Heterologous expression of these proteins in *Xenopus* oocytes has been accomplished by injecting the appropriate mRNA messages. The electrophysiological and pharmacological responses of these oocytes are well characterized. By selecting these targets, the efficiency of the translation process can be evaluated. The presence of any electrophysiological response bodes well for the unnatural translation process in general. If the channels produced using both the natural and artificial tRNAs produce similar electrophysiological responses, this would suggest the desired amino acid was incorporated at the appropriate site with a high degree of selectivity. In other words, the mutated tRNA delivered its original amino acid, and was not reacylated.

Compound **9** was designed for use after the initial "shake down" of the expression system was complete. Aminoacylated tRNAs made from compound

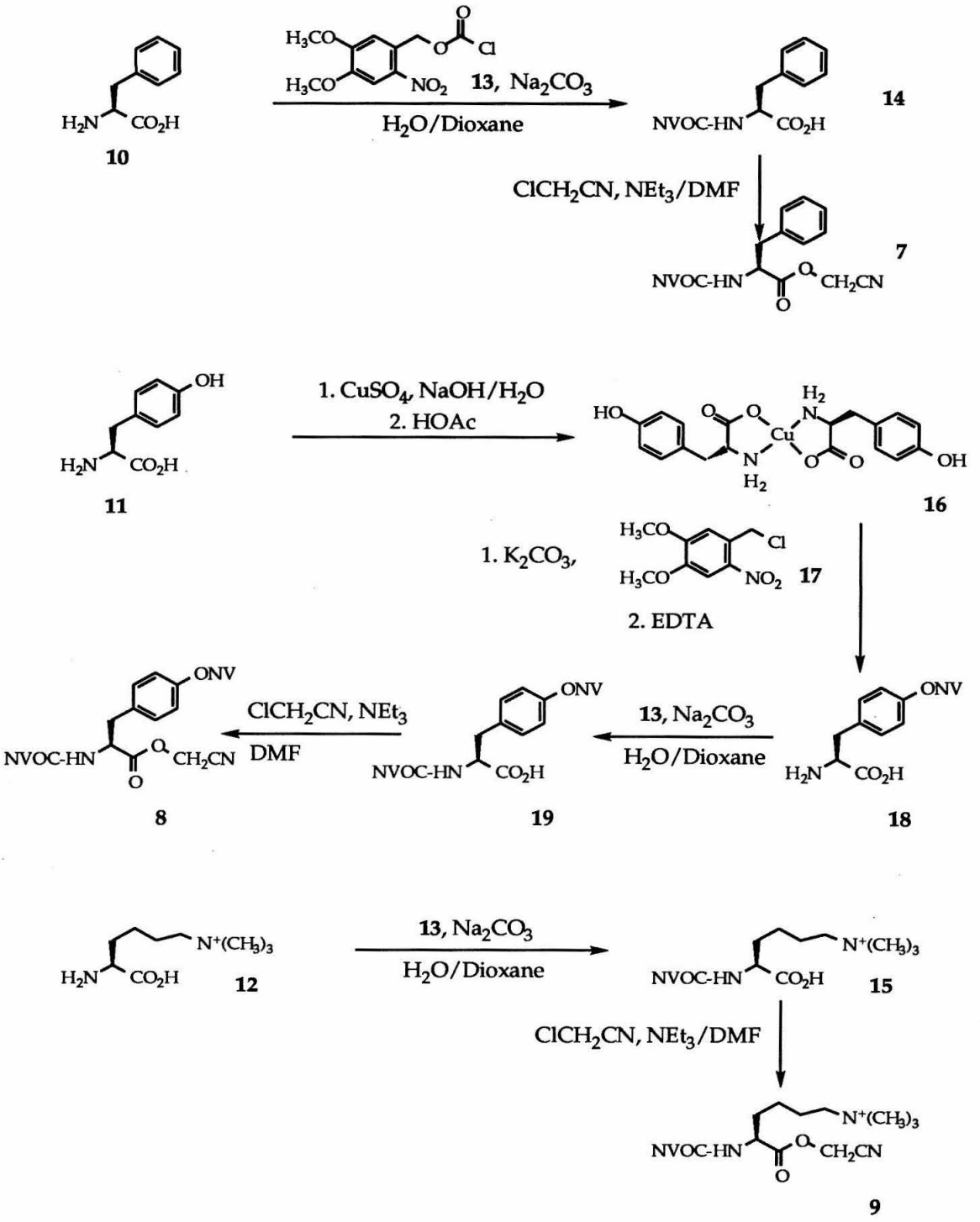


Figure 9.3. Synthesis of Protected Amino Acids

9 and co-injected with the mutated mRNA message would place a quaternary ammonium group at the binding site for acetylcholine, itself a quaternary ammonium compound. This protein might be expected to gate itself, an unusual property for a ligand-gated ion channel.

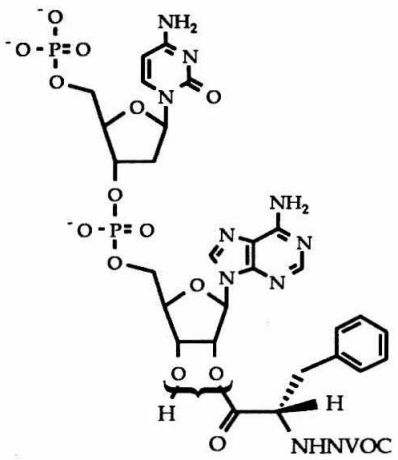
The compounds **7-9** were each synthesized in a similar fashion, as outlined in figure 9.3. Compounds **7** and **9** were produced in two steps from phenylalanine **10** and trimethyllysine **12** respectively. These compounds were first treated with nitroveratryloxycarbonyl chloride **13** in dioxane/water, using sodium carbonate as a base. The resulting carbamates **14** and **15** were then reacted with chloroacetonitrile in DMF, using triethylamine as a base to produce **7** and **9**. In order to facilitate both the cyanomethylation and the aminoacylation reactions leading to **8**, the side chain of tyrosine must be protected. This was accomplished by the complexation of tyrosine with copper to generate **16**, followed by reaction with nitroveratryl chloride[67] **17** in basic solution to produce compound **18**. The nitroveratryl ether was selected, since it could be removed photolytically in the same step as the NVOC group. Reaction of **18** with **13** to synthesize **19**, followed by treatment with chloroacetonitrile as described above, produced compound **8**.

The dCA fragment has been aminoacylated with both compounds **7** and **8**. The procedures and characterizations of the resulting compounds **20** and **21** are described in the experimental section of this chapter.

Preliminary Findings

It has been found that injection of only the modified mRNA encoding for the α subunit along with those for the β, γ, and δ subunits fails to produce functional

AChRs in *Xenopus*. Injections of the mRNAs along with Schultz's tRNAs both uncharged and charged with tyrosine produced functional AChRs. The results suggest the viability of the method, however, they also indicate recharging of the tRNA. The tRNA designed here at Caltech has not been adequately tested. Future results are eagerly anticipated.



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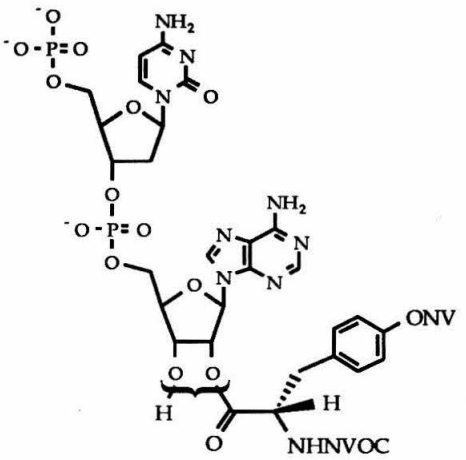

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Experimental Section

NMR spectra were recorded on JEOL JNM GX-400, Bruker AM-500 or General Electric QE-300 spectrometers. Routine spectra were referenced to the residual proton signals of the solvents and are reported in ppm downfield of 0.0 as δ values. Melting points were determined on a Thomas Hoover melting point apparatus and are corrected. HPLC was performed on a Waters dual 510 pump liquid chromatograph system equipped with a Waters 490E wavelength detector. UV spectra were taken using a Beckman DU-640 spectrometer. Mass spectral analyses were performed at the University of California, Riverside.

Compounds **3, 10, 11,** and **12** were available from commercial sources. Compounds **2, 5, 7, 14, 16,** and **17** were prepared as described in the literature.[72] In the case of compound **2,** however, it was found that the product was more easily recrystallized from ethyl acetate rather than from benzene, as reported.

4-N-Benzoyl-2'-deoxycytidylyl(3',5'-cyanoethylphosphate)-6-N,6-N,2'-O,3'-O-tetrabenzoyladenosine (4). 6-N,6-N,2'-O,3'-O-Tetrabenzoyladenosine **2** (876 mg, 1.28 mmol) was placed in a 250 mL three-necked round-bottomed flask fitted with a stopper, a septum, and an inert gas adapter. The flask was purged with argon and charged with 30 mL of dry acetonitrile. 4-N-Benzoyl-5'-O-(4,4'-dimethoxytrityl)-2-deoxycytidinyl-3'-[(2-cyanoethyl)-(N,N-diisopropyl)]phosphoramidite **3** (1.00 g 1.22 mmol) was dissolved in 10 mL of dry acetonitrile and added to the main reaction vessel. Tetrazole (214 mg, 3.05 mmol) was placed in a separate 25 mL pear-shaped flask. This flask was stoppered with a septum and purged with argon. The tetrazole was then dissolved in 10 mL of dry acetonitrile. Both the tetrazole and the adenosine solutions were cooled to 0 °C . The tetrazole solution was then added to the

adenosine solution using a make-shift cannula of surgical tubing. The solution was then allowed to warm to room temperature, and stirred for two hours.

A solution of iodine (403 mg, 1.59 mmol) and pyridine (200 μL, 2.47 mmol) in 6 mL of 2:1 THF/H_2O was added dropwise to the adenosine/phosphoramidite reaction mixture. Ten minutes after the addition was complete, all solvent was removed in vacuo. The residue was dissolved in chloroform, extracted with a 1% (w/w) aqueous solution of sodium bisulfite and with a saturated aqueous sodium chloride. The organic solution was then dried with sodium sulfate and filtered through a pad of Celite.

Toluenesulfonic acid (493 mg, 2.59 mmol) dissolved in 15 mL of 1:1 MeOH/CH_2Cl_2 was added to the chloroform solution , and the resulting solution was allowed to stir for thirty minutes. It was then extracted with chilled aqueous saturated sodium bicarbonate. The organic solution was then dried with sodium sulfate, filtered, and the solvent was removed in vacuo. The residue was chromatographed over silica gel using a 1:2 to 1:3 petroleum ether/acetone eluent gradient containing 0.2% triethylamine (v/v) to afford the desired product **4** (866 mg, 0.77 mmol, 63%). Characterization of the product by ^1H and ^{31}P NMR resulted in spectra similar to those reported for **4** in the literature.[17h]

4-N-Benzoyl-5'-(di-2-cyanoethylphosphate)-2'-deoxycytidylyl(3',5'-cyanoethylphosphate)-6-N,6-N,2'-O,3'-O-tetrabenzoyladenosine (6). Compound **4** (822 mg, 0.73 mmol) and tetrazole (102 mg, 1.46 mmol) were placed in a 25 mL round-bottomed flask. The flask was capped with a septum, purged with argon, and charged with 10 mL of dry acetonitrile. Into a separate flask was placed di(2-cyanoethyl)-N,N-diisopropylaminophosphoramidite **5** (247 mg, 0.91 mmol). This flask was then sealed with a septum, purged with argon,

and charged with 2 mL of dry acetonitrile. Both solutions were cooled to 0 °C, then the phosphoramidite solution was added to the tetrazole/dinucleotide solution. The reaction mixture was warmed to room temperature and allowed to stir overnight. A solution of iodine (276 mg, 1.09 mmol) and pyridine (170 μL, 2.10 mmol) in 3 mL of 2:1 THF/H_2O was added dropwise to the solution. Ten minutes after the addition was complete, all solvent was removed in vacuo. The residue was dissolved in chloroform, and consecutively extracted with aqueous solutions of 1% sodium bisulfite and saturated sodium chloride. The organic solution was separated, dried with sodium sulfate, filtered, and concentrated. The residue was chromatographed over silica gel, using a 1:2 to 1:3 petroleum ether/acetone eluent gradient containing 0.2% triethylamine (v/v), to yield the desired product **6** (382 mg 82%). The material is then used directly in the next step.

5′-phospho-2′-deoxycytidylyl(3′,5′)adenosine (1). Compound **6** (127 mg, 0.096 mmol), concentrated ammonium hydroxide (2.5 mL), and a 1:9 solution of dioxane/methanol (5 mL) were placed in a Kontes flask containing a stir bar. The flask was sealed and heated to 55 °C for 24 hours. The flask was then cooled to room temperature , and the solvent was removed. The residue was mixed with 2 mL of aqueous 25 mM ammonium acetate (pH 5). The resulting suspension was sonicated and the remaining off-white precipitate (benzoic acid) removed by filtration through a 0.2 μM UNIFLO filter. The clear solution was then applied to a Partisil M20 10/25 ODS-3 HPLC column. A linear elution gradient of 0 to 50 % acetonitrile in 25 mM aqueous ammonium acetate (pH 5) with a flow rate of 8 mL per minute eluted **1** after approximately 19 minutes. The solution of this purified compound was then frozen and lyophilized. The residue was redissolved in doubly distilled water, frozen, and lypohilized a

second time. The ^1H NMR spectrum of the isolated material in D_2O was in good agreement with that reported previously for the compound **1**.[17h] FAB-MS of the material showed a weak ($M-H^+$) signal at 635 as expected.

6-Nitroveratryloxycarbonyl chloride (13). 6-Nitroveratryl alcohol (869 mg, 4.08 mmol) was placed into a 100 mL three-necked round-bottomed flask fitted with a stopper, a septum, and an argon gas adapter. Dry methylene chloride (40 mL) was added to the flask, after it had been charged with an inert argon atmosphere. Pyridine (330 μL, 4.08 mmol) was injected into the flask, and the solution was cooled to 0 °C. Into a separate flask was placed triphosgene (414 mg, 1.37 mmol). The flask was capped with a septum and purged with argon. The white triphosgene solid was then dissolved in 10 mL of dry methlyene chloride and the solution cooled to 0 °C. The triphosgene solution was added dropwise to the veratryl alcohol solution. The reaction mixture was shielded from light with aluminum foil and allowed to stir for three hours. Hexane was then added directly to the reaction mixture, which was passed over a short column of silica gel, using 3:1 methylene chloride/ hexane as the eluent. The appropriate fractions were collected and concentrated. The resulting off-white residue was recrystallized from toluene at 60 °C to yield compound **13** (635 mg, 2.30 mmol, 56%): ^1H NMR ($CDCl_3$) δ 7.76 (s, 1H), 6.98 (s, 1H), 5.72 (s 2H), 3.99 (s, 3H), 3.95 (s, 3H); mp 129-130 °C, literature value 125-127 °C.

N-(Carbo-6-nitroveratryloxy)-N_ε,N_ε,N_ε-trimethyl-L-lysine (15). N_ε,N_ε,N_ε-trimethyl-L-lysine (75 mg, 99% purity, 80% amino acid content, 0.32 mmol) was dissolved in 900 μL of water. Sodium carbonate monohydrate (50 mg, 0.40 mmol) was added to the solution. Nitroveratryloxycarbonyl chloride **13** (88 mg, 0.32 mmol) dissolved in 1.1 mL of dioxane was added. The reaction mixture was then stirred for approximately two hours until the gas evolution had ceased.

Water (5 mL) was then added to the solution. It was then frozen and lyophilized. Purification via HPLC, using a Whatman Magnum 9 column (50 cm, Partisil 10, ODS-3) and a linear gradient eluent of 0 to 50% acetonitrile in 25 mM (pH 7) aqueous ammonium acetate (v/v) over 45 minutes at a flow rate of 6.75 mL per minute, afforded the desired product **15**. The product eluted after about 28 minutes. Solutions of the compound were frozen and lyophilized. The residue was then dissolved in doubly distilled water, frozen, and lyophilized a second time. Purification in this fashion should afford the product in its zwitterionic form. Eighty five milligrams of material were obtained: ^{1}H NMR (D_2O) δ 7.59 (s, 1H), 6.96 (s, 1H), 5.23 (AB, J = 12, Δν = 56 Hz, 2H), 3.79 (s, 3H), 3.75 (s, 3H), 3.13 (m, 2H), 2.92 (s, 9H), 1.66 (m, 2H), 1.59 (m, 2H), 1.25 (m, 2H).

N-(Carbo-6-nitroveratryloxy)-N$_\varepsilon$,N$_\varepsilon$,N$_\varepsilon$-trimethyl-L-lysine Cyanomethyl Ester (9). Compound **15** (80 mg, 0.18 mmol) was placed in a dry 5 mL round-bottomed flask. The flask was immediately stoppered and its contents dissolved in 2 mL of anhydrous DMF. Chloroacetonitrile (35 μL, 0.55 mmol) and triethylamine (63 μL, 0.45 mmol) were added, and the reaction mixture was allowed to stir for three days. The DMF was then removed using a rotary evaporator. The residue was purified by HPLC. Using a Whatman Magnum 9 column (50 cm, Partisil 10, ODS-3) and a linear gradient eluent of 0 to 100% acetonitrile in water (v/v) over 45 minutes at a flow rate of 6.75 mL per minute, the desired product eluted after approximately fifteen minutes. The solution of **9** was then frozen and lyophilized. The residue was dissolved in H_2O and passed through a Dowex-1X2-200 ion exchange column (chloride form) to yield **9** as its chloride salt: ^{1}H NMR (D_2O) δ 7.59 (s, 1H), 6.94 (s, 1H), 5.25 (AB, J = 12, Δν = 3 Hz, 2H), 4.80 (s, 2H), 4.18 (m, 1H), 3.81 (s, 3H), 3.75 (s, 3H), 3.15 (m, 2H), 2.94 (s,

9H), 1.82 (m, 1H), 1.67 (m, 3H), 1.31 (m, 2H); HRMS 467.2116, calculated for $C_{21}H_{31}N_4O_8$ 467.2141.

O-(6-Nitroveratryl)tyrosine (18). The copper (II) complex of tyrosine **16** (636 mg, 1.5 mmol) and potassium carbonate (415 mg, 3.0 mmol) were dissolved in 25 mL of a 30% water/70% DMF solution. 6-Nitroveratryl chloride **17** (764 mg, 3.3 mmol) was added as a solid to the solution. After 10 hours, the resulting solid was filtered off and washed consecutively with DMF, water, and acetone. This solid was then dissolved in 50 mL of a hot solution of 1:1 H_2O/EtOH. EDTA monohydrate (558 mg, 1.5 mmol) was added and the hot solution stirred for 30 minutes. After this time, the solid was filtered off and the filtrate allowed to cool to room temperature. The filtrate was stirred vigorously during this period to avoid congealing of the solution. The desired product **18** precipitated out of the solution as a yellow powder (312 mg, 28% yield): ^{1}H NMR (DMSO-d_6) δ 7.70 (s, 1H), 7.32 (s, 1H), 7.18 (d, J = 10, 2H), 6.94 (d, J = 10, 2H), 5.36 (s, 2H), 3.86 (s, 6H), 3.30 (m, 1H), 3.06 (m , 1H), 2.76 (m, 1H).

N-(Carbo-6-nitroveratryloxy)-O-(6-Nitroveratryl)tyrosine (19). Compound **18** (304 mg, 0.81 mmol) and sodium carbonate monohydrate (226 mg, 0.82 mmol) were dissolved in 6 mL of a 1:1 solution of dioxane/H_2O. 6-Nitroveratryloxycarbonyl chloride **13** (226 mg, 0.82 mmol) dissolved in 3 mL of dioxane was added dropwise to the tyrosine solution. After the addition was complete, the reaction was allowed to stir at room temperature for 1 hour. The solution was poured onto methylene chloride and extracted with 1N aqueous sodium bisulfate. The organic solution was dried with magnesium sulfate, filtered, and concentrated. Recrystallization of the residue from ethyl acetate yielded 320 mg of the desired compound **19** as a yellow solid: ^{1}H NMR ($CDCl_3$) δ 7.59 (s, 1H), 7.53 (s, 1H), 7.16 (s, 1H), 7. 00 (d, J = 10, 2H), 6.85 (s, 1H), 6.78 (d, J =

10, 2H), 5.29 (AB, J = 16, Δν = 38, 2H), 5.26 (s, 2H), 4.34 (m, 1H), 3.79 (s, 3H), 3.78 (s, 3H), 3.76 (s, 6H), 3.01 (m, 1H), 2.83 (m, 1H).

N-(Carbo-6-nitroveratryloxy)-O-(6-Nitroveratryl)-L-tyrosine Cyanomethyl Ester (8). Into a 5 mL round bottomed-flask was placed compound **19** (300 mg, 0.49 mmol). The flask was capped with a septum, and the solid was dissolved in 1 mL of anhydrous DMF. Triethylamine (140 μL, 1.0 mmol) and chloroacetonitrile (93 μL, 1.47 mmol) were added, and the reaction mixture was allowed to stir for 24 hours. The solution was then poured onto chloroform and extracted with aqueous 1N sodium bisulfate. The organic solution was dried with magnesium sulfate, filtered, and concentrated. The residue was chromatographed over silica gel, using an eluent gradient of 0 to 10% ethyl acetate in chloroform, to yield **8**: ^{1}H NMR ($CDCl_3$) δ 7.76 (s, 1H), 6.68 (s, 1H), 7.32 (s, 1H), 7.08 (d, *J* = 10, 2H), 6.96 (d, *J* = 10, 2H), 6.91 (s, 1H), 5.48 (AB, J = 16, Δν = 45, 2H), 5.44 (s, 2H), 5.22 (d, *J* = 10, 1H), 4.75 (AB, J = 16, Δν = 57, 2H), 4.68 (m, 1H), 3.98 (s, 3H). 3.96 (s, 6H), 3.92 (s, 3H), 3.10 (m, 2H).

5′-Phospho-2′-deoxycytidylyl(3′,5′)-2′(3′)-O-[N-carbo-(6-nitroveratryloxy)-L-phenylalanyl]-adenosine (20). Compound **1**, previously reacted with 2.2 equivalents of tetrabutylammonium hydroxide, frozen and lyophilized (12 mg, 0.10 mmol), was placed in a 5 mL volumetric flask fitted with a septum. Compound **7** (23 mg, 0.51 mmol) and anhydrous DMF (400 μL) were added and the solution stirred for three hours. The solution was then mixed with 3 mL of aqueous 25 mM ammonium acetate (pH 4.5). The resulting solution was then filtered, frozen and lyophilized. The residue was purified by HPLC, using a Partisil M20 10/25 ODS-3 HPLC column with a linear eluent gradient of 0 to 60 % acetonitrile in 25 mM ammonium acetate (pH 4.5) over 20 minutes at a flow rate of 8 mL per minute. The desired product eluted after twelve minutes.

Further purification was carried out using a Whatman Magnum 9 column (50 cm, Partisil 10, ODS-3). Using an eluent gradient of 0 to 100% acetonitrile in 10 mM aqueous acetic acid over 50 minutes at a flow rate of 2 mL per minute, the product **1** was eluted after 18 minute. Characterization by UV spectroscopy indicated a strong absorbance at 350 nm. FAB-Mass Spectroscopy showed a parent ion M^+ at 1022 as expected for this compound.

5'-phospho-2'-deoxycytidylyl(3',5')-2'(3')-O-[N-(carbo-6-nitroveratryloxy)-O-(6-Nitroveratryl)-L-tyrosyl]-adenosine (21). Compound **1**, previously reacted with 2.2 equivalents of tetrabutylammonium hydroxide, frozen, and lyophilized (12 mg, 0.10 mmol), was placed in a 5 mL volumetric flask fitted with a septum. Compound **8** (36 mg, 0.55 mmol) was added to the flask, and all solids were dissolved in 1.0 mL of anhydrous DMF. After 3 hours, 4 mL of a 2:1 mixture of 25 mM ammonium acetate (pH 4.5)/acetonitrile was added to the flask. The solution was then frozen and lyophilized. The residue was taken up in 3 mL of a 9:1 solution of 25 mM ammonium acetate (pH 4.5)/acetonitrile. The resulting suspension was filtered through 0.2 μM UNIFLO filters. The filtrate was used to purify the desired compound by HPLC, using a Whatman Magnum 9 column (50 cm, Partisil 10, ODS-3). Using a linear eluent gradient of 0 to 90% acetonitrile in 25 mM ammonium acetate (pH 4.5) at a flow rate of 6.8 mL per minute, the desired compound **21** eluted after 26 minutes. Further purification was carried out using the same column. Using a linear eluent gradient of 0 to 90% acetonitrile in 10 mM aqueous acetic acid at a flow rate of 6.8 mL per minute, the desired compound again eluted after 26 minutes. Characterization by UV spectroscopy indicated a strong absorbance at 350 nm. FAB-Mass Spectroscopy showed a parent ion M^+ at 1233 as expected.

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