MOLECULAR TUBES: SYNTHETIC RECEPTORS FOR THE RECOGNITION AND SENSING OF LIPIDS

A Dissertation in Chemistry

by

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ABSTRACT

The design, synthesis and binding characteristic of a novel class of molecular receptors are described. Naphthalene subunits were adjoined to construct molecular tubes with defined cavities. These receptors exhibited a fluorescent response to titration with acyl lipid guests. One of the tubes exhibited shape selective recognition of straight chain lipid guests against lipids with branching or unsaturation. Analysis of the characteristics of these receptors enabled design revisions for the preparation of a second generation series of tubes which resulted in improved binding characteristics and fluorescent response.
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For Camille and April
Chapter 1: Molecular Recognition

1.1 Overview

The field of molecular recognition\(^1\) encompasses efforts to understand selective non-covalent interactions at the molecular level and to prepare molecular systems which exhibit this kind of behavior. These interactions may be inter- or intra- molecular. The applications of molecular recognition are varied and include drug delivery systems, molecular reactors, nanotechnology, materials, and chemical sensors.

Chemical sensors\(^2\)\(^-\)\(^4\) are small molecules which produce a detectable response to the presence of a specific compound, termed the analyte. Chemical sensors are often targeted toward biologically relevant analytes for the purpose of elucidating the mechanisms of various biological machinery.

Chemical sensing differs from molecular recognition in that the term “molecular recognition” does not imply a change in signal output and the term “chemical sensing” does not imply a non-covalent interaction, although much of chemical sensing draws from and is molecular recognition. The work reported here can be characterized as host-guest chemistry, molecular recognition, or chemical sensing, as it satisfies the requirements of the definitions of each of these.
1.2 Molecular Recognition and Host-Guest Chemistry

The field of molecular recognition concerns the non-covalent interactions which govern selective associations between chemical groups\(^1\). These types of interactions are crucial in biological systems. The interactions between biological receptors and their substrates are molecular recognition events. Various biological structures result from and are defined by non-covalent interactions, as with the double helix of DNA and the bilayer structure of some cell membranes. Synthetic materials and molecular devices are also influenced and sometimes based on molecular recognition.

The development of molecular recognition has resulted in an increased understanding of biological systems and has been integral to the advancement of the technologies of medicinal and materials chemistries. Host-guest chemistry is a subdiscipline of molecular recognition which is differentiated somewhat from molecular recognition as a whole by the definitions of the host and the guest\(^{1,5-7}\). A host is defined as a chemical species having converging binding sites which bind a guest. In other words, a host is a receptor which binds a guest. A host-guest complex (H\(\cdot\)G) is formed by the inclusion of a guest within the binding site of the host.

The formation of a host-guest complex is an equilibrium process involving the desolvation of the guest and the host binding site and the formation of host-guest interactions as well as new solvent-solvent interactions (Scheme 1). The equilibrium for association is the association constant \(K_a\), defined mathematically by equation 1:

\[
K_a = \frac{[H_m\cdot G_n]}{[H]^m [G]^n}
\]  

(1)
where \( m \) is the number of host molecules in the \( H\cdot G \) and \( n \) is the number of guest molecules.

**Scheme 1:** Schematic representation of host-guest equilibrium

\[
\begin{align*}
\text{H} & \quad + \quad \text{G} \\
\text{H} + \text{G} & \quad \xleftrightarrow{} \quad \text{HG} + \cdot
\end{align*}
\]

\( \cdot = \text{solvent} \)

Successful guest-host interactions depend on an overall decrease in the Gibbs free energy of the system. Thus, entropy and enthalpy must be considered for several interactions. The binding of guest by a host entails the elimination of certain interactions – namely host-solvent and guest-solvent interactions – and the introduction of new interactions: solvent-solvent and host-guest. Therefore, it is possible that a binding event may be driven by a decrease in enthalpy or an increase in entropy. It is not necessary that both occur, so long as there is a net decrease in the free energy. The manner by which this is achieved is a combination of shape, size and electrostatic complementarity between the interacting species. Obviously, a host must contain a cavity of the appropriate size and shape to bind a given guest and vice-versa. Electrostatic complementarity requires that the location and electronics of the binding sites of a host be compatible with its guest.

The available binding interactions include hydrogen bonding, hydrophobic forces, dipole-dipole, dipole-ion, metal-ligand and aromatic-aromatic interactions. Hydrogen
bonding, while quite strong in less polar solvents, is very weak in aqueous environments due to competition with solvent molecules. Host-guest systems based on hydrogen bonding and designed to be used in aqueous media often require levels of preorganization which are unreasonable for synthetic hosts, though they are ubiquitous in the natural world. This is the case due to solvent interference – water is a powerful hydrogen bond donor and acceptor – which is disruptive to hydrogen bond based interactions, although there are some hydrogen bonding interactions which are useful in aqueous solution.

The field of host-guest chemistry was spawned from research on cyclic polyethers, known as crown ethers, which were found to be effective receptors for metal ions. Numerous polyethers of this class were reported, the most well known of which is commonly called 18-crown-6 (1). Compounds such as 1 were found to form complexes with salts by inclusion of cations in the center of the macrocycle where they could experience ion-lone pair interactions with the oxygen atoms, whose lone pairs point to the center of the macrocycle (for K^+ in CH₃OH, log K_a = 6.1).⁸⁻¹⁰ The importance of preorganization could be deduced from this, as cyclic polyethers favor orientation of the oxygen atoms around the metal ion. By comparison, the acyclic analog 2 showed far less propensity to form complexes with cations (for K^+ in CH₃OH, log K_a = 2.2)¹⁰.
Drawing from the concept crown ethers, similar compounds were devised and prepared to further explore the principles involved in molecular recognition and to broaden the scope of this chemistry. Cryptands such as 3 (Scheme 2) were developed by Lehn and were reported to bind cations with greater affinity than Pederson’s crown ethers.\textsuperscript{11} However, crystal structures of compounds 1\textsuperscript{12,13} and 3\textsuperscript{14} in the absence of guest indicated that these compounds were collapsed such that no cavity was present and the binding sites were not convergently arranged. Thus, with these systems, a reorganization of the host molecule is required before a guest can be accommodated.

**Scheme 2:** Complexation of metal ions by crown compounds 1 and 3.

![Diagram of complexation](image)

<table>
<thead>
<tr>
<th>Log $K_a$</th>
<th>Na$^+$</th>
<th>K$^+$</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3</td>
<td>6.7</td>
<td>CH$_3$OH</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Log $K_a$</th>
<th>Li$^+$</th>
<th>Na$^+$</th>
<th>K$^+$</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>$&gt;8$</td>
<td>$&gt;8$</td>
<td>CH$_3$OH</td>
<td></td>
</tr>
<tr>
<td>10.6</td>
<td></td>
<td></td>
<td>D$_2$O sat. CDCl$_3$</td>
<td></td>
</tr>
</tbody>
</table>

Compound 4 (Scheme 3) was designed as a receptor for Na$^+$ and Li$^+$ and prepared to examine this issue.\textsuperscript{15,16} With this ligand, the presence of a cavity is enforced, as is the convergency of the oxygen lone pairs about the cavity. With compound 4, Na$^+$ and Li$^+$
were bound with much greater affinities than either 1 or 3. On the other hand, K\(^+\) is too large to fit within the cavity and is therefore not bound. The term spherand was coined to describe this type of preorganization, wherein the binding sites and cavity are rigidly fixed in place.

**Scheme 3**: Complexation of metal ions by spherand 4.

![Scheme 3: Complexation of metal ions by spherand 4.](image)

<table>
<thead>
<tr>
<th>Log (K_a)</th>
<th>Li(^+)</th>
<th>Na(^+)</th>
<th>K(^+)</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.8</td>
<td>14.1</td>
<td>-</td>
<td>D(_2)O sat. CDCl(_3)</td>
<td></td>
</tr>
</tbody>
</table>

The importance of shape complementarity to the formation of stable host-guest complexes can be inferred upon examination of cryptand 5.\(^{17}\) This hexaprotonated form 6 exhibits the appropriate size and electrostatic properties to be a suitable host for halide complexation. However, the elliptical shape of the cavity it possesses makes it a poor match for spherical halide guests. Complexes of 6 with Cl\(^-\), Br\(^-\) and I\(^-\) are formed, however distortion of the host is required which results in destabilization of these complexes. On the other hand, the linear azide ion is a more appropriate shape to fit within the cavity and forms a much more stable complex than do the halides. The fluoride ion is sufficiently small in diameter so that it does not distort the host as the other
halides do and is also a more potent hydrogen bond acceptor. Thus, the $K_a$ is significantly larger in the case of $F^-$ than it is with the other halides.

Anion binding with host 6

<table>
<thead>
<tr>
<th>Anion</th>
<th>$K_a$ $(H_2O)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F^-$</td>
<td>4.1</td>
</tr>
<tr>
<td>$Cl^-$</td>
<td>3.0</td>
</tr>
<tr>
<td>$Br^-$</td>
<td>2.6</td>
</tr>
<tr>
<td>$I^-$</td>
<td>2.2</td>
</tr>
<tr>
<td>$N_3^-$</td>
<td>4.3</td>
</tr>
</tbody>
</table>

The early studies on ion complexation by crown ethers, cryptands, spherands, and related macrocycles served to effectively demonstrate the principles and possibilities of host-guest chemistry. A natural progression of the field was the recognition of organic guests. Shape is of increased significance in the recognition of organic guests as the range of possibilities is very broad. The degree of shape complementarity between host and guest can greatly affect binding affinity and binding selectivity.

The importance of shape complementarity can be illustrated by reports on non-polar isosteres for nucleosides, which indicated that shape was the governing factor in the differentiation of nucleoside during DNA replication. Unnatural nucleoside 7, a non-polar isostere of the nucleoside thymidine 8, was prepared to probe the role of size and shape in DNA replication.18,19 This synthetic guest was found not to have significant hydrogen bonding ability, even in chloroform, and almost perfectly mimics the size and shape of T. DNA replication studies were undertaken in which the F was included in an oligonucleotide with an RNA primer. Treatment of the oligonucleotide with the Klenow fragment of DNA polymerase 1 in equimolar mixtures of dATP, dCTP, dGTP, and dTTP
resulted in insertion of the natural base pair adenine (dATP) with efficiency and fidelity rivaling the control experiments with the natural nucleoside.

Similar studies were performed for isosteres of the other bases, for incrementally different sized isosteres, and the reverse experiments were performed replicating DNA using fully natural oligonucleotides and inserting the corresponding non-polar isosteres. While helping to develop an understanding of DNA replication, these experiments also highlight the importance of shape selectivity in molecular recognition.

It can be seen that shape complementarity is an important component of molecular recognition. The constraints on shape may be varied by choosing to have greater or fewer degrees of constraint built into a host. The remainder of this chapter will review hosts used for recognition of organic guests, organizing them by the level of constraint.

1.2.1 Molecular Tweezers and Clips

Molecular tweezers, also known as molecular clips, are compounds which have a cleft shaped cavity which is typically supported by some scaffold, rather than the macrocycles discussed above. With tweezer compounds, size selectivity is limited to one
dimension– the thickness of the guest. In other words, the length and width of a guest will not inhibit it from occupying the cavity, but the thickness must be compatible with the receptor. The scaffold often must be rigid in order to achieve preorganization. Tweezer compound 9 features rigidly spaced acridine groups fixed in place by a dibenzacridine spacer. The face to face distance of the acridine arms is 7.24 Å, which falls within the limits (6.4 – 7.6 Å) required for the intercalation of aromatic groups. The binding properties of 9 were studied by NMR and it was found to complex with trinitrofluorene (TNF) in CDCl₃.

![Diagram of 9 and 9•TNF](image)

Tweezers 10 and 11 are from a class of compounds which can be synthesized in a divergent manner from a few simple starting materials to produce numerous host compounds with a variety of cleft shapes and sizes. These tweezers were found to form host-guest complexes with varying types of guests. Tweezer 11 complexes Cs⁺ to form 11•Cs⁺, demonstrating the ability of the tweezer to distort from the low energy state of the vacant host in order to accommodate a guest. In this case, favorable cation-arene interactions are maximized by this distortion. Complexation of tetracyanobenzene (TCB) by a demethylated derivative (12) of tweezer 10 forms 12•TCB, which demonstrates to
an even greater extent that bond angles can and will be distorted in order to achieve a favorable host-guest complex. The tweezer walls, which are splayed apart to 14.5 Å at the ends in the absence of a guest for 10, are only 6.5 Å apart in complex $12\cdot\text{TCB}$, as the walls pinch together in order to allow for favorable $\pi-\pi$ interactions between the guest and each of the host walls.
Tweezer 13 (Scheme 4) was prepared for the purpose of selective recognition of catecholamines. The incorporated polar functionalization serves to fix the biphenyl arms in a conformation producing a cleft and aids in the formation of host-guest complexes. Intramolecular H-bonding between the proximal phenazine nitrogen and the amide hydrogens results in a low energy conformation in which the biphenyls are arranged at a distance of ~7 Å. The cleft is suitably arranged for binding catechols via π stacking and H-bonding. The terminal phosphonate groups can make charge-charge and hydrogen bonding interactions. In $d_4$-methanol, the catecholamines norepinephrine (nor-ep), epinephrine and dopamine were bound, but simpler guests such as catechol, phenethylamine, ethanolamine and lysine were not.

**Scheme 4:** Molecular tweezer for norepinephrine recognition.
1.2.2 Cyclophanes

Cyclophanes are macrocyclic compounds containing aryl rings within the macrocycle. Thus the cavities formed are more restrictive than the cavities of tweezers in the sense that these hosts are shape selective for guests in two dimensions, rather than one.

Whitlock prepared a series of naphthalene diol based rigid cyclophanes such as 14 (Scheme 5). A number of these cyclophanes exhibited high binding affinities for certain phenols in nonaqueous solvents. Host 14 exhibits selectivity for \( p \)-nitrophenol in CDCl\(_3\) over other guests, indicating that size and shape, as well as H-bond donating ability, are factors governing the selectivity of the host.

**Scheme 5:** Cyclophane receptor for phenols.

Water soluble tetramine analogue 15 (Scheme 6) was prepared to examine complexation characteristics in D\(_2\)O.\(^{27}\) The \(^1\)H NMR chemical shift data indicated the complexation of phenol. Control experiments with diamine 16 gave no evidence of
binding. Next this host was analyzed for enzyme mimic traits. The acetyl transfer reaction between 15 and acetyl ester 18 was studied in D₂O under neutral conditions and the reaction was found to be facile, resulting in acetyl transfer to the amino groups. Competition studies involving 16 or phenethylamine (17) resulted in almost complete acyl transfer to 15 with only trace acylation of amine 16 or phenethylamine. Thus, the reaction was accelerated by complexation of the substrate, which allows for a pseudo-intramolecular transfer of the acetate to one of the pendent amines.

**Scheme 6:** Artificial enzyme mimics.
1.2.3 Bowls and Extended Bowls

Molecular bowls are macrocyclic compounds having a closed or restricted opening at one end to make a bowl shaped cavity. Such compounds now bear a cavity with a defined opening in two dimensions and a defined depth, which can be altered by extending the walls of the bowl. Resorcarenes are resorcinol-derived bowl shaped cavitands. The basic parent structure is shown (21) where R can be a variety of alkyl groups. Calixarenes are similar compounds derived from the condensation of phenols and an aldehyde. The parent compound is calix-[4]-arene (22).
Ungaro prepared functionalized calixarenes which were used to bind various ammonium guests in aqueous solution. In the case of $N,N,N$-trimethylanilinium (TMA), host compound 23 (Scheme 7) bound the $-N^+(CH)_3$ group whereas host 24 bound the phenyl ring. In the case of complexation of benzyltrimethylammonium (TMB) with host, an equilibrium is observed for 24•TMB between one state in which the ammonium group is bound and one in which the phenyl ring is bound.

**Scheme 7:** Recognition of anilinium compounds by calixarenes.

![Scheme 7 Diagram](attachment://Scheme_7.png)
Cavitands of the basic structure 27 were originally reported by Cram. These resorcarene-derived compounds may be described as equilibrating between a closed “vase-like” conformation (27, structure at right), in which the diazanaphthalene rings are axial and form a cylindrical entryway into a larger diameter spherical cavity, and an open “kite-like” conformation, in which the diazanaphthalene rings are equatorial. The equilibrium is controlled by the system parameters; solvent, temperature, variation of the extended aromatic walls, and functionalization of the outer edge, and these may be selected or tuned to achieve a desired equilibrium. It was recognized that the cavity of the vase-like conformation should be useful for binding of organic guests and data were obtained demonstrating this phenomenon in solution, solid-state and gas states.

Rebek prepared water soluble cavitand host 28 and experimented with the binding of organic substrates by these hosts in aqueous environments. Cavitands with benzimidazole walls decorated with carboxylic acid groups were prepared and examined for their recognition properties in aqueous solution. This cavitand is stable in the vase-like conformation in water due to solvent bridged hydrogen bonds which form between
the benzimidazole nitrogens. The deep binding pocket was found to be well suited for the recognition of tetramethyl ammonium (29) as well as choline (30) and acetyl choline (31) (28•31). In part, this is due to the proper match in size and shape of the bowl-shaped base of the cavity with the spherically shaped tetra-alkyl ammonium moiety of the guest. Larger quaternary ammonium substrates are unable to fit within the cavity and are not bound. Hydrophobic effects also play a role in the favorable complexation. Additionally, the polarizable aromatic rings which form the walls of the host form highly favorable contacts with the methyl groups, which encapsulate and shield most of the positive charge on the nitrogen from coming into contact with the water solvent. The receptor was found to be selective against L-carnatine (32), which presumably is repelled in basic aqueous solution by unfavorable interactions between the host and guest carboxylate groups. Curiously, while most H•G studied exhibited favorable enthalpic and entropic terms, the binding of choline resulted in a loss of entropy. The authors could not account for this result.
1.2.4 Fully Enclosed Hosts

Full enclosure of a guest within a host places an absolute restriction on the size and shape of potential guests. Not only must a guest be of appropriate dimensions to reside within the cavity, but entrance into the cavity must often occur through portals which can place additional restrictions. Indeed, in some cases of covalently formed hosts which fully enclose a defined space, the solvent encapsulated during formation is permanently trapped inside the host. Such hosts are called carcerands. Similar to carcerands are hemicarcerands, which display semi-permanent retention of guests.
However, with hemicarcerands it is possible to exchange guests without breaking bonds. Self assembly (*vide infra*) is a strategy which can be employed to generate hosts which fully enclose a defined space, yet allow for exchange of guests.

Cram and coworkers prepared host compounds 33 and 34, which feature tightly enclosed cavities accessible by three small portals and suitable for binding only very small molecules (ca. 2 heavy atoms). Crystal structures were obtained for each host in which a single solvent molecule (CH$_3$OH or CH$_3$CN) was found to occupy the cavity. On the other hand, when 33 was crystallized from CH$_2$Cl$_2$ and hexanes there was no discernable guest in the crystal structure, which was consistent with the expectation that the cavity was too small to accommodate CH$_2$Cl$_2$. Moreover, the observed crystal structure of host 33 with its cavity vacant was identical to its crystal structure with an included solvent molecule. Hence, it was established that these hosts had a very rigid structure.

The rigid structure of hosts 33 and 34 coupled with their inability to accept CH$_2$Cl$_2$ or CHCl$_3$ as guest meant that while dissolved in such solvents, the hosts would remain open and their cavities empty. The complexation of guests by these hosts was studied by $^1$H NMR and it was found that gasses such as O$_2$, N$_2$, and CO$_2$, in addition to
small molecules such as CH₃OH, H₂O, and CH₃CN, were efficiently complexed by these hosts. On the other hand, C₂H₅OH was found to be a very poor guest as even after 1 week in neat C₂H₅OH at room temperature, 90% of the host remained vacant.

Self assembly is a process by which molecular subunits arrange themselves in solution to produce large supramolecular structures. These processes are driven by reversible interactions such as molecular recognition and coordination chemistry, allowing formation of the thermodynamically favored products. Therefore, with care in design, the use of self assembly can enable the preparation of large chemical structures at a minimum synthetic cost for a variety of purposes, host-guest chemistry being one. Because the structures are formed reversibly, guest exchange can occur even without the presence of portals in the fully formed host.

Biscatechol ligand 35 has been found to form supramolecular coordination cage complexes of stoichiometry M₄L₆ with several different metal salts in the presence of tetraethyl ammonium salts₃¹ (Scheme 8). These complexes enclose one ammonium guest within a cube shaped space and have a formal charge of -12. The tetramethylammonium cation used in templating the assembly can be exchanged for other guests and studies with these hosts have shown that they bind a variety of guests and in particular they bind positively charged guests strongly.₃²,₃₃ They have also been examined for use as artificial enzymes. Orthoformate esters are known to be hydrolyzed by acid catalysis but are stable under basic conditions. When M is Ga³⁺ (36), a catalytic amount of the complex was shown to promote quantitative hydrolysis of orthoformate esters in aqueous solution at pH 11.₃⁴ Thus, the strongly negatively charged host creates a microenvironment wherein the pH of substrates are modulated such that even weakly basic compounds are
protonated under moderately basic conditions. $^{31}$P NMR data indicated that simple alkyl phosphines are protonated within the cage, giving further evidence for this pH modulation effect. This is not unlike known examples of natural enzymes which can dramatically alter the inherent reactivity of substrates, including modulation of pH by up to 12 $pK_a$ units.$^{35-37}$

**Scheme 8:** Self assembly of molecular host 36 by coordination chemistry. For CPK model, counter ions omitted for clarity. For stick model, ligands differentiated by color coding and hydrogens and counter ions omitted for clarity.
Scheme 9: Catalytic hydrolysis of orthoformate esters.

\[
\text{HC(OR)}_3 + \text{H}_2\text{O} \xrightarrow{\text{1 mol % 36}} \xrightarrow{\text{pH = 11}} \text{H}^\text{OR} \xrightarrow{} \text{H}^\text{O}^-.
\]

1.3 Conclusions

The field of molecular recognition studies weak, non-covalent interactions that result in stable assemblies of molecules. The stability of these assemblies is dependent on several factors including the size and shape complementarity of the interacting components. Shape selectivity can be an effective strategy in designing host-guest systems and a number of conceptual approaches may be employed in expanding or limiting the extent to which shape selectivity is used to achieve desired selectivity.
Chapter 2: Chemical sensing

2.1 Overview

The ability to monitor biological systems at the molecular level and in real time provides important information which is useful for a variety of medical applications. Chemical sensing\textsuperscript{2-4,7} entails the use of small molecules which provide a detectable response upon a specific interaction with a targeted analyte. Oftentimes, the principles of molecular recognition are used to prepare receptors for this purpose. A major goal is to introduce these chemical sensors (chemosensors) into live cells in order to gain information about cellular or sub-cellular structure and function. An ideal sensor would enable real-time observation of biological processes without interfering with overall function - i.e., it must be non-destructive.

An appropriate binding affinity and high selectivity are goals when developing sensors. The binding affinity should match the average concentration of the analyte in solution so that both increases and decreases in analyte concentration can be reported. High selectivity is usually important as there are typically numerous substrates which may compete with the targeted analyte for binding the sensor. These undesirable binding events must be limited in order to ensure that the response corresponds to the concentration of the analyte.

For biological applications, a reversible interaction between chemical sensor and its target analyte is imperative. Reversible interactions enable dynamic monitoring of
biological systems. In other words, it is necessary to sense both increases and decreases in concentration.

### 2.2 Fluorescence as a Method for Detection

Fluorescence is a phenomenon in which a molecule absorbs a photon of light and subsequently emits a photon of longer wavelength than the one it absorbed. Fluorescent emission in the visible range is a favored technique for biological sensing as it has advantages over other methods. The limits of detection for fluorescence are orders of magnitude lower than for other methods which makes this technique much more sensitive for the detection of analytes at low concentration. Furthermore, it is frequently possible to choose an excitation wavelength which will penetrate media impenetrable by other methods and will not result in cell damage or interfere with cell functions. These qualities make fluorescence readout a useful strategy in designing chemosensors for visualizing bio-molecules in cells.

The process of fluorescence is portrayed graphically in a Jablonski diagram (Figure 1). An electron in its ground state $S_0$ may be excited to a higher vibrational level of singlet excited state (e.g. $S_1$ or $S_2$). At this point, one of a number of processes may follow. The molecule may undergo internal conversion - the relaxation to the lowest energy excited state. Following internal conversion, the electron may return to a higher vibrational level of the ground state, followed by relaxation to the ground state. In this case, return to the ground state is spin allowed and occurs with the emission of a photon. The $\Delta E$ for excitation is thus greater than the $\Delta E$ for transition to the ground state, and the
photon emitted is longer in wavelength ($\lambda$) than the photon absorbed. This difference in $\lambda$ is the Stokes shift and the process is fluorescence.

**Figure 1: Jablonski diagram**

Absorption of a photon does not always produce a fluorescent emission. Other processes may occur which result in the relaxation of a fluorophore to its ground state. The ratio of photons emitted to photons absorbed is the quantum efficiency ($\Phi_F$) which can serve as an indication of how fluorescent a compound is. Quenching involves the transfer of energy from the fluorophore to another entity. It is possible for transfer of energy to occur to another part of the excited molecule or to another chemical species, such as solvent. These non-radiative processes lead to lower $\Phi_F$. 
Phosphorescence may occur if the S\textsubscript{1} state undergoes intersystem crossing to the triplet excited (T\textsubscript{1}) state. This results in the inversion of the spin of the electron in the excited state so that the unpaired electrons in the S\textsubscript{0} and T\textsubscript{1} states have the same spin. Therefore, return to the ground state is spin forbidden, and the process occurs over much longer time periods. Because T\textsubscript{1} lifetimes are so long, phosphorescence usually does not occur in solution. Instead, the T\textsubscript{1} state is quenched by some other process.

2.2.1 Modulation of Fluorescence

Fluorescence modulation is a widely favored strategy for sensing, but combining a binding event with a signal transduction is not trivial. A number of phenomena are commonly used to incorporate a change in fluorescence with the binding of an analyte by a sensor, including: photoinduced electron transfer (PET) quenching, \pi-system modulation, monomer-excimer switching, and environment sensitivity. These strategies are discussed below.

2.2.1.1 PET Quenching

PET quenching occurs when excitation of a fluorophore is followed by transfer of an electron from the HOMO of a nearby quenching element into the singly occupied S\textsubscript{0} orbital of the excited fluorophore (Figure 2). The return of the excited electron to the ground state is blocked, and the fluorophore returns to the ground state by electron transfer to the SOMO of the quenching element. PET quenching is possible when the
HOMO of a quenching element is slightly higher in energy than the ground state LUMO of a fluorophore.

The interruption of PET quenching is a method for modulation of signal intensity (Figure 3). One strategy for fluorescent sensing using PET quenching is an array consisting of a fluorophore linked to a receptor by some spacer. Binding of an analyte by the receptor changes the HOMO energy of the receptor. Judicious choice of a receptor and fluorophore can enable either an on or off response.

Figure 2: Diagram of PET quenching.
Czarnick has reported a series of anthracene based sensors which operate based on the principle of PET quenching. While anthracene itself is fluorescent, functionalized anthracenes bearing PET quenching groups can have very low fluorescence. Heavy atoms and amines possess lone pairs which are capable electron transfer agents that serve to quench fluorescence. In some cases, such as with amines and thionyl groups, it is possible to chelate the atom lone pairs, thereby stabilizing the atom and reducing its oxidizability to the extent that PET is deactivated and a fluorescence increase is observed. This strategy was employed in designing sensor 37. The benzylic amines of the tris(3-aminopropyl)amine groups adorning the 1,8 positions of this sensor quench the fluorescence of the anthracene fluorophore. Chelation to a molecule of pyrophosphate results in a strong hydrogen bond between the benzylic amines and the hydroxyl groups of the pyrophosphate. This association leads to a more than 2-fold...
increase in fluorescence and an assay for pyrophosphatase was developed using this sensor.

**Scheme 10:** Pyrophosphate sensing by PET quenching.

![Scheme 10: Pyrophosphate sensing by PET quenching.](image)

**2.2.1.2 Pi System Modulation**

Modification of fluorophore π systems by disruption or extension is a useful general method for signal transduction. Tsien and coworkers developed the fura-2 chemosensor (38) for calcium which exemplifies some important characteristics of these types of systems. The fura-2 sensor is selective for Ca$^{2+}$ against other metal ions, including Mg$^{2+}$. Chelation of a Ca$^{2+}$ ion occurs by four carboxylates, two anilines and two phenoxy oxygens resulting in a significant blue shift of the absorption maximum with only a very slight shift of the emission maximum. The modulation of fluorescence
is a result of the fixing of the torsional angles of the aniline and phenoxy ether oxygen of the fluorophore. This fixing of torsional angles inhibits delocalization of the heteroatom lone pairs with the $\pi$ system of the fluorophore. Lone pair interaction by the aryl ether oxygen with the calcium ion may also contribute to $\text{Ca}^{2+}$ binding.

**Scheme 11:** Ratiometric calcium sensing through manipulation of $\pi$ system.

Another key feature of this system is the high fluorescence of both the $\text{Ca}^{2+}$-free and $\text{Ca}^{2+}$-bound states coupled with the shift in the absorption spectrum and a roughly fixed excitation maximum. This allows for the ratiometric determination of the $\text{Ca}^{2+}$ concentration by comparison of the relative concentrations of free and bound sensor. Ratiometric sensing entails a wavelength modulation for absorption or excitation rather than an amplitude modulation, and thus the ratio of bound to unbound sensor can be determined from the ratio of their signals and hence analyte concentration can be extrapolated as well. Ratiometric sensors are particularly useful because they alleviate a number of problems which can hamper non-ratiometric sensors in cell studies, such as variations in excitation source intensity and difficulties in knowing the actual concentration of the sensor.
2.2.1.3 Excimers and Exciplexes

An exciplex (excited state complex) results from the transfer of energy from a fluorophore in an electronically excited state to another nearby fluorophore to form a complex, followed by emission. This typically results in emission at a longer wavelength than that of the fluorophore alone. The term excimer (excited state dimer) designates that both fluorophores are the same species. Formation or prevention of excimers and exciplexes is a strategy for coupling a binding event to a signal transduction. This strategy has been used extensively in chemical sensing.

For example, Kim, et. al. reported excimer based cation sensor 39 (Scheme 12), which could be switched to high or low fluorescence depending on the analyte. The sensor was constructed using 1,3-alternate calix[4]arene as a scaffold, with appended pyrene and crown-5 units. The resultant probe exhibited fluorescent excimer and monomer emission from the pyrenes. Results from the titration of 39 with different metal ions in CH$_3$CN were not general, but certain metals –Na$^+$, Ag$^+$, Pb$^{2+}$, Zn$^{2+}$, Rb$^+$ -were found to quench excimer fluorescence, while other metals –Li$^+$, K$^+$, Cs$^+$, Mg$^{2+}$ -led to enhancement of excimer emission with a corresponding decrease in monomer emission. In cases where the excimer fluorescence was extinguished, it was proposed that the metal ions were bound by the amide carbonyl groups, causing the mechanical separation of the pyrenes. On the other hand, complexation of ions by the crown-5 unit should rigidify the system, stabilizing the excimer and allowing more efficient energy transfer between the pyrene rings. Additionally, there is an energy preference to release a bound Pb$^{2+}$ ion in
favor of binding $K^+$. Therefore, it was found that excimer emission could be restored to a sensor saturated with $\text{Pb}^{2+}$ by adding $K^+$. 

Scheme 12: Excimer based metal ion sensing.
2.2.1.4 Environment sensitive probes

Environment sensitive probes are chemosensors that are sensitive to solvent polarity. Environment sensitivity results from reorganization of solvent molecules in the solvent cage surrounding the excited state of a fluorophore. Excitation of a fluorophore induces a change in dipole moment, the extent of which varies depending on the structure of the fluorophore. Solvent relaxation lowers the energy of the excited state, resulting in a red shift in the emission spectrum or quenching of fluorescence or both. The dye 7-(p-methoxybenzylamino)-4-nitrobenz-2-oxo-1,3-diazole (MBD) is highly fluorescent in nonpolar media, but quantum yields decrease as solvent polarity is increased. In water, MBD is almost non-fluorescent. These types of sensors are useful for probing polarity gradients in membranes, enzyme active sites, and sensing conformational changes in enzymes which induce polarity changes.

Table 1: Fluorescence of MBD in various solvents.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>λ max (nm)</th>
<th>φ</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Butanol</td>
<td>534</td>
<td>0.442</td>
</tr>
<tr>
<td>1-Propanol</td>
<td>534</td>
<td>0.448</td>
</tr>
<tr>
<td>Ethanol</td>
<td>535</td>
<td>0.358</td>
</tr>
<tr>
<td>Methanol</td>
<td>538</td>
<td>0.215</td>
</tr>
<tr>
<td>Water (3% ethanol)</td>
<td>553</td>
<td>0.005</td>
</tr>
</tbody>
</table>
2.3 Conclusions

Fluorescent chemical sensing is a maturing field which has already had an important impact on biological research. Many useful chemosensors have been developed from a few basic strategies which have allowed the monitoring of biological function at the molecular level. The development of new sensors is still needed in order to meet the continuing demands of biological research.
Lipids have an extensive and complex role in biology. In the most basic regard, lipids serve as structural components, forming the permeable boundaries between the intracellular and extracellular matrices of cells and some organelles. The roles of lipids in membranes can be highly complex and specific, influencing protein function and forming specialized structures. Lipids play a crucial role in a variety of signaling processes as well, acting as both first and second messenger molecules and in some cases having a neuroregulatory function. Furthermore, certain lipids have been implicated in disease states such as atherosclerosis. The following section reviews some of the various roles of lipids.

3.2 Lipids in Membranes

Lipids are the building blocks of cell membranes and serve to define the physical characteristics of the membranes. Membranes are formed as bilayers wherein polar head groups interface with the aqueous cytosol and extracellular fluid and non-polar hydrocarbon tails are oriented toward the center of the bilayer, forming the hydrophobic membrane core. The stabilizing force that maintains a lipid bilayer is the hydrophobic effect. In essence, the hydrophobic effect is the tendency of nonpolar molecules to
aggregate in aqueous solution because of the high entropic cost of solvating a non-polar molecule while maintaining the hydrogen bond network of the solvent. At low concentrations, the entropic cost of aggregation is high enough that freely solvated lipids are observed. Above a certain concentration – the critical micelle concentration – the formation of aggregates becomes entropically more favorable. The critical micelle concentration is dependent on the nature of the lipid and the nature of the solvent.

The physical properties of a lipid -i.e., shape, rigidity, and size –play a crucial role in membrane function. Membrane lipids can be classified under three general categories according to their molecular shape: inverted cone, cylindrical, and cone. Cylindrical shaped lipids, whose hydrophilic head group and hydrophobic tail have similar diameters, form stable bilayers above their critical micelle concentration in aqueous solution. The physical nature of such a bilayer can be either the gel state (L_β), which is rigid, or fluid state (L_α), which is supple. While some lipids tend to form gel state bilayers and others form fluid state bilayers, it is the bulk properties of all the components of a bilayer which determine its fluidity or rigidity. Cell membranes are fluid and composed mainly of cylindrical lipids, of which the most common are phosphotidyl choline (PC) based lipids.

Inverted cone shaped lipids are those in which the polar head group is greater in diameter than the lipid tail. These lipids form spherical micelles with a hydrophobic core above their critical micelle concentration. Cone shaped lipids are those in which the polar head group is smaller in diameter than the lipid tail. These lipids form inverted micellar structures having an aqueous core lined by the polar head groups with lipid tails aggregating around the perimeter. Cone shaped and inverted cone shaped lipids
destabilize lipid bilayers and these destabilizing properties have function in tuning the bulk membrane properties and in controlling microdomains.

Within each class of lipid, the length, substitution and unsaturation pattern of the hydrocarbon chain affect the physical properties of a lipid. Longer chains increase the viscosity of a membrane but also destabilize the bilayer. Unsaturation can alter the nature of a lipid from cylindrical to cone shaped. Moreover, temperature influences the effects of an acyl chain, and different acyl chains are affected differently by temperature.

3.2.1 Membrane Function and Microdomains

Bilayer membranes consist of an inner and outer leaflet. The inner leaflet is the lipid monolayer which is in contact with the cytosol and the outer leaflet is in contact with the extracellular matrix. Cell membranes were once visualized using the fluid mosaic model, which essentially suggested that the constituents of the cell membrane were uncontrolled and homogenously distributed over the circumference of the cell. The current understanding suggests that the lipid distribution within a membrane is controlled rather than random. Membranes are dynamic, and flip-flop and lateral diffusion of lipids in membranes is known to occur. Lateral diffusion is diffusion within a single leaflet, whereas flip-flop diffusion occurs between leaflets. Lipid composition of the outer leaflet is different from the inner leaflet and the lipid distribution is enzymatically controlled. The major lipid components of cell membranes are sphingolipids, glycophospholipids and cholesterol (Figure 4). Sphingolipids are composed of the amino alcohol sphingosine, a fatty acid, and a head group. Glycophospholipids are based on a
glycerol backbone, two fatty acids and a phosphate based head group. A major physical difference between sphingolipids and glycophospholipids is that sphingolipids generally have saturated alkyl

**Figure 4:** Cell membrane lipids.

Glycophospholipids

\[
\begin{align*}
R^1 & = \text{Saturated acyl group;} \\
R^2 & = \text{Unsaturated acyl group;} \\
R^3 & = \text{Polar head group}
\end{align*}
\]

Sphingolipids

\[
\begin{align*}
R^1 & = \text{Polar head group;} \\
R^2 & = \text{Saturated acyl group}
\end{align*}
\]

Cholesterol
chains and glycoprophospholipids generally have one alkyl and one alkenyl chain. For this reason, they have very different packing properties. Cholesterol is a tetracyclic sterol lipid. Its shape disrupts close packing of lipids, decreasing membrane rigidity. At the same time its rigidity inhibits motion of lipid alkyl chains, decreasing membrane fluidity. The result of this is that cholesterol stabilizes the liquid ordered state of membranes while inhibiting transition to a gel or liquid disordered state.

3.2.1.1 Lipid Rafts

Sphingolipids and cholesterol are found primarily in the outer leaflet and several glycoprophospholipids are found only in the inner leaflet. It is believed that aggregates of lipids are formed within leaflets which differ in makeup and physical properties from the bulk membrane. These aggregates, termed lipid rafts,\textsuperscript{44-46} are believed to participate in a wide array of cellular processes.

Lipid rafts have been observed by various indirect techniques, although their lifetimes and origins have not been established. Rafts tend to be rich in sphingolipids and cholesterol and are ordered around membrane bound proteins. The high concentrations of cholesterol and sphingolipids in rafts impart higher viscosity than the surrounding bulk membrane. This property is the source of the term “raft”, as it implies an ordered domain “floating” in a fluid bilayer. The specificity of the lipid concentration may be explained by the tendency of cholesterol to associate with the saturated alkyl chains of sphingolipids as well as the capacity of sphingolipid headgroups to participate in intermolecular hydrogen bonding. Whether rafts are formed in order to accommodate
proteins in membranes, or whether membrane bound proteins associate with certain lipids has yet to be determined. However, rafts do aid in the interface of the proteins and membranes. In addition to stabilizing proteins in membranes, rafts have a direct relationship with the function of membrane proteins, which are responsible for cell signaling and cellular uptake. There are two factors contributing to lipid raft influence on protein function. First, there is a direct effect of chemical interaction between the protein and certain closely associated lipids. Second, protein function depends on the bulk effect that the lipids in the raft have on the microdomain. These effects include the order and curvature of the microdomain, which have a profound effect on protein function. In addition to their role in cellular function, rafts have been found to be a point of entry for a number of pathogens \(^{47,48}\) including viruses, \(^{49-51}\) bacteria, \(^{52}\) parasites, and prions.\(^{53}\)

### 3.2.1.2 Caveolae

Related to rafts are the caveolae \(^{44,54-56}\) (little caves), a lipid subdomain characterized as flask shaped invaginations in membranes and generally expressing integral proteins known as caveolins on the intracellular surface. There are three known caveolins: caveolin-1,2,3. Caveolae are rich in cholesterol and sphingolipids and cholesterol is known to associate strongly with caveolins, maintaining association even in the presence of harsh detergents. While originally observed as invaginations, caveolae have also been shown to exist separate from the membrane as single vesicles or in clusters and have been observed to form elongated tubes and trans-cellular channels.\(^{54}\) These architectures enable a number of cellular functions including, in the general sense,
endocytosis and transcytosis. These have emerged as extremely important functions. Endocytosis via caveolae is clathrin free, and thus differs from the classical concept of endocytosis via clathrin coated vesicles in that the contents of caveolae derived vesicles are not degraded by lysomes. This is important for the endo- and trans-cytosis of molecules and macromolecules such as folate, chemokines, and immunoglobulin A, which must be transported while maintaining their integrity.

A number of pathogens take advantage of the security found within caveolae. For example, HIV is known to employ caveolae for transcytosis, RSV enters the cell and subsequently the endoplasmic reticulum via caveolae, and a number of bacteria use them to enter the cell and reside there. This last example implies that a variety of bacteria actively control the lipid constitution of the caveolae they inhabit, as the lipid constitution of these vesicles is dynamic and must be maintained in order to avoid fusion with lysomes.

3.3 Lipids as Signaling Agents

Lipids have been identified as having diverse roles in cell signaling pathways and a few examples are discussed here. Degradation of glycolipids by enzymes results in the generation of second messengers in the form of diacylglycerols, phosphatidic acids, and modified head groups. These signaling molecules are involved in electrolyte release and protein modulation and effect a number of processes such as cell proliferation, apoptosis, smooth muscle contraction and inflammation.
Sphingomyelin is converted to ceramides and sphingosines in order to control proteins and enzymes which affect diverse cellular activity such as cell growth and differentiation. Sphingosine (44) can also be phosphorylated to sphingosine-1-phosphate (S1P, 45) which can be dephosphorylated to regenerate sphingosine. S1P has numerous roles and, in fact, sphingosine and S1P have opposing effects on a number of functions. The balance between them is used to regulate such events as cell growth and suppression of apoptosis, which are favored by S1P, and cell growth arrest and enhancement which are favored by sphingosine.59

\[
\text{OH} \\
\text{NH}_2 \\
\text{OH} \\
\text{NH}_2 \\
\text{OPO}_3^-_2
\]

Fatty acids are used in signaling and in some cases have been recognized as having neuroregulatory function, whether by actively participating as a neurotransmitter or by deactivation of neuroregulatory pathways. Anandamide (46) is a cannabinoid neurotransmitter which acts as an agonist for cannabinoid receptors CB1 and CB2. It affects memory, sleep, appetite and pain perception. Oleamide (47) was discovered to have neuromodulatory activity.64,65
Oleamide induces physiological responses similar to those of anandamide, namely impaired motor activity, body temperature depression, pain sensory inhibition, and catalepsy. It has also displayed psychotropic activity which has been characterized as subdued anxiety and increased sociability. Oleylethanolamide (48), while more structurally similar to anandamide than is oleamide, does not bind cannabinoid receptors. It does operate as a signaling molecule though, affecting appetite and weight.66

3.4 Oxidized Phospholipids

In the past decade, oxidized phospholipids have been found to play a significant role in vascular disease. These phospholipid products result from myeloperoxidase (MPO) oxidation of phospholipids in low density lipoproteins (LDL).67-69 LDL are transport structures for cholesterol and triglycerides having a cholesterol and phospholipid monolayer shell with a single embedded Apo-B100 protein. Within this
outer shell is a core consisting of triacylglycerol and cholesteryl esters. Oxidation of monolayer phospholipids results in expression of a variety of oxidized products on the lipoprotein surface. A number of oxidized phospholipids bind to macrophage scavenger receptor CD36, inciting formation of arterial inflammation and lesions, resulting in atherosclerosis. Podrez, et. al. reported the preparation and isolation of a series of MPO oxidized LDL phospholipids which were found to be potent ligands of CD36 (49-52). 70

Interestingly, the hydroxy analogs of these compounds, which were also products of the MPO oxidation (54-57), exhibited decreased binding affinity for the CD36 receptor, indicating the importance of the ketone functional group.
3.5 Conclusions

Lipids constitute a biologically important class of compounds. The function of these compounds in biological systems can be indirect, as in the physical properties of membrane lipids and the effects of membrane fluidity and curvature on membrane proteins. Lipids can also have more direct function, as in their roles as messenger molecules. Methods for the selective recognition or sensing of lipids could play an important role in the biological study of many of these important systems.
Chapter 4: Lipid Recognition

4.1 Cholesterol recognition

4.1.1 Cyclodextrins

The cyclodextrins (CD) are oligosaccharide-based cyclophanes derived from glucose. These cyclophanes are water soluble and have a hydrophobic interior. These compounds have been used to solublize lipophilic substrates in aqueous environments for a variety of applications. The natural cyclodextrins are α, β and γ cyclodextrin consisting of six, seven and eight glucose units, respectively. These compounds were the subject of studies on the extraction of cholesterol from cell membranes. It was found that β-cyclodextrin (58) was markedly better at solublizing cholesterol than were α and γ cyclodextrins.
Cholesterol extraction studies demonstrated that β-cyclodextrin could be used for the selective cholesterol depletion of erythrocytes, removing cholesterol from the cell membranes quantitatively at ~10mM concentration. On the other hand, α and γ cyclodextrins showed little capacity for cholesterol extraction. The process of extraction was shown to be reversible by experiments with [³H]cholesterol-labeled erythrocytes. Exposure of these erythrocytes to β-cyclodextrin solution resulted in extraction of [³H]cholesterol into solution. Once the extraction process reached equilibrium, unlabelled erythrocytes were introduced to the suspension and reabsorption of the [³H]cholesterol was observed. This work demonstrated the selective recognition of cholesterol over other lipids by β-cyclodextrin, the reversible extraction of cholesterol from cell membranes by β-cyclodextrin and how β-cyclodextrin might be useful for cellular studies.

Following the establishment of β-cyclodextrin as the preeminent tool for cholesterol depletion in cell studies, chemically modified β-cyclodextrins were examined to determine their usefulness. Hydroxypropyl-β-cyclodextrin (HβCD) and methyl-β-cyclodextrin (MβCD) result from alkylation of the free hydroxyl groups. HβCD, which has enhanced hydrophilicity, was found to be superior to β-cyclodextrin for the aqueous solubilization of lipids. However, MβCD was reported to outperform either HβCD or β-cyclodextrin in cholesterol extraction, having greater specificity for cholesterol over acyl lipids. In that work, Fahrenholz and co-workers found that incubation of cells with 100mg/mL MβCD resulted in a change of the cholesterol/phospholipid ratio to 0.02 from 0.65 in untreated cells. As was previously reported, an equilibrium is established and cholesterol is exchanged dynamically between the cell membrane and inclusion
complexes in solution. Thus, by increasing the concentration of the cholesterol-MβCD inclusion complex, the equilibrium could be shifted to partially or completely restore the cholesterol content of cell membranes. The researchers went on to demonstrate that oxytocin receptor function in guinea pig uterine cells was highly dependent on membrane cholesterol content. Depletion of cholesterol from these cells induced the transition from a high binding affinity state to a low binding affinity state for these receptors.

Cyclodextrins have also been shown to be useful for acyl lipid depletion in thylakoid membranes. In these studies, α-CD was found to favor slightly the removal of saturated acyl lipids over unsaturated lipids and showed no significant propensity for protein or chlorophyll extraction. The success of these experiments was of course dependent on the absence of steroid compounds as membrane components and competing substrates.

Breslow prepared a number of cyclodextrin dimers for the ditopic binding of hydrophobic substrates. Dimer 59 was designed for the enhanced binding of cholesterol relative to β-cyclodextrin. The depth of the cavity of β-cyclodextrin is ca. 7.8 Å, whereas the head to tail length of cholesterol is ca. 15 Å. Therefore, it was anticipated that two units of β-cyclodextrin linked together by a very short linker would cooperatively bind one molecule of cholesterol. Indeed, cholesterol was bound with a $K_a$ two orders of magnitude greater than β-cyclodextrin alone. 59 was also shown to outperform a β-cyclodextrin polymer which had been used commercially for the sequestration of cholesterol.
4.1.2 Synthetic receptors for cholesterol

Cyclophane 60 was reported by Deiderich as a receptor for flat aromatic compounds. The distance between the bridging oxygens of each diphenyl methane unit is 8.4 Å, making an effective cavity for the tight binding of aromatic guests.

In seeking to prepare receptors for steroid inclusion, a redesign of the host would be necessary, as these saturated polyalicyclic targets are more cylindrical and have a larger cross sectional area than simple aromatic substrates. Receptors 61 and 62 were prepared to account for this. By substituting two naphthalene rings for phenyl rings, the
distance between bridging oxygens was increased to between 10.4 and 11.0 Å, depending on the conformation of the host.

![Chemical structure](image1)

![Chemical structure](image2)

The capacity for these hosts to bind various guests, including simple alicyclic substrates as well as bile acids and steroids, was examined in mixtures of D$_2$O and CD$_3$OD. Cholesterol derived substrates were included axially within the cavities of 61 and 62. Calorimetric titrations showed this complexation to be enthalpically driven with entropic compensation. Higher binding affinities were observed for receptor 61 in complexing neutral steroid guests due to the solvating groups of receptor 62, which serve
to increase the polarity in proximity to the cavity and thus make it a less hydrophobic receptor than 61. Additionally, the least polar guest - lithocholic acid (Table 2, 63e) - formed the most stable complex, whereas cholic acid (63a), which has a hydroxyl group on both its B and C rings, formed the least stable complex. These hydroxyl groups must be desolvated in order to be included within the cavity and may enforce unfavorable conformational changes. For this reason, guests bearing a single hydroxyl group on either ring B or C (63b-c) produced complexes of a stability value which was intermediate to those formed with lithocholic and cholic acid.

Table 2: Table of steroidal guests.

<table>
<thead>
<tr>
<th>Steroid</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>a Cholic acid</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
<td>CO₂H</td>
</tr>
<tr>
<td>b Deoxycholic acid</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>CO₂H</td>
</tr>
<tr>
<td>c Chenodeoxycholic acid</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>CO₂H</td>
</tr>
<tr>
<td>d Ursodeoxycholic acid</td>
<td>H</td>
<td>H</td>
<td>OH</td>
<td>CO₂H</td>
</tr>
<tr>
<td>e Lithocholic acid</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>CO₂H</td>
</tr>
<tr>
<td>f sodium glycochenodeoxycholate</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>R₁</td>
</tr>
<tr>
<td>g 3-[(3-cholamidopropyl)dimethylammonio]-l-propanesulfonate</td>
<td>OH</td>
<td>H</td>
<td>OH</td>
<td>R₂</td>
</tr>
</tbody>
</table>

R₁ = CONHCH₂COO⁻ Na⁺  R₂ = CONH(CH₂)₃N⁺(CH₃)₂-(CH₂)₃SO₃⁻
The cationic appendages of 62, while somewhat destabilizing to the inclusion of neutral guests, result in additional stabilization of complexes with guests bearing anionic groups (63f-g) due to favorable ion pairing interactions. In these cases, complexes formed with 62 were found to be more stable than those formed with 61. Both cyclophanes were found to be selective against smaller substrates such as adamantane carboxylic acid (64) and camphor (65), which do not completely fill the cavity.

While 61 and 62 proved to be useful receptors for steroids, they did not solublize cholesterol or cholesterol derived compounds in aqueous solution without a co-solvent like methanol. With a view toward cholesterol binding and further understanding of steroid recognition, tricyclic cyclophanes 66 and 67 were prepared.82-84 These receptors are essentially extensions of receptor 61 and possess cavities of dimensions similar to those of natural steroid binding proteins. The tighter cavity of 66 was found to be more suitable for unsaturated steroids whereas the more spacious 67 is selective for fully saturated steroids. Both 66 and 67 were found to be capable of solublizing cholesterol in aqueous solution.
4.2 Alkane receptors

Cavitand 68 was first reported by Gibb as a receptor for a variety of hydrophobic guests.\textsuperscript{85,86} Originally reported studies were for the complexation of halogenated alkanes. Adamantyl iodide in particular formed a stable complex with 68 due to the complementarity of the bowl shape of the receptor and the spherical form of the adamantane as well as favorable interactions between the iodine atom and the interior benzylic hydrogens. Later reports divulged that 68 dimerized non-covalently in aqueous solution to form capsules in the presence of simple unbranched alkane guests.\textsuperscript{87,88}
Butane gas was extracted into a solution of 68 in buffered D₂O without the aid of agitation to form a 2:2 complex. Examination of the complexation properties of 68 with a series of n-alkane guests (propane through octadecane) revealed that stable capsular complexes were formed with guests as large as heptadecane. Octadecane was solubilized by 68 but stable capsules were not observed. Guests larger than octane formed 2:1 complexes and guests smaller than octane formed 2:2 complexes while a mixture of 2:1 and 2:2 complexes were observed for octane itself. A competition study demonstrated the proof of concept for using this system to separate hydrocarbon gasses. Upon exposure to a headspace mixture of 1:1 propane and butane, only the encapsulation of butane was observed, resulting in a propane enriched headspace. However, the source of this selectivity has yet to be made clear. Indeed, the structural nature of the encapsulation of alkanes by 68 is not yet well understood.

Rebek recently reported the binding of normal alkanes using water soluble benzimidazole based cavitand 28. As was previously discussed (vide supra), this cavitand forms solvent bridged hydrogen bonds in water between the imidazole groups to favor the vase conformation and the carboxylate groups repel guests such as carnitine
which, if bound, would require close spatial proximity of the guest’s negative charge to the host’s negative charges. Thus, the zwitterionic dodecylphosphocholine was not expected to form a complex with 28 by burial of the ammonium moiety deep within the cavity of the host. Indeed, what was observed was the burial of the alkyl chain in the cavity. Moreover, while depth of the cavity is sufficient to bind six carbons of an extended alkyl chain, eight carbons were observed to be bound. It can be surmised from this that the alkyl chain must be coiled within the cavity. Coiling of an alkyl chain induces significant amounts of gauche strain, ca. 0.5 kcal·mol⁻¹ for each turn. However, the cavity is not sufficiently tight to bind alkyl chains in the extended conformation with complete expulsion of solvent. Therefore, the complete expulsion of water from the hydrophobic interior more than compensates for this strain energy. These complexes were observed using ¹H NMR by the characteristic upfield shift of the alkane protons which shifted as much as 5 ppm. The complexes were also characterized by nOe spectroscopy.
Rebek, et. al. have reported the formation of capsules by cavitand 69. The imides which make up the rim of the cavity form bifurcated hydrogen bonds to produce the container (Scheme 13). These containers were observed by $^1$H NMR in mesitylene-$d_{12}$ in the presence of suitable guest compounds, but in the absence of such a guest capsules were not observed. A large number of guests could be bound in this way, so long as they fit within the confines of the capsule. A strict limit was found on the length of the guest, which could not exceed the length of the interior space of the capsule. It was found that alkanes made good guests in this system. The driving forces for encapsulation in this system are the inability of the solvent to act as a guest and the network of hydrogen bonds that is only possible with formation of a capsule. Therefore, suitable guests are bound within the cavity as the alternatives are the presence of a vacuum within the capsule or the disruption of the hydrogen bonding. In some cases, alkanes were bound in the lowest energy extended conformation, as was the case with C$_{10}$. In the case of C$_{12}$ and C$_{14}$, the interior of the capsule is of insufficient length to accommodate the alkane in the fully extended conformation and these guests must coil, incurring strain energy from
**Scheme 13:** Capsules formation by dimerization of cavitand 69. Guest and R groups omitted in model structure for clarity.

![Diagram of Scheme 13](image)

**Figure 5:** Model representation of dodecane guest in capsule host. Cutaway CPK graphical representation of 69\textsubscript{2}\textcdot C\textsubscript{12}H\textsubscript{26} - dodecane guest in molecular capsule. R groups omitted and guest colored gold for clarity.

![Diagram of Figure 5](image)
gauche interactions. Fourteen carbons was found to be the upper limit for alkane guest size as C\textsubscript{15} was not encapsulated.

Rebek later reported the reversible expansion of these capsules with glycoluril based spacers\textsuperscript{90} Again, studies were done by NMR in mesitylene-\textit{d}\textsubscript{12}. It was observed that, in the presence of excess glycoluril 70\textit{a} or 70\textit{b}, a number of guests which

**Scheme 14:** Reversible addition of glycoluril spacers to form elongated capsules. R groups, Ar groups, guest and one glycoluril unit omitted for clarity.

\[\begin{align*}
69 & \quad + \quad \text{Guest} \\
70\text{a} & \quad \text{Ar} = C_6H_4\text{p}-OC_8H_{17} \\
70\text{b} & \quad \text{Ar} = C_6H_4\text{p}-C_{12}H_{25}
\end{align*}\]

had previously been found not to bind were bound in capsules. In these cases capsule formation incorporated four units of either glycoluril present. Studies with both glycolurils present indicated formation of multiple types of capsules having incorporated different combinations of the two glycolurils. This expansion increases the length and
volume of the capsules by 7 Å and ~200 Å³ respectively. Normal alkanes as large as C₂₀ are encapsulated by this system.

The glycoluril spacer 72 was prepared to probe pH controlled expansion and contraction of molecular capsules. Addition of glycoluril 72 resulted in expansion of capsule 69₂•C₁₄H₃₀, allowing for an extended conformation of guest tetradecane. Bubbling HCl gas into solution caused the precipitation of the glycoluril as its HCl salt, inducing contraction of the capsule and coiling of the guest. Addition of TEA to this suspension liberated the glycoluril and allowed reformation of the extended capsule. This process was also found to be repeatable.

\[ \text{N} \quad \text{N} \quad \text{O} \quad \text{O} \quad \text{N(C₄H₉)₂} \quad \text{N(C₄H₉)₂} \]

4.3 Conclusions

Lipid recognition is an important goal which could aid in the development of tools for biological studies and other useful materials. Research into recognition of biologically important lipids had been limited almost exclusively to the steroids. The advances in this area have been instrumental in developing the biology of cell membranes
and as medicinal agents. On the other hand, selective receptors for acyl lipids are currently unavailable despite their potential for use in a similar fashion. Receptors for simple alkanes are limited and do not distinguish between n-alkanes and other alkane guests. Therefore, methods for the selective recognition of n-alkanes remain an important but unfulfilled goal.
Chapter 5: Molecular Tubes for the Shape Selective Recognition and Sensing of Lipids.

5.1 Designing a Molecular Tube

5.1.1 Introduction

The origin of the molecular tubes project was in the preparation of naphthalene based cavitands for host-guest studies.* During the course of this work a reaction for the preparation of bicyclized naphthalene cleft 74 (Scheme 15) from 2,7-dihydroxynaphthalene (73) was found in the literature. Consideration of the physical characteristics of 74 indicated that molecules of this type might be useful for the synthesis of molecular tubes. The bicyclic bridge that links the aromatic rings together locks them in a rigid conformation such that the edges of the naphthalenes meet in nearly perpendicular fashion. The aromatic rings constitute large, flat, rigid surfaces that would function suitably as the walls of the tube. Due to the inherent fluorescence of naphthalenes, the potential existed that such tubes could act fluorescent sensors. Aromatic substitution would provide a convenient way to modify these types of molecules in a controlled way. Thus, molecular tubes were conceptualized wherein two bis-naphthalene molecules would be linked together so as to form a cavity which could

* - Berkeley J. Shorthill and this author contributed equally to the work presented in sections 5.1.1 through 5.2.3.
be useful for molecular recognition (Figure 6). This connectivity could result in two isomers designated syn and anti.

In order to achieve the desired linkage we envisioned adding linkers (X in compound 75, Figure 6) to compound 74 via aromatic substitution. It was known that the regiochemistry of aromatic substitution of the naphthalenes in 74 would favor substitution at the 8-position almost exclusively. Thus, 2,6-dihydroxy naphthalene was

**Scheme 15:** Formation of naphthalene clefts.

![Scheme 15](image)

**Figure 6:** Conceptualized molecular tube.

![Figure 6](image)

chosen as the starting structure (Scheme 16). This substitution pattern would enable aromatic substitution at the desired 5-position. Unfortunately, it was reported in the
original paper that 2,6-, 1,5- and 2,3-naphthalene diols tended to form polymers under the reported conditions. It was postulated in that paper that the free phenols of 73 were inhibited from further interaction by sterics, which was not the case with the other diols examined.

Despite concerns over polymerization, our initial foray into these types of systems was the dimerization of 2,6-naphthalene diol (76) with malonaldehyde bis(dimethyl)acetal (Scheme 16). This reaction was found to proceed in an ether/TFA solvent system to produce the desired bicyclic compound 77 in low yield. Attempts to formylate compound 77 returned only starting material under a number of conditions. By protecting the phenols as methyl ethers, the formylation by treatment with dichloromethylmethyl ether and TiCl4 was possible.93
Scheme 16: Model studies on naphthalene chemistry.

The sequence was optimized as shown in scheme 17. Mono-methylation of diol 76 gave naphthol 81, which resulted in increased solubility relative to the dihydroxy compound 76. Dimerization could then be conducted with CH2Cl2 as co-solvent, giving a higher yield of 79. Having established an efficient route to model compound 79, the electrophilic aromatic substitution (EAS) characteristics were further probed. In addition to formylation, bromination and bromomethylation were found to proceed cleanly and in high yield. At this time, it became necessary to examine possible linker motifs in order to target structures which should be useful for binding studies.
**Scheme 17:** Optimization and further exploration of model chemistry.

```
\[ \text{76} \xrightarrow{\text{CH}_3\text{I, NaH, DMF, 30\%}} \text{81} \xrightarrow{TFA, DCM, 60\%} \text{79} \]
```

**Scheme 18:** Studies on EAS reactions of compound 79.

```
\[ \text{79} \xrightarrow{\text{Conditions and results: See Table 3}} \]
```

**Table 3:** Conditions and results for EAS of cleft compound 79.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Conditions</th>
<th>X</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>TiCl₄, CHCl₂OCH₃, CH₂Cl₂</td>
<td>CHO</td>
<td>88%</td>
</tr>
<tr>
<td>82</td>
<td>(CH₂O)x, HBr, HOAc, CH₂Cl₂ⁱ⁴</td>
<td>CH₂Br</td>
<td>87%</td>
</tr>
<tr>
<td>83</td>
<td>Br₂, HOAc, CH₂Cl₂ⁱ⁴</td>
<td>Br</td>
<td>98%</td>
</tr>
</tbody>
</table>
5.1.2 Evaluation of prospective linkers

Several basic guidelines were considered while evaluating linking group possibilities. Certainly, for a linker to be considered, it should produce a useful structure. In the general sense, this means structures with cavities which would be suitable for binding a host. Our initial goal was lipid recognition. Specifically, we sought to demonstrate shape selective recognition of saturated, unbranched lipids. Thus, structures were evaluated with these targets in mind. Additionally, the linker should be chemically stable and synthetically accessible.

Structures were modeled by computation\textsuperscript{95-97} and with plastic Corey-Pauling-Koltun (CPK) models. Inspection of models revealed that tubes having linkers with an odd number of atoms incurred significantly less strain than tubes having linkers with an even number of atoms. Generally, an even number of atoms in a linker necessitates gauche interactions and requires greater distortion of bond lengths and angles.
After considering the length of the linker as it effects the stability of the system, it was then necessary to consider the volume of the cavity as determined by linker length. For the purpose of targeting lipids, three atom linkers were believed to be optimum based of evaluation of the modeling (Figure 7). The use of a three atom linker (84b) would provide a cavity which would be well matched to the volume occupied by a normal alkyl chain in the extended conformation. Deviations such as unsaturation or branching would create portions of the chain that were not properly matched to the available cavity, and thus should be bound with less affinity. A one atom linker (84a) would result in too tight a cavity for effective binding and for steric reasons may be synthetically inaccessible. A number of linkers having five atoms (84c) would be plausible and would result in interesting tube molecules. However, these types of linkers would result in a cavity large

**Figure 7:** Tubes with varying linker lengths: A one atom linker (84a) with bound acetylene; a three atom linker (84b) with bound alkane; a five atom linker (84c) with bound decalin.
enough to accommodate a number of types of guests and we anticipated that little selectivity would be observed. Tubes having three atom linkers were thus our initial focus.

A number of three atom linkers were examined by molecular modeling, several of which are shown in scheme 18. The modeling indicated that there were essentially two possible conformations which could result from the proposed connectivity. The desired conformation was an open tube, such as the one depicted in structure 84b (Figure 7). It was also possible for the tube to collapse by bringing the tube halves together. By calculation, some linkers resulted in open structures (c, d, e) while others resulted in collapsed structures (a, b) as the lowest energy conformation (Scheme 19). The later conformation was confirmed by x-ray analysis of 86a.\textsuperscript{98} Our assumption was that tubes which preferred an open conformation would be more useful for the recognition of lipids, as binding of a lipid within the tube would not necessitate a change in conformation. Therefore, we chose to pursue linkers which were found by molecular modeling to produce an open conformation.
Scheme 19: Planned tube connectivity and possible linkers.

\[ X = \text{[Diagram of various linkers]} \]

85

86

86a
Amide linked tubes were selected as the initial targets because modeling indicated that the syn isomer would be reasonably rigid and posses an open cavity and because they appeared to be synthetically accessible and chemically robust targets (Figure 8). The syn and anti isomers were both found to be somewhat flexible by modeling, and the anti isomer in particular was found to be susceptible to collapse. Nevertheless, the calculated lowest energy conformation of either host bore an open cavity which had the appropriate shape for inclusion of alkanes. Collapse of the tube was less favorable as a result of the carbonyls adjacent to two of the naphthalenes in this motif. Collapsing the tube would require positioning one of the carbonyls inside the cavity, which was found to be an unfavorable conformation. As shown, the syn amide linked tube appears capable of opening into a bowl shape via rotation about the amide linkers (Figure 8, c). However, alkylation of the phenols would prevent this process via steric repulsion.
Figure 8: Models of amide linked tubes: a) syn isomer of amide linked tube, end on view b) anti isomer of amide linked tube, end on view c) syn isomer of amide linked tube, side view d) anti isomer of amide linked tube, side view. R groups are omitted for clarity. Structures a and c were minimized by molecular dynamics simulation in chloroform (Amber 7).
5.2 Amide Linked Molecular Tubes

5.2.1 Synthesis of Amide Linked Tubes

The retrosynthetic plan was to access these host compounds via the known formylation chemistry (Scheme 20). Coupling of an amine (88) and a carboxylic acid (89) would result in an amide linked tube. The acid and amine coupling partners could both be synthesized from the formylated compound 90.

Scheme 20: Retrosynthesis of amide linked tube.

In addition to this basic strategy, a slight modification of the established chemistry was necessary to address the requirement of water solubility for this system.
Latent water solubilizing groups were incorporated by first mono-alkylating 2,6-dihydroxynaphthalene with methyl bromoacetate and NaH (Scheme 21). The resulting naphthol 91 was cyclized as previously described to produce dimer 92 in 76% yield. Formylation of 92 was accomplished by treatment with TiCl₄ and α,α-dichloromethylmethyl ether in methylene chloride to yield the 5,5'-diformylated compound 93 regiospecifically in 98% yield.

**Scheme 21:** Synthesis of amide linked tube; preparation of diformylated cleft.

From this formylated compound, both coupling partners for the synthesis of the lactam were derived. Diacid compound 95 was prepared by oxidation of the aldehyde under mild conditions using NaOClO and sulfamic acid in water/THF (Scheme 22). The diacid was cleanly prepared by this method in 95% yield. The other coupling partner, the dibenzylic amine di-TFA salt 96, was prepared by reductive amination of the dialdehyde.
with \( t\)-butoxycarbamate, TES and TFA, followed by deprotection with TFA in CH\(_2\)Cl\(_2\), to give compound 96 in 66% yield.

**Scheme 22: Synthesis of coupling partners.**

Coupling of acid 95 and amine 96 was accomplished using the Py-BOP\(^{100}\) coupling reagent and diisopropylethylamine (DIEA) in DMF (Scheme 23). This reaction was
Scheme 23: Formation of amide linked tube 98 from precursors 95 and 96.

\[
\begin{align*}
\text{95} & \quad + \quad \text{96} \\
\text{PyBOP} & \quad \text{DIEA, DMF} \\
\text{35\%} & \\
\text{97} & \\
\text{NaOH, H}_2\text{O} & \rightarrow \quad \text{MeOH, THF} \\
\text{95\%} & \\
\text{98} & \\
\text{R} = \text{CH}_2\text{CO}_2\text{OH} & \quad \text{R} = \text{CH}_2\text{CO}_2\text{CH}_3
\end{align*}
\]

found to proceed in 35\% yield of syn (97a) and anti (97b) stereochemistry combined.

The purified mixture of syn and anti tubes was subjected to saponification conditions,
producing the tetracarboxylate tubes 98 as a mixture in high yield. The syn and anti isomers were then separated by reverse phase HPLC.

2D NMR spectroscopy was used to assign the syn and anti isomers of tube 98. The protons of each tube were assigned based on COSY and NOESY NMR. The assignment of syn versus anti was made using NOESY. There were specific nOe’s that were anticipated which would distinguish between syn and anti 98. Figure 9 shows the relevant portion of the NOESY spectra of the syn (98a) isomer. The indicated nOe clearly indicates compound 98a as the syn isomer and is not observed in the NOESY spectrum of 98b. A weak nOe in the NOESY spectrum of the compound 98b which is not present in that of 98a, supports the assignment of compound 98b as the anti isomer (Figure 10).

**Figure 9:** Relevant portion of NOESY spectrum of 98a. Red frame indicates the nOe depicted in the structure at right.
5.2.2 Recognition of Alkane Guests

The saponified hosts were found to be suitably soluble in aqueous D$_2$O for examining the binding properties with carboxylate guests via $^1$H NMR. Thus, solutions of 98a and 98b (40 mM Na$_2$CO$_3$ in D$_2$O) were prepared and titrated with decanoic acid and 4-methyloctanoic acid.$^{101}$ Upon addition of guest, proton signals were observed upfield from their positions for free guest. Larger shifts were observed with 98a than with 98b and larger shifts were observed with decanoic acid than with 4-methyloctanoic acid. The complexation of decanoic acid by host 98a resulted in four broad peaks in the
range from 0 to -2 ppm (Figure 11). Aromatic shielding this substantial is consistent with inclusion of the guest within an aromatic pocket and is well documented in the literature. Therefore, the data indicates that host 98a and decanoic acid form an inclusion complex in aqueous solution where some portion of the alkane is within the cavity of the tube. The spectrum for the same guest with host 98b show guest proton signals shifted upfield, but the shifts are less dramatic resulting in signals at ca. 0.7 and -0.2 ppm. Hence, guests do not experience significant aromatic shielding with host 98b. The guest 4-methyloctanoate does not experience significant shielding by either host 98a or 98b.

**Figure 11:** NMR data from titration of 98a with decanoic acid in 40mM Na$_2$CO$_3$ in D$_2$O: a) decanoic acid only; b) host 98a only; c) 98a + decanoic acid.
Due to the inherent fluorescence of the naphthalene rings, hosts 98a and 98b had potential to act as fluorescent sensors. It was believed that the expulsion of water from the open tube upon binding of a guest should result in an environment-sensitive fluorescent response by the naphthyl groups and an increase in fluorescence. Although no change in UV absorption was observed for either isomer upon addition of lipids, both isomers showed significant changes in fluorescence upon addition of fatty acids. Both the association constant and the change in fluorescence were highly dependent on the guest and the isomer (Table 4). For the anti isomer 98b, strong fluorescence quenching (42-89%) was observed for all hydrophobic guests (Figure 12) except the two and four carbon acids (entries 1 and 2) and the cyclic acid (entry 10). The association constants varied uniformly per the size and hydrophobicity of the guest, with more hydrophobic guests binding more tightly.

In the case of the syn isomer 98a, a pronounced difference in affinity pattern was observed. For simple straight chain saturated lipids, the observed trend corresponded with that observed for the anti isomer (Figure 13). Introduction of branching in the middle of the chain inhibited binding (entry 8). However, branching at the end of a long chain was tolerated (entry 9). This is expected based on models which indicate that host 98a cannot accept 4-methyl-octanoic without significant steric strain. With 8-methyl-nonanoic acid, this strain is released by fully threading the tube, such that only unbranched alkane fills the cavity.
**Table 4:** Association Constants and Fluorescence Changes of Sensors 98a and 98b with Lipid Guests (20 mM HEPES; pH = 8.4; [98] = 10^{-5} M; \( \lambda_{ex} = 365 \text{ nm} \); \( \lambda_{em} = 396 \text{ nm} \)). \( I_{sat} \) is the fluorescence intensity at saturation taken from the fit of the titration data; \( I_0 \) is initial fluorescence intensity.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Guest</th>
<th>Sensor 98a</th>
<th>Sensor 98b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( K (M^{-1}) )</td>
<td>( I_{sat}/I_0 )</td>
</tr>
<tr>
<td>1</td>
<td>Acetic acid</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Butyric acid</td>
<td>17</td>
<td>1.25</td>
</tr>
<tr>
<td>3</td>
<td>Hexanoic acid</td>
<td>100</td>
<td>1.14</td>
</tr>
<tr>
<td>4</td>
<td>Octanoic acid</td>
<td>250</td>
<td>0.39</td>
</tr>
<tr>
<td>5</td>
<td>Decanoic acid</td>
<td>3,500</td>
<td>0.48</td>
</tr>
<tr>
<td>6</td>
<td>Dodecanoic acid</td>
<td>18,000</td>
<td>0.47</td>
</tr>
<tr>
<td>7</td>
<td><em>trans</em>-3-Octenoic acid</td>
<td>92</td>
<td>0.61</td>
</tr>
<tr>
<td>8</td>
<td>4-Methyl-octanoic acid</td>
<td>71</td>
<td>1.05</td>
</tr>
<tr>
<td>9</td>
<td>8-Methyl-nonanoic acid</td>
<td>1,600</td>
<td>0.57</td>
</tr>
<tr>
<td>10</td>
<td>Cyclohexane carboxylic acid</td>
<td>470</td>
<td>7.1</td>
</tr>
<tr>
<td>11</td>
<td>Triethylene glycol</td>
<td>63</td>
<td>1.83</td>
</tr>
<tr>
<td>12</td>
<td>1-Heptanol</td>
<td>4,900</td>
<td>0.28</td>
</tr>
<tr>
<td>13</td>
<td><em>cis</em>-4-Hepten-1-ol</td>
<td>1,200</td>
<td>1.32</td>
</tr>
<tr>
<td>14</td>
<td>Heptyl amine</td>
<td>16,000</td>
<td>0.42</td>
</tr>
</tbody>
</table>
**Figure 12:** Fluorescence titration of compound 98a with octanoic acid in buffer (20 mM HEPES; pH = 8.4; [98a] = 10^{-5} M; \lambda_{ex} = 365 \text{ nm}): a) Fluorescence emission spectra as a function of added octanoate, b) titration data at \lambda_{em} = 396 \text{ nm}.

![Fluorescence emission spectra](image1.png)

![Titration data](image2.png)

**Figure 13:** Fluorescence titration of compound 98b with octanoic acid in buffer (20 mM HEPES; pH = 8.4; [98b] = 10^{-5} M; \lambda_{ex} = 365 \text{ nm}): a) Fluorescence emission spectra as a function of added octanoate, b) relative fluorescence (\lambda_{em} = 396 \text{ nm}) of host 98b vs added octanoate.

![Fluorescence emission spectra](image3.png)

![Relative fluorescence](image4.png)
Alkenes in the chain produce slightly lower binding constants with the cis-alkene (compare entries 12 and 13) being tolerated less well than the trans-alkene (entries 4 and 7). The sp² C of the alkenes occupy significantly less space than the corresponding methylene units of alkanes. Thus, alkenes do not fill the cavities of the tubes, resulting in poor steric interactions with the sides of the tubes (Figure 14). Finally, affinity increased from anionic to neutral to cationic guests as expected (entries 4, 12, and 14).

**Figure 14:** CPK models of *cis*-alkene, *trans*-alkene and alkane.

The unusual feature of the fluorescence data for the syn isomer (98a) is the type of fluorescence change observed, wherein some guests induce a fluorescence quenching and some induced a fluorescence enhancement. Examination of Table 4 reveals that in
the case of 98a, only straight chain lipids which can thread completely through the tube produced a quenched fluorescence (39-72%) similar to that seen in 98b. Guests with branching, rings, or cis-alkenes produced mild to marked fluorescent enhancements (5-710%) (Figure 15). Triethylene glycol, which is hydrophilic and contains gauche ether dihedral angles, was bound poorly and produced a fluorescence increase. Even short-chain lipids (entries 2 and 3) which cannot span the length of the sensor gave a fluorescence increase. Thus, sensor 98a possesses an unusual selectivity for long, straight-chain hydrophobic lipids.

**Figure 15:** Titration of compound 98 with 4-methyloctanoate (20 mM HEPES; pH = 8.4; [98] = 10^{-5} M; \lambda_{ex} = 365 nm): a) Fluorescence titration of compound 98a with 4-methyloctanoic acid; b) Fluorescence titration of compound 98b with 4-methyloctanoic acid.

The dichotomy in the fluorescent response of 98a most likely results from the probability that the tube prefers a collapsed conformation in aqueous environments. Therefore, guests which thread the tube cause a conformational change resulting in
fluorescence quenching. Guests which cannot thread the tube bind the shallow cavity present in the collapsed tube, thereby inciting environment sensitive fluorescent enhancement. On the other hand, the anti isomer 98b has an effective tube length of only one benzene ring, compared to the effective length of a full naphthalene ring in the case of 98a. Therefore, it is easier for short, branched or unsaturated guests to thread 98b because a shorter length of lipid lies within the cavity. Thus, steric strain incurred in binding of certain guests by 98a might not be incurred in the binding of them by 98b and the lipids which cannot thread 98a may still cause a change in conformation from collapsed to open for 98b.

Stoichiometry of the H•G was determined by the continuous variation method, also known as the Job experiment. In this experiment, the total concentration of host (cₘ) and guest (c₉) are held constant (cᵣ = cₘ + c₉) and the host to guest ratio is varied while some spectroscopic observable is recorded. The ratio of total host to total guest at the maximum of the observable denotes the binding stoichiometry. This technique was originally developed for absorption spectroscopy, however any technique that produces an observable which is proportional to the concentration of complex in solution can be used. When following guest binding by fluorescence quenching, one cannot construct a Job plot using only the fluorescence intensity as the observable because the fluorescence intensity is not proportional to the concentration of complex in solution. In other words, with a fluorescent host as one moves from 100% host to 0% host, the fluorescence goes down due to guest binding as well as the decrease in host concentration. Thus, an inflection point is never seen. It can be possible to determine stoichiometry by NMR, but in the case of host 98, aggregation effects became significant at concentrations necessary to observe signals in the ¹H NMR spectrum. Therefore, it was necessary to develop a method to construct a Job plot using fluorescence quenching. For the observable, the
fraction of fluorescence quenched (Q = 1 - I/I₀) was used, since this value is related to the ratio of bound to unbound host. Multiplying Q by the mole fraction of host (χ) produces a value proportional to the concentration of H•G. For each point of the Job plot, a sample of host 1 was prepared at the appropriate concentration (c_h) and the fluorescence recorded (I₀). A concentrated solution of guest was added such that the final concentration of guest (c_g) gave the correct total concentration and the volume of the sample was not significantly changed. The fluorescence of this sample was recorded (I) and Q was calculated for each point. Thus, the Job plot was constructed by plotting Qχ versus χ (Figure 16) which gave a maximum at nearly 0.5 mole fraction of host, indicating a 1:1 stoichiometry of binding.

**Figure 16:** Job plot of compound 98a and dodecanoic acid: 20 mM HEPES buffer, pH=8.4, c_t = 4 x 10⁻⁴ M, λ_ex = 365 nm, λ_em = 396 nm.
5.2.3 Conclusions

The pair of diastereomeric molecular tubes 98a and 98b represent competent sensors for all types of lipids since they recognize only the hydrophobic surface and no other functional group. In particular, the syn isomer 98a reports on the presence of hydrophobic compounds with shape selective recognition by fluorescent quenching for straight-chain lipids and fluorescent enhancement for other lipids. To our knowledge, this type of spectroscopic selection between simple lipids based only on the shape of their hydrophobic surface is unprecedented.
5.3.1 Redesign of Molecular Tubes

Based on the success of hosts 98a and 98b, the tube design was reexamined with the intent to optimize lipid binding and fluorescence response. It was postulated that the amide linker, while rigid in comparison to other linkers initially examined, was flexible enough to allow the tube to collapse in aqueous solvent. In such a case, the conformational changes invoked upon the tube by binding resulted in fluorescence decrease. It was believed that a more rigid linker which could reliably set the host into the desired tube shaped conformation would lead to the expected environment sensitive fluorescence increase.

Creating a rigidly open tube would also allow us to explore the effect of rigidity on binding affinities. It was anticipated that the greater preorganization of a more rigid tube would result in higher binding affinities and increased selectivity. Forcing the tube into an open conformation should result in a greater enthalpic drive for complexation since water molecules inside the cavity cannot participate in hydrogen bonding. In many instances, entropy is the dominant factor in the hydrophobic effect. This is because water-hydrocarbon interactions are actually favorable, but not as favorable as water-water interactions. However, water has the ability to organize itself around many hydrocarbon solutes in such a fashion that the overall enthalpy change is negative. So the solvation of hydrocarbon is often enthalpically favorable, but at an entropic price. These water-water interactions are greatly limited for solvent molecules occupying the tight cavity defined by the tube, so the expulsion of water from the tube should be enthalpically favorable as well as entropically favorable. Moreover, a rigidly preorganized tube should exhibit greater selectivity for guests having shape which is highly compatible with the cavity of the tube. Whereas more flexible tubes would possess greater adaptability to the shape of the guest and would be less selective.
A number of possible molecular architectures were considered and tube Figure 17 was chosen as the second generation series of molecular tubes. The allyl linker succeeds in preorganization where the majority of other linkers fail because there is no rotation around a double bond. Torsional rotation between each atom of the linkers is necessary to collapse the tube. Hence, the allyl linker was targeted in order to test the hypotheses regarding the binding affinities and fluorescence characteristics of the first generation molecular tubes.

**Figure 17:** a) Structure of allyl linked tube, b and c) Molecular models of syn allyl linked tube. R groups are omitted for clarity.
5.4 Allyl Linked Molecular Tubes

5.4.1 Synthesis of Allyl Linked Tubes

The retrosynthesis entailed preparing two naphthalene clefts as coupling partners and conjoining them via palladium catalyzed coupling to form the tube (Scheme 24). It was anticipated that Claisen chemistry could be used to install allyl groups in the peri positions of the bis-naphthalene intermediate which could be converted via olefin cross metathesis to vinyl boronic esters for Suzuki coupling chemistry.

**Scheme 24:** Retrosynthesis of allyl coupled tube.

A model system was prepared first in order to explore the coupling reaction. Alkylation of 2,6-dihydroxynaphthalene with allyl bromide gave compound 102 in 38% yield (Scheme 25). This was followed by dimerization to afford the allylated bis-naphthalene cleft 103 in 74% yields. Refluxing cleft compound 103 in \( N,N- \)
diethylaniline produced Claisen rearranged product cleanly, however this compound was found to be sensitive and degraded readily. Therefore, it was found that it was best to alkylate without further purification following the rearrangement. In this case, methylation using iodomethane gave compound 104 in 86% over two steps. Treatment of 104 with Grubbs’ 2nd generation metathesis catalyst and vinyl pinacol boronate in refluxing DCM produced the olefin metathesis product 105 in 65% yield with the desired $E,E$ stereochemistry$^{110}$.

**Scheme 25:** Synthesis of compounds for model tube coupling studies.
A cursory screening of conditions for the Suzuki coupling of boronic ester 105 with dibromo compound 83 (Scheme 26) revealed that using phosphine ligand S-phos,\textsuperscript{111,112} Pd(OAc)$_2$, CsF as the activator and refluxing in THF produced the model tube compounds 107\textit{a} and 107\textit{b} in 7\% combined yield (Table 5).

**Scheme 26**: Examination of coupling conditions with model system.
Table 5: Conditions and results for suzuki coupling of model compounds 83 and 105.

<table>
<thead>
<tr>
<th>Base</th>
<th>Solvent</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>K₂CO₃</td>
<td>THF</td>
<td>0</td>
</tr>
<tr>
<td>t-BuOK</td>
<td>THF</td>
<td>0</td>
</tr>
<tr>
<td>K₃PO₄</td>
<td>DMF</td>
<td>0</td>
</tr>
<tr>
<td>K₃PO₄</td>
<td>THF</td>
<td>0</td>
</tr>
<tr>
<td>CsF</td>
<td>THF</td>
<td>~7</td>
</tr>
</tbody>
</table>

*Reactions were run with Pd(OAc)₂ and ligand 106 in addition to solvent and base as indicated

Having had some success with the model system, work was begun to prepare tubes with water solublizing groups without any further optimization of the coupling conditions. Compound 108 was prepared in 31% yield by mono-alkylation of dihydroxynaphthalene with ethyl bromoacetate (Scheme 27). Bicyclization of compound 108 to give naphthalene cleft compound 109 proceeded in 82% yield. This species was then brominated to give the 5,5’ dibrominated cleft 110 in 99% yield. Cleft compound 111 was synthesized in two steps in 88% yield from compound 103 by Claisen rearrangement followed by alkylation with methyl bromoacetate (Scheme 28). Boronic ester coupling partner 113 was synthesized by cross metathesis with vinyl pinacol boronate via the Neolyst M1¹¹³ catalyst (112). Thus, coupling partners were readily prepared with the requisite latent water solublizing groups which could be unmasked following the macrocyclization step.
Initially, the conditions which gave some success in the model case worked for the coupling of compounds 107 and 110 as well in preparing the allyl linked tube 116 (Scheme 31). However, the coupling was found to be sensitive to the conditions and did not always reliably result in product. Therefore, coupling reactions were extensively screened in order to formulate a more robust set of conditions (Table 6). Trifluoroborate compound 114 (Scheme 29) and iodo compound 115 (Scheme 30) were prepared as alternative coupling partners as well. Ultimately, it was found that an excess of the activator had a deleterious effect. By being careful to use only a slight excess of CsF, the coupling reaction could be run routinely (entry 15).
**Scheme 28:** Synthesis of boronic acid ester coupling partner 111.

1. $N,N$-diethylaniline reflux
2. $\text{Br} - \underset{\text{THF, $\text{K}_2\text{CO}_3$}}{\rightarrow} \text{OMe}$

88% over 2 steps

**Scheme 29:** Preparation of trifluoroborate coupling partner 114.

$\text{BF}_3\cdot\text{K}^+$
**Scheme 30:** Iodination of cleft compound 109.

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

\[\text{109} \quad \text{I2, AgO}_2\text{CCF}_3, \quad \text{DCM, MeNO}_2 \quad \text{77\%}
\]

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

\[\text{115}
\]

**Scheme 31:** Macrocyclization reactions for the preparation of allyl linked tubes 116.

\[\begin{align*}
\text{R'O} & \quad \text{R'O} \\
& \quad \text{RO}
\end{align*}\]

\[\text{110} \quad X = \text{Br} \quad \text{115} \quad X = \text{I}
\]

\[\begin{align*}
\text{RO} & \quad \text{RO} \\
& \quad \text{RO}
\end{align*}\]

\[\begin{align*}
\text{113} & \quad M = \text{Bpin} \\
\text{114} & \quad M = \text{BF}_3^{-}
\end{align*}\]

\[\begin{align*}
\text{R} & \quad \text{R'} \\
& \quad \text{R'}
\end{align*}\]

\[\begin{align*}
\text{116a} & \quad \text{116b}
\end{align*}\]

Conditions and results: see Table 6

\[\begin{align*}
\text{R} & \quad \text{R'} \\
& \quad \text{R'}
\end{align*}\]

\[\begin{align*}
\text{116a} & \quad \text{116b}
\end{align*}\]
Table 6: Representative coupling conditions for Suzuki reaction.

<table>
<thead>
<tr>
<th>Entry</th>
<th>X</th>
<th>M</th>
<th>Pd Source</th>
<th>Ligand</th>
<th>Pd : L</th>
<th>Base^a</th>
<th>Solvent</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Br</td>
<td>Bpin</td>
<td>Pd$_3$dba$_3$</td>
<td>S-Phos</td>
<td>1 : 2</td>
<td>CsF</td>
<td>THF</td>
<td>34%</td>
</tr>
<tr>
<td>2</td>
<td>Br</td>
<td>Bpin</td>
<td>Pd$_3$dba$_3$</td>
<td>S-Phos</td>
<td>1 : 4</td>
<td>CsF</td>
<td>THF</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Br</td>
<td>Bpin</td>
<td>Pd(OAc)$_2$</td>
<td>S-Phos</td>
<td>1 : 2</td>
<td>CsF</td>
<td>THF</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Br</td>
<td>Bpin</td>
<td>Pd(OAc)$_2$</td>
<td>S-Phos</td>
<td>1 : 2</td>
<td>CsF 1 THF : 2 PhH</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Br</td>
<td>Bpin</td>
<td>Pd(OAc)$_2$</td>
<td>S-Phos</td>
<td>1 : 1</td>
<td>CsF</td>
<td>THF</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Br</td>
<td>Bpin</td>
<td>Pd(OAc)$_2$</td>
<td>S-Phos</td>
<td>1 : 2</td>
<td>Ag$_2$CO$_3$</td>
<td>THF</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Br</td>
<td>Bpin</td>
<td>dppf PdCl$_2$CH$_2$Cl$_2$</td>
<td>dppf</td>
<td>1 : 1</td>
<td>CsF</td>
<td>THF</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>I</td>
<td>Bpin</td>
<td>Pd$_3$dba$_3$</td>
<td>S-Phos</td>
<td>1 : 2</td>
<td>CsF</td>
<td>THF</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>I</td>
<td>Bpin</td>
<td>Pd$_3$dba$_3$</td>
<td>S-Phos</td>
<td>1 : 4</td>
<td>CsF</td>
<td>THF</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Br</td>
<td>BF$_3$</td>
<td>Pd(PPh$_3$)$_3$</td>
<td>-</td>
<td>-</td>
<td>Cs$_2$CO$_3$</td>
<td>THF (0.4 % H$_2$O)</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>Br</td>
<td>BF$_3$</td>
<td>Pd(PPh$_3$)$_3$</td>
<td>-</td>
<td>-</td>
<td>Cs$_2$CO$_3$</td>
<td>THF (1 % H$_2$O)</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>Br</td>
<td>BF$_3$</td>
<td>Pd(PPh$_3$)$_3$</td>
<td>-</td>
<td>-</td>
<td>Cs$_2$CO$_3$</td>
<td>DMF (1 % H$_2$O)</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>Br</td>
<td>BF$_3$</td>
<td>Pd(PPh$_3$)$_3$</td>
<td>-</td>
<td>-</td>
<td>Cs$_2$CO$_3$/KOtBu</td>
<td>THF (5% MeOH)</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>Br</td>
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<td>Pd(PPh$_3$)$_3$</td>
<td>-</td>
<td>-</td>
<td>Cs$_2$CO$_3$/KOtBu</td>
<td>THF (10 % MeOH)</td>
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<tr>
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<td>S-Phos</td>
<td>1 : 2</td>
<td>CsF°</td>
<td>THF</td>
<td>38%</td>
</tr>
</tbody>
</table>

^a With CsF, an excess was used (>6 eq) unless otherwise noted. ^b This yield was not reproducible. ^c 4.5 eq.

The two isomers were separated by flash chromatography on silica gel. As with the amide linked tubes, distinguishing nOe’s were expected for the syn and anti isomers. However, NOESY experiments did not result in data identifying either isomer – the pertinent nOe’s were not present in the spectrum of either isomer. This lack of cross tube enhancement likely resulted from the tube being fixed in an open conformation (compared to the amide), a situation which augered well for recognition studies. Selective 1-D nOe spectroscopy offers greater sensitivity than NOESY and was used to
establish compound 116a as the syn isomer (Figure 18) and 116b as the anti isomer (Figure 19).

**Figure 18:** Selective 1-D NOESY spectrum identifying compound 116a as the syn tube.
Saponification of the esters of 116a or 116b with NaOH in water/THF/methanol gave quantitative conversion to the tetracarboxylates 116a and 116b, respectively (Scheme 32). Solubility in D₂O (40 mM Na₂CO₃) was insufficient for NMR experiments and binding was studied by fluorescence spectroscopy.
Scheme 32: Saponification of esters for 116a and 116b.

\[
\begin{align*}
\text{R} & = \text{CH}_2\text{CO}_2\text{CH}_3 \\
\text{R}' & = \text{CH}_2\text{CO}_2\text{C}_2\text{H}_5
\end{align*}
\]

5.4.2 Recognition of Guests by Allyl Linked Tubes

As in the case of the amide linked tubes, 117a and 117b were titrated with various lipid guests. Titrations were performed in buffered water (20mM HEPES) at pH 8.4 with host concentration of 1 μM. Significant changes in the fluorescence emission were observed for most guests (Table 7). For each host, and in contrast to the amide linked tubes, fluorescence was enhanced upon addition of guest in most cases (Figures 20, 21). Notably, fluorescence quenching was only observed for the syn isomer 117a when it was titrated with amine (Figure 22) or olefin guests (entries 6, 12, 13) but for all other guests fluorescence enhancement was observed. This is consistent with the known quenching properties of amines\textsuperscript{38,114} and alkenes. In the case of the latter, it is known that conjugated alkenes and dienes have a considerable capacity to quench fluorescence via charge transfer.\textsuperscript{115} However, mono-alkenes generally function much less effectively as quenchers,\textsuperscript{116} although in some cases they have been shown to quench fluorescence.
Table 7: Association Constants and Fluorescence Changes of Sensors 117a and 117b with Lipid Guests (20 mM HEPES; pH = 8.4; [117] = 5x10^-7 M; λ_ex = 365 nm; λ_em = 410 nm). I_sat is the fluorescence intensity at saturation taken from the fit of the titration data; I_0 is initial fluorescence intensity.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Guest</th>
<th>Allyl Linked K_a (M^-1)</th>
<th>I_sat/I_0</th>
<th>Syn (117a) K_a (M^-1)</th>
<th>I_sat/I_0</th>
<th>Anti (117b) K_a (M^-1)</th>
<th>I_sat/I_0</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Butyric acid</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Hexanoic acid</td>
<td>800</td>
<td>1.54</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Octanoic acid</td>
<td>17,000</td>
<td>1.78</td>
<td>5,400</td>
<td>1.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Decanoic acid</td>
<td>140,000</td>
<td>1.97</td>
<td>120,000</td>
<td>1.46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Dodecanoic acid</td>
<td>590,000</td>
<td>1.79</td>
<td>350,000</td>
<td>1.79</td>
<td></td>
<td></td>
</tr>
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<td>6</td>
<td>trans-3-Octenoic acid</td>
<td>1,300</td>
<td>0.61</td>
<td>7,200</td>
<td>0.61</td>
<td></td>
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<td>7</td>
<td>4-Methyl-octanoic acid</td>
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<td>2.02</td>
<td>5,800</td>
<td>1.44</td>
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<td>8-Methyl-nonanoic acid</td>
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<td>13,000</td>
<td>1.61</td>
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</tr>
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<td>9</td>
<td>Cyclohexane carboxylic acid</td>
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<td>-</td>
<td>157</td>
<td>1.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Triethylene glycol</td>
<td>20</td>
<td>1.52</td>
<td>-</td>
<td>-</td>
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<td></td>
</tr>
<tr>
<td>11</td>
<td>1-Heptanol</td>
<td>420,000</td>
<td>1.10</td>
<td>240,000</td>
<td>1.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>cis-4-Hepten-1-ol</td>
<td>39,000</td>
<td>0.64</td>
<td>28,000</td>
<td>1.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Heptylamine</td>
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<td>0.63</td>
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<td>2.03</td>
<td>4,400,000</td>
<td>1.71</td>
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</table>

We attribute the significant quenching observed here to the close contact of the bound alkene guest with the naphthalene fluorophores. Similarly, the fluorescence enhancement observed for the addition of heptanol is substantially less than the other saturated guests (entry 11). Alcohols have been shown to have some ability to quench fluorescence via PET,\textsuperscript{120} and this may partially compensate for the environment sensitive fluorescence enhancement which results from expulsion of water. As expected, the longer straight chain lipids were bound with higher affinity than were shorter lipids.
(entries 2-5). The greater hydrophobic surface of these lipids renders them less soluble in water. Moreover, the additional length allows for greater freedom of motion in the bound state. There is some entropic cost associated with forming the host-guest complex. However, longer guests have greater translational freedom along the axis of the tube cavity while still filling the cavity than do the shorter guests. Thus, the entropic cost associated with formation of the host-guest complex should be less for longer guests than for shorter guests. Branching or unsaturation resulted in significantly lower binding affinity than the corresponding straight chain lipids (entries 6, 7, 12). As was the case with the amide linked tube, an exception to this trend is terminal branching of a long chain saturated guest. The cyclic and hydrophilic guest were bound poorly by 117a (entries 9,10) and a binding constant could not be determined for cyclohexane carboxylic acid. Higher binding affinity was observed in going from anionic to neutral and from neutral to cationic guests (entries 3, 11, 13). The stoichiometry of the complex was found to be 1:1 using the Job method.

Similar results were observed for anti isomer 117b, although there were some notable differences in the trends. For 117b, only the trans-alkene (entry 6) caused fluorescence quenching, while all other guests produced fluorescence enhancement. While 117b was found to be highly selective against the cis-alkenyl guest (entry 12), the binding affinities for internally branched and trans-alkenyl guests were similar to that of the corresponding straight chain guest (entries 6,7). The maximum fluorescence changes were also lower in general for 117b than for 117a. As with the case of the anti isomer of the amide linked tube, these effects may be attributed to the limited effective tube length of 117b in comparison with the syn allyl linked tube. The shorter cavity has less of a
capacity for shape selectivity because a shorter length of lipid lies within the cavity, and
fragments which fit poorly may remain outside of cavity in the bound state. The shorter
cavity also explains the lack of fluorescence quenching with most guests in that there is
usually host fluorophore which is not in close contact with the quencher. Moreover,
lower fluorescence responses relative to the syn isomer result from the fact that fewer
water molecules must occupy the tube in the unbound state. Hence, the environment
sensitive fluorescence increase observed upon expulsion of the quenching water
molecules is diminished.

**Figure 20:** Fluorescence titration of compound **117a** with octanoic acid in buffer (20 mM
HEPES; pH = 8.4; [**117a**] = 10^{-6} M; \( \lambda_{ex} = 365 \) nm): a) Fluorescence emission as a
function of added octanoic acid; b) Fit of the titration data at \( \lambda_{em} = 410 \) nm to a single site
binding isotherm.
**Figure 21:** Fluorescence titration of compound 117b with octanoic acid in buffer (20 mM HEPES; pH = 8.4; [117b] = 10^{-6} M; \lambda_{ex} = 365 \text{ nm}): a) Fluorescence emission as a function of added octanoic acid; b) Fit of the titration data at \lambda_{em} = 410 \text{ nm} to a single site binding isotherm.

![Fluorescence titration of compound 117b with octanoic acid](image)

**Figure 22:** Fluorescence titration of compound 117 with heptylamine in buffer (20 mM HEPES; pH = 8.4; [117] = 10^{-6} M; \lambda_{ex} = 365 \text{ nm}): a) Fluorescence emission of compound 117a upon titration with heptylamine; b) Fluorescence emission of compound 117b upon titration with heptylamine.

![Fluorescence titration of compound 117 with heptylamine](image)
The allyl linked tube was designed to test the effects of increased preorganization on the system. It was anticipated that this would effect selectivity, affinity, and fluorescence modulation. The results obtained by fluorescence titrations confirmed the predicted effects on each of these aspects. Indeed, the binding affinities with hosts 117a and 117b are larger than they are for 98a and 98b in almost all cases and the allyl linked tubes show greater shape selectivity for straight chain lipids than the amide linked tubes as well.

For syn amide linked tube 98a, association constants for saturated guests are on the same order of magnitude as their corresponding branched or unsaturated, differing by a maximum factor of 4 (Table 4; entries 12, 13). Syn allyl linked tube 117a was substantially more selective, exhibiting roughly an order of magnitude difference in association constants for saturated guests versus their corresponding branched or unsaturated guests. The inherent flexibility of the amide linker allows for conformational changes that will accommodate guests which fit within the cavity but do not possess a shape complementary to the cavity. Indeed, it is probable that this is true for all guests which are included under aqueous conditions as 98a is likely collapsed in aqueous solvent. However, the cavity of 117a is strictly confined to a conformation which is highly complementary to normal alkanes, but less so to other guests, such as those containing unsaturation or branching. The limited flexibility of the allyl linker relative the amide linker renders them less capable of accommodating the form of branched and unsaturated guests. Nevertheless, inclusion of a guest is clearly a favorable alternative to inclusion of water. Thus, the preorganization imparted by the allyl linker results in
greater selectivity for the unbranched saturated guests as well as larger binding constants for branched and unsaturated guests relative to amide linked tube 98a.

The comparison of the anti-linked tubes parallels that of the syn linked tubes. Association constants were higher for anti allyl linked tube 117b than they were for anti amide linked tube 98b and fluorescence enhancement was observed for 117b in most cases whereas quenching was observed for 98b. Again, these effects are attributed to the rigid preorganization of the allyl linked tube in comparison to the more flexible amide linked tube. Also, 117b exhibited significant selectivity for straight chain lipids versus the cis alkene, while 98b exhibited no significant selectivity. However, neither 117b or 98b exhibited significant difference in binding affinity against trans-alkenyl or internally branched lipids. As previously discussed, this is attributable to the short effective length of the tubes.

5.4.3 Conclusions

Revision of the structure of amide linked molecular tubes 98 resulted in the design of allyl linked molecular tubes 117. Tubes 117 were prepared using a double Suzuki coupling as the key step to form the macrocyclic tube. These host compounds behaved predictably based on an increase in the preorganization of the system which resulted in higher binding constants and increased selectivity relative to the amide linked series. Also observed was the desired positive fluorescence change upon the addition of most guests. The fluorescence modulation observed was predicted based on the premise of environment sensitive fluorescence.
Chapter 6: Ongoing Projects

6.1 Propyl linked tube

6.1.1 Overview and Synthesis of Propyl Linked Molecular Tubes

While the allyl linked tube was designed to test the effects of preorganization on the system, a propyl linked tube (118a and 118b) was devised as a control (Figure 23). Propyl linkers would have greater flexibility than the amide linkers, which are of intermediate rigidity relative to allyl and propyl groups, and thus a propyl linked tube would be complimentary to the series.

Figure 23: Model structures of propyl linked tubes 118a and 118b.

It was thought that these tubes might be accessible via Suzuki chemistry and studies on a model system commenced. To that end, it was determined that allyl compound 104 could be quantitatively hydroborated to primary alkyl borane 119 via
treatment with 9-BBN (Scheme 33). Borane 119 was subsequently reacted with bromide 83 under Suzuki coupling conditions. Initial results appeared to be promising as the required propyl signals were observed in the $^1$H NMR spectra of the product, but the yield was low and the product was resistant to purification. Attempts were made to optimize the coupling reaction (Table 8) but examination of NMR data for the crude products of these reactions demonstrated that better conditions could not be developed. Moreover, the NMR data of product obtained exhibited broad peaks, which might have resulted from slowly interconverting conformers of the tube, but made definitive characterization elusive. NMR experiments at high and low temperature did not result in resolution of these peaks, and it was concluded that the cyclized product was not observed, but rather oligomers of Suzuki coupling products.

**Scheme 33:** Attempts to synthesize propyl linked tubes by alkyl Suzuki coupling.
**Table 8**: Representative conditions for alkyl suzuki coupling.

<table>
<thead>
<tr>
<th><strong>Pd Source</strong></th>
<th><strong>Ligand</strong></th>
<th><strong>Base</strong></th>
<th><strong>Solvent</strong></th>
<th><strong>Results</strong></th>
</tr>
</thead>
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<td>Pd(PPh₃)₄</td>
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<td>THF</td>
<td>Oligomers</td>
</tr>
<tr>
<td>Pd(PPh₃)₄</td>
<td>Pd(PPh₃)₄</td>
<td>K₃PO₄</td>
<td>THF</td>
<td>Oligomers</td>
</tr>
<tr>
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<td>S-Phos</td>
<td>CsF</td>
<td>THF</td>
<td>Oligomers</td>
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<tr>
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<td>dppf</td>
<td>CsF</td>
<td>THF</td>
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</tr>
<tr>
<td>Pd₂dba₃</td>
<td>PCy₃</td>
<td>K₃PO₄</td>
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</tr>
<tr>
<td>Pd(OAc)₂</td>
<td>PCy₃</td>
<td>NaOH</td>
<td>THF</td>
<td>Oligomers</td>
</tr>
</tbody>
</table>

While the alkyl Suzuki coupling reaction did not result in the desired propyl linked tube, the allyl linked tube had been prepared and so the reduction of the allyl groups to propyl groups was explored (Scheme 34). Hydrogenation of 116b with H₂ and Pd/C returned starting material, even at 750 psi. However, reaction of 116b with TFA and TES resulted in clean reduction to the propyl linked tube 121b in 75% yield. Similar results were achieved when the syn host 116a was subjected to these conditions (76% yield). Attempts to reduce the saponified tubes by this method were less straightforward. The saponified tubes were less soluble in the solvent system used for the tetra ester, and so additional TFA was added to fully dissolve the starting material. This reaction was not as clean as reduction of compounds 116a and 116b, but the reaction was not optimized.
Scheme 34: Reduction of allyl linked tube 116b.

Table 9: Attempts at the reduction of the allyl linkers of 116b.
6.1.2 Conclusions

A synthesis of propyl linked tubes 120 has been discovered. The desired tubes 121 should be readily accessible from 120. These compounds have not yet been fully characterized, nor has the route been optimized. Tubes 121 would be complementary to the amide (98) and allyl (117) linked series and would help to define the effects of preorganization on this system in terms of binding affinity, selectivity and fluorescent response.
6.2 Towards Anthracene Based Tubes.

6.2.1 Design of Anthracene Based Tubes.

Anthracene based molecular tubes were designed to explore the effect of varying the depth of the cavity. An anthracene based tube would be able to accommodate two additional methylene units (Figure 24). A longer tube may result in higher binding constants and greater selectivity for unbranched alkyl lipids. A feature of primary importance though, results from the anticipated linker connectivity as shown in Figure 25. The linker will be appended to the 9-position of the anthracene. In this case, both the anti and the syn tubes should have viable tube shapes. In both of the previous cases the anti tubes, which account for half of the synthesized receptor, were poorly selective receptors. It is anticipated that for anthracene based tubes, both isomers will be viable receptors. However, in the case of the anti linked tube, there are two functionalized phenols at each tube end, whereas in the case of the syn linked tube, all of the phenols at one end. Thus, the syn and anti anthracene tubes have essentially the same shape and bear cavities of the same shape, but the ends of these tubes will be functionalized differently. This results in alternatives in terms of functionalization which is not available with the naphthalene based tubes. Taken in conjunction with the possibility of further substitution alpha to the acetal bridge, this would be important in developing tubes for more advanced applications.
Allyl and amide linked tubes were targeted first, as the chemistry for synthesizing them had already been developed in the case of the naphthalene based hosts (Scheme 35).
Unfortunately, some of the chemistry did not directly translate to the anthracene system (Scheme 36). Compound 128 could be prepared by a literature method. However, conditions for a clean mono-alkylation could not be found.

**Scheme 35:** Retrosynthesis of anthracene based molecular tubes.
**Scheme 36:** Attempted mono-alkylation of 2,6-dihydroxyanthracene.

![Chemical structure of 128](image1)

Mono-alkylation of anthraflavic acid (130) proceeded to give compound 131 (Scheme 37). However, attempts the to reduce compound 131 to anthracene 132 were unsuccessful, resulting in either return of starting material or decomposition.

**Scheme 37:** Further attempts to prepare mono-alkylated 2,6-dihydroxyanthracene.

![Chemical structure of 130, 131, and 132](image2)

Finally, a model system was developed in order to circumvent the issues presented thus far and examine the aromatic substitution chemistry of the anthracene compounds. Mono-benzylation of anthraflavic acid gave compound 133 in 38% yield. This species could be quantitatively reduced to anthracene compound 134 (Scheme 38). Dimerization of compound gave anthracene cleft 135 in moderate yield. It was hoped that aromatic substitution on cleft compound 135 would result in functionalization at the 9 position, as that is the most reactive position in anthracene. However, when 135 was subjected to formylation conditions\(^{121,122}\), a mixture of regioisomers was observed with
formylation occurring at the 5 and 9 positions due to electron donation by the alkoxy group at position 6. Substitution at the 9 position was favored by a ratio of 1.6:1 and formylation was performed at low temperature to try to enhance this ratio, but this resulted in considerably longer reaction time, without significantly improving regioselectivity.\textsuperscript{123}

**Scheme 38:** Model studies on aromatic substitution of anthracene clefts.

Because the regiochemistry of the system could not be controlled by the experimental conditions, it was planned to influence it by steric blocking or electronic deactivation of the 5 positions. The triisopropyl silyl (TIPS) ether \textsuperscript{137} was prepared in order to examine steric blocking as a method for regiocontrol of this system (Scheme 39). After several attempts though, the bicyclization reaction had only resulted in
decomposition. The doubly protected silyl ether 139 was prepared in order to test the steric blocking plan without further investment toward the synthesis of cleft compound 138 (Scheme 40). Formylation of doubly TIPS protected anthracene 139 failed to give the desired regioselectivity but instead resulted in a 1.8:1 ratio of formylation at the 9- and 5-positions respectively. It was thus concluded that sterically blocking the 5 position was likely not a viable route to 9,9’ disubstituted anthracene clefts.

**Scheme 39:** Efforts toward TIPS protected anthracene clefts.
When it became apparent that steric blocking would not be successful, efforts were focused on electronic deactivation as a means to regioselective substitution at the 9 position. Triflation of anthracene diol 128 with phenyltriflimide was a straightforward entry into this strategy, giving the monotriflated product 141 in 39% yield (Scheme 41). Compound 141 in turn underwent the bicyclization reaction to give anthracene cleft 142 in reasonably good yield.
Unfortunately, triflate groups proved to be too deactivating and formylation was not possible, even under forcing conditions. On the other hand, bromination with Br₂ and HOAc regiospecifically produced the 9,9′ dibromo compound 144 in good yield (Scheme 42). Attempts to convert the dibromo compound to the diformylated compound via lithium halogen exchange were fruitless, plagued by incomplete reaction of BuLi with the starting material and a lack of reactivity of the aryl lithiate and DMF. Palladium catalyzed couplings were not explored with 144 as adequate selectivity between the bromide and triflate groups was not anticipated. It was thought that this issue might be addressed via the 9,9′ dichloro compound 145, but the attempted chlorination prove unsuccessful.
Based on the partial success of the triflate chemistry, it was believed that deactivation by a less electron withdrawing sulfonate could generate the desired regioselectivity for the bromination, but would allow for the use of palladium chemistry for further elaboration and cyclization. The known protected sulfonyl chloride 146, derived from taurine, was thought to be a promising candidate for this approach. The commercially available trifluoroethanesulfonyl chloride 125 (147) was used in model studies for this strategy (Scheme 43).
Similar reactivity was observed for this model system as was observed for the triflate system. Mono sulfonation proceeded in 38% yield to give compound 148. Bicyclization of compound 148 gave 149 in 65% yield. Finally, bromination produced the desired 9,9’ dibrominated compound 150 in 70% yield.

Scheme 43: Model system for taurine protected anthracene clefts.

Further exploration of this chemistry awaits. The diallyl compound should be accessible via palladium catalyzed coupling with an allyl reagent\textsuperscript{126} (e.g., tetraallyl tin\textsuperscript{127}). Once the diallyl compound is available, cross metathesis and suzuki coupling should yield allyl linked anthracene based tubes (Scheme 44). This chemistry should be extendable to the synthesis of water soluble anthracene based tubes 152.
Scheme 44: Proposed retrosynthesis of allyl linked anthracene tubes using dibromo compound 150.
6.2.2 Conclusions

Progress has been made toward the synthesis of anthracene based molecular tubes. Anthracene based tubes should provide insight into the importance of tube length for lipid binding characteristics. Moreover, both syn and anti linked tubes should exhibit useful recognition characteristics yet would provide different platforms by which to further this technology. Future work on this project should be to work out the allylation reaction on the model system, afterward studies should begin on the actual system.
APPENDIX

EXPERIMENTAL PROCEDURES

General Fluorescence Procedures. For fluorescence titrations, solutions were prepared in the indicated solvent. Fluorescence spectra were acquired on a Shimadzu RF-5301 PC spectrofluorimeter at ambient temperature. Titration data was fit to a 1:1 binding isotherm\textsuperscript{128} using the software program Prism 3.03.\textsuperscript{129} Guests binding with dissociation constants less than $10^{-6}$ were fit to a 1:1 binding isotherm using the quadratic equation shown below.

$$Y = \frac{B_{\text{max}}((k+r+X) - \sqrt{(k+r+X)(k+r+X) - 4rX})}{2r}$$

NMR Titration of Compounds 98a and 98b. For the NMR work, guest concentrations approached (but did not exceed) their CMC values, so no quantitative data was taken from these experiments.

General Synthetic Procedures. All reactions were carried out in dried glassware under argon or nitrogen atmosphere unless otherwise noted. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl under argon or nitrogen immediately before use. Dichloromethane (DCM) was distilled from calcium hydride under argon or nitrogen immediately before use. Flash chromatography\textsuperscript{130} was performed with 32-63 μm silica gel. All melting points are uncorrected. NMR spectra were recorded on a
Bruker AMX-250, DPX-300, DRX-300, AMX-360, DRX-400, AMX-500 or DRX-500 in CDCl₃ using TMS as a reference unless otherwise indicated.
Compound 91: NaH (1.37 g, 60% in mineral oil, 34 mmol) was added to a solution of 2,6-dihydroxynaphthalene (5.0 g, 31 mmol) in DMF (155 mL). After stirring for 1 hour, methyl bromoacetate (3.25 mL, 34 mmol) was added via syringe, and the resulting mixture was stirred for 16 h. DMF was removed in vacuo, and the remaining residue was partitioned between EtOAc and 10% HCl. After collecting the organic layer, the aqueous phase was extracted with additional EtOAc (2 X 200 mL). The combined organic layers were dried over Na$_2$SO$_4$ and filtered. After removal of the solvents in vacuo, purification by chromatography (5% Et$_2$O/DCM) gave the mono-alkylated product 91 (2.39 g, 33%) as a white solid. $^1$H NMR (300 MHz, MeOD): $\delta$ 7.60 (d, 1H, $J$ = 9.3) 7.57 (d, 1H, $J$ = 9.4) 7.07 (m, 4H), 4.77 (s, 2H), 3.80 (s, 3H). $^{13}$C NMR (75 MHz, MeOD) $\delta$ 171.6,155.3, 155.1, 132.2, 130.4, 129.4, 128.9, 119.8, 119.7, 110.1, 108.5, 66.2, 52.6. IR (neat, cm$^{-1}$) 3554, 1741, 1604. HRMS for M+H$^+$ calcd for C$_{13}$H$_{13}$O$_4$ 233.0808, found 233.0810.
Compound 92: Malonaldehyde bis(dimethylacetal) (1.1 mL, 6.7 mmol) was added dropwise over 5 min to a solution of mono-alkylated product 91 (2.39 g, 10 mmol) and TFA (18 mL) in DCM (120 mL). After the addition was complete, the mixture was stirred 4 h under a drying tube (CaCl₂). The reaction was poured over crushed ice, followed by the slow addition of 33 mL 6 M NaOH. The pH of the resulting mixture was brought to ca. 8 by addition of sat. NaHCO₃. Following extraction with DCM (3 X 125 mL), the combined organic layers were washed with sat. NaHCO₃ (1 X 500 mL), dried over Na₂SO₄, and filtered. Solvents were removed *in vacuo* and the residue was purified by silica chromatography (0.5-1% Et₂O/DCM) to give compound 92 (1.96 g, 76 %) as a white solid (m.p.: 176-178 °C). \(^1\)H NMR (300 MHz, d₂-DCM): δ 8.49 (d, 2H, \(J = 9.3\)), 7.48 (d, 2H, \(J = 8.9\)), 7.29 (dd, 2H, \(J = 9.3, 2.7\)), 7.11 (d, 2H, \(J = 8.9\)), 7.00 (d, 2H, \(J = 2.7\)), 6.24 (s, 1H), 5.28 (s, 1H), 4.69 (s, 4H), 3.77 (s, 6H), 2.42 (t, 2H, \(J = 2.6\)). \(^{13}\)C NMR (90 MHz, d₂-DCM) δ 169.7, 154.7, 149.6, 131.1, 127.8, 127.4, 125.1, 119.6, 119.5, 118.9, 109.2, 92.1, 66.0, 52.6, 27.3, 23.5. IR (neat, cm⁻¹ 2954, 1759, 1604). HRMS for M+H⁺ calcd for C₂₉H₂₅O₈ 501.1549, found 501.1546.
**Dialdehyde 93:** A solution of compound 92 (910 mg, 1.8 mmol) in DCM (50 mL) was cooled to 0 °C. Following addition of α,α-dichloromethyl methyl ether (0.64 mL, 7.2 mmol), TiCl₄ (6.2 mL, 6.2 mmol, 1.0 M in DCM) was added via syringe. After 1 h at 0 °C, the resulting mixture was warmed to room temperature for 2.5 h. Ice was added to the reaction mixture, which was then poured into a sat. solution of NaHCO₃. Following extraction with EtOAc (2 X 75 mL), the combined organic layers were dried over Na₂SO₄, and filtered. The residue obtained after removal of solvents *in vacuo* was filtered on silica (50% Et₂O/50% DCM) to give the dialdehyde 93 (991 mg, 98%) as a yellow solid. 

**¹H NMR (300 MHz, d₂-DCM):** δ 10.9 (s, 2H), 9.03 (d, 2H, J = 9.4), 8.72 (d, 2H, J = 9.5), 7.28 (dd, 4H, J = 9.5, 1.7), 6.28 (s, 1H), 5.29 (s, 1H), 4.88 (s, 4H), 3.80 (s, 6H), 2.47 (t, J = 2.6). 

**¹³C NMR (75 MHz, d₂-DCM):** δ 192.3, 169.2, 160.7, 150.1, 131.3, 128.1, 127.4, 125.9, 122.5, 119.2, 119.0, 114.4, 92.0, 67.0, 52.9, 27.2, 23.4. IR (neat, cm⁻¹ 2955, 1759, 1672). HRMS for M+H⁺ calcd for C₃₁H₂₅O₁₀ 557.1442 found 557.1460.
**Diacid 95**: A solution of dialdehyde 93 (0.200 g, 0.36 mmol) in acetone (7 mL), THF (7 mL), and H2O (14 mL) was cooled to 0 °C. Sulfamic acid (0.209 g, 2.2 mmol) and sodium chlorite (0.203 g, 1.8 mmol, 80%) were added. The mixture was slowly warmed to room temperature and kept there for 14 h. After addition of sat. aqueous ammonium chloride, the product was extracted with EtOAc (2 X 25 mL). The combined organic layers were dried over Na2SO4 and filtered. After removal of the solvents *in vacuo*, the residue was purified by eluting through a short column of silica (5/1/94 MeOH/HOAc/DCM). The diacid (95) (0.200 g, 95%) was obtained as a pale yellow solid (m.p.: 170°C dec.). 1H NMR (300 MHz, MeOD): δ 8.68 (d, 2H, J = 9.5), 7.61 (d, 2H, J = 9.2), 7.35 (d, 2H, J = 9.4), 7.17 (d, 2H, J = 9.1), 6.26 (s, 1H), 5.43 (s, 1H), 4.84 (s, 4H), 3.78 (s, 6H), 2.40 (s, 2H). 13C NMR (75 MHz, 2:1 MeOD/DCM) δ 170.9, 170.7, 151.2, 150.2, 127.8, 127.5, 126.7, 125.2, 121.9, 120.9, 119.8, 115.5, 92.5, 67.6, 52.6, 27.3, 23.6. IR (neat, cm⁻¹ 4500-1800, 1710). HRMS for M+Li⁺ calcd for C13H24O12Li 595.1428 found 595.1394.
132

ppm (H1)

78.40
72.20

4500 4000 3500 3000 2500 2000 1500 1000 cm⁻¹
**Boc-protected diamine 94:** t-Butyl carbamate (0.206 g, 1.8 mmol) was added to a solution of dialdehyde 93 (0.163 g, 0.29 mmol) in MeCN (4 mL) and DCM (1.5 mL). To this mixture was added triethylsilane (0.28 mL, 1.8 mmol) and TFA (0.09 mL, 1.2 mmol). The resulting mixture was stirred for 16 h (adding DCM to maintain solubility, if necessary). Following addition of saturated aqueous NaHCO₃, the aqueous layer was extracted with DCM (3 X 25 mL). The combined organic were dried over Na₂SO₄ and filtered. After removal of solvents, the residue was purified by chromatography (0→10% Et₂O/DCM). The BOC protected diamine 94 (0.147 g, 66%) was obtained as an off-white solid (m.p.: 103°C dec.). ¹H NMR (300 MHz, d₂-DCM): δ 8.50 (d, 2H, J = 9.4), 8.03 (d, 2H, J = 9.3), 7.24 (d, 2H, J = 10), 7.20 (d, 2H, J = 9.6), 6.26 (s, 1H), 5.30 (s, 3H), 4.82 (s, 2H), 4.81 (s, 2H), 4.73 (dd, J = 5.4, 2.6), 3.78 (s, 6H), 2.43 (s, 2H), 1.37 (s, 18H). ¹³C NMR (75 MHz, d₂-DCM) δ 170.3, 156.2, 152.7, 149.6, 129.5, 127.8, 124.8, 124.6, 123.2, 120.1, 119.4, 114.6, 92.1, 79.3, 67.1, 52.8, 35.4, 28.6, 27.4, 23.5. IR (neat, cm⁻¹ 3414, 2976, 1755, 1703, 1600). HRMS for M+H⁺ calcd for C₄₁H₄₇N₂O₁₂ 759.3123 found 759.3159.
Sensor 98: TFA (1mL) was added to a solution of Boc-protected diamine 94 (0.075 g, 0.099 mmol) in DCM (2 mL). After stirring for 16 h, the solvents were removed in vacuo (<35 °C). CHCl₃ (5mL) was added and solvents were again removed in vacuo (<35 °C) to give diamine 96. The remaining bis(TFA) salt of the diamine was dried under high vacuum for 24 h to remove any residual TFA. The dry diamine was taken up in DMF (10 mL) (flask A). In a separate flask (B), diacid (95) (0.058 g, 0.099 mmol) was combined with PyBOP (0.206 g, 0.40 mmol) and Hunig’s base (0.17 mL, 0.99 mmol) in DMF (10 mL). In a third flask (C) was placed DMF (20 mL). Using a double syringe pump, the contents of flask A and flask B (separate syringes) were added dropwise to flask C over a period of 1.5 h. The resulting mixture was stirred for 16 h. Solvents were removed in vacuo, and the resulting residue was taken up in DCM (50 mL). The organic layer was washed with HCl (50 mL, 10%) followed by sat. aqueous NaHCO₃ (50 mL) and dried over Na₂SO₄. Following removal of the solvent in vacuo, the residue was purified by column chromatography (EtOAc) to give the tetramethyl ester 97 as a mixture of diastereomers (0.038 g, 35%).
Sodium hydroxide (0.5 mL, 6 M) was added to a solution of the above tetramethyl ester (0.030 g, 0.027 mmol) in MeOH (1 mL) and THF (1 mL). After stirring overnight, the reaction was quenched with 10% HCl (5 mL), extracted with a mixture of MeOH/DCM (5 X 10 mL), and dried over Na₂SO₄. Solvents were removed in vacuo to give the final sensor 98 (0.027 g, 95%) as a crude mixture of diastereomers. The two isomers were separated by reverse-phase HPLC (ramping from 15-25% acetonitrile in phosphate buffer (pH 7.5) over 30 min.). After multiple injections, like fractions were combined and extracted with MeOH/DCM. After drying with Na₂SO₄, solvents were removed in vacuo to give the separate isomers. Tritration with CCl₄ was often required to remove residual aliphatic contaminants. Assignment of syn versus anti was made by 2D NMR (COSY/NOESY).

**98a** (syn): ¹H NMR (500 MHz, d₆-DMSO) : δ 8.56 (d, 2H, J = 9.6), 8.52 (d, 2H, J = 9.6), 7.72 (d, 2H, J = 9.3), 7.51 (d, 2H, J = 9.3), 7.19 (d, 2H, J = 9.5), 7.15 (d, 2H, J = 9.2), 7.11 (d, 2H, J = 9.5), 7.10 (d, 2H, J = 9.1), 6.32 (s, 1H), 6.27 (s, 1H), 5.60 (s, 2H), 4.98 (dd, 2H, J = 13.1, 8.4), 4.67 (m, 8H), 4.53 (d, 2H, J = 13.36), 2.54 (bs, 2H), 2.43 (bs, 2H). ¹³C NMR (125 MHz, d₆-DMSO): δ 171.1, 170.6, 165.3, 165.1, 152.3, 150.3, 148.8, 148.7, 148.4, 128.0, 126.9, 126.7, 126.1, 126.0, 125.2, 124.2, 123.3, 121.9, 120.8, 120.6, 120.0, 119.6, 119.1, 118.9, 115.2, 114.5, 90.6, 90.4, 66.9, 66.1, 33.1, 25.2, 25.0, 22.4, 21.3. IR (neat, cm⁻¹) 3401, 2924, 1724, 1633. HRMS for (M-H⁻) calcd for C₅₈H₄₁N₂O₁₈ 1053.2360, found 1053.2325.
98b (anti): $^1$H NMR (500 MHz, d$_6$-DMSO): $\delta$ 8.49 (d, 2H, $J = 9.2$), 8.48 (d, 2H, $J = 9.3$), 7.87 (bs, 2H), 7.54 (d, 2H, $J = 9.3$), 7.38 (d, 2H, $J = 9.1$), 7.07 (d, 2H, $J = 9.4$), 7.01 (d, 2H, $J = 9.4$), 6.98 (d, 2H, $J = 9.2$), 6.92 (d, 2H, $J = 9.1$), 6.28 (s, 1H), 6.28 (s, 1H), 5.55 (s, 1H), 5.53 (s, 1H), 5.21 (dd, 2H, $J = 13.9$, 6), 4.58 (m, 6H), 4.45 (d, 2H, $J = 16.2$), 4.36 (d, 2H, $J = 11.1$), 2.53 (s, 2H), 2.49 (s, 2H, obscured by d$_6$-DMSO peak). $^{13}$C NMR (125 MHz, d$_6$-DMSO): $\delta$ 170.8, 170.3, 165.5, 151.5, 149.9, 148.7, 148.3, 128.7, 126.7, 126.6, 126.2, 125.6, 124.9, 124.3, 123.1, 122.9, 119.5, 119.3, 118.9, 114.7, 114.0, 90.7, 66.2, 65.9, 33.371, 29.0, 21.3. IR (neat, cm$^{-1}$) 3376, 3202, 2922, 1723. HRMS for (M-H$^-$) calcd for C$_{58}$H$_{41}$N$_2$O$_{18}$ 1053.2360, found 1053.2367.
R = CH₂CO₂H

98a

COSY
Naphthol 108: NaH (3.56 g, 60% in mineral oil, 89 mmol) was added to a solution of 2,6-dihydroxynaphthalene (13.4 g, 90 mmol) in DMF (400 mL). After stirring for 45 min., methyl bromoacetate (10.0 mL, 90 mmol) was added via syringe, and the resulting mixture was stirred for 16 h. The solvent was removed in vacuo, and the residue was partitioned between EtOAc and 10% HCl. After collecting the organic layer, the aqueous phase was extracted with additional EtOAc (2 X 200 mL). The combined organic layers were dried over Na₂SO₄ and filtered. After removal of the solvents in vacuo, purification by chromatography (1→3% Et₂O/DCM) gave the mono-alkylated product (6.78 g, 31%) as a white solid (m.p.: 133-134°C). ¹H NMR (300 MHz, CDCl₃) δ: 7.61 (d, 1H, J = 8.6 Hz), 7.59 (d, 1H, J = 8.9 Hz), 7.19 (dd, 1H, J = 9.0, 2.6 Hz), (m, 2H, J = 9.3 Hz), 5.013 (s, 1H), 4.71 (s, 2H), 4.30 (q, 2H, J = 7.1 Hz), 1.31 (t, 3H, J = 7.1 Hz); ¹³C NMR (75 MHz, CDCl₃) δ: 169.1, 154.3, 152.2, 130.4, 129.4, 128.1, 119.2, 118.3, 109.7, 107.5, 65.6, 61.5, 14.2. IR (neat, cm⁻¹): 3383, 2981, 1732, 1604, 1442, 1387, 1206, 1160, 1074. HRMS for M⁺ calcd for C₁₄H₁₄O₄ 246.0892, found 246.0891.
Compound 109: Hydroxynaphthalene 108 (14.94 g, 60.7 mmol) was dissolved in nitromethane (150 mL), DCM (50 mL) and TFA (50 mL). 1,1,3,3-tetramethoxypropane (6.0 mL, 36.4 mmol) was added via syringe pump over 1 hour while stirring. Stirring was continued for an additional 3 hours, after which the reaction was quenched with excess saturated aqueous Na₂CO₃. The organic layer was extracted with DCM, dried with MgSO₄, and the solvent was evaporated *in vacuo*. The crude product was purified by filtering through a plug of silica (1 EtOAc : 1 DCM) followed by recrystallization from EtOAc to give compound 109 (13.2 g, 82%) as a white solid (m.p.: 155-156 °C).

1H NMR (300 MHz, CDCl₃) δ: 8.45 (d, 2H, J = 9.3 Hz), 7.44 (d, 2H, J = 8.9 Hz), 7.29 (dd, 2H, J = 9.3, 2.7 Hz), 7.11 (d, 2H, J = 8.9 Hz), 6.98 (d, 2H, J = 2.7 Hz), 6.23 (d, 1H, J = 1.2 Hz), 5.23 (s, 1H), 4.65 (s, 4H), 4.26 (t, 4H, J = 7.2 Hz), 2.38 (t, 2H, J = 2.5 Hz) 1.27 (t, 6H, J = 7.1 Hz). 13C NMR (75 MHz, CDCl₃) δ 169.7, 154.7, 149.6, 131.1, 127.8, 127.4, 125.1, 119.6, 119.5, 118.9, 109.2, 92.1, 66.0, 52.6, 27.3, 23.5. IR (neat, cm⁻¹): 2981, 1756, 1605, 1389, 1202, 1082, 1013. HRMS for M⁺ calcd for C₃₁H₂₈O₈ 528.1784, found 528.1785.
EtO

EtO

109

10 9 8 7 6 5 4 3 2 1 ppm

1.00 1.02 1.00 0.89 0.89 1.07 1.06 0.98 3.00

100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 20 15 10 5

4000 3500 3000 2500 2000 1500 1000 500 Wavenumbers (cm^{-1})
Bisnaphthalene compound \textbf{109} (2.87 g, 5.4 mmol) was dissolved in DCM (50 mL) and nitromethane (50 mL). The solution was cooled in an ice bath. HOAc (0.05 mL, 0.87 mmol) and Br$_2$ (0.65 mL, 12.6 mmol) were added. The solution was stirred for one hour and warmed to room temperature for an additional 2 hours. The reaction was quenched with excess saturated aqueous Na$_2$S$_2$O$_3$. The organic layer was extracted with DCM, dried with Na$_2$SO$_4$ and the solvent was removed. The crude product was purified by chromatography (SiO$_2$, DCM) followed by treatment with activated carbon to give compound \textbf{110} (3.52 g, 94%) as a white solid (m.p.: 175-178°C).

\textsuperscript{1}H NMR (300 MHz, CDCl$_3$) $\delta$: 8.42 (d, 2H, $J = 9.3$ Hz), 8.08 (d, 2H, $J = 9.3$ Hz), 7.26 (d, 4H, $J = 9.2$ Hz), 6.28 (d, 1H, $J = 1.1$ Hz), 5.25 (s, 1H), 4.77 (s, 4H), 4.28 (q, 4H, $J = 7.1$ Hz), 2.43 (t, 2H, $J = 2.5$ Hz), 1.29 (t, 6H, $J = 7.1$ Hz). $\textsuperscript{13}$C NMR (75 MHz, CDCl$_3$) $\delta$: 168.7, 151.0, 149.8, 129.4, 128.2, 127.3, 123.1, 120.5, 118.3, 115.9, 112.1, 91.4, 67.3, 61.5, 26.8, 23.2, 14.2. IR (neat, cm$^{-1}$): 1756, 1599, 1214, 1108, 1085. HRMS for M+H$^+$ calcd for C$_{31}$H$_{27}$O$_8$Br$_2$ 685.0073, found 685.0064.
Naphthol 103: 2,6-dihydroxynaphthalene (8.68 g, 53.8 mmol) was dissolved in DMF (250 mL). K$_2$CO$_3$ (8.2 g, 59.3 mmol) was added to this solution, followed by allyl bromide (4.7 mL, 54.0 mmol) and the mixture was stirred 48 hours. The solution was diluted with DCM and extracted with 1M NaOH. The combined aqueous extracts were acidified with 6M HCl and extracted with EtOAc. The combined organic extracts were dried with Na$_2$SO$_4$ and the solvent was removed. The crude product was purified by flash chromatography on silica (1 Hexanes/3 DCM → 10% EtOAc/DCM) to give 103 4.12 g, 38%) as a white solid (m.p.: 94°C).

$^1$H NMR (250 MHz, CDCl$_3$) δ: 7.61 (d, 1H, $J = 8.5$ Hz), 7.52 (d, 1H, $J = 8.7$ Hz), 7.15 (dd, 1H, $J = 8.8$, 2.6 Hz), 7.10-7.04 (m, 3H), 6.19-6.04 (m, 1H), 5.46 (dd, 1H, $J = 17.3$, 1.6 Hz), 5.31 (dd, 1H, $J = 10.5$, 1.4 Hz), 5.13 (s, 1H), 4.62 (dt, 2H, $J = 5.3$, 1.5 Hz). $^{13}$C NMR (75 MHz, CDCl$_3$) δ: 155.0, 151.8, 133.3, 129.9, 129.7, 128.5, 127.8, 119.6, 118.1, 117.8, 109.7, 107.4, 69.0. IR(neat, cm$^{-1}$): 3413, 1630, 853. HRMS for M$^+$-1 calcd for C$_{13}$H$_{11}$O$_2$ 199.0759, found 199.0760.
Compound 103: Allyloxynaphthol 102 (10.5 g, 53.4 mmol) was dissolved in nitromethane (200 mL) and TFA (50 mL). The solution was cooled in an ice bath. 1,1,3,3-tetramethoxypropane (5 mL, 30.4 mmol) was added via syringe pump (2.7 mL/hr) while stirring. Stirring was continued for an additional 2.5 hours, then quenched with saturated aqueous Na₂CO₃. The organic layer was extracted with DCM, dried with Na₂SO₄, and the solvent was evaporated in vacuo. The crude product was purified by chromatography (SiO₂, 50% → 10% hexanes/DCM) to give 103 (8.5 g, 74%) as a white solid (m.p.: 197-199°C).

¹H NMR (250 MHz, CDCl₃) δ: 8.47 (d, 2H, J = 9.3 Hz), 7.47 (d, 2H, J = 8.9 Hz), 7.26 (dd, 2H, J = 9.3, 2.7 Hz), 7.12 (d, 2H, J = 8.9 Hz), 7.05 (d, 2H, J = 2.7 Hz), 6.25 (d, 1H, J = 1.3 Hz), 6.16-6.01 (m, 2H), 5.43 (dd, 2H, J = 17.2, 1.6 Hz), 5.31-5.26 (m, 3H), 4.59 (dd, 4H, J = 5.3, 1.2 Hz), 2.43 (t, 2H, J = 2.6 Hz). ¹³C NMR (75 MHz, CDCl₃) δ: 154.7, 148.7, 133.2, 130.7, 127.2, 126.4, 124.2, 118.9, 118.8, 118.7, 117.7, 108.6, 91.4, 68.9, 26.9, 22.9. IR(neat, cm⁻¹): 1602, 1514, 1383, 1234, 1107. HRMS for M⁺ calcd for C₂₉H₂₄O₄: 436.1675, found 436.1675. Elemental analysis calcd %C 79.80, %H 5.54, Found %C 79.53, %H 5.66.
Compound 111: Compound 103 was refluxed in N,N-diethylaniline (5 mL) for 45 min. The solution was cooled and poured into 3M HCl (50 mL) and extracted with EtOAc. The organic extracts were washed with 3M HCl (x1), sat. NaCl (x2), dried over Na₂SO₄ and the solvent was removed. The crude product was dissolved in DMF (10 mL). To the solution was added K₂CO₃ and methyl bromoacetate (0.1 mL, 0.89 mmol) and the solution was stirred overnight. The mixture was poured into H₂O, and extracted with EtOAc. The extracts were dried with Na₂SO₄ and the solvent removed. The crude product was purified by flash chromatography on silica (50% → 5% hexanes / DCM) to yield compound 111 (142 mg, 88%) as a white solid (m.p.: 144-145°C).

\[^1\text{H} \text{NMR (300MHz, CDCl}_3]\]: δ: 8.45 (d, 2H, \text{J} = 9.4 \text{ Hz}), 7.76 (d, 2H, \text{J} = 9.3 \text{ Hz}), 7.23 (d, 2H, \text{J} = 9.3 \text{ Hz}), 7.17 (d, 4H, \text{J} = 9.2 \text{ Hz}), 6.26 (s, 1H), 6.03-5.90 (m, 2H), 5.29 (s, 1H), 4.96 (dd, 2H, \text{J} = 3.4, 1.6 \text{ Hz}), 4.92 (dd, 2H, \text{J} = 12.3, 1.6 \text{ Hz}), 4.73 (s, 4H), 3.85 (d, 4H, \text{J} = 5.7 \text{ Hz}), 3.80 (s, 6H), 2.41 (s, 2H). \[^{13}\text{C} \text{NMR (75 MHz, CDCl}_3]\]: δ: 169.8, 155.2, 151.6, 151.5, 148.8, 129.5, 127.5, 124.5, 122.8, 122.7, 119.1, 118.7, 116.8, 91.3, 82.9, 67.3, 52.1, 31.4, 27.0, 24.7, 23.0. IR (neat, cm\(^{-1}\)) : 2954, 1759, 1600, 1519, 1403, 1210, 1107,
1059. HRMS for $\text{M}^+$ calcd for $\text{C}_{35}\text{H}_{32}\text{O}_8$ 580.2097, found 580.2098. Elemental analysis calcd %C 72.40, %H 5.56, Found %C 72.48, %H 5.50.
Allyl compound 111 (698 mg, 1.2 mmol), vinyl pinacolboronate (1.25 g, 8.1 mmol), and Neolyst 1 (50 mg, 0.05 mmol) were placed in a 100 mL round bottom flask under N₂, dissolved in 50 mL DCM, and brought to reflux. Additional quantities of Neolyst 1 (49 mg, 0.05 mmol) and vinyl pinacolboronate (0.91 g, 5.9 mmol) were dissolved in 5 mL DCM each and added to the reaction via syringe pump over several hours. The solvent was removed after 24 hrs and the crude product was purified by flash chromatography on silica gel (1 → 7% EtOAc / DCM) followed by precipitation from PhH with hexanes to give compound 113 (704 mg, 70 %) as a white solid (m.p.: 204-205 °C)

\(^1\)H NMR (300 MHz, CDCl₃) δ: 8.43 (d, 2H, J = 9.4 Hz), 7.68 (d, 2H, J = 9.3 Hz), 7.21 (d, 2H, J = 9.4 Hz), 7.15 (d, 2H, J = 9.2 Hz), 6.73 (dt, 2H, J = 17.9, 5.6 Hz), 6.25 (s, 1H), 5.28 (s, 1H), 5.27 (d, 2H, J = 2.7 Hz), 4.71 (s, 4H), 3.95 (t, 4H, J = 2.3 Hz), 3.80 (2, 6H), 2.42 (s, 2H) 1.17 (s, 12H). \(^13\)C NMR (75 MHz, CDCl₃) δ: 169.8, 155.2, 151.6, 151.5, 148.8, 129.5, 127.5, 124.5, 122.8, 122.7, 119.1, 118.7, 116.8, 91.3, 82.9, 67.3, 52.1, 31.4, 27.0, 24.7, 23.0. IR (neat, cm⁻¹): 2977, 1761, 1633, 1361. HRMS for M⁺ calcd for C₄₇H₄₇B₂O₁₂ 832.3801, found 832.3802.
Compound 116: Bromide 110 (44.1 mg, 0.064 mmol), boronate ester 113 (53.9 mg, 0.065), CsF (43.9 mg, 0.289 mmol), Pd2dba3 (3.7 mg, .004 mmol) and S-Phos (7.1 mg, .017 mmol) where placed in a 100 mL round bottom flask with a reflux condenser. The system was evacuated under vacuum and refilled with N2 (x3). Freshly distilled THF 50 (mL) was added. The system was evacuated under vacuum and refilled with N2 (x3). This mixture was then refluxed over 48 hrs. The solvent was removed and the crude product was purified by flash chromatography on SiO2 (0-5% Et2O / DCM) to afford 16b (anti, 14 mg, 19%) as a white solid (m.p.: 235°C dec.) and 16a (syn, 14 mg, 19%) as a white solid (m.p.: 195°C dec.).

116a: 1H NMR (500mHz, CD2Cl2) δ: 8.23, (d, 2H, J = 9.4 Hz), 8.09 (d, 2H, J = 9.3 Hz), 7.77 (t, 4H, J = 8.2 Hz), 7.10 (d, 2H, J = 9.2 Hz), 7.07 (d, 2H, J = 9.3 Hz), 6.96-6.91 (m, 4H), 6.88 (d, 2H, J = 9.2 Hz), 6.20 (s, 1H), 6.17 (s, 1H), 5.43-5.37 (m, 2H), 5.27 (s, 1H), 5.22 (s, 1H), 4.78-4.47 (m, 8H), 4.20-4.13 (m, 6H), 3.84 (dd, 2H, J = 15.7, 4.7), 3.79 (s, 6H), 2.60 (s, 1H), 2.52 (s, 1H), 1.20, (t, 2H, J = 7.2 Hz). 13C NMR (75 MHz, CD2Cl2) δ: 170.3, 169.3, 151.5, 150.5, 149.2, 149.1, 136.1, 128.4, 128.3, 127.7, 127.5, 125.1, 124.5,
124.1, 123.3, 123.1, 122.8, 122.6, 119.3, 119.2, 118.8, 118.5, 115.8, 116.3, 91.1, 90.9, 68.0, 67.3, 61.1, 52.1, 29.6, 26.0, 25.6, 22.4, 14.0. IR (Neat, cm\(^{-1}\)): 2976, 1756, 1598, 1401, 1207, 1109, 1062. IR (neat, cm\(^{-1}\)): 2976, 1755, 1598, 1518, 1401, 1207, 1109, 1062. HRMS for M\(^+\) +1 calcd for C\(_{66}H_{57}O_{16}\) 1105.3647, found 1105.3643.

\(116b\): \(^1\)H NMR (300mHz, CD\(_2\)Cl\(_2\)) \(\delta\): 8.23, (d, 2H, \(J = 9.4\) Hz), 8.07 (d, 2H, \(J = 9.3\)Hz), 7.77 (d, 4H, \(J = 8.2\)Hz), 7.07 (d, 2H, \(J = 9.3\) Hz), 7.00-6.90 (m, 4H\(_s\)), 6.84 (d, 2H, \(J = 9.2\) Hz), 6.20 (s, 2H), 5.43-5.36 (m, 2H), 5.23 (s, 1H), 4.65 (d, 4H, \(J = 9.2\) Hz), 4.57 (s, 4H), 4.37 (dd, 2H, \(J = 14.1\), 4.1), 4.19-4.03 (m, 4H\(_s\)), 3.75 (s, 6H\(_s\)), 3.63 (dd, 2H, \(J = 14.4\), 9.4), 2.60 (s, 1H), 2.49 (s, 1H), 1.07, (t, 2H, \(J = 11.9\) Hz). \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\): 169.7, 169.0, 150.6, 150.5, 149.0, 149.0, 136.8, 129.0, 128.5, 127.3, 126.9, 125.0, 124.2, 123.7, 123.6, 122.9, 122.8, 118.9, 118.9, 118.7, 118.1, 114.1, 113.7, 91.2, 91.0, 67.1, 67.0, 60.9, 52.1, 29.2, 26.1, 25.6, 22.5, 22.4, 13.3. IR (Neat, cm\(^{-1}\)): 2975, 1758, 1598, 1400, 1209, 1108, 1062. HRMS for M\(^+\) +1 calcd for C\(_{66}H_{57}O_{16}\) 1105.3647, found 1105.3625.
$R = \text{CH}_3\text{CO}_2\text{CH}_3$
$R' = \text{CH}_2\text{CO}_2\text{C}_2\text{H}_5$

116a
$R = \text{CH}_3\text{CO}_2\text{CH}_3$

$R' = \text{CH}_3\text{CO}_2\text{C}_2\text{H}_5$

**116b**
Host 117a: Compound 116a (3.24 mg, 0.003 mmol) was placed in a 4mL vial with a stir bar and fitted with a septum screw cap. The vial was purged with N₂. THF (1.3mL), CH₃OH (0.65mL), and NaOH (0.65mL 6M) were added and the contents were stirred and sparged with N₂ for 15 minutes and then stirred thereafter for an additional 75 minutes. The vial was cooled in a methanol/ice bath, and quenched with chilled aqueous TFA (0.65mL, 6M). The mixture was transferred to a separatory funnel containing chilled water. The aqueous phase was made slightly acidic with aqueous TFA (6M) and extracted into DCM/THF (x3). The extracts were rinsed with water (x2), then brine and dried over Na₂SO₄. The solvent was removed in vacuo at room temperature. The crude material obtained was triturated (x2) with a 1:1 mixture of DCM and pentane to afford host 117a (2.9 mg, 97%) as a white solid (m.p.: 182°C dec.).

¹H NMR (300mHz, CDCl₃, CD₃OD) δ: 8.27, (d, 2H, J = 9.4 Hz), 8.12 (d, 2H, J = Hz), 7.76 (d, 2H, J = Hz), 7.73 (d, 2H, J = Hz), 7.11 (d, 2H, J = Hz), 7.06 (d, 2H, J = Hz), 6.92 (m, 6H), 6.24 (s, 1H), 6.20 (s, 1H), 5.50-4.41 (m, 2H), 5.30 (s, 1H), 5.25 (s, 1H), 4.73-4.55 (m, 8H), 4.16 (dd, 2H, J = 15.4, 8.9), 2.65 (s, 1H), 2.57 (s, 1H). ¹³C NMR (125 MHz, CDCl₃, CD₃OD) δ: 174.2, 173.7, 151.2, 150.3, 149.2, 149.0, 136.6, 128.6, 128.4, 127.3, 126.8, 124.7, 123.5, 123.4, 123.2, 123.1, 122.8, 122.5, 119.6, 119.4, 119.3, 118.7, 114.7, 112.7, 91.4, 91.3, 67.7, 66.8, 29.3, 26.1, 25.7, 22.6, 22.6. IR (neat, cm⁻¹): 2928,
1731, 1598, 1517, 1401, 1215, 1061. HRMS for M$^+$+1 calcd for C$_{60}$H$_{45}$O$_{16}$ 1021.2708, found 1021.2711.

R = CH$_2$CO$_2$H

117a
Host 117b: Compound 116b (2.66 mg, 0.002 mmol) was placed in a 4mL vial with a stir bar and fitted with a septum screw cap. The vial was purged with N₂. THF (1.3mL), CH₃OH (0.65mL), and NaOH (0.65mL 6M) were added and the contents were stirred and sparged with N₂ for 15 minutes and then stirred thereafter for an additional 75 minutes. The vial was cooled in a methanol/ice bath, and quenched with chilled aqueous TFA (0.65mL, 6M). The mixture was transferred to a separatory funnel containing chilled water. The aqueous phase was made slightly acidic with aqueous TFA (6M) and extracted into DCM/THF (x3). The extracts were rinsed with water (x2), then brine and dried over Na₂SO₄. The solvent was removed in vacuo at room temperature. The crude material obtained was trituated (x2) with a 1:1 mixture of DCM and pentane to afford host 117b (2.4mg, 98%) as a white solid (m.p.: 192°C dec.).

\(^1\)H NMR (300mHz, CDCl₃, CD₃OD) δ: 8.22 (d, 2H, J = 9.4 Hz), 8.06 (d, 2H, J = 9.4 Hz), 7.80 (d, 2H, J = 9.3 Hz), 7.75 (d, 2H, J = 9.3 Hz), 7.02 (m, 6H), 6.95 (d, 2H, J = 9.3 Hz), 6.88 (d, 2H, J = 9.2 Hz), 6.16 (s, 2H), 5.46 (m, 2H), 5.25 (s, 2H), 4.65 (s, 2H), 4.63 (s, 2H), 4.52 (s, 2H), 4.51 (s, 2H), 4.39 (dd, 2H, J = 15.0, 3.8 Hz), 3.72 (dd, 2H, J = 14.8, 8.8 Hz), 2.62 (s, 2H), 2.52 (s, 2H) \(^1\)C NMR (75 MHz, CDCl₃, CD₃OD) δ: 171.9, 171.9, 151.0, 150.9, 149.2, 137.3, 129.3, 128.9, 127.6, 127.6, 126.3, 125.2, 124.1, 124.0, 123.6, 123.1, 123.0, 122.8, 119.2, 119.2, 119.0, 118.3, 115.0, 114.4, 91.4, 91.2, 67.4, 66.9, 29.3,
26.3, 25.8, 22.5  IR (neat, cm\(^{-1}\)): 2920, 1732, 1595, 1515, 1459, 1402, 1211, 1105, 1059.

HRMS for M\(^{+}\)+1 calcd for C\(_{60}\)H\(_{45}\)O\(_{16}\) 1021.2708, found 1021.2726.

R = CH\(_{3}\)CO\(_{2}\)H

117b
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(125) Attempts to use other sulfonates were not as straight forward.


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