12-15-2012

Water-Soluble Deep-Cavity Cavitands: Synthesis, Molecular Recognition, and Interactions with Phospholipid Membranes

Sarah E. Ioup

University of New Orleans, sewhisenhunt@gmail.com

Follow this and additional works at: http://scholarworks.uno.edu/td

Recommended Citation
Water-Soluble Deep-Cavity Cavitands: Synthesis, Molecular Recognition, and Interactions with Phospholipid Membranes

A Dissertation

Submitted to the Graduate Faculty of the
University of New Orleans
in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy
in
Chemistry

by

Sarah Elizabeth Ioup

B.S. University of Chicago, 2003

December, 2012
Acknowledgements

I offer many thanks to my advisor, Professor Bruce C. Gibb, for introducing me to a fascinating field of chemistry and for his knowledge, insight, guidance, and patience throughout the course of my studies. I also thank the members of my research committee, Professor Jeffery T. Davis, Professor Matthew A. Tarr, and Professor Mark L. Trudell.

I am also grateful to current and former Gibb group members for their research insights, suggestions, and assistance and for their moral support and friendship. The generous assistance of Corinne Gibb, the resident Gibb lab and NMR expert, was especially valuable. Special thanks also to Professor Davis and his research group for hosting me in his laboratory and teaching me new research techniques and for valuable insights into a new area of research for our group.

Most of all, I thank my family, without whom this endeavor would have been impossible. I thank my husband Elias for his never-ending love, support, patience, and faith in me and our daughter Layla for brightening every day.
Contents

List of figures ................................................................. viii
List of schemes ............................................................... ix
List of tables ................................................................. x
Abbreviations ................................................................. xi
Abstract ............................................................................ xii

1 Introduction 1
1.1 Noncovalent interactions .............................................. 2
  1.1.1 Ion-ion interactions ................................................. 2
  1.1.2 Ion-dipole interactions ........................................... 3
  1.1.3 Dipole-dipole interactions ....................................... 4
  1.1.4 Hydrogen bonding .................................................. 4
  1.1.5 π effects ............................................................... 5
  1.1.6 Induced-dipole interactions ...................................... 7
1.2 The hydrophobic effect ................................................ 8
1.3 Water-soluble molecular hosts ....................................... 10
  1.3.1 Cyclophanes .......................................................... 10
  1.3.2 Cyclodextrins ......................................................... 12
  1.3.3 Cucurbit[n]urils .................................................... 14
  1.3.4 Metal-coordinated hosts ......................................... 15
  1.3.5 Cavitands ............................................................. 18
1.4 Interactions between phospholipid membranes and membrane-active compounds 28
  1.4.1 Biological membranes ............................................. 28
  1.4.2 Synthetic models of biological membranes ....................... 29
  1.4.3 Types of membrane-active compounds ......................... 31

2 Synthesis of water-soluble deep-cavity cavitands 39
2.1 Introduction ............................................................... 39
2.2 Improved synthesis of octaacid ....................................... 40
2.3 Synthesis of cationic cavitands ....................................... 44
2.4 Discussion ................................................................. 49

3 Molecular recognition of new water-soluble deep-cavity cavitands 51
3.1 Introduction ............................................................... 51
3.2 Binding analysis by NMR .............................................. 51
3.3 Binding of organic guest molecules ................................... 54
  3.3.1 Formation of 1:1 host:guest complexes with 1-adamantanecarboxylic acid 54
  3.3.2 Formation of 2:1 host:guest capsules with dodecane .................... 59
3.4 Binding of anions .......................................................... 62
  3.4.1 OPy-Cl binding of anions .......................................... 63
  3.4.2 OTA-Cl binding of anions ......................................... 66
  3.4.3 OAm-HCl binding of anions ....................................... 68
3.5 Discussion ................................................................. 69
List of Figures

1 Crown ether complex with Na⁺ ........................................... 4
2 Carboxylic acid dimer formed by hydrogen bonding. .................. 5
3 π-π stacking geometries. .................................................. 7
4 Structures of cyclophane 1 and guest molecule durene. ................. 11
5 Structures of cyclophanes 2. ............................................. 12
6 Structures of native cyclodextrins and schematic illustration of cyclodextrin structural features ........................................... 13
7 Structure of cucurbit[n]uril. .............................................. 14
8 Binding constants (log $K_a$) versus chain length $m$ for binding of H(CH$_2$)$_m$NH$_3^+$ and H$_3$N(CH$_2$)$_m$NH$_3^+$ by CB[6]. ....................................................... 15
9 X-ray crystal structure of 3 along with water oxygen atoms. ORTEP drawing of 10 water oxygen atoms inside cage 3. ............................. 17
10 Resorcin[4]arene 7 and suitable guests. ...................................... 19
12 Structure of cavitand 12. .................................................. 20
13 Structures of deep-cavity cavitands 13 and 14. Cavitands 13 and 14 in the vase conformation. .................................................. 21
14 Structure of deep-cavity cavitand 15 and energy-minimized structures of 15-SDS complex ......................................................... 22
16 Schematic representation of binding of octaacid with a guest molecule to form a 1:1 complex or 2:1 capsule. ................................. 24
17 Structure and space-filling model of octaacid 20. ......................... 25
18 Hofmeister series for anions. .............................................. 26
19 Structures of G3 21, octol 22, and TEMOA 23. ......................... 27
20 Generic phospholipid structure with common headgroup and fatty acid substituents. ...................................................... 28
21 Structures of important membrane sterols. ................................. 29
22 Schematic illustration of different design strategies for synthetic ion channels and pores. ......................................................... 34
23 Structures of mobile carrier ionophores prodigiosin, isophthalamide carriers 24, and chlapod 25. ................................................ 35
24 Stylized representation of a di-walled molecular umbrella. Proposed mechanism of transmembrane transport by a di-walled molecular umbrella. ...................................................... 36
25 Structures of molecular umbrellas 26 and 27 used to effect transmembrane transport of glutathione. Structure of glutathione .......... 37
26 Illustration of AMP interaction with a multicellular organism cell membrane versus with a bacterial cell membrane. ......................... 38
27 Summary of procedure for octol 22 purification from new octaacid 20 synthesis. ................................................................. 39
28 $^1$H NMR spectra of octol 22 made via the old and new methods. ...... 43
29 $^1$H NMR spectra of OAm-HCl 36 in D$_2$O and CD$_3$OD. ............... 48
30 $^1$H NMR spectra of OTA-Cl 38 in D$_2$O and CD$_3$OD. ................. 49
32 $^1$H NMR spectra of OPy-Cl 39 in D$_2$O and CD$_3$OD. .............................. 50
33 Generic cavitatnd with proton labels. ....................................................... 52
34 $^1$H NMR spectra of OPy-Cl titration with adaCO$_2$H. ............................... 55
35 Bound guest region of COSY NMR spectrum of OPy-Cl-adaCO$_2$H complex. .... 55
36 $^1$H NMR spectra of OPy-Cl-adaCO$_2$H dilution study. ............................... 56
37 $^1$H NMR spectra of OPy-Cl-adaCO$_2$H dilution study. ............................... 57
38 $^1$H NMR spectra of OPy-Cl dilution study. .............................................. 58
39 $^1$H NMR spectra of OAm-HCl titration with adaCO$_2$H. ............................... 59
40 $^1$H NMR spectra of free OPy-Cl and (OPy-Cl)$_2$·dodecane capsule. ............... 60
41 $^1$H NMR spectra of OPy-Cl·adaCO$_2$H dilution study. ............................... 61
42 $^1$H NMR spectra of OPy-Cl·adaCO$_2$H dilution study. ............................... 62
43 $^1$H NMR spectra of free OAm-HCl and (OAm-HCl)$_2$·dodecane capsule. ......... 63
44 $^1$H NMR spectra of OPy-Cl with each Hofmeister salt. ................................ 64
45 Plots of OPy-Cl$_j$ peak ∆$\delta$ as a function of Cl$^-$ and Br$^-$ concentration. .... 66
46 $^1$H NMR spectra of OTA-Cl with each Hofmeister salt. ................................ 67
47 Binding isotherm of OTA-Cl $b$ ∆$\delta$ as a function of NaI concentration. ...... 68
48 $^1$H NMR spectra of OAm-HCl with each Hofmeister salt. ................................ 70
49 Structure of octapropylsulfonate cavatand 40. ......................................... 76
50 Structures of hexylene-linked and phenylene-linked octaacid dimers 41 and 42. .... 76
51 Structures of POPC, ANTS, and DPX. Schematic representation of liposome leakage experiments. ................................................................. 77
52 Simulations of $Q_{in}$ as a function of $f_{out}$ for the performed requenching experiment. 78
53 Host-induced leakage of POPC LUVs as a function of time. ............................. 80
54 OPS-induced leakage of POPC LUVs as a function of time. ............................. 81
55 Leakage of POPC LUVs by free OPS, OPS·adaCO$_2$H complex, (OPS)$_2$·progesterone capsule. ................................................................. 83
56 Results of requenching experiment to determine mechanism of membrane leakage by OPS, OA, and TEMOA. ................................................................. 84
57 $^1$H NMR spectra of free TEMOA and TEMOA with one equivalent of POPC. ....... 85
58 Mechanistic model for perturbation of phospholipid membranes by AMPs. ....... 87
59 Structures of DPPC and POPG. ................................................................. 90
60 COSY NMR spectrum of OAm-HCl. ........................................................... 97
61 COSY NMR spectrum of OTA-Cl. .............................................................. 98
62 COSY NMR spectrum of OPy-Cl. .............................................................. 100
63 $^1$H NMR spectra of OTA-Cl titration with adaCO$_2$H. .................................. 102
64 COSY NMR spectrum of OPy-Cl-adaCO$_2$H complex. .................................. 103
65 COSY NMR spectrum of OTA-Cl-adaCO$_2$H complex. .................................. 104
66 COSY NMR spectrum of OAm-HCl-adaCO$_2$H complex. ................................ 105
67 $^1$H NMR spectra of OTA-Cl-adaCO$_2$H dilution study. ................................ 106
68 $^1$H NMR spectra of OAm-HCl-adaCO$_2$H dilution study. ................................ 106
69 $^1$H NMR spectra of free OTA-Cl and (OTA-Cl)$_2$·dodecane capsule. ............... 107
70 Bound host region of COSY NMR spectrum of (OPy-Cl)$_2$·dodecane capsule. .... 108
71 COSY NMR spectrum of (OTA-Cl)$_2$·dodecane capsule. ................................ 109
72 COSY NMR spectrum of (OAm-HCl)$_2$·dodecane capsule. ............................ 110
73 $^1$H NMR spectra of (OTA-Cl)$_2$·dodecane dilution study. ............................ 111
The \( \text{H} \) NMR spectra of \((\text{OAm-HCl})_2\)-dodecane dilution study. .......................... 111
The \( \text{j} \), \( \text{b} \), and \( \text{s} \) peak regions of \( \text{H} \) NMR spectra of \( \text{OPy-Cl} \) with \( \text{NaF} \). ......................... 113
Binding isotherms for titration of \( \text{OPy-Cl} \) with \( \text{NaF} \). .......................... 114
The \( \text{j} \), \( \text{b} \), and \( \text{s} \) peak regions of \( \text{H} \) NMR spectra of \( \text{OPy-Cl} \) with \( \text{Na}_2\text{SO}_4 \). .......................... 115
Binding isotherms for titration of \( \text{OPy-Cl} \) with \( \text{Na}_2\text{SO}_4 \). .......................... 115
The \( \text{j} \), \( \text{b} \), and \( \text{s} \) peak regions of \( \text{H} \) NMR spectra of \( \text{OPy-Cl} \) with \( \text{NaOAc} \). .......................... 116
Binding isotherms for titration of \( \text{OPy-Cl} \) with \( \text{NaOAc} \). .......................... 116
The \( \text{j} \), \( \text{b} \), and \( \text{s} \) peak regions of \( \text{H} \) NMR spectra of \( \text{OPy-Cl} \) with \( \text{NaCl} \). .......................... 117
Binding isotherms for titration of \( \text{OPy-Cl} \) with \( \text{NaCl} \). .......................... 118
The \( \text{j} \), \( \text{b} \), and \( \text{s} \) peak regions of \( \text{H} \) NMR spectra of \( \text{OPy-Cl} \) with \( \text{NaNO}_3 \). .......................... 119
Binding isotherms for titration of \( \text{OPy-Cl} \) with \( \text{NaNO}_3 \). .......................... 119
The \( \text{j} \), \( \text{b} \), and \( \text{s} \) peak regions of \( \text{H} \) NMR spectra of \( \text{OPy-Cl} \) with \( \text{NaBr} \). .......................... 120
Binding isotherms for titration of \( \text{OPy-Cl} \) with \( \text{NaBr} \). .......................... 121
The \( \text{j} \), \( \text{b} \), and \( \text{s} \) peak regions of \( \text{H} \) NMR spectra of \( \text{OPy-Cl} \) with \( \text{NaClO}_3 \). .......................... 122
Binding isotherms for titration of \( \text{OPy-Cl} \) with \( \text{NaClO}_3 \). .......................... 122
The \( \text{j} \), \( \text{b} \), and \( \text{s} \) peak regions of \( \text{H} \) NMR spectra of \( \text{OPy-Cl} \) with \( \text{NaI} \). .......................... 123
Binding isotherms for titration of \( \text{OPy-Cl} \) with \( \text{NaI} \). .......................... 123
The \( \text{j} \), \( \text{b} \), and \( \text{s} \) peak regions of \( \text{H} \) NMR spectra of \( \text{OPy-Cl} \) with \( \text{NaSCN} \). .......................... 124
Binding isotherms for titration of \( \text{OPy-Cl} \) with \( \text{NaSCN} \). .......................... 124
The \( \text{j} \), \( \text{b} \), and \( \text{s} \) peak regions of \( \text{H} \) NMR spectra of \( \text{OPy-Cl} \) with \( \text{NaClO}_4 \). .......................... 125
Binding isotherms for titration of \( \text{OPy-Cl} \) with \( \text{NaClO}_4 \). .......................... 125
The \( \text{j} \), \( \text{b} \), and \( \text{s} \) peak regions of \( \text{H} \) NMR spectra of \( \text{OTA-Cl} \) with \( \text{NaF} \). .......................... 127
Binding isotherms for titration of \( \text{OTA-Cl} \) with \( \text{NaF} \). .......................... 128
The \( \text{j} \), \( \text{b} \), and \( \text{s} \) peak regions of \( \text{H} \) NMR spectra of \( \text{OTA-Cl} \) with \( \text{Na}_2\text{SO}_4 \). .......................... 129
Binding isotherms for titration of \( \text{OTA-Cl} \) with \( \text{Na}_2\text{SO}_4 \). .......................... 130
The \( \text{j} \), \( \text{b} \), and \( \text{s} \) peak regions of \( \text{H} \) NMR spectra of \( \text{OTA-Cl} \) with \( \text{NaOAc} \). .......................... 131
Binding isotherms for titration of \( \text{OTA-Cl} \) with \( \text{NaOAc} \). .......................... 131
The \( \text{j} \), \( \text{b} \), and \( \text{s} \) peak regions of \( \text{H} \) NMR spectra of \( \text{OTA-Cl} \) with \( \text{NaCl} \). .......................... 132
Binding isotherms for titration of \( \text{OTA-Cl} \) with \( \text{NaCl} \). .......................... 132
The \( \text{j} \), \( \text{b} \), and \( \text{s} \) peak regions of \( \text{H} \) NMR spectra of \( \text{OTA-Cl} \) with \( \text{NaNO}_3 \). .......................... 133
Binding isotherms for titration of \( \text{OTA-Cl} \) with \( \text{NaNO}_3 \). .......................... 133
The \( \text{j} \), \( \text{b} \), and \( \text{s} \) peak regions of \( \text{H} \) NMR spectra of \( \text{OTA-Cl} \) with \( \text{NaBr} \). .......................... 134
Binding isotherms for titration of \( \text{OTA-Cl} \) with \( \text{NaBr} \). .......................... 135
The \( \text{j} \), \( \text{b} \), and \( \text{s} \) peak regions of \( \text{H} \) NMR spectra of \( \text{OTA-Cl} \) with \( \text{NaClO}_3 \). .......................... 136
Binding isotherms for titration of \( \text{OTA-Cl} \) with \( \text{NaClO}_3 \). .......................... 136
The \( \text{j} \), \( \text{b} \), and \( \text{s} \) peak regions of \( \text{H} \) NMR spectra of \( \text{OTA-Cl} \) with \( \text{NaI} \). .......................... 137
Binding isotherms for titration of \( \text{OTA-Cl} \) with \( \text{NaI} \). .......................... 138
The \( \text{j} \), \( \text{b} \), and \( \text{s} \) peak regions of \( \text{H} \) NMR spectra of \( \text{OTA-Cl} \) with \( \text{NaSCN} \). .......................... 139
Binding isotherms for titration of \( \text{OTA-Cl} \) with \( \text{NaSCN} \). .......................... 139
The \( \text{j} \), \( \text{b} \), and \( \text{s} \) peak regions of \( \text{H} \) NMR spectra of \( \text{OTA-Cl} \) with \( \text{NaClO}_4 \). .......................... 140
Binding isotherms for titration of \( \text{OTA-Cl} \) with \( \text{NaClO}_4 \). .......................... 140
## List of Schemes

1. Self-assembly of coordination cage 3. ........................................... 16
2. Diels-Alder reaction of triphenylene 4 with maleimide 5 inside coordination cage 3 to obtain *endo* adduct 6. ........................................... 17
4. Original synthesis of octaacid 20. ........................................... 40
5. Improved synthesis of octaacid 20. ........................................... 41
6. Proposed synthesis of an octa-electrophilic cavitand. ........................................... 45
7. Failed attempts to synthesize an octa-electrophilic cavitand. ........................................... 45
8. Synthesis of octamesylate cavitand 35. ........................................... 46
9. Synthesis of new water-soluble cavitands 36, 38, and 39 from octamesylate 35. ........................................... 47
<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Characteristics of some common supramolecular interactions.</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Properties of hydrogen bonds.</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>Comparison of organic solvent washes to purify octol 22 made via new octaacid synthesis.</td>
<td>42</td>
</tr>
<tr>
<td>4</td>
<td>Association constants for OPy-Cl binding of anions.</td>
<td>65</td>
</tr>
<tr>
<td>5</td>
<td>Association constants for OTA-Cl binding of anions.</td>
<td>67</td>
</tr>
<tr>
<td>6</td>
<td>Host-induced turbidity changes in POPC LUVs.</td>
<td>82</td>
</tr>
<tr>
<td>7</td>
<td>All calculations of association constants for OPy-Cl binding of anions.</td>
<td>112</td>
</tr>
<tr>
<td>8</td>
<td>All calculations of association constants for OTA-Cl binding of anions.</td>
<td>126</td>
</tr>
</tbody>
</table>
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$-CD</td>
<td>$\alpha$-cyclodextrin</td>
</tr>
<tr>
<td>$\text{adaCO}_2\text{H}$</td>
<td>1-adamantanecarboxylic acid</td>
</tr>
<tr>
<td>AMP</td>
<td>antimicrobial peptide</td>
</tr>
<tr>
<td>ANTS</td>
<td>8-aminonaphthalene-1,3,6-trisulfonic acid disodium salt</td>
</tr>
<tr>
<td>$\beta$-CD</td>
<td>$\beta$-cyclodextrin</td>
</tr>
<tr>
<td>BLM</td>
<td>black lipid membrane</td>
</tr>
<tr>
<td>CB</td>
<td>cucurbit$[n]$uril</td>
</tr>
<tr>
<td>CD</td>
<td>cyclodextrin</td>
</tr>
<tr>
<td>COSY</td>
<td>correlation NMR spectroscopy</td>
</tr>
<tr>
<td>CPP</td>
<td>cell-penetrating peptides</td>
</tr>
<tr>
<td>DCC</td>
<td>deep-cavity cavitand</td>
</tr>
<tr>
<td>DMF</td>
<td>$N,N$-dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethyl sulfoxide</td>
</tr>
<tr>
<td>DPC</td>
<td>dodecyl phosphatidylcholine</td>
</tr>
<tr>
<td>DPX</td>
<td>$p$-xylene-bis($N$-pyridinium bromide)</td>
</tr>
<tr>
<td>EYPC</td>
<td>egg yolk phosphatidylcholine</td>
</tr>
<tr>
<td>FRET</td>
<td>fluorescence resonance energy transfer</td>
</tr>
<tr>
<td>$\gamma$-CD</td>
<td>$\gamma$-cyclodextrin</td>
</tr>
<tr>
<td>GSH</td>
<td>glutathione</td>
</tr>
<tr>
<td>GUV</td>
<td>giant unilamellar vesicle</td>
</tr>
<tr>
<td>HDA</td>
<td>hexylene-linked octaacid dimer 41</td>
</tr>
<tr>
<td>ITC</td>
<td>isothermal titration calorimetry</td>
</tr>
<tr>
<td>LUV</td>
<td>large unilamellar vesicle</td>
</tr>
<tr>
<td>MLV</td>
<td>multilamellar vesicle</td>
</tr>
<tr>
<td>MsCl</td>
<td>methanesulfonyl chloride</td>
</tr>
<tr>
<td>Ms$_2$O</td>
<td>methanesulfonic anhydride</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>OA</td>
<td>octaacid 20</td>
</tr>
<tr>
<td>OAm</td>
<td>octaamine 36</td>
</tr>
<tr>
<td>OPS</td>
<td>octapropylsulfonate 40</td>
</tr>
<tr>
<td>OPy</td>
<td>octapyridinium salt 39</td>
</tr>
<tr>
<td>OTA</td>
<td>octatrimethylammonium salt 38</td>
</tr>
<tr>
<td>PBr$_3$</td>
<td>phosphorous tribromide</td>
</tr>
<tr>
<td>PDA</td>
<td>phenylene-linked octaacid dimer 42</td>
</tr>
<tr>
<td>POPC</td>
<td>1-palmitoyl-2-oleoyl-$sn$-glycero-3-phosphocholine</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecylsulfate</td>
</tr>
<tr>
<td>SOCl$_2$</td>
<td>thionyl chloride</td>
</tr>
<tr>
<td>SUV</td>
<td>small unilamellar vesicle</td>
</tr>
<tr>
<td>TEMOA</td>
<td>tetra endo-methyl octaacid 23</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>UWL</td>
<td>unstirred water layer</td>
</tr>
</tbody>
</table>
Abstract

Water-soluble deep-cavity cavitands provide a rare opportunity to study self-assembly driven by the hydrophobic effect. These molecular hosts dimerize in the presence of certain guest molecules to form water-soluble molecular capsules. These systems have given rise to numerous novel chemical phenomena and have potential use in drug delivery. The host octaacid (OA) has been particularly well-characterized, but studies are limited to basic pH because of limited host solubility.

Herein we report an improved synthesis of OA and the syntheses of three new water-soluble deep-cavity cavitands. The new hosts are soluble at neutral pH, increasing relevance for biological studies. The new syntheses are versatile enough to apply to the synthesis of additional water-soluble cavitands in the future. We also describe preliminary characterization of the molecular recognition properties of the new hosts. Binding of organic guest molecules to form 1:1 host:guest complexes and 2:1 host:guest capsules was qualitatively similar to that of OA. However, binding of anions spanning the Hofmeister series revealed interesting new behavior. The new hosts bound a wider range of anions inside the hydrophobic pocket with much higher association constants. Moreover, external binding of several anions to the cavitand pendant feet was observed.

Looking towards biological applications, we desired to learn how these molecules interact with phospholipid membranes. Six water-soluble cavitands were tested for their ability to permeabilize liposomal POPC membranes. One host showed very high potency in permeabilizing membranes, while three other hosts showed moderate activity. Host binding of POPC was found to be at least one factor in host-induced permeabilization. A requenching assay to determine leakage mechanism strongly supported all-or-none leakage, whereby some vesicles lose all contents while others lose none. These results suggest that these cavitands induce partial transient leakage of vesicles by the formation of transient membrane pores. These findings show potential for the use of these hosts as drug delivery carriers, antimicrobial compounds, and tools in membrane alteration studies.

Keywords: supramolecular chemistry, phospholipid membrane, anion receptor, host-guest chemistry, cavitand, membrane permeabilization.
1 Introduction

Supramolecular chemistry is described by Jean-Marie Lehn, one of the pioneers in the field, as the “chemistry of molecular assemblies and of the intermolecular bond,” or more succinctly as “chemistry beyond the molecule.” The molecular components of supramolecular assemblies must possess the structural elements that lead to the favorable noncovalent interactions on which assembly relies. Hence, the task of the supramolecular chemist is to design molecules with enough structural “information” to undergo the assembly process spontaneously. It may seem intuitive that self-assembly of individual molecules into one supermolecule would be entropically penalized and that assembly must therefore be enthalpically driven (Equation 1). Yet, this is not always true, and supramolecular processes that are entropically driven are abundant in nature. Therefore, while many (perhaps most) synthetic self-assembling systems are enthalpically driven and rely on strong, highly directional noncovalent forces such as hydrogen bonding or metal coordination, this is not the only strategy available.

\[ \Delta G = \Delta H - T\Delta S \]  

Self-assembly in organic solvent is generally driven by enthalpically favorable noncovalent interactions, since the solvent does not compete significantly with these interactions. In water, such forces are typically insufficient to drive assembly because they cannot effectively compete with water’s own ability to participate in such interactions. (Examples in nature of assembly-driving hydrogen bonding in water are possible because the hydrogen bonding array is shielded from the water solvent. Synthetic systems, however, have not reached this level of sophistication.) Self-assembly of synthetic systems in water usually relies on either metal coordination, a very strong noncovalent force, or the hydrophobic effect. The research described here involves deep-cavity cavitands that self-assemble in water in a process that is driven by the hydrophobic effect.
1.1 Noncovalent interactions

Noncovalent intermolecular forces are less important in the research reported here than the hydrophobic effect. Nonetheless, they do play some role and should not be overlooked. Moreover, knowledge of these forces is necessary to understand why they play a relatively small role in this work and in supramolecular chemistry in water in general. These interactions are summarized in Table 1 and discussed further below. All of these interactions rely at least in part on electrostatic interaction. Here, this is defined as strictly Coulombic attraction or repulsion between whole or partial charges without change to the participating species.

<table>
<thead>
<tr>
<th>Interaction</th>
<th>Directionality</th>
<th>Bond energy (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ion-ion</td>
<td>Nondirectional</td>
<td>100-350</td>
</tr>
<tr>
<td>Ion-dipole</td>
<td>Slightly directional</td>
<td>50-200</td>
</tr>
<tr>
<td>Dipole-dipole</td>
<td>Slightly directional</td>
<td>5-50</td>
</tr>
<tr>
<td>Hydrogen bond</td>
<td>Directional</td>
<td>4-120</td>
</tr>
<tr>
<td>(\pi-\pi)</td>
<td>Directional</td>
<td>2-50</td>
</tr>
<tr>
<td>Cation- and anion-(\pi)</td>
<td>Directional</td>
<td>5-80</td>
</tr>
<tr>
<td>Van der Waals</td>
<td>Nondirectional</td>
<td>&lt; 5</td>
</tr>
</tbody>
</table>

### 1.1.1 Ion-ion interactions

Oppositely charged ions are attracted to each other through electrostatic interaction. An ion pair exists when oppositely charged ions are in close enough proximity that the energy resulting from their electrostatic attraction is greater than the thermal energy available to separate them. In other words, ions remain paired if the time required for Brownian motion to separate them is greater than the lifetime of their association. Ion pairing and ion solvation can be viewed as competing routes to lower the Gibbs free energy of the solution. The strength of the ion pairing interaction is inversely dependent on the dielectric constant of the medium, \(\varepsilon\), as illustrated by Coulomb’s law (Equation 2), which defines the potential energy \(E\) between two charges \(q_1\) and \(q_2\) at a distance \(r\) (\(\varepsilon_0\) is the permittivity of the vacuum):

\[
E = \frac{1}{4\pi\varepsilon_0} \frac{q_1 q_2}{r}
\]
Equation 2 is actually an imperfect description of the energy resulting from an ion-ion interaction, because there are other factors involved, such as the ability of the solvent to solvate the ions and the size and shape of the ions. Nonetheless, it illustrates the importance of dielectric constant of the solvent as a factor in the strength of an ion pairing interaction; indeed, in the gas phase ($\varepsilon = 0$) ion-ion interactions can be worth over 100 kcal/mol. Conversely, ion pairing interactions in water ($\varepsilon = 78$) tend to be quite weak, and indeed many salts are soluble in water.$^3$

### 1.1.2 Ion-dipole interactions

The potential energy between a dipole fixed in space of dipole moment $\mu$ and a charge $q_2$ at angle $\theta$ is inversely proportional to the square of the distance $r$ between charge and dipole, as described by Equation 3 (assuming $r$ is much greater than the length of the dipole):

$$E = \frac{\mu q_2 \cos \theta}{4\pi\varepsilon\varepsilon_0 r^2}$$  \hspace{1cm} (3)

While this is an imperfect description of actual ion-dipole interactions, it illustrates the importance of several factors: the medium, the distance between ion and dipole, and the orientation of ion and dipole. As with ion-ion interactions, ion-dipole interactions are inversely proportional to the dielectric constant of the medium and are therefore much weaker in water than in organic solvents. Compared to ion-ion interactions, the strength of ion-dipole interactions decreases more rapidly as the distance between the participating species decreases.$^3$

The dissolution of salts in water is often accompanied by a significant release of heat, which is due in part to the large attractive force between the water molecules and salt ions, an ion-dipole interaction.$^3$ The complexation of metal cations by crown ethers is a supramolecular example of an ion-dipole interaction (Figure 1). Coordinative bonds between nonpolarizable metal cations...
and hard bases are another type of ion-dipole interaction that is important in supramolecular chemistry, both in organic and aqueous media (vide infra).\textsuperscript{1}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{crown_ether.png}
\caption{Crown ether complex with Na\textsuperscript{+}.}
\end{figure}

1.1.3 Dipole-dipole interactions

As with ion-ion and ion-dipole interactions, the strength of dipole-dipole interactions is dependent on the dielectric constant of the medium and the distance between the participating species. The orientation of the participating species is also a factor, as with ion-dipole interactions. These relationships are illustrated in Equation 4, which describes the energy $E$ between two fixed dipoles that are parallel and in the same plane:

$$E = -\frac{\mu_1 \mu_2 (3 \cos^2 \theta - 1)}{4 \pi \varepsilon \varepsilon_0 r^3}$$

where $\mu_1$ and $\mu_2$ are the dipole moments of the dipoles, $\theta$ is the angle between a dipole and a line connecting the dipoles, $\varepsilon$ is the dielectric constant of the medium, and $r$ is the distance between the two dipoles ($r$ is much greater than the length of the dipoles). $E$ is inversely proportional to the cube of $r$, making the interaction quite sensitive to the distance between dipoles. While Equation 4 is an imperfect descriptor of ion-dipole interactions, it serves to illustrate the factors that influence the strength of these interactions and their relative importance.\textsuperscript{3}

1.1.4 Hydrogen bonding

The hydrogen bond is a specific type of dipole-dipole interaction in which a hydrogen covalently bonded to a more electronegative atom is attracted to a neighboring dipole. A hydrogen bond is
commonly written as D−H···A, where D−H signifies the donor and A signifies the acceptor. Both D and A are usually electronegative atoms such as oxygen or nitrogen. The hydrogen bond is both fairly strong and highly directional, making it an extremely useful noncovalent interaction in supramolecular chemistry. Lehn even described it as the “masterkey interaction in supramolecular chemistry”. The formation of carboxylic acid dimers is an example of a simple supramolecular assembly that arises due to hydrogen bonding (Figure 2).

![Figure 2: Carboxylic acid dimer formed by hydrogen bonding.](image_url)

Hydrogen bonds vary widely in strength, length, and geometry, as illustrated in Table 2. Strong hydrogen bonds are comparable to covalent bonds in strength. Moderate strength hydrogen bonds are formed between neutral donor and acceptor groups through electron lone pairs. They have a slightly bent geometry. Weak hydrogen bonds typically involve such donors as C−H groups and tend to be highly nonlinear. Despite the low bond energy of weak hydrogen bonds, their effect on structure stabilization can be significant in large numbers. While many factors influence hydrogen bond strength, solvent plays the largest role. A solvent that is itself capable of hydrogen bonding, as are many polar solvents, will generally render hydrogen bonds between solutes thermodynamically neutral. Hydrogen bonds that do form in hydrogen bonding solvents are usually shielded in some way from the solvent; for example, a hydrogen bond in the interior of a protein dissolved in water is usually worth 0.5 to 1.5 kcal/mol, whereas the same hydrogen bond in bulk water is usually worth 0 kcal/mol. Hydrogen bonds are ubiquitous in nature and are responsible for determining the shape of many proteins, for substrate recognition for many enzymes, and (along with π-π interactions) for the double helix structure of DNA.

1.1.5 π effects

The electrons in π systems comprise regions of negative charge that are capable of engaging in electrostatic interactions, termed π effects. The strength of these interactions varies widely.
Table 2: Properties of hydrogen bonds (A—H = hydrogen bond acid, B = hydrogen bond base).

<table>
<thead>
<tr>
<th>Bond energy (kJ/mol)</th>
<th>Strong</th>
<th>Moderate</th>
<th>Weak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bond lengths (Å)</td>
<td>60-120</td>
<td>16-60</td>
<td>&lt;12</td>
</tr>
<tr>
<td>H···B</td>
<td>1.2-1.5</td>
<td>1.5-2.2</td>
<td>2.2-3.2</td>
</tr>
<tr>
<td>A···B</td>
<td>2.2-2.5</td>
<td>2.5-3.2</td>
<td>3.2-4.0</td>
</tr>
<tr>
<td>Bond angles (°)</td>
<td>175-180</td>
<td>130-180</td>
<td>90-150</td>
</tr>
<tr>
<td>Examples</td>
<td>Proton sponge</td>
<td>Alcohols</td>
<td>Bifurcated bonds</td>
</tr>
<tr>
<td></td>
<td>HF complexes</td>
<td>Biological molecules</td>
<td>C—H hydrogen bonds</td>
</tr>
</tbody>
</table>

Cation-π interactions  Cation-π interactions result from the interaction of a cation with the face of a simple π system. The power of this interaction to influence molecular recognition (both synthetic and natural) has only recently been appreciated. Electrostatics are considered to play a large role in cation-π interactions. Aromatic systems such as benzene possess a quadrupole moment, and ion-quadrupole interactions are possible just as ion-dipole interactions are. However, it should be noted that an ion-quadrupole interaction is not sufficient to quantitatively describe the cation-π interaction. Like other strongly electrostatic interactions, cation-π interactions are dependent on the polarity of the medium and are thus strongest in the gas phase and stronger in organic solvents than in water. However, the weakening effect of an aqueous medium is not as pronounced as with other electrostatic interactions. Cation-π interactions are important in molecular recognition of both synthetic and natural systems. An example of a molecular recognition event in nature that is dependent on cation-π interactions is the binding of acetylcholine to the nicotinic acetylcholine receptor. Dougherty and colleagues have demonstrated cation-π interactions in a number of synthetic systems in aqueous media.

Anion-π interactions  The existence of favorable anion-π interactions seems counterintuitive, and indeed research into this type of interaction has only developed recently. Anion-π interactions involve electron-deficient aromatic systems, and much of the evidence for these interactions has arisen from research surrounding the development of anion receptors. In one example of this interaction, electron deficient aromatic compounds such as 1,2,4,5-tetracyanobenzene were shown to form charge transfer complexes with halides.
**Polar-π interactions**  Molecules possessing a π system with a quadrupole moment are capable of electrostatically favorable interactions with polar molecules that are analogous to π hydrogen bonds. For instance, benzene will bind water such that the hydrogen atoms are in proximity to the benzene face. In the gas phase, the binding energy of benzene with water is 1.9 kcal/mol, with ammonia is 1.4 kcal/mol, and with the NH₂ of aniline is 1.6 kcal/mol.³

**π-π interactions**  The term “π stacking” is often applied to interactions between aromatic systems, implying that direct stacking of aromatic systems is energetically favorable. While aromatic systems can have energetically favorable interactions, direct stacking (Figure 3, left) is unfavorable due to electrostatic repulsion between the electron clouds. Edge-to-face geometry (Figure 3, right) is favorable because it places the partial negative charge on the face of one aromatic ring in contact with the partial positive charge on the edge of another aromatic ring.³ This interaction is responsible for the lubricant properties of graphite.¹ Displaced geometry (Figure 3, middle) is also favorable because there is some alignment of regions of positive and negative electrostatic potential. Moreover, in water, the alignment of hydrophobic regions is favorable.³ This type of interaction occurs between nucleobase pairs in DNA and helps to stabilize the double helix structure of DNA.¹

![Figure 3: π-π stacking geometries.](image)

1.1.6 **Induced-dipole interactions**

Polar molecules and ions can interact with nonpolar molecules to induce a dipole, creating an induced-dipole interaction. These interactions are much weaker than those previously discussed, and are more dependent on the distance between the participating species, r. For an ion-induced
dipole, the energy of interaction is dependent on $r^{-4}$, and for a dipole-induced dipole, the distance dependence is $r^{-6}$. A nonpolar molecule that is momentarily polarized can also induce a dipole in a nonpolar molecule, creating an induced-dipole-induced-dipole interaction, also known as a van der Waals interaction. The distance dependence of this interaction is roughly $r^{-6}$. The strength of these interactions is also dependent on the polarizability of the nonpolar molecule.\(^3\) Although these interactions are weak in comparison to other noncovalent interactions, they are not insignificant, and they can be important in the formation of inclusion complexes.\(^1\)

### 1.2 The hydrophobic effect

The hydrophobic effect has the power to drive self-assembly just as the previously discussed intermolecular forces do, although it is not a force itself. In simplest terms, the hydrophobic effect is the tendency of oil and water to separate.\(^8,9\) It can be a powerful driving force for self-assembly in water and plays a crucial role in biological molecular recognition. Protein folding, phospholipid bilayer structure and small molecule binding by biological receptors are all dependent on the hydrophobic effect. There are two different manifestations of the hydrophobic effect: the low solubility of hydrocarbons in water and the tendency of hydrophobes to aggregate in water. Studies of the former focus on $\Delta G^\circ$ of transfer of small organic molecules from the gas phase into water. Studies of the latter typically focus on $\Delta G^\circ$ of association and/or binding. Generally, $\Delta G^\circ$ of transfer correlates with the surface area of the solute, such that typical calculations of $\Delta G^\circ$ translate into 1.2 kcal/mol of destabilization per methylene group. In order to minimize the surface area that must be solvated, hydrophobic solutes in water spontaneously aggregate and adopt conformations that minimize surface area. For example, $n$-butane in the gas or liquid phase exists in a 70:30 anti:gauche equilibrium, but in water, the equilibrium shifts to 55:45. Studies of aggregation generally involve amphiphilic molecules like surfactants. It should be noted that studies of these two manifestations often reach conflicting conclusions.\(^3\)

The origin of the hydrophobic effect can be divided into enthalpic and entropic components. The enthalpic component involves the stabilization of high-energy water molecules inside
a hydrophobic cavity by their release into the bulk water solvent. These waters are high-energy inside the cavity because they cannot interact strongly with the cavity walls, while in bulk water they are stabilized by strong interactions with other water molecules.\(^1\) The strong intermolecular interactions between water molecules cause water to have a high surface tension. This translates into a large energetic penalty for creating a cavity in the water, which is necessary to dissolve a hydrophobe, and the very weak interactions between the water and the hydrophobic solute do not compensate for this energetic penalty. Water-water and, to a lesser extent, hydrophobe-hydrophobe interactions are stronger than water-hydrophobe interactions, further driving segregation. While enthalpic factors are important in explaining the hydrophobic effect, processes driven by the hydrophobic effect are often minimally enthalpically favorable or even penalized, especially at ambient temperature.\(^3\) Therefore, processes that are driven by the hydrophobic effect often must be entropically favorable.

The entropic component of the hydrophobic effect involves the reduction in the number of “holes” in the bulk water that result from the solvation of individual hydrophobic molecules; the order in the water solvent required to solvate two hydrophobic molecules (to create two holes) is greater than the order required to solvate one hydrophobic aggregate (to create one hole).\(^1\) The solvation of hydrophobes by water requires changes in the structure of water. While liquid water is more structured than other solvents, it is only maximally hydrogen bonded (four hydrogen bonds per water molecule) in solid form. While ice is enthalpically favorable to liquid water because of the greater number of hydrogen bonds, it is entropically unfavorable because of its greater order. Liquid water that is in contact with a hydrophobic solute loses hydrogen bonds and must compensate by strengthening the remaining ones. Hence, the water in direct contact with a hydrophobic solute is more “ice-like” than the bulk water. The result is often enthalpy-entropy compensation: hydrophobe solvation causes a net enthalpic gain, due to strengthened water-water interactions, and a net entropic penalty, due to increased order in the solvating water. If one hydrophobic aggregate is solvated, rather than two hydrophobic molecules, then fewer water-water interactions will be affected, and the solvation process becomes roughly enthalpically neutral or even unfavorable.
This also means that fewer water molecules must order themselves around the hydrophobic solutes and become ice-like, making the process entropically favorable. At ambient temperatures, the hydrophobic effect is often entropically driven, but at higher temperatures, enthalpy effects often dominate entropy effects. In general, a negative change in heat capacity is a more reliable indicator of the hydrophobic effect than a positive change in entropy. The extent to which heat capacity is affected depends on hydrophobic surface area; if association results in decreased hydrophobic surface area, then heat capacity will decrease.\(^3\)

Although processes driven by the hydrophobic effect are abundant in nature, supramolecular chemists have a long way to go in harnessing this effect to drive molecular recognition and self-assembly. There are numerous molecular hosts for which binding is in some part driven by the hydrophobic effect, as will be discussed in the next section. However, there are very few synthetic self-assembling systems for which assembly is driven by the hydrophobic effect, and the work of the Gibb group has been pioneering in this field (vide infra).

1.3 Water-soluble molecular hosts

The past few decades have seen a rapid increase in the number of synthetic water-soluble molecular hosts. The major categories of water-soluble hosts that possess a hydrophobic cavity in which one or more guest molecules can bind are described here. Simple molecular hosts like crown ethers and hosts with an open shape such as molecular tweezers are not included in this discussion. Additionally, the discussion is limited to hosts that freely associate and dissociate with guests in solution, thus excluding hosts such as carcerands.

1.3.1 Cyclophanes

Technically the term “cyclopane” applies to any organic molecule with a bridged aromatic system,\(^1\) which includes many host molecules defined here in separate categories. This section encompasses cyclophanes that do not belong to other host categories. These macrocycles are comprised of multiple (hetero)arene moieties covalently linked together. Stetter and Roos were among the
first to recognize the potential of cyclophanes to form inclusion complexes with organic molecules and believed they had observed 1:1 complexation of benzidine cyclophanes with benzene and dioxane, although X-ray analysis decades later showed that the “guest” molecules were actually located between the cyclophane molecules in the crystal lattice. Koga et al. later synthesized a new series of cyclophanes by replacing the benzidine units with 4,4’-diaminodiphenylmethane units. Host 1 (Figure 4) was water-soluble at acidic pH and was proven by NMR and fluorescence spectroscopic evidence to bind nonpolar guests inside its hydrophobic cavity. An X-ray crystal structure of the 1:1 complex of 1 with durene provided the first reported example of a crystalline complex of a water-soluble cyclophane with a hydrophobic guest.

![Figure 4: Structures of cyclophane 1 and guest molecule durene.](image)

Numerous other cyclophanes have been made water-soluble by the attachment of various solubilizing functional groups, including pyridinium, ammonium, carboxylic, and phosphonic groups and saccharides. The pyrenophanes 2 (Figure 5) are substituted with various water solubilizing groups that impart moderate water solubility. The cationic hosts 2 possess multiple recognition sites, the hydrophobic cavity and the positively charged substituents, making them ideal hosts for guests with multiple functional groups, including nucleotides. Nucleotides bound such that the arene portion bound inside the cavity due to hydrophobic and π-π interactions, while the anionic phosphate groups bound with the host ammonium groups due to electrostatic interactions. Binding strength greatly increased with guest charge; for example, $K_a$(AMP) = 1.9 × 10³ M⁻¹, $K_a$(ADP) = 5.3 × 10³ M⁻¹, and $K_a$(ATP) = 1.0 × 10⁶ M⁻¹. These hosts provide an excellent example of the power of multivalent interactions to enhance binding strength; the organization resulting from the hydrophobic binding event provides a high effective molarity that allows elec-
trostatic binding to occur despite the strong competition of the water solvent.\textsuperscript{16}

![Figure 5: Structures of cyclophanes 2.](image)

### 1.3.2 Cyclodextrins

Cyclodextrins (CDs) are cyclic oligosaccharides comprised of D-glucopyranoside units linked by 1,4-glycosidic bonds. CDs are cheaply produced by the degradation of starch with certain enzymes, helping to make them the most studied class of molecular host and facilitating their wide use in a range of applications, including pharmaceuticals, cosmetics, food products, and chemical analysis.\textsuperscript{1} CDs are usually comprised of 6, 7, or 8 glucose units and named $\alpha$-CD, $\beta$-CD, or $\gamma$-CD, respectively (Figure 6, left). They are toroidal in shape with two distinct rims, termed “primary” and “secondary” based on the presence of primary and secondary hydroxyl groups (Figure 6, right). The hydroxyl groups impart hydrophilicity to the exterior of a CD molecule and make CDs water-soluble, while the interior cavity is relatively nonpolar. Interestingly, $\beta$-CD is far less soluble in water than $\alpha$-CD and $\gamma$-CD, with a solubility of only 18.5 g/L at 25°C, versus 145 g/L for $\alpha$-CD and 232 g/L for $\gamma$-CD.\textsuperscript{1} The most accepted explanations of the low solubility of $\beta$-CD are that the seven-fold symmetry of aggregated $\beta$-CD interrupts the hydrogen-bonded structure of water,\textsuperscript{17} and that the intramolecular hydrogen bonds at the secondary face limit interactions with the water solvent.\textsuperscript{18} Indeed, methylation of the $\beta$-CD secondary hydroxyl groups yields a far more water-soluble compound. The native CDs have been functionalized in a number of ways at the hydroxyl groups at both faces.\textsuperscript{19,20}
The hydrophobicity of the CD cavity allows these molecules to form inclusion complexes with a wide variety of hydrophobic guest molecules, generally in a 1:1 fashion, although more complex assemblies are also possible. Binding can be the product of a number of driving forces, including steric fit, release of high-energy water from the cavity, the hydrophobic effect, van der Waals interactions, dispersive forces, dipole-dipole interactions, electrostatic interactions, and hydrogen bonding.\textsuperscript{21} The importance of steric effects is tempered by the toroidal shape of the CD; a guest molecule can still bind with only a portion of the molecule actually inside the host cavity. The release of high-energy water involves enthalpy, while the hydrophobic effect involves entropy. The release of high-energy water from the cavity into the bulk water is enthalpically favorable, while inclusion of a hydrophobic guest molecule inside the cavity is typically entropically favorable. Connors has argued that because the CD cavity is actually semipolar, not nonpolar, complexation is not always a “classical” hydrophobic interaction for which $\Delta S^\circ$ is positive.\textsuperscript{21} Therefore, binding events that result in favorable hydrophobic interactions should not be assumed to be entropically favorable.

Figure 6: (Left) Structures of native cyclodextrins. (Right) Schematic illustration of cyclodextrin structural features.
1.3.3 Cucurbit[n]urils

Cucurbit[n]urils (CBs) are a class of macrocycle synthesized by the acid-catalyzed condensation of \( n \) units of glycoluril with formaldehyde, of the general structure shown in Figure 7.\textsuperscript{22,23} CB[6] was first reported in 1905,\textsuperscript{24} but was not fully characterized until almost eighty years later.\textsuperscript{25} In 2000, Kim and coworkers modified the original reaction conditions for CB synthesis to obtain CB\([n]\), where \( n = 5 \) to 9.\textsuperscript{26} CB[10] was later synthesized as an inclusion complex with CB[5],\textsuperscript{27} and subsequently CB[10] was isolated by displacing CB[5] with melamine diamine, which was removed via acylation and washing.\textsuperscript{28} CBs possess a barrel-shaped hydrophobic cavity 9 Å deep with polar carbonyl groups around the portals. From CB[5] to CB[8], the internal diameter of the cavity ranges from 4.4 to 8.8 Å and the portal diameter 2.4 to 6.9 Å.\textsuperscript{1} CB[6], CB[7], and CB[8] have approximately the same cavity size as \( \alpha \)-CD, \( \beta \)-CD, and \( \gamma \)-CD, respectively.\textsuperscript{29}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{cucurbitnuril.png}
\caption{Structure of cucurbit[n]uril.}
\end{figure}

CBs are able to bind neutral and charged organic guest molecules and metal ions, generally with binding affinities that equal or exceed those seen with cyclodextrins and crown ethers. Binding is driven by both the hydrophobic effect and by electrostatic interactions; the carbonyl groups surrounding each cavity portal comprise regions of partial negative charge that can participate in strong ion-dipole interactions with cationic guests. This is clearly evident upon viewing the electrostatic potential map of CB[7] (Figure 8). Note that in contrast, \( \alpha \)-CD has little to no partial charge near its binding site. For neutral guests and metal cations, association constants are moderately higher than those of cyclodextrins for the former and crown ethers for the latter, and CBs exhibit low selectivity between these guests.\textsuperscript{23} With cationic guest molecules, much stronger and more selective binding is exhibited. Favorable ion-dipole interactions between host and guest are responsible for high binding affinities, while the rigid structure of the host imparts binding.
selectivity. This is well illustrated by comparing the binding constants of alkyl amines and alkane diamines by CB[6].\(^{30}\) The binding strength of these guests is highly dependent on chain length, as the plot of \(\log K_a\) as a function of chain length makes evident (Figure 9).

![Image](image_url)

**Figure 8:** Electrostatic potential maps for (a) CB[7] and (b) \(\alpha\)-CD. Reprinted with permission from Lee, J. W.; Samal, S.; Selvapalam, N.; Kim, H.-J.; Kim, K. Acc. Chem. Res. **2003**, 36, 621-630. Copyright 2003 American Chemical Society.

![Image](image_url)

**Figure 9:** Binding constants (log \(K_a\)) versus chain length \(m\) for binding of \(\text{H(CH}_2\text{)}_m\text{NH}_3^+\) (○) and \(^+\text{H}_2\text{N(CH}_2\text{)}_m\text{NH}_3^+\) (△) by CB[6]. From\(^{23}\) reprinted with permission.

### 1.3.4 Metal-coordinated hosts

As noted above, the use of metal-ligand coordination has been an important strategy in creating self-assembling systems in water. The Fujita group has synthesized numerous metal-coordinating supramolecular hosts that have been used to effect a variety of phenomena. The tetrahedral \(M_6L_4\)
coordination cage 3 is one of the most widely studied of these hosts; it is assembled from four tridentate ligands, six metal ions, and six ancillary ligands that cap the metal corners (Scheme 1).\textsuperscript{31} Most work has involved the use of palladium or platinum ions as the metals and ethylenediamine derivatives as the ancillary ligands. Host 3 has been used in a number of ways, including to selectively bind peptides,\textsuperscript{32} to study the intermolecular interactions of radicals,\textsuperscript{33} to induce novel reactivity by acting as a yoctoliter-sized reaction flask,\textsuperscript{34} to act as an enclosed space to effect photo-dimerizations,\textsuperscript{35} and to prevent reaction by storing a reactive species.\textsuperscript{36}

Scheme 1: Self-assembly of coordination cage 3.

One example of how cage 3 can effect different reactivity from that seen in free solution is in the Diels-Alder reaction between triphenylene 4 and maleimides (Scheme 2).\textsuperscript{34} Pericyclic reactivity of 4 has never been reported in solution. However, when 4 and the dienophile $N$-cyclohexylmaleimide 5 were entrapped in host 3 and heated, the \textit{endo} adduct 6 was formed in quantitative yield. Other normally non-reactive arenes were found to undergo similar reactions. In some cases, the product was only stable while sequestered inside the host, showing the power of the host to inhibit reactivity. Furthermore, when similar reactions were carried out with a host containing chiral diamines as the ancillary ligands, enantioselectivity could be achieved in the reactions inside the host.\textsuperscript{37} Good enantiomeric excess was found to be dependent on the maleimide having sufficient steric bulk on the $N$-alkyl group.
Scheme 2: Diels-Alder reaction of triphenylene 4 with maleimide 5 inside coordination cage 3 (represented by gray circle) to obtain endo adduct 6.

Interestingly, X-ray crystallography and neutron diffraction studies revealed that in the absence of guest, water inside empty cage 3 assembles into an adamantoid (H₂O)₁₀ cluster quite similar to the smallest unit of naturally occurring I_c-type ice (Figure 10). This structure was even present at room temperature. The stability of this structure was found to result from H₂O · · · π interactions between the water lone electron pairs and the tris-pyridyl ligands. The authors propose that this is possible because coordination of the ligands to the metal ions renders the ligands electron deficient. These findings suggest that the molecular recognition by host 3 is entropically driven, since guest binding results in the “melting” and release of this highly structured water. These finding contribute to the understanding of how water behaves at the interface with hydrophobic solutes.

1.3.5 Cavitands

A cavitand is here defined as a synthetic macrocycle with an interior concave surface to which one or more guest molecules can bind. The earliest cavitands of this type were shallow and flexible and thus formed short-lived complexes. Over time, researchers synthesized more sophisticated cavitands with more recognition features, leading to complexes with greater kinetic and thermodynamic stability and greater binding selectivity. Cavitands are generally based on resorcin[4]arenes, synthesized through the acid-catalyzed condensation of resorcinol with a variety of aldehydes (Scheme 3). The choice of aldehyde allows the synthesis of rescorin[4]arenes with different “feet” (the R groups in Scheme 3).

![Scheme 3: General synthesis of resorcin[4]arenes.](image)

Schneider et al. synthesized a resorcin[4]arene capable of guest binding by deprotonating the methyl-footed resorcin[4]arene with NaOH, forming a tetraanionic cavitand stabilized by four intramolecular hydrogen bonds (Figure 11). Cavitand bound small tetraalkylammonium salts with association constants in the $10^4$ to $10^5$ M$^{-1}$ range in an exchange process that was fast on the NMR time scale. Such strong binding was attributed to electrostatic interaction between the negative charge on the host upper rim and the positive charge of the guest ammonium groups (tert-butyl alcohol bound weakly at ca. 10 M$^{-1}$).

Hosts with larger, more rigid cavities were later synthesized by “bridging” resorcin[4]arenes with bromochloromethane, which connected the hydroxyl groups at the upper rim with methylene spacers (Figure 12). The subsequent attachment of solubilizing groups either at the upper rim or at the pendant feet on the lower rim produced a range of cavitands capable of binding guest molecules. Several examples of bridged resorcin[4]arene cavitands with functionalization at the
upper rim are shown in Figure 12. The simple hosts 8 and 9 possess cavities of limited size and functionality, and were only capable of binding cesium cation. The isophthalate-functionalized host 10, octaanionic under basic conditions, was found to bind cationic guests such as $N,N,N,4$-tetramethylbenzenecarboxonium iodide with association constants in the $10^1$ to $10^3$ M$^{-1}$ range. The tetracationic pyridinium-functionalized host 11 was found to bind $p$-cresol and $p$-toluenesulfonate with $K_a$ of $1.1 \times 10^2$ and $5.2 \times 10^2$ M$^{-1}$, respectively, with fast binding kinetics on the NMR time scale.

An example of a cavitand with solubilizing groups at the feet is the ethylene bridged cavity 12 (Figure 13). Host 12 is functionalized with four amidinium groups at the upper rim.
and four polyethylene glycol chains at the feet, which impart water solubility. Guests 5-methoxy- and 5-nitroisophthalate bound to form 1:2 host:guest complexes in D₂O and 1:1 host:guest complexes in borate buffer. The evidence suggested that the 1:2 complexes were stabilized by favorable electrostatic interactions between both guests and the amidinium groups. In borate buffer, the borate ions associated strongly with the amidinium groups, preventing binding of a second guest molecule. In TRIS/HCl-buffered D₂O, 1:1 binding with several nucleotides was observed, again due to favorable electrostatic interactions between negatively charged guest functionalities and the positively charged cavitand upper rim. The adenine derivatives cAMP, AMP, ADP, and ATP were the strongest binding nucleotides, with binding strength correlating strongly to guest charge. Binding of all guest molecules with 12 was shown to be enthalpically driven, the result of favorable polar and nonpolar host-guest interactions.

![Figure 13: Structure of cavitand 12.](image)

Cavitands with even deeper and larger cavities, termed “deep-cavity cavitands” were synthesized by the Rebek group by bridging resorcin[4]arenes with electron-poor aromatic rings. The water-soluble cavitands 13 and 14 (Figure 14), two examples of such hosts, possess eight amide groups at the upper rim and four ammonium groups at the feet that impart water solubility. In water, the free hosts adopt a kite conformation with $D_{2d}$ symmetry, likely to minimize contact of lipophilic surfaces with water. Upon inclusion of a suitable guest molecule, these hosts rearrange into a $C_{4v}$ vase conformation (Figure 14). The large energy barrier between the free and bound host conformations enhances the kinetic stability of the host-guest complexes. Consequently, guest exchange is slow on the NMR time scale, and therefore large ($\geq 3$ ppm) host and
guest $^1$H NMR peak shifts are observed upon complexation. Various suitable guests were found, most consisting of a hydrophobic center with one polar functional group, and modest binding affinities (up to $1.4 \times 10^2 \text{ M}^{-1}$) were observed.

![Diagram of deep-cavity cavitands](image)

**Figure 14:** (Left) Structures of deep-cavity cavitands 13 and 14. (Right) Cavitands 13 and 14 in the vase conformation.

The water-soluble deep-cavity cavitand 15 (Figure 15) was synthesized via modification of a resorcin[4]arene with carboxylate-substituted benzimidazole. Even in the absence of a guest, this cavitand adopts a $C_{4v}$ symmetric vase conformation in water as a result of hydrogen bonding between the benzimidazole nitrogens and four water molecules and the presence of a THF molecule inside the cavity (a remnant from the synthesis). Choline was found to bind inside 15, but larger quaternary ammonium salts such as tetrapropyl ammonium salt were not. However, the long guests dodecyl phosphatidylcholine (DPC) and sodium dodecylsulfate (SDS) unexpectedly bound with 15 at sub-micellar concentrations with the long alkyl chain coiled into a helix inside the cavity and the polar head group exposed to the solvent (Figure 15). Binding of these guests was energetically favorable due to (1) burial of the large hydrophobic alkyl chain surfaces inside the cavity, (2) C–H–π interactions between guest and host that are only possible in the coiled conformation, and (3) an ideal guest volume for the cavity size. Combining cavitand 15 with SDS above its critical micellar...
concentration caused a reversal in the host and guest roles and inclusion of 15 inside the SDS micelle. Even inside the micelle, 15 retained its molecular recognition properties, albeit with a reduction in association constants of approximately one order of magnitude.

Figure 15: (Left) Structure of deep-cavity cavitand 15. (Right) Energy-minimized structures of 15-SDS complex with one host wall removed for clarity: (A) Space-filling model of partially coiled SDS. (B) SDS with five guauche conformations inside the cavity. (C) SDS in extended conformation. From Trembleau, L.; Rebek, J., Jr. Science 2003, 301, 1219-1220. Reprinted with permission from AAAS.

One interesting property of some cavitands is the ability to form molecular capsules via self-assembly and encapsulation of one or more guest molecules. In organic solvents, capsule formation (and other types of self-assembly) is typically driven by strong, highly directional noncovalent interactions between host molecules, usually hydrogen bonding or metal-ligand coordination. (For a comprehensive review of molecular encapsulation in organic solvent, the reader is directed to reference 52.) However, in water, hydrogen bonds are ineffective in self-assembly because they are usually “neutralized” by the water solvent, as previously discussed. Metal-ligand coordination is a strong enough force to be useful in water, and the previous section described molecular cage complexes that self assemble in water due to this interaction. The hydrophobic effect is the other primary tool in designing water-soluble self-assembling molecular capsules.

A rare example of self-assembly in water driven by electrostatic attraction was reported by the Reinhoudt group. This group synthesized a series of water-soluble calix[4]arenes functionalized at the upper rim with either amidinium, sulfonate, or carboxylate groups (Figure 16, left). The
combination of 16 and 17 in water resulted in the precipitation of a dimeric heterocapsule, despite the fact that both monomers were water-soluble. The assembly process was driven by favorable electrostatic interactions between the positive and negative upper rims of each host. In methanol, the 1:1 complex remained in solution, encapsulating one of the host propyl feet as the guest.\textsuperscript{53} The monomers were altered to enhance water solubility of the capsule: the ethylene glycol feet of 16 were lengthened to produce host 18, and the sulfonate groups of 17 were replaced with carboxylate groups to produce host 19 (Figure 16, left). The effort was successful, and the heterodimeric assembly 18·19 was found to be soluble in water at pH 9 (Figure 16, right).\textsuperscript{54} This capsule also encapsulated one host propyl foot, in addition to the methyl groups of the alanine functionalities on 19. Complex 18·19 was also found to bind several cationic guests with fast exchange on the NMR time scale.\textsuperscript{55}

![Figure 16: (Left) Structures of calix[4]arenes 16-19. (Right) Depiction of water-soluble heterodimeric assembly 18·19.](image)

The Gibb group has synthesized resorcin[4]arene-based water-soluble deep-cavity cavitands that, driven by the hydrophobic effect, self-assemble in aqueous media into dimeric molecular capsules in the presence of one or more hydrophobic guest molecules (Figure 17).\textsuperscript{56} These cavitands also bind amphiphilic guests to form 1:1 complexes in which the hydrophilic portion
of the guest is positioned at the mouth of the cavity, where it is exposed to the aqueous solvent (Figure 17).57 (For reviews of research on Gibb group cavitands, see references 58 and 59.)

![Figure 17: Schematic representation of binding of octaacid (blue bowl) with a guest molecule (orange sphere) to form a 1:1 complex or 2:1 capsule.](image)

The first of these hosts to be synthesized was the octaacid (OA) 20 (Figure 18).56 This molecule has a bowl shape with three rows of aromatic rings that are linked together. The third row deepens and rigidifies the hydrophobic cavity and also forms a wide hydrophobic rim at the mouth of the cavity, which is approximately 8 Å deep and 8 Å wide.60 Eight carboxylic acid groups, four on the outer edge of the cavity rim and four at the pendant feet, impart solubility in basic aqueous solutions. Studies by the Ramamurthy group of OA complexes with nine fluorescent guests showed that the fluorescence emission spectra of capsule-forming guests were characteristic of dry aromatic solvents, and hence that the inside of the OA capsule is dry.61 Molecular dynamics computer simulations were performed to study water inside the cavity of free OA60 and revealed that the cavity contains 0 to 7 water molecules, with 4.4 on average. These water molecules possess fewer hydrogen bonds than bulk water molecules, allowing faster orientational motion, while translational motion in restricted by the walls of the cavity. Despite the fact that these waters are higher energy than the bulk water and that solvation of the cavity is entropically unfavorable, the solvation process is thermodynamically favorable, with \( \Delta G \sim -5 \text{ kcal/mol} \). This is because interactions between OA and water inside the cavity and between water inside the cavity and bulk water are enthalpically favorable. It was also found that as the small hydrophobic guest ethane approaches the cavity, water inside the cavity is expelled, indicating a “triggered dissociative” exchange mechanism.
OA has been shown to encapsulate rigid guests such as steroids, small guests such as hydrocarbon gases, flexible guests such as \( n \)-alkanes, and moderately polar guests such as polyethylene glycol derivatives. The thermodynamic stability of many of these capsules is remarkably high; for instance, estradiol was found to bind with a minimum apparent association constant of \( 1 \times 10^8 \text{ M}^{-1} \). OA has also been shown to form 1:1 complexes with several amphiphilic guests that exhibit high association constants with slow exchange on the NMR time scale. Several cyclic and acyclic aliphatic carboxylic acids were found to bind with OA with association constants ranging from \( 10^3 \) to \( 10^6 \text{ M}^{-1} \). The key criteria for a guest to bind strongly and with a slow exchange rate are shape and size that are complementary to the host cavity and hydrophobicity. For example, the long hydrophobic guest cholesterol fails to form a well-defined complex, the small hydrophobic guest propane forms a fast-exchanging 2:2 capsule, and the moderately hydrophilic guest \( \text{CH}_3(\text{CH}_2\text{CH}_2\text{O})_3\text{CH}_2\text{CH}_2\text{OH} \) forms a kinetically unstable 2:1 capsule with a low (relative to other OA capsules) association constant of 2500 M\(^{-1}\).

Research involving OA carried out by the Gibb group and numerous other groups has shown the ability of this molecule to effect a variety of interesting new chemical phenomena. OA in aqueous solution was found to extract hydrocarbon gas molecules from the gas phase to form 2:2 capsules and to effect the separation of butane and propane gases via preferential binding of butane. Numerous studies have involved the use of the OA capsule as a yoctoliter-sized reaction vessel. The ability of capsular OA to allow unique photochemical conversions and to prevent photochemical reaction has been particularly well-documented. The capsule-forming ability of
OA was also used to achieve the kinetic resolution of constitutionally isomeric long-chain esters. OA in hydrolytic solution was combined with pairs of esters that normally hydrolyze at the same rate, causing the stronger-binding ester to hydrolyze at a much slower rate than the weaker-binding ester.\(^6\) OA has also been shown to bind cationic molecules externally, presumably due to electrostatic interactions with the carboxylates at the feet.\(^7\) External binding of a cationic ferrocene was used by the Kaifer group to mediate the electrochemistry of encapsulated ferrocene.\(^8\)

Recently, binding studies of OA with the sodium salts of anions spanning the Hofmeister series revealed the ability of chaotropic anions to bind inside the hydrophobic pocket.\(^9\) Hofmeister first observed over a century ago that certain salts decrease the solubility of proteins, while others increase their solubility.\(^7, 10\) Anions and cations can be ranked according to their propensity to increase or decrease protein solubility to create the Hofmeister series, with kosmotropes decreasing protein solubility and chaotropes increasing protein solubility (Figure 19). This effect is more pronounced with anions than with cations. The Hofmeister effect is not yet fully understood, and there are conflicting explanations for its origin. Towards explaining this effect, direct anion-protein interactions have been studied, especially interactions with specific peptide groups that result in protein fold destabilization.\(^11\) Several studies have suggested that chaotropic anions are capable of weak interactions with hydrophobic groups.\(^12–14\) Gibb et al. discovered that the chaotropic anions perchlorate, isothiocyanate, chlorate, iodide, and nitrate bind inside the OA hydrophobic pocket, providing the first direct evidence of such an interaction. Anion binding competed with the binding of a hydrophobic guest and altered the thermodynamics of that binding event. This work suggested that chaotropic anion binding with a hydrophobic surface is responsible for the ability of these anions to disrupt protein structure and induce a molten globule state.

\[
\text{F}^- \sim \text{SO}_4^{2-} > \text{OAc}^- > \text{Cl}^- > \text{Br}^- > \text{NO}_3^- > \text{ClO}_3^- > \text{I}^- > \text{ClO}_4^- > \text{SCN}^- \\
\]

### Kosmotropes
- Salting out of proteins
- Decreased protein solubility
- Increased protein stability

### Chaotropes
- Salting in of proteins
- Increased protein solubility
- Decreased protein stability

**Figure 19:** Hofmeister series for anions.
OA was the first of these cavitands to be synthesized and is the most heavily studied, but other cavitands of this type have also been made. The dendronized water-soluble cavitand 21 (Figure 20), also known as G3, was derived from the octol precursor to OA, 22 (Figure 20). G3 is coated with eight three-generation hydroxyl-terminated aliphatic polyester dendrons, imparting a coat of 128 hydroxy groups, making it electronically neutral and soluble at physiological pH. This host exhibited similar binding behavior to OA but somewhat lower binding affinities, which was attributed to (1) the ability of the dendrons to bind and thus compete with another guest and (2) the less-solvated nature of the hydrophobic rim, decreasing the desolvation of the rim that occurs upon capsule formation. More recently, tetra endo-methyl octaacid 23 (Figure 20), also known as TEMOA, was synthesized by replacing the four endo hydrogen atoms at the rim of the OA cavity with methyl groups. The endo methyl groups constrict the cavity portal and reduce the host tendency to dimerize, resulting in interesting new binding behaviors. For instance, binding studies with \( n \)-alkanes showed an unusual non-monotonic assembly profile, whereby binding with methane through \( n \)-butane and \( n \)-hexane through \( n \)-octane did not result in host dimerization, while binding with \( n \)-pentane and \( n \)-nonane through \( n \)-tetradecane did result in host dimerization. Additionally, mixtures of OA and TEMOA in the presence of \( n \)-alkanes were found to exhibit self-sorting behavior, where non-statistical mixtures of homo- and heterocapsules were observed. Self-sorting was attributed to the different propensity of OA and TEMOA to form capsules, depending on the size of the guest. Additional cavitands have been synthesized by other group members, some of which were used in the study of host-membrane interactions (vide infra).

**Figure 20:** Structures of G3 21, octol 22, and TEMOA 23.
1.4 Interactions between phospholipid membranes and membrane-active compounds

1.4.1 Biological membranes

Phospholipid bilayers constitute the main structure of biological membranes. Phospholipids are amphiphilic molecules comprised of a polar headgroup containing a phosphate group and long, lipophilic tails made up of esterified fatty acids (Figure 21). The fatty acids (R\textsuperscript{2} and R\textsuperscript{3} in Figure 21) can vary in length and degree of saturation. The phosphate group is esterified with an additional group (R\textsuperscript{1} in Figure 21), which can vary in charge and thus determine the overall charge of the phospholipid. Some common headgroup substituents and acyl chains are shown in Figure 21. The amphiphilic nature of phospholipids allows them to assemble into supramolecular structures; in the case of bilayer membranes, the molecules align themselves into two leaflets, with the polar headgroups exposed to the aqueous medium to form the membrane inner and outer surfaces and the fatty acid chains forming the interior of the membrane. This hydrophobic interior, known as the insulator regime, is 30-35 Å thick and has a polarity similar to that of hexane. The mid-polar regime and headgroup are about 20 Å thick, giving the typical bilayer a total thickness of 50-55 Å.

![Figure 21: Generic phospholipid structure with common headgroup and fatty acid substituents.](image)

While phospholipids provide the basic structure of biological membranes, proteins and sterols are also major membrane components. Cholesterol is a major component of eukaryotic cells; among other functions, it serves to enhance the mechanical strength of membranes and helps to modulate membrane phase behavior.\textsuperscript{82} Ergosterol is the equivalent in fungal membranes and stigmasterol and β-sitosterol are the equivalents in plant membranes (Figure 22).
Under normal conditions, a membrane serves as a barrier and container. The bilayer is permeable to small molecules, including water and O₂, by passive diffusion. It is impermeable to ions and large polar molecules (e.g., glucose), so transport of these species occurs through the action of proteins that either enable transport by diffusion down a concentration gradient (passive transport) or against a concentration gradient (active transport). Compounds that enable transmembrane ion transport are known as ionophores and can either act as mobile carriers of ions or can form ion channels.

The fluid mosaic model is the currently accepted model describing membrane structure, although it continues to evolve as understanding of biological membranes improves. According to this model, the phospholipid bilayer is a fluid structure through which proteins can diffuse. Phospholipid molecules can move laterally within a leaflet. Phospholipid flip-flop is also possible, whereby a phospholipid translocates to the opposite leaflet, but this occurs on a much longer time scale. Membrane fusion, in which two different bilayers fuse to form a single new structure, occurs in biological cells under certain circumstances, or it can occur artificially. The fusion can occur with only one leaflet or with both, in which case the aqueous contents intermix.

1.4.2 Synthetic models of biological membranes

Artificial membranes are important in membrane research, as they are simplified versions of biological membranes that are easier to study. These studies generally fit two broad classes: those that use planar lipid membranes and those that use vesicles. Planar lipid membranes, also known as black lipid membranes (BLMs), are useful in studies of ion transport. Experiments using BLMs are designed as follows: two chambers are separated by an aperture that contains a single phos-
pholipid bilayer, and one chamber contains the ionophore under study. A potential is established across the bilayer, and the flow of current across the bilayer is measured. The formation of ion channels is shown by step-changes in current across the bilayer. This technique is sensitive enough to show the formation of a single ion channel.

Vesicles, also known as liposomes, are spherical structures with phospholipid bilayer walls. Multilamellar vesicles (MLVs) have multiple bilayer walls, giving them an onion-like structure. Unilamellar vesicles have walls comprised of a single bilayer and are classified by size: small unilamellar vesicles (SUVs) are less than 100 nm in diameter, large unilamellar vesicles (LUVs) are 100-1000 nm in diameter, and giant unilamellar vesicles (GUVs) are greater than 1 \( \mu \)m in diameter. Unlike BLM experiments, vesicle-based experiments examine change to membrane function as a bulk phenomenon. The phospholipid composition of vesicles can be tailored to suit the experiment; for instance, the choice of acyl chains will affect membrane fluidity and thickness, while the choice of headgroup will determine membrane charge. Often, cholesterol is included in the composition to more closely mimic animal cells. The contents of the vesicles and the extravesicular solution can also be tailored to the researcher’s needs. The liposomes are simply prepared using the desired intravesicular buffer solution, and the extravesicular solution is exchanged with the desired buffer by such procedures as gel filtration or dialysis.

A variety of different membrane alterations can be observed using vesicles, such as permeability changes, ion transport, fluidity changes, phospholipid flip-flop, and membrane fusion. There are numerous techniques to observe these phenomena, including fluorescence-based methods, differential scanning calorimetry, isothermal titration calorimetry, nearest neighbor recognition, and NMR. Following are examples of some important techniques for studying several membrane phenomena using fluorescence spectroscopy. This is by no means an exhaustive review of the different techniques available.

**Membrane permeabilization** Since vesicle membranes are normally impermeable to charged molecules, vesicles can be prepared containing a quenched fluorophore, where leakage relieves
the quenching. Because of the high sensitivity of fluorescence spectroscopy, leakage of even tiny amounts of fluorescent marker can be observed.  

**Ion transport**  Fluorescence-based methods with vesicles are often used to study ion transport. Generally, the vesicles are loaded with a pH- or ion-sensitive dye, and transport is signified by a change in fluorescence emission.

**Membrane fusion**  Membrane fusion results in the mixing of lipids in the membranes involved and sometimes results in mixing of vesicle contents. Lipid mixing can be observed through the use of fluorescent-labeled lipids, where one sample of vesicles is prepared with labeled lipids and the other without. When the samples are combined, fusion is evinced by alteration of the lipid fluorescent properties. Fluorescent labels include fluorescence resonance energy transfer (FRET) donor-acceptor pairs, self-quenching labels, and excimer forming labels. Membrane fusion that results in mixing of vesicle contents can be monitored by the use of two sets of vesicles loaded with different contents that, when mixed, either exhibit fluorescence quenching or fluorescence enhancement.

### 1.4.3 Types of membrane-active compounds

An enormous range of compounds can alter membranes in a variety of ways. Structurally, these compounds are very diverse, but all are amphiphilic. Here, several broad types of compounds will be discussed, some of which are more heavily based on structure, some of which are more heavily based on function. This discussion is not intended to be an exhaustive review of membrane-active compounds, but rather to introduce the reader to the diverse ways in which amphiphilic compounds can interact with membranes and alter their function.

**Membrane-active cyclodextrins**  Cyclodextrins are molecular hosts that are useful in drug delivery, as noted in Section 1.3.2. CDs can improve drug performance in several ways, including increasing solubility, improving bioavailability, and enhancing stability. The effectiveness of
many hydrophobic drugs is mitigated by low solubility and poor bioavailability. However, this same hydrophobicity enables them to form inclusion complexes with hydrophilic cyclodextrins, often improving drug solubility and bioavailability. Water at the surface of a phospholipid bilayer, the unstirred water layer (UWL), is more highly structured than bulk water, and is difficult for a hydrophobic drug to penetrate. A CD-drug complex can penetrate the UWL far more easily and deliver the drug to the membrane surface, where it can cross by passive diffusion. Since the formation of the CD-drug complexes is dynamic, the drug is available for its intended action once the complex dissociates. For drugs that are unstable and prone to reaction before they are able to reach their target, the CD can bind with the drug and protect it from the reactive medium. Further information on the use of CDs in drug delivery is available in numerous review articles; these include general discussions on the topic and discussions of the history of CDs in drug delivery, the mechanism of drug release from CD-drug complexes, the improvement of oral drug delivery, and the toxicity of CDs used for drug delivery.

CDs that are most suitable for drug delivery have minimal interaction with biological membranes and are not technically membrane-active compounds. However, CDs are capable of binding with phospholipids, cholesterol, and other membrane components and therefore have the potential to damage biological membranes when used in drug delivery. For this reason, there exists much research focused on disruption of phospholipid membranes by CDs. An illustrative example was carried out by Nishijo and coworkers, who compared the ability of various CDs to cause leakage of LUVs comprised of different phospholipids. The more hydrophilic CDs except α-CD did not cause significant leakage, while α-CD and the more hydrophobic CDs (methylated β-CDs) caused moderate to extensive leakage, depending on the phospholipid used. Further studies using differential scanning calorimetry indicated that the methylated β-CDs actually penetrated the membrane bilayer while the more hydrophilic α-CD adsorbed onto the membrane surface, where it was able to extract phospholipid molecules. Piel et al. compared the ability of various CDs to induce leakage of soy PC LUVs made with and without cholesterol. Normally, cholesterol enhances membrane stability and gives some resistance to detergents. However, in this study,
the LUVs containing cholesterol were more susceptible to leakage by hydrophobic CDs than the LUVs without cholesterol. The authors attributed these findings to the greater binding affinity of cholesterol and, to a lesser extent, soy PC to the permeabilizing CDs than to the benign CDs.

In some applications, CD-membrane interaction is actually desired. As molecular hosts, CDs can be used to manipulate membrane contents by extracting membrane components, depositing guest molecules into membranes, and even shuttling compounds between membranes. This behavior makes CDs useful tools in membrane studies and can have pharmaceutical and therapeutic applications. For instance, Ikeda and colleagues found that γ-CD could be used to enrich liposomal and cell membranes with C70, which can serve as a substrate in photodynamic therapy. The success of this process relied on the instability of the γ-CD-C70 complex; similar experiments with C60 were less successful because stronger binding reduced the degree to which C60 was deposited into the membrane. In an example of CD-mediated extraction of membrane components, Zhong et al. found that β-CDs could cause the selective desorption of palmitic acid over cholesterol from Langmuir monolayers, and that selectivity could be tuned based on the functionalization of the CD. Brunaldi et al. used methyl-β-CD to extract fatty acids from and deposit fatty acids into liposomal and cellular membranes, making methyl-β-CD a possible tool in membrane studies that is superior to albumin, which is usually used. Furthermore, CDs have been used to manipulate the cholesterol content in cells and to mediate cholesterol transport between liposomes. In all applications involving CD-membrane interactions, proper choice in CD and experimental conditions is essential in controlling the desired interactions or lack thereof.

**Ion channel-forming amphiphiles** Biological membranes are normally impermeable to ions; in nature, ion transport occurs in a selective manner via proteins that form ion channels and ion pumps. Ion channels allow passive transport down a concentration gradient, while ion pumps cause active transport against a concentration gradient. Certain synthetic amphiphilic compounds are also able to form ion channels that span the width of a membrane, allowing passive diffusion of ions across the normally impermeable phospholipid membrane, ideally in a selective manner.
For a comprehensive discussion of these compounds, the reader is directed to several recent reviews. While it is often difficult to determine the actual structure of a synthetic ion channel, channel-forming molecules can be strategically designed according to several design motifs, as illustrated in Figure 23. In the simplest motif, a single molecule can form a unimolecular ion channel. Supramolecular ion channels can be formed from linear monomers that assemble into barrel-stave pores, hoop-shaped monomers that stack to form barrel-hoop pores, or smaller monomers that assemble to form barrel-rosette pores. Micellar pores are a more complex type of pore that tends to be transient and poorly organized. Antimicrobial peptides are thought to sometimes cause the formation of micellar pores, as are detergents at low concentrations.

**Figure 23:** Schematic illustration of different design strategies for synthetic ion channels and pores. Figure reprinted from Matile, S.; Som, A.; Sordé, N. *Tetrahedron* **2004**, *60*, 6405-6435, with permission from Elsevier.

Compounds that form ion channels are structurally quite diverse, but all are amphiphilic and in their active form are positioned such that the hydrophobic portions are exposed to the membrane interior and the hydrophilic portions are exposed to the aqueous medium. Ion channel-forming compounds can be peptide-based, crown ether-based, octiphenyl-based, bile acid-based, calixarenes, cyclodextrins, and other structural types.

**Mobile carrier ionophores** Transmembrane ion transport can also occur via mobile carriers that bind the ion(s) in solution, cross the membrane, and release the ion(s) into the solution on the other side. Therefore, these carriers must possess a binding site and sufficient hydrophobicity to cross a phospholipid membrane. It should be noted that the distinction between a carrier ionophore and a channel-forming ionophore is not always clear, and that these compounds can behave differently.
at different concentrations and under different conditions. Ionophores from nature include valinomycin, which transports potassium cation, and a group of compounds called prodigiosins, which transport chloride via H⁺/Cl⁻ symport (see Figure 24 for the structure of the parent prodigiosin). The prodigiosins inspired the synthesis of dipyrrrole and tripyrrrole\textsuperscript{114} and pyrrolecarboxamide\textsuperscript{115} ionophores that transport HCl. Davis et al. found that prodigiosins also transport bicarbonate via HCO₃⁻/Cl⁻ antiport and synthesized a series of isophthalamides that do the same (24 in Figure 24).\textsuperscript{116} Another group of synthetic anionophores, the cholapods, are cholic acid derivatives functionalized with urea. The cholic acid moiety provides the lipophilicity necessary to traverse the membrane, while the urea moieties are hydrogen bond donors that serve as a binding site. Cholapods transport chloride via Cl⁻/NO₃⁻ exchange and do not transport cations. Cholapod 25 (Figure 24) exchanges Cl⁻ for NO₃ at a ratio of 1:25 000 steroid to lipid.\textsuperscript{117}

![Figure 24: Structures of mobile carrier ionophores prodigiosin, isophthalamide carriers 24, and cholapod 25.](image)

**Molecular umbrellas** Regen and colleagues have developed a class of “amphomorphic” compounds known as molecular umbrellas, which present a hydrophobic exterior in hydrophobic environments and a hydrophilic exterior in hydrophilic environments (Figure 25a).\textsuperscript{118} The molecular umbrella structure consists of two or more facially amphiphilic “walls” connected to a central scaffold, which possesses a “handle” to which can be attached a hydrophilic agent that the umbrella can transport across a phospholipid bilayer. Bile acids are used for the walls, while polyamines such as spermine and spermidine are used for the scaffold. Molecular umbrellas have been shown to transport hydrophilic peptides, nucleotides, and oligonucleotides across liposomal membranes by passive diffusion, and are active below a 1:100 umbrella:lipid ratio. The hypothetical mechanism
for this behavior is illustrated in Figure 25b. The molecular umbrella approaches the membrane in the exposed conformation (phase A), then adsorbs onto the membrane surface by aligning the hydrophilic faces of the walls with the polar headgroups and the hydrophobic faces of the walls with the hydrophobic lipid interior (phase B). The umbrella enters the membrane interior in the shielded conformation (phase C) and translocates to the opposite leaflet (phase D), then leaves the membrane in the same way that it entered (phases E and F), carrying the hydrophilic agent to the solution on the other side of the membrane.

Figure 25: (a) Stylized representation of a di-walled molecular umbrella bearing a hydrophilic agent (gray oval) in exposed and shielded conformations. (b) Proposed mechanism of transmembrane transport by a di-walled molecular umbrella. Reprinted with permission from Janout, V.; Regen, S. L. Bioconjugate Chem. 2009, 20, 183-192. Copyright 2009 American Chemical Society.

In an early study, the molecular umbrellas 26 and 27 (Figure 26a) were used to transport glutathione (GSH) (Figure 26b) across liposomal membranes. The spermidine scaffold is coupled to two cholic acid walls via amide bonds, while the 5-thiol(2-nitrobenzoyl) handle allows both easy release of the glutathione cargo through thiolate-disulfide interchange and monitoring of this release by UV absorption. In umbrella 27, the cholic acid hydroxyls were sulfated, thus greatly increasing the overall hydrophilicity of the molecule in exposed conformation and minimizing the possibility of transport due to simple diffusion of the umbrella in any conformation. Molecular umbrellas show great promise as drug delivery agents.
Antimicrobial peptides Antimicrobial peptides (AMPs) are cationic, amphipathic polypeptides that act as broad-spectrum microbicidies that exhibit little or no cytotoxic activity against host cells. Naturally occurring AMPs are widespread in the plant and animal kingdoms and have likely played a fundamental role in the survival of many species. The microbial targets of AMPs have been remarkably unable to develop resistance to these compounds. While AMPs are very diverse in structure, they are all amphipathic in the active form as a result of clustering of cationic and hydrophobic residues. The activity of AMPs is dependent on fundamental differences between plant and animal cell membranes and the target microbial cell membranes. Plant and animal membranes typically have an outer leaflet comprised of zwitterionic lipids, while lipids with negatively charged headgroups are primarily located in the inner leaflet (Figure 27). Conversely, bacterial membranes (one major class of microbe targeted by AMPs) contain negatively charged lipids in both bilayer leaflets. This difference results in much stronger binding of AMPs to bacterial membranes than to host membranes due to electrostatic interactions between the cationic portions of the AMP and the negatively charged surface of the membrane. It is unclear precisely how this binding results in microbial death, but it is often associated with membrane permeabilization.

The structure-function relationship of AMPs remains poorly understood. Very few such relationships have been elucidated, and the existence of specific amino acid sequences or pep-

tide structures has not been correlated with AMP activity.121 Wimley et al. argue that activity is instead dependent on “interfacial activity,” defined as “the ability of a molecule to bind to a membrane, partition into the membrane-water interface, and to alter the packing and organization of the lipids”,122 which is dependent on amino acid composition and physical chemical properties. In this context, the necessity of AMP amphiphilicity is obvious; it allows the peptide to partition into the membrane in a disruptive fashion, thus deforming the bilayer and interrupting the hydrophobic interior.
2 Synthesis of water-soluble deep-cavity cavitands

2.1 Introduction

The synthesis of OA 20 (Figure 18) marked an important turning point in our group’s research, allowing the study of molecular recognition in water. The unique properties of this molecule have led to the study of fascinating chemical phenomena, but expanding the arsenal of water-soluble deep-cavity cavitands would truly unleash the potential of this avenue of research. Research on TEMOA 23 (Figure 20), which has exhibited entirely new self-assembly behavior, illustrates this point. One obvious and versatile approach to synthesizing new water-soluble cavitands is alteration of the “coat” of water-solubilizing functional groups at the cavity rim and pendant feet. In the case of OA, these are the eight carboxylic acid groups, which impart moderate solubility at basic pH. By coating the cavitand with different functionalities, one could synthesize cavitands with different properties. Thus, cavitands with greater solubility across the pH range could be accessible, facilitating a wider range of studies and possibly increasing the biological relevance of these compounds. Access to cavitands of different electrical charges would allow the study of the effect of host charge on binding behavior and on interactions with phospholipid membranes. Moreover, multiple cavitands could make possible the formation of heterocapsules, whereby two different cavitands encapsulate one or more guest molecules.

A step in this direction was taken with the synthesis of the dendronized water-soluble cavitand G3 21 (Figure 20). While G3 is an important molecule, the synthesis is fairly long and not readily adaptable to the synthesis of different water-soluble cavitands. Ideally, any syntheses of new cavitands should be similarly efficient to that of OA and should be versatile, allowing the synthesis of multiple cavitands with minimal changes to the overall synthesis. With these criteria in mind, three new water-soluble cavitands were synthesized from the same compound, octol 22, the precursor to OA (Figure 20). These syntheses are comparable to that of OA in efficiency and also benefit from recent improvements to the synthesis of OA (vide infra). Moreover, they are readily adaptable to the synthesis of additional water-soluble cavitands in the future.
2.2 Improved synthesis of octaacid

The original synthesis of OA $^{20}$ is remarkably efficient considering the molecule’s complexity, comprising eight non-linear steps and four chromatographic purifications (Scheme 4). This synthesis was shortened to seven non-linear steps and one chromatographic purification, and a minor impurity present from the original synthesis was eliminated. $^{123}$ The original synthesis started with the acid-catalyzed condensation of resorcinol and 2,3-dihydrofuran to yield resorcinarene $^{28}$, which was “bridged” with 3,5-dibromobenzal bromide to afford octabromide cavitand $^{29}$ in crude form. The four hydroxy groups at the “feet” of $^{29}$ were benzylated with benzyl bromide, and the resulting benzylated octabromide $^{30}$ was purified by column chromatography. Cavitand $^{30}$ was then “weaved” with 3,5-dihydroxybenzyl alcohol via an eight-fold Ullmann ether coupling to form the deep-cavity cavitand $^{31}$, which was purified by column chromatography. Deprotection of $^{31}$ with hydrogen and palladium/carbon afforded octol $^{22}$, which was oxidized to yield OA $^{20}$.

Scheme 4: Original synthesis of octaacid $^{20}$.

As research involving OA progressed, the motivation to make the synthesis more efficient grew. Compounding this desire was the discovery that the final step of oxidation of octol $^{22}$ did
not go to completion and thus gave a small amount of heptaacid byproducts. A multi-person effort focused on shortening the overall synthesis, minimizing chromatographic purification, and removing heptaacid byproducts. The resulting improved synthesis of OA is shown in Scheme 5. Minor modifications to the synthesis of 28 improved yield. Synthesis of 29 remained unchanged, but the workup was modified such that it was obtained in pure form. The benzylation of 29 to 30 was bypassed; rather, 29 was weaved directly with 3,5-dihydroxybenzyl alcohol to obtain octol 22 in crude form. Because of low solubility of 22, purification via column chromatography was bypassed, and the crude product was oxidized to form crude OA. In order to purify OA of both heptaesters and impurities from impure 22, OA was esterified in acidic ethanol to afford octaester 32, which was purified by column chromatography. Finally, pure 32 was hydrolyzed to yield pure OA.

The author’s contribution to this effort centered on the isolation of octol 22 in sufficiently pure form for oxidation to octaacid 20. This allowed elimination of two reactions, the benzylation of 29 to 30 and the deprotection of 31 to 22, and the elimination of two chromatographic purifications, those of 30 and 31. Since the lone column in the new OA synthesis is performed at the
end, octol 22 needed to be isolated in pure enough form such that any impurities could be removed in the purification of octaester 32. This task proved surprisingly difficult due to the low solubility of octol 22 and the large amount of byproduct associated with its formation. It was found that the success of the weaving reaction using 29 was similar to that observed using 30, and thus an extensive investigation of different workup procedures was performed. It was found that, as in the original procedure, Celite filtration was the most effective way to remove insoluble material, primarily copper oxide and polymerized product. Tetrahydrofuran and residual pyridine from the reaction ensured that the desired product remained in solution at this stage. After this step, numerous methods of removing the remaining soluble impurities were explored that involved different combinations of washing with dilute HCl, washing with organic solvents, product precipitation, and product recrystallization. Initially, the post-filtration product was washed with dilute HCl to remove copper impurities, but the step was omitted after it was found to be unnecessary. Precipitation was accompanied by unacceptable product loss, while recrystallization from water/pyridine gave no improvement in product purity. Washing with organic solvent was found to be the most facile and effective purification method, and numerous solvents were compared (Table 3).

<table>
<thead>
<tr>
<th>Solvent</th>
<th>% Retention</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>40</td>
<td>good</td>
</tr>
<tr>
<td>Acetone</td>
<td>32</td>
<td>good</td>
</tr>
<tr>
<td>CHCl₃</td>
<td>98</td>
<td>moderate</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>70</td>
<td>moderate</td>
</tr>
<tr>
<td>THF</td>
<td>29</td>
<td>moderate</td>
</tr>
<tr>
<td>Ethanol</td>
<td>19</td>
<td>moderate</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>75</td>
<td>poor</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>36</td>
<td>moderate</td>
</tr>
<tr>
<td>Diethyl ether</td>
<td>86</td>
<td>poor</td>
</tr>
<tr>
<td>2-butanone</td>
<td>45</td>
<td>good</td>
</tr>
<tr>
<td>Chlorobenzene</td>
<td>89</td>
<td>poor</td>
</tr>
<tr>
<td>CH₂Cl₂</td>
<td>68</td>
<td>poor</td>
</tr>
</tbody>
</table>

For each wash, 30 to 100 mg of post-filtration product was sonicated 5 min in 10-20 mL of solvent, filtered, and briefly rinsed with solvent. Purity was estimated based on ¹H NMR spectra in DMSO-d₆. All samples were obtained from the same reaction.
Washing with chloroform clearly resulted in the best combination of purification and product retention; while it removed impurities less effectively than some other solvents, it caused almost no loss in product, and a second wash was found to improve the purity of the final product. The final workup procedure is summarized in Figure 28 (see the Experimental Section for a detailed description). Briefly, the reaction mixture was dried under reduced pressure to remove pyridine, resuspended in THF, filtered through Celite to remove insoluble material, and washed with chloroform twice to remove soluble impurities. Thus the final workup procedure was both effective and facile because it exploited the solubility properties of the product. A comparison of the NMR spectra of pure 22 made via the original procedure and crude 22 made via the new procedure is shown in Figure 29. Judging by NMR, the impurities in crude 22 appeared to be partially or incorrectly woven byproducts, which were removed with the purification of octaester 32.

Figure 28: Summary of procedure for octol 22 purification from new octaacid 20 synthesis.

Figure 29: $^1$H NMR spectra of octol 22 in DMSO-d$_6$ made via the (1) old method and (2) new method.
It should be noted that the new synthesis of 22 not only facilitates synthesis of OA but also facilitates the syntheses of new water-soluble cavitands. Crude octol 22 can now be made in two fewer steps and with two fewer chromatographic purifications than before and was found to be suitable for use in the syntheses of new water-soluble cavitands (vide infra). Additionally, 22 can be purified via acetylation with acetic anhydride, chromatographic purification of the resulting octaacetate, and hydrolysis to afford pure 22 (this work was not performed by the author). Synthesis of pure 22 via this route is one column shorter than the original synthesis of pure 22.

2.3 Synthesis of cationic cavitands

Octol 22 was envisioned to be a useful precursor to an octa-electrophilic cavitand (Scheme 6), which would allow the synthesis of a wide range of water-soluble cavitands via nucleophilic substitution with water-solubilizing substrates. This conversion proved surprisingly difficult. The presence of eight functional groups magnified any yield decrease due to incomplete reaction and increased the number of possible byproducts, making purification more difficult than with a single functional group transformation. Additionally, the extremely low solubility of 22 in most organic solvents greatly limited suitable reaction conditions and gave a tendency towards polymerization or incomplete reaction. Numerous methods were tried that gave low yields or byproducts that were difficult to remove or both, even after extensive investigation of different reaction conditions. Many attempts led to complex product mixtures resulting from combinations of the intended reaction, unreacted alcohol, and, in some cases, unexpected side reactions (Scheme 7). Typically, yields of the desired product were extremely low and often could not be separated from the byproducts.

Among the different unsuccessful methods tried were tosylation of 22 to 33 with p-toluenesulfonyl chloride, bromination of 22 to 34 with thionyl bromide or phosphorous tribromide, and mesylation of 22 to 35 with methanesulfonyl chloride. Attempted tosylation of 22 led to complex product mixtures from either no reaction, the intended tosylation, or pyridinium salt formation from reaction with the pyridine solvent. Reaction of 22 with PBr$_3$ led to polymerization due to the poor solubility of 22 in solvents compatible with PBr$_3$. Bromination with SOBr$_2$...
was also unsuccessful; initially, bromination of the aromatic rings near the rim of the cavity was observed. This was prevented by adding anisole as a Br⁺ scavenger, but incomplete reaction ultimately made this method unsuitable. Mesylation of 22 with MsCl gave rise to a significant amount of chlorination instead of mesylation due to nucleophilic displacement of the mesylate by chloride, generated after reaction of 22 with MsCl. Even after an exhaustive search of different bases and reaction conditions, mesylation with MsCl never afforded 35 in reasonable yields.

**Scheme 6:** Proposed synthesis of an octa-electrophilic cavitand.

**Scheme 7:** Failed attempts to synthesize an octa-electrophilic cavitand.
Finally, mesylation with methanesulfonic anhydride was tried. Unlike MsCl, this reagent
avoided the generation of any reactive byproducts, and so the only challenge remaining to contend
with was incomplete reaction. After experimentation with different reaction conditions, a synthesis
was devised that produced 35 in 60% yield (Scheme 8). THF was found to be the best solvent;
while 22 is only sparingly soluble in THF, enough material can dissolve to allow the reaction to
start, which eventually leads to complete solubility, since the product is freely soluble in THF. A
low temperature at the start of the reaction, relatively dilute conditions, and slow addition of Ms$_2$O
was found to minimize polymerization. Conveniently, this reaction can be carried out using crude
22 made via the new synthesis of OA (see above) with an overall yield of 20% from 29 to 35,
compared to an overall yield of 25% from 29 when pure 22 is used.

Scheme 8: Synthesis of octamesylate cavitand 35.

Access to octamesylate 35 greatly enhances our group’s ability to synthesize new water-
soluble cavitands. The compound is extremely versatile, as it can be reacted with any number
of water-solubilizing nucleophiles to create a water-soluble cavitand. This approach has already
led to the synthesis of three new water-soluble hosts (Scheme 9). All three hosts possess eight
ammonium salt functional groups, making all three water-soluble at neutral and acidic pH. These
cationic hosts are complimentary to the anionic OA, and their solubility at neutral pH makes them
more relevant for biological studies.

Octaamine HCl 36 (OAm-HCl) was synthesized via a two step azidation and reduction.
(Initially, the direct synthesis of 37 from 22 was attempted by a few different methods for one-
step azidation of alcohols. These efforts were either completely unsuccessful$^{124,125}$ or only gave
Scheme 9: Synthesis of new water-soluble cavitands 36, 38, and 39 from octamesylate 35.

Partial conversion. Octamesylate 35 was converted to octaazide 37 in good yield by simple nucleophilic substitution with sodium azide. Staudinger reduction of 37 with triphenylphosphine and workup with aqueous HCl yielded 36 as the HCl salt. While the yield is modest, the reaction was performed at a small scale, and improved yield is expected at a larger scale. This compound is highly water soluble at acidic and neutral pH. The NMR spectrum in CD$_3$OD clearly shows sharp peaks consistent with pure product, but the NMR in D$_2$O shows numerous extra and broad peaks, indicative of aggregation of the monomeric host (Figure 30). This is not surprising, as the ammonium groups are capable of hydrogen bonding, and so hydrogen bonding between different
host molecules and even within one host is possible. This characteristic unfortunately makes this host less useful for studies of guest binding by NMR.

![Figure 30: $^1$H NMR spectra of OAm-HCl 36 in (bottom) D$_2$O and (top) CD$_3$OD. Refer to Scheme 9 for proton assignments.](image)

The syntheses of octatrimethylammonium chloride 38 (OTA-Cl) and octapyridinium chloride 39 (OPy-Cl) are both one-step processes whereby the eight mesylate groups in 35 are replaced with a water-solubilizing group via nucleophilic substitution with trimethylamine and pyridine, respectively. These compounds are produced in moderate to good yield on a small scale and are expected to have excellent yields on a larger scale since there are no byproducts to remove. Neither compound requires purification by column chromatography, which is fortunate since such purification would likely be extremely difficult given the nature of these compounds. Both are converted to the chloride salt by ion exchange chromatography with Dowex, a process easily adaptable to other counterions. Both OTA-Cl and OPy-Cl are highly water soluble at acidic and neutral pH. Unlike OAm-HCl, NMR spectra in D$_2$O for both compounds show relatively sharp peaks; evidently the inability of either host to form hydrogen bonds prevents aggregation (Figure 31 and Figure 32). This property makes both compounds ideal for extensive guest binding studies by NMR.
2.4 Discussion

Studies of OA 20 are now more accessible thanks to a shorter and easier new synthetic procedure that yields a purer product. This procedure also makes the synthesis of octol 22 more efficient, which facilitates the synthesis of the new water-soluble hosts OAm-HCl 36, OTA-Cl 38, and OPy-Cl 39. Importantly, the syntheses of these new hosts all start from the same precursor, octamesylate 35, a versatile compound that can be used to synthesize many more water-soluble hosts in the future. The addition of these hosts to the family of Gibb group cavitands greatly diversifies the types of studies that can be performed. These cavitands are now available in anionic (OA 20 and TEMOA 23), neutral (G3 21), and cationic (OAm-HCl 36, OTA-Cl 38, and OPy-Cl 39) form, and together are soluble across the pH range. The molecular recognition properties of these new hosts will be described in the next section.

Figure 31: \(^1\)H NMR spectra of OTA-Cl 38 in (bottom) D\(_2\)O and (top) CD\(_3\)OD. Refer to Scheme 9 for proton assignments.
Figure 32: $^1$H NMR spectra of OPy-Cl 39 in (bottom) D$_2$O and (top) CD$_3$OD. Refer to Scheme 9 for proton assignments.
3 Molecular recognition of new water-soluble deep-cavity cavitands

3.1 Introduction

The new hosts OPy-Cl 39, OTA-Cl 38, and OAm-HCl 36 have the same basic cavitand structure as OA but have different hydrophilic functionalities at the rim and feet, so we were interested to compare the binding behavior of these new hosts to that of OA. As discussed in Section 1.3.5, OA is known to bind hydrophobic and amphiphilic guest molecules and chaotropic anions inside the hydrophobic cavitand pocket. Anion binding with the new hosts was of particular interest, since the cationic functional groups coating these hosts afford the possibility of enhanced binding due to favorable electrostatic interactions between anion and host.

3.2 Binding analysis by NMR

The binding behavior of OAm-HCl, OTA-Cl, and OPy-Cl was investigated using $^1$H NMR spectroscopy, a technique that is a valuable tool in host-guest chemistry because binding causes a change in the environment of host and guest protons. Binding by OA and related cavitands causes guest peaks to undergo a significant (up to 4 ppm) upfield shift due to shielding by the $\pi$ electrons of the cavity walls, with peak shift increasing with depth of binding. Certain protons in the cavitand also experience large peak shifts (see Figure 33 for host proton labeling). Not surprisingly, the $b$ protons, the four benzal protons pointing inward at the base of the cavity, are particularly good indicators of binding inside the pocket. Shifts in the $c$ and $d$ proton peaks are often indicative of capsule formation due to the location of these protons at the rim of the cavity. The $g$, $j$, and other protons can also undergo NMR peak shifts upon guest binding inside the pocket due to their location. Additionally, external binding at the cavitand feet can cause peak shifts in the $j$ and $s$ protons.
Host-guest complexation is an equilibrium between free host $H$, free guest $G$, and host-guest complex $H_nG_m$. For a 1:1 host-guest binding event, this equilibrium is described by Equation 5,

$$H + G \rightleftharpoons HG$$

and the association constant $K_a$ is obtained using Equation 6,

$$K_a = \frac{[HG]}{[H][G]}$$

where larger $K_a$ indicates stronger binding. Analysis of binding events by NMR differs depending on the kinetics of binding; an exchange process that is slow on the NMR time scale will result in distinct “free” and “bound” peaks, while an exchange process that is fast on the NMR time scale will result in a single peak that is an average of free and bound host or guest. In the case of slow binding, the concentrations of free and bound host and guest are obtained by peak integration and the association constant $K_a$ is easily calculated using Equation 6. In the case of fast binding, a titration must be carried out, whereby increasing amounts of guest are added to the host solution until binding is saturated. The resulting peak shift $\Delta\delta_{obs}$ is plotted against total guest concentration $G_t$ to obtain a binding isotherm, which can be fitted to Equation 7 to obtain $K_a$, where $\Delta\delta_{max}$ is the maximum peak shift at full complexation and $H_t$ is the total host concentration.$^{127}$
\[
\Delta \delta_{obs} = \frac{\Delta \delta_{max}}{2} \left( \frac{K_a G_t - K_a H_t - 1 + \sqrt{(1 - K_a G_t + K_a H_t)^2 + 4K_a G_t}}{1} \right)
\]  
(7)

Analysis of higher order binding events is more complicated. However, to calculate \(K_a\) for a 2:1 cavitand-guest system, it is acceptable to model capsule formation as a 1:1 binding event between an empty capsule \(H_2\) and guest \(G\) (Equation 8).\textsuperscript{56}

\[
H_2 + G \rightleftharpoons H_2G
\]  
(8)

Thus, for capsule formation with slow exchange on the NMR time scale, the apparent binding constant \(K_{app}\) is calculated with Equation 9. It should be noted, however, that \(K_{app}\) does not reflect the monomeric state of the host in the absence of guest.

\[
K_{app} = \frac{[H_2G]}{[H_2][G]}
\]  
(9)

While NMR provides structural information regarding binding, the technique’s relatively low sensitivity limits the range of association constants that can be determined. Generally, binding constants between 0.1 and \(10^6 \text{ M}^{-1}\) can be obtained through the means described above. An association constant above \(10^4 \text{ M}^{-1}\) must be determined either under dilute conditions (requiring long acquisition times) or with competitive binding experiments. In the case of slow binding, this is because free host will only be present in amounts visible by NMR under dilute conditions. In the case of fast binding, the binding isotherm will reach saturation too quickly at normal NMR concentrations to allow accurate calculation of \(K_a\). Association constants above \(10^6 \text{ M}^{-1}\) are associated with a high degree of error.\textsuperscript{127}
3.3 Binding of organic guest molecules

Preliminary binding studies of OPy-Cl, OTA-Cl, and OAm-HCl with the organic guest molecules 1-adamantanecarboxylic acid (adaCO$_2$H) and dodecane were carried out. These guests were chosen because they exhibit strong binding with OA with slow exchange on the NMR time scale, and their structures allow for relatively straightforward analysis of complexation by NMR. When combined with OA, the amphiphilic adaCO$_2$H forms a 1:1 complex, while dodecane forms a 2:1 capsule. Since these hosts have the same cavitand structure as OA but are coated with positively charged functionalities instead of negatively charged ones, it was expected that these hosts would bind both guests in a similar fashion as OA, but that adaCO$_2$H could bind more strongly due to favorable electrostatic interactions between the guest carboxylate and the positive functional groups at the host cavity rim.

3.3.1 Formation of 1:1 host:guest complexes with 1-adamantanecarboxylic acid

AdaCO$_2$H is the strongest-binding known guest for OA that binds 1:1, with $K_a = 4.7 \times 10^6$ M$^{-1}$. The hydrophobic adamantane portion has a shape that is highly complementary to the shape of the host hydrophobic pocket, while the hydrophilic carboxylic acid group orients outward at the mouth of the cavity, where it is solvated by the aqueous medium. Titrations of adaCO$_2$H into OPy-Cl, OTA-Cl, and OAm-HCl in D$_2$O showed binding behavior that is qualitatively similar to adaCO$_2$H binding by OA. Figure 34 shows the NMR spectra for the titration experiment with OPy-Cl. Binding is evident by the large upfield shift in the guest peaks and the large shift of the host $b$ peak. Peak integration at one equivalent of guest and the appearance of free guest peaks above one equivalent of guest confirm 1:1 binding stoichiometry. The presence of distinct free and bound host peaks at 0.5 equivalents of guest indicate that complexation is slow on the NMR time scale. Guest peak assignment by COSY NMR shows that the guest binds with the adamantane portion inside the pocket and the carboxylic acid pointing out of the cavity towards the water solvent (see Figure 35; the entire spectrum is shown in the Experimental Section). The absence of visible free host at one equivalent of adaCO$_2$H indicates that the guest is strongly bound.
Figure 34: $^1$H NMR spectra of 0.5 mM OPy-Cl in D$_2$O with (1) 0, (2) 0.5, (3) 1.0, and (4) 1.5 equivalents of adaCO$_2$H. Bound host peaks are indicated by an asterisk. Refer to Figure 33 for host peak labels.

Figure 35: Bound guest region of COSY NMR spectrum of 0.5 mM OPy-Cl in D$_2$O with 1.5 equivalents of adaCO$_2$H.
No free host was visible even at 5 μM host with one equivalent of guest (Figure 36). If 5% error in peak integration is assumed (i.e., 5% maximum of free host), then a minimum association constant of $4 \times 10^6$ M$^{-1}$ can be obtained with Equation 6. In conducting the dilution experiment to obtain $K_a$, it was noticed that certain host peaks, particularly the $j$ and $s$ peaks, shifted to new positions that did not correlate with the free host at 0.5 mM (compare spectrum 3 to spectra 1 and 2 in Figure 37). It was hypothesized that the chloride counterions were binding externally to the host feet at 0.5 mM host and that the peak shifts at 5 μM host were due to the much lowered proportion of bound host that would be expected at the lower concentration. To test this hypothesis, NaCl solution was added to the diluted sample such that the amount of Cl$^-$ complexation to the feet would be approximately the same as the original sample at 0.5 mM, according to Equation 6 (this assumes that Cl$^-$ binding at the feet is independent of adaCO$_2$H binding inside the pocket). Indeed, after addition of NaCl, the $j$ and $s$ peaks did return to roughly their position before the dilution (compare spectra 2 and 4 in Figure 37).

![Figure 36](image)

**Figure 36:** $^1$H NMR spectra of OPy-Cl in D$_2$O: (1) 0.5 mM OPy-Cl, (2) 0.5 mM OPy-Cl with 1.0 equivalent adaCO$_2$H, (3) 5 μM OPy-Cl with 1.0 equivalent of adaCO$_2$H. Bound host peaks are indicated by an asterisk. Refer to Figure 33 for host peak labels.
Figure 37: $^1$H NMR spectra of OPy-Cl in D$_2$O: (1) 0.5 mM OPy-Cl, (2) 0.5 mM OPy-Cl with 1.5 equivalents adaCO$_2$H, (3) 5 µM OPy-Cl with 1.5 equivalents of adaCO$_2$H, (4) 5 µM OPy-Cl with 1.5 equivalents of adaCO$_2$H and 900 equivalents of NaCl. Bound host peaks are indicated by an asterisk. Refer to Figure 33 for host peak labels.

The possibility that Cl$^-$ binding at the feet causes dilution-induced host peak shifts was explored further with a dilution study of OPy-Cl in the absence of guest. Comparison of NMR spectra of OPy-Cl at a range of concentrations showed a progressive upfield shift of the $j$ and $s$ peaks with decreasing concentration (spectra 1-4 in Figure 38). NaCl was then added to the most dilute sample in an amount that, according to Equation 6, would give the same proportion of complexation as the most concentrated sample, assuming 1:1 binding of Cl$^-$ to host. Upon addition of NaCl, the $j$ and $s$ peaks shifted to the same positions as the most concentrated sample (compare spectra 1 and 5 in Figure 38). These results are further evidence that the Cl$^-$ counterions bind to the host feet with fast kinetics, causing the $j$ and $s$ peaks to shift as a function of host concentration. (Since Cl$^-$ binding is much weaker than adaCO$_2$H binding (vide infra), its effect
on binding of this guest is presumed to be negligible.) The subject of anion binding externally to
the host feet will be discussed further in Section 3.4.

![Figure 38: Selected regions of the $^1$H NMR spectra of OPy-Cl in D$_2$O at (1) 500 µM, (2) 250 µM, (3) 125 µM, (4) 75 µM, and (5) 75 µM with 43.2 equivalents of NaCl.](image)

A titration experiment of adaCO$_2$H into OTA-Cl gave the same results as with OPy-Cl. The guest bound inside the cavity with the carboxylic acid pointing outward into the water solvent. Binding was slow on the NMR time scale and strong; a dilution experiment gave a minimum $K_a$ of $4 \times 10^6$ M$^{-1}$. As with OPy-Cl, dilution of the OTA-Cl·adaCO$_2$H complex was accompanied by a shift of the host $j$ and $s$ peaks. This was again attributed to weakened chloride binding at the feet upon dilution, although this was not confirmed for this host. NMR spectra for the titration, peak assignment by COSY NMR, and dilution NMR spectra can be found in the Experimental Section.

Complexation of OAm-HCl with adaCO$_2$H caused the NMR peaks to sharpen dramatically, indicating that aggregation of the monomeric host was disrupted. The NMR spectra for the titration experiment are shown in Figure 39. Binding of adaCO$_2$H with OAm-HCl appeared to be fast on the NMR time scale, as evidenced by the merging of free and bound host peaks at 0.5 equivalents of guest. Binding was also strong; the host peaks were fully shifted with only one equivalent of guest, indicating that binding was saturated even without excess guest. However, a minimum association constant could not be calculated since the complex appeared to be fast-exchanging. The
guest orientation in the complex is also the same as with OPy-Cl and OTA-Cl (see the Experimental Section for the COSY NMR spectrum confirming guest orientation). Upon dilution of the complex to 5 µM host, the host \( j \) and \( s \) peaks shifted, as was observed with the other two hosts, again presumably because of weakened Cl\(^-\) binding to the feet, although this was not confirmed (see the Experimental Section for these NMR spectra).

Figure 39: \(^1\)H NMR spectra of 0.5 mM OAm-HCl in D\(_2\)O with (1) 0, (2) 0.5, (3) 1.0, and (4) 1.5 equivalents of adaCO\(_2\)H. Bound host peaks indicated by an asterisk. Refer to Figure 33 for host peak labels.

3.3.2 Formation of 2:1 host:guest capsules with dodecane

OA is known to self-assemble with dodecane to form a 2:1 host-guest capsule of high thermodynamic stability that is kinetically stable on the NMR time scale.\(^{63}\) Dodecane binds inside OA in an extended conformation, with each methyl group anchored at the base of each cavity. All three new hosts were qualitatively found to bind dodecane in the same manner. Figure 40 compares the
NMR spectra of free OPy-Cl and the OPy-Cl capsule with dodecane. The shift of the host $b$ peak is indicative of binding inside the pocket, while the shifts of the $c$ and $d$ peaks are indicative of capsule formation, as these protons are located at the rim of the cavity. Peak integration confirms 2:1 host:guest stoichiometry. The guest peaks are shifted far downfield (free dodecane appears at 0.9 and 1.3 ppm), indicating binding deep inside the cavity, and the staggering of the peaks for each methylene group suggests an extended conformation of the guest, with the methyl groups positioned at the base of each cavity. This conformation was confirmed by COSY NMR; the guest region of this spectrum is shown in Figure 41 (for the host region, see the Experimental Section).

Figure 40: $^1$H NMR spectra of 0.5 mM OPy-Cl in D$_2$O with (1) no guest and (2) excess dodecane. Bound host peaks are indicated by an asterisk. Refer to Figure 33 for host peak labels.

Figure 41: Bound guest region of COSY NMR spectrum of 0.5 mM OPy-Cl in D$_2$O with excess dodecane.
The absence of visible free host in the NMR spectrum of the capsule (Figure 40) indicates strong binding; at 5 \( \mu \text{M} \) host, integration of the free and bound host \( b \) peaks showed 40% bound host, allowing the qualitative conclusion that binding is very strong (Figure 42). The insolubility of dodecane in DMSO and other non-competitive NMR solvents prohibits \( K_{app} \) calculation using Equation 9; because dodecane cannot be titrated as a solution, its concentration in the host solution is unknown. More suitable solvents for dodecane compete as guests, and adding a dodecane solution to the NMR tube and evaporating the solvent would also result in some evaporation of dodecane. The only way to obtain \( K_{app} \) for dodecane is to carry out the dilution experiment several times to get a range of \( K_{app} \) values, which would still be associated with a high degree of error. As with the OPy-Cl-adaCO\(_2\)H complex, host peaks shifts were observed upon dilution, especially the \( j \) and \( s \) peaks (Figure 42). This was again attributed to Cl\(^-\) binding at the feet.

**Figure 42:** \(^1\)H NMR spectra of OPy-Cl in D\(_2\)O: (1) 0.5 mM OPy-Cl, (2) 0.5 mM OPy-Cl with excess dodecane, (3) 5 \( \mu \text{M} \) OPy-Cl with excess dodecane. Bound peaks indicated by an asterisk. Refer to Figure 33 for host peak labels.

The OTA-Cl binding experiment with dodecane yielded all of the same observations as with OPy-Cl: a 2:1 capsule was formed with high thermodynamic and kinetic stability, and the guest bound in an extended conformation. At 0.5 mM host, no free host was visible, while at 5 \( \mu \text{M} \) host,
41% of host was bound, indicating very strong binding. The dilution experiment also indicated \( \text{Cl}^- \) binding at the host feet, as evidenced by shifts in the host \( j \) and \( s \) peaks. NMR spectra of free OTA-Cl versus (OTA-Cl)_2·dodecane capsule, the COSY NMR spectrum to identify peaks, and the NMR spectra for the dilution experiment are located in the Experimental Section. OAm-HCl also was found to bind with dodecane to form a 2:1 capsule (Figure 43), with the guest adopting an extended conformation (see the Experimental Section for peak identification by COSY NMR). The sharpened NMR peaks of the bound host show that capsule formation disrupts aggregation of monomeric OAm-HCl. As with OPy-Cl and OTA-Cl, binding is strong enough that no free host was visible by NMR at 0.5 mM host. Dilution to 5 \( \mu \)M host gave a very poor quality spectrum in which many of the free host peaks were either not visible or too broad to allow accurate integration (see Experimental Section). Dilution also caused large shifts in the host \( j \) and \( s \) peaks, which was attributed to weakened \( \text{Cl}^- \) binding at the feet.

![Figure 43: \(^1\text{H NMR spectra of 0.5 mM OAm-HCl in D}_2\text{O with (1) no guest and (2) excess dodecane. Bound host peaks are indicated by an asterisk. Refer to Figure 33 for host peak labels.}](image)

### 3.4 Binding of anions

As noted in Section 1.3.5, OA was tested for binding with anions spanning the Hofmeister series and was found to bind the chaotropic anions \( \text{ClO}_4^- \), \( \text{SCN}^- \), \( \text{I}^- \), \( \text{ClO}_3^- \), and \( \text{NO}_3^- \) inside the cavitand pocket with the following association constants: \( \text{ClO}_4^- \), 95 M\(^{-1}\); \( \text{SCN}^- \), 33 M\(^{-1}\); \( \text{I}^- \), 11 M\(^{-1}\); \( \text{ClO}_3^- \),
Considering that OPy-Cl, OTA-Cl, and OAm-HCl possess the same hydrophobic pocket as OA, it was predicted that these new hosts would also bind chaotropic anions inside the pocket. Also, because the new hosts are coated with eight positively charged functional groups, it seemed likely that favorable electrostatic interactions between the anion and the four cationic groups at the rim would enhance binding strength. Moreover, the presence of four cationic groups at the host feet gave the possibility of external anion binding at the feet, also driven by favorable electrostatic interactions. Indeed, external binding of cationic violegens by OA due to electrostatic interactions has already been reported (vide supra).70,71

Preliminary binding studies were performed for the three new hosts with the sodium salts of anions spanning the Hofmeister series: NaF, Na₂SO₄, NaOAc, NaCl, NaNO₃, NaBr, NaI, NaClO₃, NaSCN, and NaClO₄. Several of the anions were found to bind in some form, either inside the cavitand pocket, externally at the feet, or at both sites, although in some cases, the identification of binding site(s) was inconclusive. In all cases, binding was fast on the NMR time scale so that when possible, association constants were calculated according to the binding model for fast 1:1 binding (Equation 7). The \( b \), \( j \), and \( s \) peaks were the most useful indicators of binding inside the pocket. The \( b \) protons point inside the pocket and experience a drastic change in environment upon binding, while the \( j \) and \( s \) protons are presumably affected by a slight cavity deformation that occurs upon binding. Binding at the feet was indicated by shifts in the \( j \) and \( s \) peaks because of their proximity to the binding anion. Since the \( j \) and \( s \) peaks were affected by binding at both sites, conclusive assignment of binding sites was sometimes impossible.

### 3.4.1 OPy-Cl binding of anions

Figure 44 compares NMR spectra of OPy-Cl in D₂O alone and with 10 equivalents of each salt (except NaClO₄, which caused host precipitation at six equivalents). A visual comparison of the positions of the \( j \), \( b \), and \( s \) peaks allows some generalizations about binding to be made. The anions ClO₄⁻, SCN⁻, I⁻, and ClO₅⁻ caused large shifts in the \( b \) peak, and Br⁻, Cl⁻, and SO₄²⁻ caused smaller shifts, indicating that these anions bind to OPy-Cl inside the pocket. Shifts in the \( j \)
and s peaks are apparent in the presence of all anions except OAc<sup>−</sup> and F<sup>−</sup>, although at higher salt concentrations, even these anions cause small j and s peak shifts. For F<sup>−</sup>, OAc<sup>−</sup>, and NO<sub>3</sub> which caused no significant b peak shift, this could be attributed to external binding at the feet. For ClO<sub>4</sub>−, SCN<sup>−</sup>, I<sup>−</sup>, ClO<sub>3</sub>−, Br<sup>−</sup>, Cl<sup>−</sup>, and SO<sub>4</sub><sup>2−</sup>, these shifts could be attributed to binding inside the pocket, or to binding at the feet, or to binding at both sites. To assign binding sites more definitively and to obtain binding constants, it was necessary to analyze peak shift as a function of salt concentration.

![Figure 44: 1H NMR spectra of 0.5 mM OPy-Cl in D<sub>2</sub>O with each Hofmeister salt. 10 equivalents of each salt were added, except for NaClO<sub>4</sub>, which contains 4 equivalents. The j, b, and s peaks are labeled with an asterisk. In the spectra with NaSCN and NaI, the b peak is shifted underneath the water peak.](image)

For each salt titration, the peak shifts ∆δ for the b, j, and s peaks were plotted as a function of anion concentration to create binding isotherms, and association constants were obtained by fitting the curves to Equation 7. (Binding isotherms for the other peaks were created as well, but
they did not correlate with binding.) The results are presented in Table 4 (the NMR spectra, binding isotherms, and association constants for each peak are shown in the Experimental Section).

**Table 4:** Association constants for OPy-Cl binding of anions.

<table>
<thead>
<tr>
<th>Anion</th>
<th>Pocket</th>
<th>Feet</th>
</tr>
</thead>
<tbody>
<tr>
<td>F(^{-})</td>
<td>-(^a)</td>
<td>7.7</td>
</tr>
<tr>
<td>SO(_4^{2-})</td>
<td>321</td>
<td>(-)(^b)</td>
</tr>
<tr>
<td>OAc(^-)</td>
<td>-</td>
<td>16.3</td>
</tr>
<tr>
<td>Cl(^-)</td>
<td>66.5</td>
<td>121</td>
</tr>
<tr>
<td>NO(_3^-)</td>
<td>-</td>
<td>545</td>
</tr>
<tr>
<td>Br(^-)</td>
<td>918</td>
<td>(982)</td>
</tr>
<tr>
<td>ClO(_3^-)</td>
<td>324</td>
<td>(-)</td>
</tr>
<tr>
<td>I(^-)</td>
<td>n.d.(^c)</td>
<td>(2973)</td>
</tr>
<tr>
<td>SCN(^-)</td>
<td>2285</td>
<td>(-)</td>
</tr>
<tr>
<td>ClO(_4^-)</td>
<td>2982</td>
<td>(-)</td>
</tr>
</tbody>
</table>

\(^a\) A dash indicates no binding was observed. \(^b\) Parentheses indicate assignment of binding site is tentative. \(^c\) “N.d.” indicates that binding could not be determined due to peak overlap with water.

The kosmotropes F\(^-\) and OAc\(^-\) did not bind inside the pocket and bound very weakly to the feet. The mid-Hofmeister anion NO\(_3^-\) did not bind inside the pocket and bound moderately strongly to the feet. For the remaining salts, results were less clear, but some reasonable speculations can be made about binding sites. Cl\(^-\) appeared to bind weakly both inside the pocket (\(K_a\) derived from the \(b\) peak) and at the feet (\(K_a\) derived from the \(s\) peak), as evidenced from the two distinct \(K_a\) values obtained from the \(b\) and \(s\) peaks. The odd shape of the curve for the \(j\) peak shift (Figure 45a) could be due to binding at both sites (this is of course highly speculative). For Br\(^-\), fairly high binding constants were obtained for binding inside the pocket (\(b\) peak) and at the feet (\(s\) peak). As with Cl\(^-\), Br\(^-\) caused an unusual \(j\) peak shift that could be due to binding at both sites (Figure 45b). For ClO\(_4^-\), SCN\(^-\), ClO\(_3^-\), and SO\(_4^{2-}\), the association constants derived from binding isotherms for the \(b, j,\) and \(s\) peaks were roughly the same (the \(b\) peak for SCN\(^-\) was an exception; \(K_a\) was inaccurate due to overlap of the \(b\) peak with the water peak at higher salt concentrations). The tentative explanation for this observation is that binding only occurs inside the pocket for these salts. While it is possible that binding happens to occur at the feet with the same strength as inside the pocket,
the results for Cl\(^-\) and Br\(^-\) suggest that this would result in unusual binding isotherms for at least one of the peaks. Results were inconclusive for I\(^-\) because the b peak shifted underneath the water peak, so insufficient data were collected to create a valid binding isotherm for this peak.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{plot.png}
\caption{Plots of OPy-Cl j peak $\Delta \delta$ as a function of (a) Cl\(^-\) and (b) Br\(^-\) concentration.}
\end{figure}

3.4.2 OTA-Cl binding of anions

NMR spectra of OTA-Cl alone and with 10 equivalents of each salt are shown in Figure 46. Large shifts of the b peak in the presence of ClO\(_4\)^-\), SCN\(^-\), I\(^-\), and ClO\(_3\)^-\) and smaller shifts in the presence of Br\(^-\), NO\(_3\)^-\), SO\(_4\)^2-\), and OAc\(^-\) indicate that these anions bind to OTA-Cl inside the pocket. All anions caused shifts in the j and s peaks (some quite small at only 10 equivalents of salt), but as was the case with OPy-Cl, a visual assessment is not sufficient to determine whether these shifts were due to binding inside the pocket or at the feet. Binding isotherms for the shifts in the b, j, and s peaks were used to obtain association constants (Table 5) (see the Experimental Section for NMR spectra, binding isotherms, and all association constants). For some anions, it was possible to confidently identify the binding site(s), but for others the assignment is tentative.

The kosmotrope F\(^-\) bound very weakly at the feet, and the mid-Hofmeister Cl\(^-\) bound moderately weakly at the feet. Neither anion bound inside the pocket. For NO\(_3\)^-\), $K_a$ was similar for the j and s peaks and much lower for the b peak, indicating binding of NO\(_3\)^- at both sites, with
Figure 46: $^1$H NMR spectra of 0.5 mM OTA-Cl in D$_2$O with each Hofmeister salt. 10 equivalents of each salt were added. The $j$, $b$, and $s$ peaks are labeled with an asterisk.

Table 5: Association constants for OTA-Cl binding of anions.

<table>
<thead>
<tr>
<th>Anion</th>
<th>Pocket</th>
<th>$K_a$ (M$^{-1}$)</th>
<th>Feet</th>
</tr>
</thead>
<tbody>
<tr>
<td>F$^-$</td>
<td>-$^a$</td>
<td>17.1</td>
<td></td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td>326</td>
<td>(-)$^b$</td>
<td></td>
</tr>
<tr>
<td>OAc$^-$</td>
<td>6.7</td>
<td>(19.6)</td>
<td></td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>-</td>
<td>162</td>
<td></td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>90.7</td>
<td>365</td>
<td></td>
</tr>
<tr>
<td>Br$^-$</td>
<td>13.3</td>
<td>1256 (s peak), 3378 (j peak)</td>
<td></td>
</tr>
<tr>
<td>ClO$_3^-$</td>
<td>288</td>
<td>(-)</td>
<td></td>
</tr>
<tr>
<td>I$^-$</td>
<td>632$^c$</td>
<td>2055 (s peak), 4549 (j peak)</td>
<td></td>
</tr>
<tr>
<td>SCN$^-$</td>
<td>1915</td>
<td>(-)</td>
<td></td>
</tr>
<tr>
<td>ClO$_4^-$</td>
<td>2695</td>
<td>(-)</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ A dash indicates no binding was observed.  $^b$ Parentheses indicate assignment of binding site is tentative.  $^c$ Decreased accuracy due to peak overlap with water.
stronger binding at the feet. Results for the remaining anions were less definitive. I\(^{-}\) and Br\(^{-}\) gave puzzling results because analysis of all three peak shifts gave three very different binding constants. It seems likely that these anions bind at both binding sites with stronger binding at the feet, but it is unclear what would cause the \(j\) and \(s\) peaks to produce such different results. Moreover, the binding isotherm for the \(b\) peak with addition of I\(^{-}\) had a somewhat sigmoidal shape that fit poorly to the binding model (Figure 47). Therefore, with the exception of the \(K_a\) for Br\(^{-}\) binding inside the pocket derived from the \(b\) peak shift, the binding constants for Br\(^{-}\) and I\(^{-}\) are highly suspect. For ClO\(_4\)^{-}, SCN\(^{-}\), ClO\(_3\)^{-}, and SO\(_4\)^{2-}, roughly similar binding constants were obtained from analysis of all three peak shifts. (For ClO\(_4\)^{-}, SCN\(^{-}\), ClO\(_3\)^{-}, there was a greater deviation for the \(K_a\) from the \(b\) peak that was attributed to error from gaps in the data due to overlap of the \(b\) peak with the \(a\) and water peaks.) It seems likely that these anions only bind inside the pocket, although this conclusion is tentative because it is possible that these anions simply have similar affinity for the pocket and the feet. OAc\(^{-}\) was judged to bind at both sites because it was shown to bind to OPy-Cl at the feet.

![Figure 47: Binding isotherm of OTA-Cl b \(\Delta \delta\) as a function of NaI concentration.](image)

### 3.4.3 OAm-HCl binding of anions

It was previously noted that OAm-HCl in D\(_2\)O has a strong tendency to aggregate, as is evident by broad peaks in the NMR spectrum. Complexation with adaCO\(_2\)H and dodecane disrupted this aggregation, as shown by sharpened NMR peaks. Unfortunately, anion binding does not dimin-
ish OAm-HCl aggregation, making quantitative analysis of anion binding to this host impossible. Since the host forms undefined aggregates, a 1:1 binding model cannot be used to calculate association constants. Some qualitative analysis of binding was possible, but even this was made difficult or impossible for certain salts because the presence of high concentrations of salt exacerbated peak broadening.

Figure 48 shows the NMR spectra of OAm-HCl with varied amounts of each of the salts tested. Each salt was added up to 12 equivalents, but in most cases, peak broadness at that salt concentration interfered with any meaningful analysis. The spectra shown are illustrative of the peak shifts observed for each salt. The binding of chaotropes ClO$_4^-$, SCN$^-$, I$^-$, and ClO$_2^-$ inside the pocket is strongly supported by significant downfield shifts of the $b$ peak in the presence of these anions. Shifts in the $j$ and $s$ peaks were observed for these salts also, but it could not be determined whether this was due to binding inside the pocket or to additional binding at the feet. Br$^-$ caused sizable downfield shifts in the $j$ and $s$ peaks with little shift in the $b$ peak, strongly indicating binding at the feet and weak or no binding inside the pocket. The titrations with the remaining salts were inconclusive. NO$_3^-$ and SO$_4^{2-}$ caused severe peak broadening at even low concentrations, making analysis impossible. Cl$^-$, OAc$^-$, and F$^-$ showed no evidence of binding at modest salt concentrations, but peak broadness at higher salt concentrations made it impossible to rule out weak binding of these anions.

3.5 Discussion

The new hosts OPy-Cl, OTA-Cl, and OAm-HCl were shown to bind guest molecules in water in both familiar and novel ways. Binding of the organic guest dodecane was qualitatively very similar to binding of dodecane by OA. All three hosts were observed to self-assemble with dodecane to form 2:1 capsules of high thermodynamic and kinetic (relative to the NMR time scale) stability. This is not surprising, since the basic cavatand structure of these hosts is identical to that of OA, and the eight solubilizing groups would not be expected to interact differently with a neutral hydrophobic guest like dodecane. Therefore, OPy-Cl, OTA-Cl, and OAm-HCl can be expected to
bind with neutral hydrophobic guests in much the same way that OA does, although this obviously needs to be explored further. Also, for hydrophobic guests that are soluble in DMSO, such as certain steroids, $K_{app}$ could be quantified.

The new hosts bound with the amphiphilic guest ada$\text{CO}_2\text{H}$ to form 1:1 complexes of high thermodynamic stability in which the hydrophilic portion of the guest points out of the cavity mouth, where it is exposed to the water solvent. The same behavior is exhibited by OA. Also like OA, complexation with OPy-$\text{Cl}$ and OTA-$\text{Cl}$ was slow on the NMR time scale. Conversely, complexation of OAm-$\text{HCl}$ with this guest was fast on the NMR time scale. Since no other water-soluble hosts of this type have shown fast binding with ada$\text{CO}_2\text{H}$, it seems likely that an impurity is competing with ada$\text{CO}_2\text{H}$ for binding inside the pocket. The major expected difference in

**Figure 48:** $^1$H NMR spectra of 0.5 mM OAm-$\text{HCl}$ in $\text{D}_2\text{O}$ with each Hofmeister salt at varied concentrations. The $j$, $b$, and $s$ peaks are labeled with an asterisk.
adaCO$_2$H binding between OA and the new hosts is in binding strength, since there is the possibility of electrostatic attraction between the anionic carboxylate on adaCO$_2$H and the four cationic groups at the rim of each of the new hosts, which would enhance binding strength. Because of the inherent limits of NMR methods in the determination of association constants, this was not confirmed or rejected. However, association constants for adaCO$_2$H binding to OPy-Cl, OTA-Cl, and OAm-HCl could be obtained using isothermal titration calorimetry (ITC), as the upper limit of this technique is $10^9$ M$^{-1}$.$^{127}$ Additionally, further binding studies with other amphiphilic guest molecules could elucidate the influence of host and guest charges (or lack thereof in the case of neutral amphiphiles) on binding strength. If electrostatic interactions between host and guest do affect binding strength, the pH of the solution would also be a factor to consider. Potentially, the choice of host and of solvent pH could be used to tune the binding affinities of different guests.

Studies of anion binding with the new hosts revealed interesting new behaviors. It should be emphasized that because the results presented are preliminary and because some of the binding events are more complicated than those previously studied with these types of host molecules, much of the analysis of the anion binding results is speculative. However, the results clearly show that OPy-Cl, OTA-Cl, and OAm-HCl all bind in some way with certain anions. As with OA, binding of chaotropic anions inside the hydrophobic pocket was observed, with greater chaotropicity corresponding with stronger binding. However, association constants (when they could be obtained) were one or two orders of magnitude larger than those found with OA. Moreover, some mid-range Hofmeister anions and even the kosmotropic SO$_4^{2-}$ were shown to bind inside the pocket of OPy-Cl and OTA-Cl. These findings appear to affirm the ability of chaotropic anions to bind inside a hydrophobic pocket, as first reported with OA,$^{72}$ but they also suggest that the presence of four cationic functional groups around the cavitand rim enhances binding through electrostatic attraction. This additional favorable interaction could explain the enhanced binding strength of the chaotropes and the binding to some of the mid-range anions that did not bind with OA. The well-solvated kosmotropes (except SO$_4^{2-}$) and some mid-range anions are presumably unable to shed enough of their solvation shell to bind inside the pocket. It is possible that the doubly charged
SO\textsubscript{4}\textsuperscript{2−} experiences enough electrostatic attraction to the host to overcome its high heat of hydration and allow partial desolvation and binding inside the pocket.

The presence of a second binding site at the feet is an interesting development in the study of anion binding by cavitands in water, although it also complicates analysis. It is intuitive that such binding should occur, considering that the cationic functional groups present a preorganized site of positive charge. This work gives strong evidence that OPy-Cl and OTA-Cl bind F\textsuperscript{−}, Cl\textsuperscript{−}, and NO\textsubscript{3}\textsuperscript{−} at the feet and moderately strong evidence that they bind OAc\textsuperscript{−}, Br\textsuperscript{−}, and I\textsuperscript{−} at the feet. Although in some cases the association constants have a high degree of error, it appears that binding strength at this site increases as the anion becomes more chaotropic. However, the highly chaotropic anions ClO\textsubscript{4}\textsuperscript{−}, SCN\textsuperscript{−}, and ClO\textsubscript{3}\textsuperscript{−} do not appear to bind at the feet. It seems that the structure-making or structure-breaking nature of the anion is less important for binding at the feet than inside the pocket, if it is a factor at all. It is possible that binding at the feet is more dependent on the size and shape of the anion than is binding inside the pocket.

Obviously, much more investigation is needed to fully understand the binding of anions by these new hosts. One of the first issues to address is how to confidently identify the binding mode of each anion: i.e., no binding, 1:1 binding in the pocket, 1:1 binding at the feet, or 1:2 binding in the pocket and at the feet. A possible strategy for determining whether an anion binds to a host at the feet is to perform the salt titration with a very stable 1:1 host-guest complex like host-adaCl\textsubscript{2}H. This would minimize or eliminate \( j \) and \( s \) peak shifts due to pocket binding since a guest with much higher affinity would already occupy the pocket. This method would also allow a more accurate determination of \( K_a \) for binding at the feet. (However, this would not be a definitive determination of \( K_a \) unless the two binding sites were known to be independent.) In cases where 1:1 binding is confirmed, further investigation should be done into the thermodynamics of binding by creating vant Hoff plots. This would allow comparison of the thermodynamics of binding inside the pocket versus binding at the feet, as well as comparison of binding inside the pocket of these cationic hosts versus binding inside the pocket of OA. In cases where 1:2 binding is confirmed, a new binding model would need to be devised. This is a significant undertaking, since ternary binding events
are more complex than binary binding events. Moreover, the fact that the two binding sites are different adds an extra level of complexity. The cooperativity of the two sites is another factor to consider; if the two sites are interdependent and $\Delta G$ of binding for the overall process differs from the sum of $\Delta G$ of binding for the individual sites, then the binding model would need to be modified further.\textsuperscript{127} Indeed, fully describing the binding of anions that bind both inside the pocket and at the feet certainly be a challenging task.

These new host molecules present numerous opportunities for novel studies in molecular recognition. The fact that they are soluble in neutral to acidic pH makes them complementary to OA, which can only be studied at basic pH (OA aggregates and is sparingly soluble at neutral pH). The greater solubility of these hosts over a wider pH range expands the types of conditions and types of guests that can be used in binding studies. For instance, studies on the effect of pH on binding are possible with these hosts. The presence of two very different binding sites on these hosts provides an opportunity for the study of more complex binding events than have previously been investigated with cavitand hosts of this type. The ability to bind two different guests simultaneously (as was shown here by the evidence for Cl$^-$ binding at the feet of host$\texttt{-}\texttt{adaCO}_2\texttt{H}$ complexes and host$_2$-dodecane capsules) offers the potential for interesting new phenomena. The fact that these guests bind anions with some degree of specificity could have practical implication in terms of, for instance, sensing, anion transport, or water purification. Indeed, these host molecules are exciting new additions to this family of host molecule that should allow for much interesting research in the future.
4 Interactions of Water-Soluble Deep-Cavity Cavitands with Phospholipid Membranes

4.1 Introduction

Previous work has shown that OA binds with many guests that have biological and pharmaceutical relevance, including steroids\textsuperscript{56} and antimalarial compounds.\textsuperscript{128} This behavior gives OA and other water-soluble deep-cavity cavitands the potential to serve as drug delivery devices for compounds that are too reactive in free solution or have poor solubility, much in the same way that cyclodextrins are used. Focus on this goal gave rise to the obvious question of how these cavitands would interact with biological membranes. As noted in Section 1.4.3, certain CDs have been shown to interact with phospholipid membranes; for drug delivery, this is undesirable, but this property can be useful for other applications. The membrane-active compounds discussed in Section 1.4.3 are extraordinarily diverse in structure, but the one commonality of these compounds is amphiphilicity. As amphiphiles, water-soluble cavitands like OA have the potential to interact with phospholipid membranes. However, their structure is unlike any amphiphile yet studied, comprising a convex, hydrophilic exterior and a rigid hydrophobic pocket. This made the interaction of these compounds with membranes difficult to predict. Cyclodextrins are structurally the most similar class of membrane-active amphiphile since they too possess a hydrophilic exterior and a hydrophobic interior inside which hydrophobic guest molecules can bind. The major difference between CDs and our DCCs is that CDs are toroidal in shape. This allows for behavior that is impossible for our DCCs, such as embedding into the membrane to form an ion channel and threading of phospholipid acyl chains through the host cavity.

We predicted that the hydrophilic exterior of our cavitands would prevent them from embedding inside or traversing a membrane, since the membrane interior is hydrophobic. Thus, we did not expect these compounds to act as mobile carriers across the membrane or to form a persistent, organized channel inside the membrane. The studies involving hydrophilic CDs were judged to be the best guide as to what direction the research should take. As discussed above,
this body of research includes examples of CDs that cause membrane disruption\(^{97,98,129–133}\) and also CDs that manipulate membrane contents by extracting membrane components,\(^{101}\) depositing guest molecules into membranes,\(^{99,100}\) and even shuttling membrane components between membranes.\(^{102,104}\) The most logical starting point was to test for cavitand ability to cause membrane disruption. Research on membrane disruption by CDs has shown a link between disruption and CD binding with both phospholipid and sterol. Since the structure of the our cavitands appears compatible with both phospholipid and sterol binding (OA has been shown to bind cholesterol weakly and other membrane-relevant steroids such as progesterone strongly\(^{56}\)), it seemed logical to start with phospholipids only. Therefore, the available water-soluble cavitands were tested for their ability to disrupt membranes comprised only of phospholipid.

### 4.2 Experimental design

Six water-soluble deep-cavity cavitands were tested for their ability to cause membrane leakage. Three of these have already been introduced: OA \(^{20}\) (page 25), TEMOA \(^{23}\) (page 27), and G3 \(^{21}\) (page 27). The other three cavitands are octapropylsulfonate \(^{40}\) (OPS, Figure 49),\(^{134}\) hexylene-linked octaacid dimer \(^{41}\) (HDA), and phenylene-linked octaacid dimer \(^{42}\) (PDA) (Figure 50).\(^{135}\) (Unfortunately, OTA-Cl \(^{38}\) was not synthesized in time to include in these experiments. OAm-HCl \(^{36}\) and OPy-Cl \(^{39}\) are not soluble in the buffer used for these experiments, and time did not allow for additional experiments using a different buffer.) OPS has the same cavitand structure as OA, but is appended with eight sulfonic acid groups instead of eight carboxylic acids, making it soluble across the pH range except in very acidic pH. At the pH used for these studies (7.4), free OPS does not appear to aggregate. OPS has exhibited similar binding behavior to OA with slightly lower binding constants.\(^{134}\) HDA and PDA were formed by coupling two octaacid molecules with an amide-linked spacer at one of the rim carboxylates of each. Binding studies of these hosts are still under way, but in general they show a greater propensity towards capsule formation than OA.\(^{135}\) OA, TEMOA, HDA, and PDA all aggregate at neutral pH in the absence of guest, although this tendency is weaker in TEMOA. G3 is electronically neutral, while the other five hosts are anionic.
To study host-induced membrane leakage, we used LUVs comprised of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) as model membranes. POPC (Figure 51a) is a zwitterionic phospholipid that is often used to mimic animal cell membranes. Egg yolk phosphatidylcholine (EYPC) is also a popular choice of phospholipid for making liposomes that roughly mimic animal cells. It is a mixture of PC phospholipids that includes POPC and forms membranes with similar properties. Originally, we used EYPC, but we later switched to POPC since using a pure compound would facilitate NMR binding studies of the hosts with phospholipid. Employing a popular method for studying vesicle leakage, the LUVs were loaded with the fluorophore 8-aminonaphthalene-1,3,6-trisulfonic acid, disodium salt (ANTS) and the quencher p-xylene-bispyridinium bromide (DPX) (Figure 51a). In intact liposomes, ANTS fluorescence is quenched by DPX, but upon leakage of vesicle contents, ANTS quenching is relieved and leakage can be mon-
itored and quantified by measuring ANTS fluorescence emission (Figure 51b). At the end of the experiment, the detergent Triton X-100 was added to cause lysis, and maximum fluorescence was recorded. This type of experiment allows leakage to be continuously monitored and quantified. Fractional fluorescence can be expressed according to Equation 10,

\[
\text{Fractional fluorescence} = \frac{F(t) - F_0}{F_{\text{max}} - F_0}
\]  

where \( F(t) \) is fluorescence as a function of time, \( F_0 \) is fluorescence of intact liposomes, and \( F_{\text{max}} \) is fluorescence post-lysis. It should be noted that because the extent to which ANTS is quenched inside the vesicle can be affected by the leakage process itself, the fractional fluorescence is not an exact indicator of the fraction of vesicle contents leaked. However, fractional fluorescence is a very close indicator of fractional leakage.

\[\text{(a)}\] Structures of POPC, ANTS, and DPX. (b) Schematic representation of liposome leakage experiments.

This type of experiment does not provide information on the mechanism of leakage, that is whether leakage is “graded” or “all-or-none”. In graded leakage, partial leakage occurs in all vesicles in the same proportion, while in all-or-none leakage, some vesicles release all of their contents while others release none. The mechanism of leakage can be determined with the “requenching” assay.\textsuperscript{136–138} In this assay, the leakage experiment is carried out using LUVs loaded with ANTS and DPX. Once leakage has levelled off, the sample is titrated with DPX to quench external ANTS.
and allow determination of the degree of quenching of remaining internal ANTS. The technique is described by Equation 11,

\[
Q_{in} = \left[ \left(1 + K_d[\text{DPX}]_0(1 - f_{out})^\alpha \right) \left(1 + K_a[\text{DPX}]_0(1 - f_{out})^\alpha \right) \right]^{-1}
\]  

(11)

where \(Q_{in}\) is the measure of quenching of internal ANTS, \(f_{out}\) is the measure of fluorescence of external ANTS, and \(\alpha\) is the parameter of preferential release between ANTS and DPX (\(\alpha < 1\) if DPX release is preferred, \(\alpha > 1\) if ANTS release is preferred). \(K_d\) is the dynamic quenching constant for ANTS and DPX, \(K_a\) is the static quenching constant for ANTS and DPX (both of which must be determined for the experimental conditions used), and \([\text{DPX}]_0\) is the initial concentration of internal DPX. The experimental results for varying degrees of leakage are plotted to see if they more closely fit the all-or-none curve or the graded release curve. Representative theoretical curves are shown in Figure 52. The requenching assay was carried out for suitable cavitands.

![Figure 52](image_url)

**Figure 52:** Simulations of \(Q_{in}\) as a function of \(f_{out}\) for the performed requenching experiment.
4.3 Host-induced permeabilization of POPC liposomes

4.3.1 Host-induced membrane leakage: comparison of hosts

OA 20, TEMOA 23, HDA 41, PDA 42, OPS 40, and G3 21 were all tested for their ability to cause leakage of POPC LUVs at various cavity:lipid ratios (note that cavity and not molar concentration are used, to account for the two binding sites in HDA and PDA). In similar work with CDs, CD:lipid ratios are typically 1:10, 1:1, or even higher. In contrast, in research on membrane disruption by antimicrobial peptides, typical peptide:lipid ratios are 1:500 to 1:50. Indeed, apparent membrane leakage at AMP levels greater than 1:50 should not automatically be attributed to membrane permeabilization because other types of membrane perturbations are possible at high AMP levels. These include vesicle aggregation, vesicle fusion, phospholipid flip-flop, lipid phase separation, formation of non-bilayer phases, and membrane solubilization. Vesicle aggregation and fusion are particularly common when using anionic vesicles. Initially, our studies used cavity:lipid ratios ranging from 1:100 to 10:1, similar to amounts used in CD studies. A large amount of leakage was observed, so cavitands were tested at lower concentrations to learn the limit of their active range. It was found that most of the cavitands were somewhat less potent than AMPs but much more potent than membrane-permeabilizing CDs.

Plots of leakage at cavitand:POPC ratios of 1:200 and 1:50 are shown in Figure 53. In all cases where leakage occurs, the rate is high at the start of the experiment as a burst of leakage occurs, then slows drastically after a few minutes, until levelling off with incomplete leakage after 12 minutes (other experiments were carried out for up to one hour and also showed incomplete leakage). At the lower concentration, OPS causes a large amount of leakage, while all the other hosts cause almost no leakage. At the higher concentration, OA, TEMOA, and PDA cause a moderate amount of leakage, while G3 and HDA cause only small amounts of leakage. OPS remains by far the most potent leakage-causing host. (Earlier tests using higher host concentrations did show that G3 and HDA caused moderate leakage at higher concentrations.) Since OPS proved to be such a potent leakage-inducing agent, it was tested further at a range of concentrations, and
Figure 53: Host-induced leakage of POPC LUVs as a function of time at (top) 1:200 cavitand:POPC and (bottom) 1:50 cavitand:POPC.
the rate at which OPS causes leakage proved to be concentration-dependent (Figure 54). Remarkably, OPS levels as low as 1:1000 OPS:POPC still caused significant leakage. OA, TEMOA, and PDA were also found to cause leakage in a concentration-dependent manner. Conversely, increases in the concentration of G3 only caused small increases in leakage.

![Figure 54: OPS-induced leakage of POPC LUVs as a function of time.](image)

Often, these types of experiments can appear to indicate leakage when in reality the agent is causing vesicle aggregation or fusion, during which some leakage can occur.\textsuperscript{121} A simple method of testing for aggregation or fusion is to measure turbidity of the LUV suspension.\textsuperscript{139,140} A large increase in turbidity is indicative of membrane fusion or aggregation. This test was performed for each of the hosts at a 1:50 cavitand:POPC ratio, and no significant increase in turbidity was observed beyond that observed in the control experiment, presumably due to agitation and incorporation of air into the sample from stirring (Table 6).

### 4.3.2 Host-induced membrane leakage: effect of host complexation

Since one goal for our group is using these hosts to encapsulate drug molecules, we were interested to see if host-guest complexes had the same effect on membrane permeability as free, monomeric
Table 6: Host-induced turbidity changes in POPC LUVs.

<table>
<thead>
<tr>
<th>Host</th>
<th>% Turbidity t = 1 min</th>
<th>% Turbidity t = 5 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO blank</td>
<td>146</td>
<td>136</td>
</tr>
<tr>
<td>OPS</td>
<td>102</td>
<td>84</td>
</tr>
<tr>
<td>OA</td>
<td>129</td>
<td>124</td>
</tr>
<tr>
<td>TEMOA</td>
<td>138</td>
<td>130</td>
</tr>
<tr>
<td>HDA</td>
<td>142</td>
<td>141</td>
</tr>
<tr>
<td>PDA</td>
<td>152</td>
<td>143</td>
</tr>
<tr>
<td>G3</td>
<td>153</td>
<td>135</td>
</tr>
<tr>
<td>Triton X-100</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

host. OPS was studied since it is the most potent leakage-causing agent and was tested at a 1:50 OPS:POPC ratio as the monomeric free host, as a 1:1 complex with 1-adamantanecarboxylic acid, and as a 2:1 capsule with progesterone. AdaCO₂H was chosen as the 1:1 guest because it is the strongest-binding known 1:1 guest for these hosts. Analysis of OPS binding by ITC gave $K_a = 2 \times 10^6$ M$^{-1}$. Progesterone was chosen as the 2:1 guest because it is a very strong-binding guest that is also important in biological membranes. OA was previously shown to encapsulate progesterone more strongly than estradiol, which bound with a minimum $K_{app}$ of $1 \times 10^8$ M$^{-1}$.56 Judging by comparisons of OA and OPS binding that have been carried out, OPS can be expected to bind progesterone only slightly weaker than OA does.

The results are shown in Figure 55. The leakage was dramatically reduced for complexed versus free host, with the effect being strongest for the capsule. While leakage caused by the capsule levels off in a similar fashion to that of the free OPS, the leakage caused by the 1:1 complex continues to progress. It should be noted that under these conditions of very dilute host (8 µM), the complexed samples will have a fair amount of free host present. Given that $K_a = 2 \times 10^6$ M$^{-1}$ for the OPS·adaCO₂H complex, 22% of the host would be free. Assuming a rather low estimate of $K_{app}$ of $1 \times 10^7$ M$^{-1}$ for the OPS₂·progesterone capsule, 15% of the host would be free.

4.3.3 Mechanism of host-induced membrane leakage

To better understand how these cavitands are causing leakage, we carried out the requenching assay described in Section 4.2 for OPS, OA, and TEMOA to determine whether leakage was graded or
all-or-none. HDA and G3 were not used because they did not cause enough leakage at reasonable host concentrations to obtain a range of $f_{out}$ values. PDA was not used because at higher host concentrations necessary for higher $f_{out}$ values, the host causes significant quenching of ANTS fluorescence. Each host was tested at various concentrations to obtain a range of $f_{out}$ values. The results (Figure 56) are strongly supportive of an all-or-none mechanism for all three DCCs.

### 4.3.4 Studying host-phospholipid binding by NMR

Upon observing that these water-soluble cavitands induced membrane permeabilization, we hypothesized that leakage could be due at least in part to host binding of phospholipid molecules. Based on the size, shape, and hydrophobicity of the phospholipid molecule, it was expected that one of the acyl chains could bury itself into one of the host cavities. This possibility was explored using NMR binding studies. As mentioned early, EYPC was originally the phospholipid used for the liposome experiments, so binding of EYPC by OA was investigated by NMR. While the appearance of peaks far upfield supported OA binding of the ends of the EYPC acyl chains, analysis...
was difficult due to the fact that EYPC is a mixture of lipids, not a single compound. From this point on, both liposome and NMR binding experiments were carried out using POPC.

Since OPS was by far the most potent leakage-inducing host, it was first chosen for NMR binding studies with POPC. The experiment was carried out in a buffer that replicated the conditions from the liposome experiments: 10 mM sodium phosphate, 100 mM NaCl, pH 7.4. Unfortunately, OPS tends to have fairly broad peaks by NMR, which was exacerbated by the use of buffer with a high salt concentration, so results were difficult to analyze. We were able to observe the appearance of peaks far downfield (up to -3 ppm) upon addition of POPC, suggesting binding of one or both of the acyl chains, with the terminal methyl group anchored at the base of the cavity. Further analysis was not possible.

Next, TEMOA was studied instead of OPS, since this host has sharp peaks by NMR and has shown the lowest tendency to aggregate of all the carboxylic acid-coated hosts used for membrane studies. Also, borate buffer (10 mM Na₂B₄O₇) was used instead of phosphate buffer with NaCl. While this is less representative of the conditions used for the liposome experiments, TEMOA
tends to aggregate near neutral pH, and these conditions ensured minimal aggregation of TEMOA and thus sharp peaks. Figure 57 shows the NMR spectrum of free TEMOA compared to that of TEMOA with one equivalent of POPC. (It should be noted that because of the very low solubility of POPC in water, all of the POPC added to the sample is not necessarily in solution.) The appearance of new host $d$, $e$, and $b$ peaks are indicative of guest binding. The appearance of distinct peaks far upfield between 0.5 and -3.5 ppm is consistent with binding of one or more of the POPC acyl chains inside the host cavity with the terminal methyl group anchored in the base of the cavity. The presence of two distinct guest peaks at -3.4 ppm could signify that both alkyl chains of POPC bind inside the pocket, although this was not confirmed.

**Figure 57:** $^1$H NMR spectra of 1 mM TEMOA in 10 mM Na$_2$B$_4$O$_7$ with (1) no guest and (2) 1 equivalent of POPC. The label “Me” on the peaks around 1.7 ppm refers to the endo methyl groups at the rim of the cavity. Note: the three sections of the spectra do not have the same amplitude.

### 4.4 Discussion

All six hosts tested caused some degree of membrane permeabilization at varying concentrations. Hosts OA, TEMOA, and PDA caused leakage at concentrations typical for the upper range for antimicrobial peptides, while OPS caused leakage at OPS:POPC concentrations as low as 1:1000, making its activity similar to that of many AMPs. The leakage kinetics for these hosts show that an initial burst of leakage occurred at the start of the experiment, followed within minutes by a leveling off, resulting in incomplete leakage. Modeling studies of vesicle leakage have shown that
the formation of a single long-lived transmembrane pore would result in the complete leakage of vesicle contents in well under a second, a scenario that is inconsistent with the results presented here. Rather, the behavior observed here is indicative of partial transient release, where permeabilization is the result of a nonequilibrium membrane perturbation event. Furthermore, OPS, OA, and TEMOA, leakage was found to be all-or-none, yet complete leakage of vesicle contents was never observed, indicating that some vesicles undergo complete leakage while other vesicles in the exact same environment experience no leakage.

This behavior is sometimes observed with antimicrobial peptides and is attributed to the formation of transient pores (the explanation of graded leakage is less clear). Further explanation regarding AMPs is proposed by Rathinakumar et al., as illustrated in Figure 58: hundreds or thousands of peptide molecules bind to the membrane surface (A and B) and begin to partition into the membrane (C). This action can either occur in a nonpermeabilizing manner (D), or in a permeabilizing manner, such as the formation of a pore (E). Within the course of a few minutes, the system in both cases equilibrates such that the peptides are bound to the membrane surface and leakage ceases (F). AMP-membrane binding can be observed by monitoring the fluorescence of tryptophan in the peptide, and those results support this hypothesis. Unfortunately, monitoring membrane binding in this manner is not possible for our hosts, and attaching a fluorescent tag would be a nontrivial synthetic endeavor that could alter the very activity that is being tested. However, this is a reasonable hypothesis that explains the leakage caused by these hosts.

OPS-induced leakage was drastically attenuated by complexation with adaCO₂H to form a 1:1 complex and especially by complexation with progesterone to form a 2:1 capsule. Moreover, OPS and TEMOA were shown by NMR to bind with the acyl chains of POPC. These results strongly suggest that host binding with POPC plays some role in host-induced membrane permeabilization. Occupation of the host cavity with a strong-binding guest prior to exposure to the membrane would prevent binding with the less compatible and less available phospholipid to some extent. It is even possible that the leakage that did occur by OPS complexes was due to the small amount of free host present, not the complexed OPS. Host-induced leakage by phospholipid acyl
chain binding would be consistent with similar experiments exploring CD-induced membrane permeabilization. However, this explanation does not explain the difference observed between the different hosts, particular the much greater potency of OPS compared to the other five hosts. OPS has exhibited similar binding behavior to OA, suggesting that its ability to extract POPC from membranes should be similar to that of OA, yet OPS causes much greater leakage. The difference in potency between OPS and OA and possibly differences between other hosts suggests that phospholipid binding is not the only action responsible for host-induced leakage. It is also possible that
host aggregation inhibits host binding with POPC, which would explain why OPS causes greater leakage than the other anionic hosts. However, this does not explain the low activity of G3, which does not aggregate at neutral pH.

Even if we suppose that phospholipid binding is in some part responsible for host-induce leakage and that leakage is the results of transient pore formation via the mechanism proposed with AMPS, it is still difficult to envision how exactly leakage occurs. AMPs have hydrophobic and hydrophilic regions that allow them to bind with membranes and embed into them through hydrophobic and electrostatic interactions. The cavitands tested also have hydrophobic and hydrophilic regions, but the shape of these molecules does not intuitively seem compatible with membrane perturbation. Electrostatic interaction between the hydrophilic cavitand exterior and the membrane surface is certainly expected, but for an intact membrane, the hydrophobic interior is well shielded from the cavitand hydrophobic pocket. It is unclear then how the phospholipid tail, buried deep within the membrane, would gain access the the cavitand pocket. Similar experiments with CDs would be the best guide for understanding how this occurs, but such research tends to be more focused on identifying which CDs are suitable for pharmaceutical applications, and exploration into the mechanism of membrane disruption tends to be overlooked. Moreover, such experiments generally find that even membrane-active CDs are only active at concentrations much higher than those seen for most of these cavitands. Therefore, even if there were clear explanations as to how membrane-active CDs disrupt membranes, their applicability to these cavitands would be questionable.

The work discussed here is merely an introduction to this avenue of research with watersoluble deep cavity cavitands, and exciting studies certainly lie in the years ahead. The impetus for embarking on this work was the desire to use these cavitands in drug delivery. The hosts that caused less leakage, especially HDA and G3, might be promising for that application. Moreover, since complexed host was found to cause much less leakage, even hosts that do not appear to be suitable in uncomplexed form could be compatible with biological membranes when complexed with a drug. Additionally, it should be remembered that the LUVs used in these experiments are
merely membrane models that are predictive of interactions with biological membranes, and it
should not be assumed that interactions with biological membranes would be the same.

The new cationic cavitands OAm, OTA, and OPy should be an interesting addition to these
studies, complementing the current group of anionic and neutral cavitands. The results above sug-
gest that the cavitand hydrophilic coating has some influence on host-membrane interactions, so
it would not be surprising if any of these hosts exhibited different behavior from the six hosts al-
ready tested. Moreover, the positive charge of these hosts give them more potential as antimicrobial
agents, since microbial membranes exteriors typically have a net negative charge.

There is much further testing that could be performed to better understand host-induced
membrane disruption. For instance, using different sized fluorescein-labeled dextrans as fluores-
cent markers for membrane leakage could provide further evidence for or against the formation
of pores. Another possibility is testing with LUVs comprised of different phospholipids. POPC
LUVs are a common mimic of animal cell membranes, while LUVs made of a mixture of POPC
and POPG (1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1’-rac-glycerol) sodium salt, Figure 59)
are a common mimic of bacterial cell membranes. Such a comparison would help to identify cav-
itands with possible antimicrobial activity, and would be of particular interest with the cationic
cavitands. More saturated lipids tend to make less fluid bilayers. Testing with LUVs made of satu-
rated phospholipids such as DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine, Figure 59 could
be less vulnerable to host-induced leakage than POPC LUVs, since the phospholipid acyl chains
would be less accessible. Testing LUVs containing cholesterol would add an extra level of com-
plexity to these experiments; this would allow the testing of more realistic membranes, but since
OA is known to weakly bind cholesterol (and other cavitands are presumed to do the same), this
could add another possible route of membrane perturbation. Other types of membrane disruption
besides permeabilization could be studied as well, including phospholipid flip-flop and changes in
membrane fluidity.

In the longer term, it would be interesting to try to use cavitands to manipulate membrane
contents, much like some of the CDs described in Section 1.4.3. These cavitands could give rise
to new behavior not seen with CDs because of differences in binding behavior. For instance, the strongest binding guests of cavitands bind with association constants over $1 \times 10^7$ M$^{-1}$, which is much higher than is typically seen with CDs. Cavitands are also more selective hosts; for instance, OA binds estradiol very strongly ($K_{app} > 1 \times 10^8$ M$^{-1}$), whereas cholesterol is only weakly bound likely because it is too large to form a fully closed capsule. These properties could allow for more selectivity in the alteration of membrane contents than has been seen with CDs. Estrogens are a particularly attractive target, because certain estrogens are strong-binding guests with these cavitands and are present in certain membranes. These cavitands could have important therapeutic applications involving biological membranes containing estrogens. The research on host-membrane interactions presented here is only a start to this area of research, and many more interesting studies surely lie ahead.

**Figure 59:** Structures of DPPC and POPG.
5 Conclusion

The improved synthesis of octaacid (OA) has facilitated studies of this host molecule by providing easier access to a more pure product. Moreover, it has provided a more efficient route to the intermediate octol, which is also an intermediate to additional water-soluble cavitands. The synthesis of the new hosts octaamine HCl (OAm-HCl), octatrimethylammonium chloride (OTA-Cl) and octapyridinium chloride (OPy-Cl) greatly expands the range of studies that can be carried out with this class of host molecule. These cationic hosts are complimentary to the existing anionic and neutral host molecules octaacid (OA), tetra endo methyl-octaacid (TEMOA), and G3. As a group, these hosts allow studies across the pH range. Moreover, the new synthetic intermediate octamesylate has proven to be a valuable precursor to multiple new water-soluble hosts and could potentially lead to several more.

Preliminary studies of the molecular recognition properties of OAm-HCl, OTA-Cl, and OPy-Cl have shown both similarities to and differences from the binding behavior of OA. Binding of adaCO₂H and dodecane to form 1:1 host:guest complexes and 2:1 host:guest capsules respectively have shown behavior that is qualitatively similar to that of OA with these guests. Further studies are needed for full characterization, especially to investigate the possibility of enhanced 1:1 binding of anionic amphiphiles (like adaCO₂H) due to electrostatic interactions with cationic functional groups at the host rim. Conversely, binding of anions spanning the Hofmeister series showed marked differences from anion binding by OA. In addition to chaotropic anions, some mid-Hofmeister anions and one kosmotropic anion bind to the new hosts. Moreover, binding is observed both inside the hydrophobic pocket of these hosts and externally at the pendant feet. Binding constants, where calculation is possible, are significantly stronger than those observed for OA. Presumably, electrostatic interactions between the cationic substituents on the new hosts and some of the anions tested enhance the strength of binding inside the pocket and make external binding at the feet possible. Much work is needed to fully understand anion binding by these hosts.

Investigation of host-induced membrane permeabilization of POPC LUVs revealed the ability of these host molecules to induce membrane leakage with a wide range of potency between
the different hosts tested. The most active host, octapropylsulfonate (OPS) 40, caused permeabilization at concentrations typical for antimicrobial peptides. The least active hosts only caused significant leakage at much higher concentrations, more in the range seen with membrane-active cyclodextrins. A requenching assay to determine whether leakage was graded or all-or-none carried out with three of the most active hosts strongly indicated that leakage by all three hosts was all-or-none. This finding, in combination with the leakage kinetics observed, support the theory that the more active hosts cause partial transient leakage through the formation of transient pores.
6 Experimental Section

6.1 General

Reagents used for synthesis were purchased from Sigma-Aldrich, Acros, EMD, or Lancaster Synthesis. THF, DMSO, and pyridine were purchased in anhydrous form and used as is. DMF was dried over molecular sieves and degassed immediately prior to use. Lipids used for liposome preparation were purchased from Avanti Polar Lipids. ANTS and DPX were purchased from Invitrogen. Deuterated solvents were purchased from Cambridge Isotope Laboratories. All reactions were run under a nitrogen atmosphere.

NMR spectra were recorded on a 500 MHz Inova Varian NMR spectrometer with temperature regulated at 25°C unless otherwise noted. Chemical shifts are reported relative to DMSO-d$_6$ (2.50 ppm), CD$_3$OD (3.31 ppm), or D$_2$O (4.80 ppm). UV-Vis readings were carried out on a Varian Cary 500 UV-Vis-NIR spectrophotometer using quartz cuvettes. Fluorescence emission readings were carried out on a Perkin Elmer LS55 spectrophotometer using quartz cuvettes. Elemental analysis was performed by Atlantic Microlab, Inc. or Midwest Microlab, LLC. MALDI Mass spectral analysis was performed at the Laboratory for Biological Mass Spectrometry at Texas A&M University on an Applied Biosystems Voyager-DE STR instrument. Octaamine HCl 36 was the only compound for which a satisfactory mass spectrum could be obtained.

6.2 Compound synthesis

6.2.1 Modified synthesis of crude octol 22

This procedure was also reported in reference 123. A suspension of 6.84 g octabromide 29 (4.0 mmol, 1 equiv.), 3.36 g 3,5-dihydroxybenzyl alcohol (24.0 mmol, 6.0 equiv.), and 6.62 g (48.0 mmol, 12 equiv.) K$_2$CO$_3$ in 300 mL pyridine was prepared and N$_2$ gas bubbled through for 10 min while stirring. To the suspension was added 3.80 g (48.0 mmol, 12 equiv.) CuO nanopowder, and the mixture was immediately heated to vigorous reflux in a sand bath for 21 d (reaction is
unsuccessful when normal reflux is used). The reaction mixture was cooled to rt and the pyridine removed by rotovapor and drying under high vacuum for 1 h (further drying and/or exposure to air for extended time will result in greatly reduced yield). To the residue was added 250 mL THF and the mixture sonicated for 30 min. This mixture was filtered through THF-wet Celite and the filtrate solvent removed by rotovapor to give a brown solid that was dried overnight under high vacuum. To this solid was added 50 mL of CHCl₃ and the mixture sonicated for 20 min. The solid was isolated by filtration, and the rinse with CHCl₃ was repeated. The resulting solid was dried overnight under high vacuum at 120°C to yield 3.9 g crude octol 22 as an off-white powder (65% yield by weight with estimated purity of 75%, giving 45% estimated yield).

### 6.2.2 Synthesis of octamesylate 35

In a dry flask was prepared a suspension of 600 mg pure 22 (0.37 mmol, 1 equiv.) in 60 mL anhydrous THF. The suspension was sonicated 5 min, then cooled to 0°C. 1.03 mL triethylamine (7.42 mmol, 20 equiv.) was added, then 2.97 mL 2.0 M Ms₂O (5.93 mmol, 16 equiv.) in anhydrous THF (freshly prepared) was added dropwise. The mixture was stirred at 0°C for 5 min, then allowed to warm to rt. TLC (5/95 methanol/CHCl₃) after approximately 1 h showed the reaction was complete, and THF was removed by rotovapor, keeping bath temperature below 30°C. The residue was dissolved in CH₂Cl₂ (some insoluble material is normal) and run through a short silica gel plug (5/95 methanol/CHCl₃). The impure product was then purified by column chromatography, eluting first with CHCl₃, then with 1/99 methanol/CHCl₃. The resulting product was dissolved in minimal CH₂Cl₂ and precipitated with excess hexanes, collected by filtration, and washed with hexanes. The product was dried under high vacuum 12 h at 120°C to yield 499 mg of 35 as an off-white solid (60% yield). If crude 22 was used, the procedure was the same, but the final yield was lower.

¹H NMR (400 MHz, CDCl₃) δ 7.29 (s, 4H), 7.24 (d, J = 1.9 Hz, 8H), 7.00 (t, J = 2.2 Hz, 4H), 6.59 (t, J = 2.0 Hz, 4H), 6.50 (d, J = 2.1 Hz, 8H), 5.96 (s, 4H), 5.31 (s, 8H), 4.71 (t, J = 8.2 Hz, 4H), 4.51 (s, 4H), 4.37 (t, J = 5.9 Hz, 8H), 3.09 (s, 12H), 3.02 (s, 12H), 2.44 (m, 8H), 1.77 (m, 8H). Analysis calculated for C₁₀₄H₉₆O₄₀S₈: C, 55.71%; H, 4.32. Found: C, 55.68; H, 4.40.
6.2.3 Synthesis of octaazide 37

A solution of 276 mg 35 (123 \( \mu \)mol, 1 equiv.) and 70.3 mg sodium azide (1.08 mmol, 8.8 equiv.) in 6.89 mL anhydrous DMSO was stirred at room temperature for 4 d. The mixture was quenched with 15 mL water and allowed to cool to rt, then extracted with ethyl acetate three times. The organic layers were combined and washed with water twice, then washed with brine, dried with MgSO\(_4\), and rotovapped to dryness. The resulting residue was dried under high vacuum overnight (CAUTION: product should never be subjected to heat). The crude product was purified by column chromatography (85/15 CH\(_2\)Cl\(_2\)/hexanes). The purified product was dissolved in minimal CH\(_2\)Cl\(_2\) and precipitated with excess hexanes, collected by filtration, then dissolved in CH\(_2\)Cl\(_2\) and filtered to removed insoluble impurities. The solution was rotovapped and dried under high vacuum for 38 h to afford 162.8 mg of 37 as a white solid (77% yield). \(^1\)H NMR (500 MHz, CDCl\(_3\)) \( \delta \) 7.18 (d, \( J = 2.1 \) Hz, 8H), 7.16 (s, 4H), 6.99 (t, \( J = 2.2 \) Hz, 4H), 6.56 (t, \( J = 2.1 \) Hz, 4H), 6.52 (d, \( J = 1.8 \) Hz, 8H), 6.00 (s, 4H), 4.77 (t, \( J = 8.2 \) Hz, 4H), 4.52 (s, 4H), 4.46 (s, 8H), 3.42 (t, \( J = 6.4 \) Hz, 8H), 2.35 (m, 8H), 1.62 (m, 8H). Analysis calculated for C\(_{96}\)H\(_{72}\)N\(_{24}\)O\(_{16}\): C, 63.43; H, 3.99; N, 18.49. Found: C, 63.24; H, 3.89; N, 18.22.

6.2.4 Synthesis of octaamine HCl 36

To a solution of 42.6 mg 37 (23.4 \( \mu \)mol, 1 equiv.) in 3.2 mL THF was added 98.2 mg triphenylphosphine (374 \( \mu \)mol, 16.0 equiv.). Once the PPh\(_3\) was dissolved, 32 \( \mu \)L water was added and the reaction mixture was stirred at rt for 4 d. The THF was then removed by a rotovapor, and 5 mL of diethyl ether and 2 mL of 0.1 M HCl was added to the residue and stirred until all solid was dissolved. The organic layer was discarded and the aqueous layer was washed with ether three times. The aqueous layer was freeze-dried for 2 d to obtain the desired product. Further drying under high vacuum at 120\(^\circ\)C for 5 d removed most residual ether. The product was dissolved in methanol, evaporated to dryness, and dried under high vacuum at 120\(^\circ\)C for 3 d to yield 20.5 mg of 36 as a light yellow solid (46% yield). \(^1\)H NMR (500 MHz, CD\(_3\)OD) \( \delta \) 7.91 (s, 4H), 7.36 (s, 8H), 7.02 (s, 4H), 6.68 (s, 4H), 6.57 (s, 8H), 5.98 (s, 4H), 4.71 (t, \( J = 8.5 \) Hz, 4H), 4.70 (s, 4H),
4.21 (s, 8H), 3.12 (t, $J = 7.2$ Hz, 8H), 2.75 (m, 8H), 1.72 (m, 8H). $^1$H NMR (500 MHz, D$_2$O) $\delta$ 7.81 (s, 4H), 7.45 (s, 8H), 7.21 (s, 4H), 6.89 (s, 4H), 6.54 (s, 8H), 6.04 (s, 4H), 4.72 (t, $J = 8.2$ Hz, 4H), 4.55 (s, 4H), 4.33 (s, 8H), 2.66 (m, 8H), 1.74 (m, 8H). Peaks were identified by COSY NMR, as shown in Figure 60 (see Figure 30 for 1D spectrum with peak assignments). Analysis calculated for C$_{96}$H$_{96}$Cl$_8$N$_8$O$_{16}$·16 H$_2$O: C, 52.66; H, 5.89; N, 5.12. Found: C, 56.86; H, 4.78; N, 5.10 (difference from the expected elemental analysis results is attributed to a small amount of triphenylphosphine oxide, excess HCl, error in water content calculation, and possibly other trace impurities). MS (MALDI): calculated 1901.4, found 1561.2, 1633.1, and 1870.7. (Results could reflect fragmentation of the molecule and/or loss of chloride. MS analysis will be repeated.)

6.2.5 Synthesis of octatrimethylammonium chloride 38

To a solution of 101 mg 35 (45.2 µmol, 1 equiv.) in 3.35 mL dry, degassed DMF was added 1.71 mL trimethylamine (33% in ethanol, 7.2 mmol). The reaction mixture was stirred at 50°C for 2 d. The mixture was cooled to rt, and excess diethyl ether was added to precipitate the product, which was isolated by filtration, rinsed with ether, and dried under high vacuum overnight (the product should never be heated, as this will cause decomposition). The product was dissolved in minimal water, filtered through a pre-rinsed 0.45 µm PTFE syringe filter, and passed through a column of Dowex 1X8 200, chloride form. The product solution was then freeze-dried until all ice was sublimed. Grease contamination from the ion exchange column was removed by sonication with acetone and filtration. The product was dried under high vacuum 2 d to obtain 65.2 mg of 38 as a white solid (64% yield). $^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 7.97 (s, 4H), 7.59 (d, $J = 1.7$ Hz, 8H), 7.10 (s, 4H), 6.93 (s, 4H), 6.52 (d, $J = 1.2$ Hz, 8H), 5.92 (s, 4H), 4.77 (t, $J = 8.2$ Hz, 4H), 4.72 (s, 8H), 4.70 (s, 4H), 3.61 (m, 8H), 3.22 (s, 36H), 3.17 (s, 36H), 2.72 (m, 8H), 1.83 (m, 8H). $^1$H NMR (500 MHz, D$_2$O) $\delta$ 7.88 (s, 4H), 7.60 (s, 8H), 7.25 (s, 4H), 7.13 (s, 4H), 6.53 (s, 8H), 6.01 (s, 4H), 4.75 (t, $J = 8.1$ Hz, 4H), 4.67 (s, 8H), 4.60 (s, 4H), 3.54 (m, 8H), 3.21 (s, 36H), 3.11 (s, 36H), 2.67 (m, 8H), 1.86 (m, 8H). Peaks were identified by COSY NMR, as shown in Figure 61 (see Figure 31 for 1D spectrum with peak assignments). Analysis calculated for C$_{120}$H$_{144}$Cl$_8$N$_8$O$_{16}$·21 H$_2$O: C,
55.09; H, 7.17; N, 4.28; Cl, 10.84. Found: C, 55.22; H, 5.90; Cl, 9.41; N, 4.04 (difference from the expected elemental analysis results is attributed to error in water content calculation, and trace impurities, including salt and polymer).

### 6.2.6 Synthesis of octapyridinium chloride 39

A solution of 94.4 mg 35 (42.1 µmol) in 4.2 mL pyridine was stirred at 80°C for 17 h. The pyridine was removed by a rotovapor and the product dried under high vacuum for 2 h (product should never
be subjected to heat, as this will cause decomposition). To remove remaining pyridine, the product was dissolved in minimal methanol and precipitated by addition of excess diethyl ether. The solid was collected by filtration, dissolved in methanol, run through the filter, and rotovapped to dryness. This precipitation process was repeated and the product dried under high vacuum overnight to obtain the mesylate salt. This product was dissolved in minimal water, filtered through a pre-rinsed 0.45 µm PTFE syringe filter and passed through a column of Dowex 1X8 200, chloride form. The resulting product solution was freeze-dried 3 d to obtain 89.3 mg of 39 as a pale orange solid (88%
yield. $^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 9.29 (d, $J = 5.8$ Hz, 8H), 9.18 (d, $J = 5.7$ Hz, 8H), 8.67 (t, $J = 7.8$ Hz, 3H), 8.54 (t, $J = 7.8$ Hz, 3H), 8.24 (t, $J = 7.0$ Hz, 8H), 8.09 (t, $J = 7.1$ Hz, 8H), 8.03 (s, 4H), 7.51 (d, $J = 1.8$ Hz, 8H), 6.99 (s, 4H), 6.73 (s, 4H), 6.42 (s, 8H), 5.98 (s, 8H), 5.70 (s, 4H), 4.99 (t, $J = 7.0$ Hz, 8H), 4.69 (t, $J = 8.2$ Hz, 4H), 4.57 (s, 4H), 2.83 (m, 8H), 2.14 (m, 8H). $^1$H NMR (500 MHz, D$_2$O) $\delta$ 9.08 (d, $J = 5.8$ Hz, 8H), 9.00 (d, $J = 5.7$ Hz, 8H), 8.60 (t, $J = 7.8$ Hz, 4H), 8.33 (t, $J = 7.9$ Hz, 4H), 8.13 (t, $J = 7.0$ Hz, 8H), 7.98 (t, $J = 7.1$ Hz, 8H), 7.94 (s, 4H), 7.54 (s, 8H), 7.15 (s, 4H), 6.93 (s, 4H), 6.30 (s, 8H), 5.96 (s, 8H), 5.55 (s, 4H), 4.88 (t, $J = 7.2$ Hz, 8H), 4.67 (t, $J = 8.1$ Hz, 4H), 4.38 (s, 4H), 2.80 (m, 8H), 2.17 (m, 8H). Peaks were identified by COSY NMR, as shown in Figure 62 (see Figure 32 for 1D spectrum with peak assignments). Analysis calculated for C$_{136}$H$_{112}$Cl$_8$N$_8$O$_{16}$·12 H$_2$O: C, 62.48; H, 5.24; Cl, 10.85; N, 4.29. Found: C, 63.26; H, 4.87; Cl, 10.34; N, 4.21.
Figure 62: COSY NMR spectrum of 0.5 mM OPy-Cl in D$_2$O.
6.3 Binding studies

NMR binding studies for all hosts were carried out using solutions of 0.5 mM host in D$_2$O unless otherwise noted. Studies with OPy-Cl and OTA-Cl were carried out using 600 µL host solution. Studies of OAm-HCl were carried out using 500 or 600 µL of host solution.

6.3.1 Binding with adaCO$_2$H

For studies of binding with adaCO$_2$H, aliquots of a 30 mM solution of guest in DMSO-d$_6$ were added to the host solution, the sample shaken, and the spectrum recorded. The NMR spectra of titration of adaCO$_2$H into OTA-Cl are shown in Figure 63. The COSY NMR spectra of the 1:1 complexes of OPy-Cl, OTA-Cl, and OAm-HCl with adaCO$_2$H are shown in Figure 64, Figure 65, and Figure 66, respectively. For OTA-Cl and OPy-Cl, minimum association constants were obtained by recording NMR spectra of 5 µM host with 1 equivalent of guest (Figure 37 and Figure 67). In both cases, no free host was visible; assuming a maximum of 5% free host present, $K_a > 4 \times 10^6$ M$^{-1}$ was calculated for both hosts, according to Equation 6. The association constant for OAm-HCl could not be obtained in this manner (vide supra), but the NMR spectrum was recorded at 5 µM host with 1.5 equivalents of guest (Figure 68).
Figure 63: $^1$H NMR spectra of 0.5 mM OTA-Cl in D$_2$O with (1) 0, (2) 0.5, (3) 1.0, and (4) 1.5 equivalents of adaCO$_2$H. Bound host peaks are indicated by an asterisk. Refer to Figure 33 for host peak labels.
Figure 64: COSY NMR spectrum of 0.5 mM OPy-Cl in D$_2$O with 1.5 equivalents of adaCO$_2$H. Refer to Scheme 9 for host peak labels.
Figure 65: COSY NMR spectrum of 0.5 mM OTA-Cl in D$_2$O with 2.0 equivalents of adaCO$_2$H. Refer to Figure 33 for host peak labels.
Figure 66: COSY NMR spectrum of 0.5 mM OAm-HCl in D$_2$O with 1.5 equivalents of adaCO$_2$H. Refer to Figure 33 for host peak labels.
Figure 67: $^1$H NMR spectra of OTA-Cl in D$_2$O: (1) 0.5 mM OTA-Cl, (2) 0.5 mM OTA-Cl with 1.0 equivalent adaCO$_2$H, (3) 5 µM OTA-Cl with 1.0 equivalent of adaCO$_2$H. Bound host peaks are indicated by an asterisk. Refer to Figure 33 for host peak labels.

Figure 68: $^1$H NMR spectra of OAm-HCl in D$_2$O: (1) 0.5 mM OAm-HCl, (2) 0.5 mM OAm-HCl with 1.5 equivalents adaCO$_2$H, (3) 5 µM OAm-HCl with 1.5 equivalents of adaCO$_2$H. Bound host peaks are indicated by an asterisk. Refer to Figure 33 for host peak labels.
6.3.2 Binding with dodecane

For studies of binding with dodecane, a small drop of dodecane was added to the guest solution and the sample shaken and then sonicated for at least one hour before the spectrum was recorded. If the spectrum showed free host, the sample was sonicated further until no free host was visible, indicating equilibration. The NMR spectra of free OTA-Cl versus 2:1 capsule are shown in Figure 69. The COSY NMR spectra of the 2:1 capsules of OPy-Cl, OTA-Cl, and OAm-HCl with dodecane are shown in Figure 70, Figure 71, and Figure 72, respectively. The NMR spectra for dilution studies for OTA-Cl and OAm-HCl complexation are shown in Figure 73 and Figure 74.

![Figure 69: 1H NMR spectra of 0.5 mM OTA-Cl in D2O with (1) no guest and (2) excess dodecane. Bound host peaks are indicated by an asterisk. Refer to Figure 33 for host peak labels.](image-url)


Figure 70: Bound host region of COSY NMR spectrum of 0.5 mM OPy-Cl in D$_2$O with excess dodecane. Refer to Scheme 9 for host peak labels.
**Figure 71:** COSY NMR spectrum of 0.5 mM OTA-Cl in D$_2$O with excess dodecane. Refer to Figure 33 for host peak labels.
Figure 72: COSY NMR spectrum of 0.5 mM OAm-HCl in D₂O with excess dodecane. Refer to Figure 33 for host peak labels.
Figure 73: $^1$H NMR spectra of OTA-Cl in D$_2$O: (1) 0.5 mM OTA-Cl, (2) 0.5 mM OTA-Cl with excess dodecane, (3) 5 µM OTA-Cl with excess dodecane. Bound peaks are indicated by an asterisk. Refer to Figure 33 for host peak labels.

Figure 74: $^1$H NMR spectra of OAm-HCl in D$_2$O: (1) 0.5 mM OAm-HCl, (2) 0.5 mM OAm-HCl with excess dodecane, (3) 5 µM OAm-HCl with excess dodecane. Bound peaks indicated by an asterisk. Refer to Figure 33 for host peak labels.
6.3.3 Binding with anions

For each anion binding study, aliquots of salt solution in D$_2$O in the range of 30 mM to 1.2 M were added to the host solution, the sample shaken, and the NMR spectrum recorded. Association constants were obtained from the binding isotherms by curve fitting using Microsoft Excel Solver. In the following figures are displayed $^1$H NMR spectra and binding isotherms for salt titrations with OTA-Cl and OPy-Cl. For improved clarity, only the relevant portions of the NMR spectra are shown, and some spectra that were used for binding constant calculation are omitted.

OPy-Cl binding of anions The association constants derived from analysis of shifts in the $j$, $b$, and $s$ peaks are displayed in Table 7. In some cases, accuracy was reduced by factors such as peak broadness and peak overlap with other peaks or with water. In cases where multiple peaks gave satisfactory results, the $j$ peak was usually preferred because it is a relatively sharp singlet.

**Table 7:** All calculations of association constants for OPy-Cl binding of anions.

<table>
<thead>
<tr>
<th>Anion</th>
<th>$b$</th>
<th>$j$</th>
<th>$s$</th>
</tr>
</thead>
<tbody>
<tr>
<td>F$^-$</td>
<td>-$^a$</td>
<td>11.5</td>
<td>7.7</td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td>222$^b$</td>
<td>321</td>
<td>380</td>
</tr>
<tr>
<td>OAc$^-$</td>
<td>-</td>
<td>16.3</td>
<td>25.2</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>67</td>
<td>207$^c$</td>
<td>121</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>-</td>
<td>545</td>
<td>566</td>
</tr>
<tr>
<td>Br$^-$</td>
<td>918</td>
<td>n.d.$^c,d$</td>
<td>982</td>
</tr>
<tr>
<td>ClO$_3^-$</td>
<td>396$^b$</td>
<td>324</td>
<td>345</td>
</tr>
<tr>
<td>I$^-$</td>
<td>n.d.$^d,e$</td>
<td>2973</td>
<td>3319</td>
</tr>
<tr>
<td>SCN$^-$</td>
<td>881$^e$</td>
<td>2285</td>
<td>2314$^f$</td>
</tr>
<tr>
<td>ClO$_4^-$</td>
<td>2474</td>
<td>2982</td>
<td>2875</td>
</tr>
</tbody>
</table>

$^a$ A dash indicates no binding was observed. $^b$ Decreased accuracy due to peak broadness. $^c$ Low accuracy or binding constant not determined due to poor fitting. $^d$ “N.d.” indicates binding constant could not be determined. $^e$ Low accuracy or binding constant not determined due to peak overlap with water peak. $^f$ The last two data points were omitted due to peak broadness.
Figure 75: The $j$, $b$, and $s$ peak regions of $^1$H NMR spectra of 0.5 mM OPy-Cl in D$_2$O with NaF at: (1) 0, (2) 1, (3) 5, (4) 10, (5) 20, (6) 30, (7) 40, and (8) 50 equivalents.
Figure 76: Binding isotherms for titration of OPy-Cl with NaF.
**Figure 77:** The $j$, $b$, and $s$ peak regions of $^1$H NMR spectra of 0.5 mM OPy-Cl in D$_2$O with Na$_2$SO$_4$ at: (1) 0, (2) 1, (3) 3, (4) 6, (5) 10, (6) 15, (7) 20, (8) 25, and (9) 30 equivalents.

**Figure 78:** Binding isotherms for titration of OPy-Cl with Na$_2$SO$_4$. 

115
**Figure 79:** The \( j \), \( b \), and \( s \) peak regions of \( ^1H \) NMR spectra of 0.5 mM OPy-Cl in D\(_2\)O with NaOAc at: (1) 0, (2) 5, (3) 10, (4) 20, (5) 30, (6) 50, (7) 70, and (8) 90 equivalents.

**Figure 80:** Binding isotherms for titration of OPy-Cl with NaOAc.
Figure 81: The $j$, $b$, and $s$ peak regions of $^1$H NMR spectra of 0.5 mM OPy-Cl in D$_2$O with NaCl at: (1) 0, (2) 2, (3) 6, (4) 10, (5) 14, (6) 20, (7) 30, (8) 50, and (9) 70 equivalents.
Figure 82: Binding isotherms for titration of OPy-Cl with NaCl.
Figure 83: The $j$, $b$, and $s$ peak regions of $^1$H NMR spectra of 0.5 mM OPy-Cl in D$_2$O with NaNO$_3$ at: (1) 0, (2) 1, (3) 2, (4) 4, (5) 10, (6) 16, (7) 25, and (8) 40 equivalents.

Figure 84: Binding isotherms for titration of OPy-Cl with NaNO$_3$. 
Figure 85: The \( j, b, \) and \( s \) peak regions of \(^1\)H NMR spectra of 0.5 mM OPy-Cl in D\(_2\)O with NaBr at: (1) 0, (2) 0.5, (3) 1, (4) 2, (5) 5, (6) 10, (7) 16, and (8) 25 equivalents.
Figure 86: Binding isotherms for titration of OPy-Cl with NaBr.
Figure 87: The $j$, $b$, and $s$ peak regions of $^1$H NMR spectra of 0.5 mM OPy-Cl in D$_2$O with NaClO$_3$ at: (1) 0, (2) 0.5, (3) 1, (4) 2, (5) 3, (6) 5, (7) 7, (8) 10, (9) 15, and (10) 21 equivalents.

Figure 88:Binding isotherms for titration of OPy-Cl with NaClO$_3$. 
Figure 89: The $j$, $b$, and $s$ peak regions of $^1$H NMR spectra of 0.5 mM OPy-Cl in D$_2$O with NaI at: (1) 0, (2) 0.25, (3) 0.5, (4) 1, (5) 1.5, (6) 3, (7) 4, (8) 6, (9) 8, and (10) 10 equivalents. At higher salt equivalents, the $b$ peak overlaps with the water peak.

Figure 90: Binding isotherms for titration of OPy-Cl with NaI.
**Figure 91:** The $j$, $b$, and $s$ peak regions of $^1$H NMR spectra of 0.5 mM OPy-Cl in D$_2$O with NaSCN at: (1) 0, (2) 0.5, (3) 1, (4) 2, (5) 3, (6) 4, (7) 6, (8) 8, and (9) 10 equivalents. At higher salt equivalents, the $b$ peak overlaps with the water peak.

**Figure 92:** Binding isotherms for titration of OPy-Cl with NaSCN.
**Figure 93:** The $j$, $b$, and $s$ peak regions of $^1$H NMR spectra of 0.5 mM OPy-Cl in D$_2$O with NaClO$_4$ at: (1) 0, (2) 0.2, (3) 0.4, (4) 0.6, (5) 1, (6) 1.5, (7) 2, (8) 2.5, (9) 3, and (10) 4 equivalents. (Addition of 6 equiv. of salt caused host to precipitate.)

**Figure 94:** Binding isotherms for titration of OPy-Cl with NaClO$_4$. 

125
OTA-Cl 38 binding of anions  The association constants derived from analysis of shifts in the $j$, $b$, and $s$ peaks are displayed in Table 8. The same comments for anion binding by OPy-Cl apply.

Table 8: All calculations of association constants for OTA-Cl binding of anions.

<table>
<thead>
<tr>
<th>Anion</th>
<th>$b$</th>
<th>$j$</th>
<th>$s$</th>
</tr>
</thead>
<tbody>
<tr>
<td>F$^-$</td>
<td>-$^a$</td>
<td>17.1</td>
<td>14.6</td>
</tr>
<tr>
<td>SO$_4^{2-}$</td>
<td>437$^b$</td>
<td>326</td>
<td>326</td>
</tr>
<tr>
<td>OAc$^-$</td>
<td>6.7</td>
<td>19.6</td>
<td>20.8</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>-</td>
<td>162</td>
<td>115</td>
</tr>
<tr>
<td>NO$_3^-$</td>
<td>90.7$^c$</td>
<td>365</td>
<td>389</td>
</tr>
<tr>
<td>Br$^-$</td>
<td>13.3</td>
<td>3378</td>
<td>1256</td>
</tr>
<tr>
<td>ClO$_3^-$</td>
<td>457$^c,d$</td>
<td>288</td>
<td>276</td>
</tr>
<tr>
<td>I$^-$</td>
<td>632$^c$</td>
<td>2055</td>
<td>4549</td>
</tr>
<tr>
<td>SCN$^-$</td>
<td>1460</td>
<td>1915</td>
<td>2332</td>
</tr>
<tr>
<td>ClO$_4^-$</td>
<td>3856</td>
<td>2695</td>
<td>2560</td>
</tr>
</tbody>
</table>

$^a$ A dash indicates no binding was observed.  $^b$ Decreased accuracy due to poor fitting.  $^c$ Decreased accuracy due to peak overlap with water.  $^d$ Decreased accuracy due to peak broadness.
Figure 95: The $j$, $b$, and $s$ peak regions of $^1$H NMR spectra of 0.5 mM OTA-Cl in $D_2O$ with NaF at: (1) 0, (2) 5, (3) 10, (4) 15, (5) 20, (6) 30, (7) 40, (8) 50, (9) 60 equivalents.
Figure 96: Binding isotherms for titration of OTA-Cl with NaF.
Figure 97: The $j$, $b$, and $s$ peak regions of $^1$H NMR spectra of 0.5 mM OTA-Cl in D$_2$O with Na$_2$SO$_4$ at: (1) 0, (2) 1, (3) 2, (4) 4, (5) 6, (6) 10, (7) 15, (8) 20, (9) 30 equivalents.
Figure 98: Binding isotherms for titration of OTA-Cl with Na₂SO₄.
Figure 99: The j, b, and s peak regions of $^1$H NMR spectra of 0.5 mM OTA-Cl in D$_2$O with NaOAc at: (1) 0, (2) 4, (3) 10, (4) 20, (5) 30, (6) 40, (7) 60, and (8) 80 equivalents.

Figure 100: Binding isotherms for titration of OTA-Cl with NaOAc.
**Figure 101:** The $j$, $b$, and $s$ peak regions of $^1$H NMR spectra of 0.5 mM OTA-Cl in D$_2$O with NaCl at: (1) 0, (2) 3, (3) 6, (4) 10, (5) 20, (6) 30, (7) 40, (8) 60, and (9) 80 equivalents.

**Figure 102:** Binding isotherms for titration of OTA-Cl with NaCl.
**Figure 103:** The $j$, $b$, and $s$ peak regions of $^1$H NMR spectra of 0.5 mM OTA-Cl in D$_2$O with NaNO$_3$ at: (1) 0, (2) 1, (3) 3, (4) 6, (5) 12, (6) 18, (7) 30, (8) 45, and (9) 60 equivalents.

**Figure 104:** Binding isotherms for titration of OTA-Cl with NaNO$_3$.

133
Figure 105: The $j$, $b$, and $s$ peak regions of $^1$H NMR spectra of 0.5 mM OTA-Cl in D$_2$O with NaBr at: (1) 0, (2) 0.4, (3) 1, (4) 2, (5) 4, (6) 10, (7) 20.5, (8) 25, and (9) 40 equivalents.
Figure 106: Binding isotherms for titration of OTA-Cl with NaBr.
Figure 107: The $j$, $b$, and $s$ peak regions of $^1$H NMR spectra of 0.5 mM OTA-Cl in D$_2$O with NaClO$_3$ at: (1) 0, (2) 0.5, (3) 0.9, (4) 2.5, (5) 3, (6) 5, (7) 10, (8) 14, (9) 20, (10) 30, and (11) 40 equivalents.

Figure 108: Binding isotherms for titration of OTA-Cl with NaClO$_3$.
Figure 109: The $j$, $b$, and $s$ peak regions of $^1$H NMR spectra of 0.5 mM OTA-Cl in D$_2$O with NaI at: (1) 0, (2) 0.3, (3) 0.6, (4) 1, (5) 2, (6) 3, (7) 5, (8) 7, and (9) 11 equivalents.
Figure 110: Binding isotherms for titration of OTA-C1 with NaI.
Figure 111: The $j$, $b$, and $s$ peak regions of $^1$H NMR spectra of 0.5 mM OTA-Cl in D$_2$O with NaSCN at: (1) 0, (2) 0.2, (3) 0.4, (4) 0.6, (5) 1, (6) 2, (7) 3, (8) 5, (9) 7, and (10) 11 equivalents.

Figure 112: Binding isotherms for titration of OTA-Cl with NaSCN.
Figure 113: The \( j \), \( b \), and \( s \) peak regions of \( ^1\text{H} \) NMR spectra of 0.5 mM OTA-Cl in D\(_2\)O with NaClO\(_4\) at:
(1) 0, (2) 0.2, (3) 0.4, (4) 0.6, (5) 0.8, (6) 1.2, (7) 2.4, (8) 6, and (9) 10 equivalents.

Figure 114: Binding isotherms for titration of OTA-Cl with NaClO\(_4\).
6.4 Membrane experiments

6.4.1 General

Liposomes were prepared from POPC by extrusion. Approximately 3 mL of POPC solution in CHCl$_3$ was evaporated in a round bottom flask by rotovapor to form a thin lipid film, which was further dried under high vacuum for at least 2 h. The lipid film was hydrated with the appropriate intravesicular buffer by adding 1 mL of buffer to the flask and spinning the flask on a rotovapor without heat for 1 h. The mixture was then subjected to at least nine freeze-thaw cycles, vortexing for 30 s after every third cycle. The resulting multilamellar liposomes were extruded through a 100 nm polycarbonate membrane for 21 passes using an Avanti mini-extruder. (In some cases, the liposomes were first passed several times through a 200 nm membrane. This step facilitates extrusion through the 100 nm membrane, but is not necessary.) The resulting unilamellar liposome suspension was then passed through a column of Sephadex G-50 equilibrated with extravesicular buffer to remove unentrapped fluorophore. The lipid concentration of the resulting liposome suspension was determined using the Stewart Assay. Liposomes were stored in a refrigerator and used within 3-4 days of preparation.

For both leakage and requenching experiments, fluorescence was recorded with the excitation slit at 5 nm, the emission slit at 15 nm, and with a 430 nm cutoff filter. Host stock solutions were prepared in DMSO. Some hosts had low solubility and precipitated upon addition to the buffer solution; for this reason, liposomes were added to host solution. Each experiment was conducted with a 2 mL sample of 400 µM POPC under continuous stirring. Each sample contained 10 µL total of DMSO (host solution and/or plain DMSO). Vesicle lysis was caused by addition of a 10% v/v solution of Triton X-100 in extravesicular buffer.

6.4.2 Leakage experiments

For leakage experiments, LUVs were loaded with 10 mM sodium phosphate buffer with 24 mM NaCl, 12.5 mM ANTS, and 45 mM DPX at pH 7.4. The extravesicular solution was 10 mM
sodium phosphate with 102 mM NaCl at pH 7.4. Fluorescence was recorded with $\lambda_{ex} = 355$ nm and $\lambda_{em} = 513$ nm. For each leakage experiment, a solution of host (or DMSO only for control) in extravesicular buffer was prepared and recording of fluorescence emission was started. At $t = 30$ s, liposome suspension (at a volume that gave an overall concentration of 400 $\mu$M POPC) was injected into the sample. At $t = 750$ s, 200 $\mu$L Triton X-100 was injected and maximal fluorescence recorded for at least 30 s. Fractional fluorescence over the course of the experiment was calculated according to Equation 10 (page 77), with values corrected for dilution. Since $F_0$ could not be measured directly because liposomes were added to host solution, separate measurements for this value were recorded and applied to all experiments. Results reported are the average of three runs.

For leakage experiments comparing free OPS to the OPS-adaCO$_2$H complex and the OPS$_2$-progesterone capsule, the same procedure was used, but the liposomes were added to the solution of host-guest complex. These solutions were prepared one day before the experiment to ensure full equilibration. The guests were added as stock solutions in DMSO.

### 6.4.3 Requenching experiments

For requenching experiments, LUVs were loaded with 10 mM sodium phosphate buffer with 84 mM NaCl, 5 mM ANTS, and 8.1 mM DPX at pH 7.4. The extravesicular solution was 10 mM sodium phosphate with 100 mM NaCl at pH 7.4. The requenching solution was 10 mM sodium phosphate with 42 mM NaCl and 45 mM DPX at pH 7.4. The dynamic quenching constant $K_d$ and the static quenching constant $K_a$ for ANTS and DPX were determined as described in reference: $^{137}$ $K_d = 45$ M$^{-1}$ and $K_a = 307$ M$^{-1}$. Fluorescence was recorded with $\lambda_{ex} = 355$ nm and $\lambda_{em} = 520$ nm. For each requenching experiment, a solution of host in extravesicular buffer was prepared and recording of fluorescence emission was started. At $t = 30$ s, liposome suspension (at a volume that gave an overall concentration of 400 $\mu$M POPC) was injected into the sample. After 30 min, at which point leakage had levelled off, requenching solution was titrated into the sample in four 25 $\mu$L aliquots. At $t = 2140$ s, 100 $\mu$L Triton X-100 was added to cause vesicle
ysis. Results were analyzed as described in reference. Results reported are the average of two runs.

6.4.4 Liposome aggregation/fusion testing

LUVs were tested for aggregation and fusion by measuring turbidity using the same liposomes and buffers as used for the leakage experiments. For each experiment, liposomes were added to a solution of host in buffer such that cavitand:POPC ratio was 1:50, POPC concentration was 400 µM, DMSO total volume was 10 µL, and total sample volume was 2 mL. Turbidity was measured by measuring absorbance at 400 nm. Absorbance was recorded before LUVs were added to give 0% turbidity. After LUVs were added, each sample was stirred at 400 rpm for 30 s, then absorbance recorded continuously for 5 min with no stirring. The absorbance of LUVs with no host was measured separately to give 100% turbidity. Turbidity of calculated according to the equation

\[
\% \text{ turbidity} = \frac{A(t) - A_b}{A_L - A_b} \times 100
\]

where \( A(t) \) is the absorbance of the sample as a function of time, \( A_b \) is the absorbance of buffer, and \( A_L \) is the absorbance of LUVs only. Results reported are the average of two runs.
7 References


[135] Liu, S. HDA and PDA synthesis and characterization not yet published.


8 Appendix

8.1 Documentation of copyright approval for reprinted figures

Title: Cucurbituril Homologues and Derivatives: New Opportunities in Supramolecular Chemistry
Author: Jae Wook Lee et al.
Publication: Accounts of Chemical Research
Publisher: American Chemical Society
Date: Aug 1, 2003
Copyright © 2003, American Chemical Society

PERMISSION/LICENSE IS GRANTED FOR YOUR ORDER AT NO CHARGE
This type of permission/license, instead of the standard Terms & Conditions, is sent to you because no fee is being charged for your order. Please note the following:

- Permission is granted for your request in both print and electronic formats, and translations.
- If figures and/or tables were requested, they may be adapted or used in part.
- Please print this page for your records and send a copy of it to your publisher/graduate school.
- Appropriate credit for the requested material should be given as follows: "Reprinted (adapted) with permission from (COMPLETE REFERENCE CITATION), Copyright (YEAR) American Chemical Society." Insert appropriate information in place of the capitalized words.
- One-time permission is granted only for the use specified in your request. No additional uses are granted (such as derivative works or other editions). For any other uses, please submit a new request.

If credit is given to another source for the material you requested, permission must be obtained from that source.
### License Details

This is a License Agreement between Sarah Lupo ("You") and John Wiley and Sons ("John Wiley and Sons"). The license consists of your order details, the terms and conditions provided by John Wiley and Sons, and the [payment terms and conditions](#).

**Get the printable license.**

<table>
<thead>
<tr>
<th>License Number</th>
<th>3011431037228</th>
</tr>
</thead>
<tbody>
<tr>
<td>License date</td>
<td>Oct 17, 2012</td>
</tr>
<tr>
<td>Licensed content publisher</td>
<td>John Wiley and Sons</td>
</tr>
<tr>
<td>Licensed content publication</td>
<td>Angewandte Chemie International Edition</td>
</tr>
<tr>
<td>Book title</td>
<td>None</td>
</tr>
<tr>
<td>Licensed content author</td>
<td>Jason Lagona, Pratam Mukhopadihyay, Srirama Chakrabarti, Lyle Isaacs</td>
</tr>
<tr>
<td>Licensed content date</td>
<td>Jul 29, 2005</td>
</tr>
<tr>
<td>Start page</td>
<td>4844</td>
</tr>
<tr>
<td>End page</td>
<td>4870</td>
</tr>
<tr>
<td>Type of use</td>
<td>Dissertation/Thesis</td>
</tr>
<tr>
<td>Requestor type</td>
<td>University/Academic</td>
</tr>
<tr>
<td>Format</td>
<td>Print and electronic</td>
</tr>
<tr>
<td>Portion</td>
<td>Figure/table</td>
</tr>
<tr>
<td>Number of figures/tables</td>
<td>1</td>
</tr>
<tr>
<td>Original Wiley figures/tables</td>
<td>Figure 6</td>
</tr>
<tr>
<td>Will you be translating?</td>
<td>No</td>
</tr>
<tr>
<td>Order reference number</td>
<td>None</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>0.00 USD</strong></td>
</tr>
</tbody>
</table>

[Get the printable license.](#)
PERMISSION/LICENSE IS GRANTED FOR YOUR ORDER AT NO CHARGE

This type of permission/license, instead of the standard Terms & Conditions, is sent to you because no fee is being charged for your order. Please note the following:

- Permission is granted for your request in both print and electronic formats, and translations.
- If figures and/or tables were requested, they may be adapted or used in part.
- Please print this page for your records and send a copy of it to your publisher/graduate school.
- Appropriate credit for the requested material should be given as follows: "Reprinted (adapted) with permission from (COMPLETE REFERENCE CITATION). Copyright (YEAR) American Chemical Society." Insert appropriate information in place of the capitalized words.
- One-time permission is granted only for the use specified in your request. No additional uses are granted (such as derivative works or other editions). For any other uses, please submit a new request.

If credit is given to another source for the material you requested, permission must be obtained from that source.
License Details

This is a License Agreement between Sarah loup ("You") and The American Association for the Advancement of Science ("The American Association for the Advancement of Science"). The license consists of your order details, the terms and conditions provided by The American Association for the Advancement of Science, and the payment terms and conditions.

<table>
<thead>
<tr>
<th>License Number</th>
<th>3011440863474</th>
</tr>
</thead>
<tbody>
<tr>
<td>License date</td>
<td>Oct 17, 2012</td>
</tr>
<tr>
<td>Licensed content publisher</td>
<td>The American Association for the Advancement of Science</td>
</tr>
<tr>
<td>Licensed content publication</td>
<td>Science</td>
</tr>
<tr>
<td>Licensed content title</td>
<td>Helical Conformation of Alkanes in a Hydrophobic Cavity</td>
</tr>
<tr>
<td>Licensed content author</td>
<td>Laurent Trembleau, Julius Rabek, Jr.</td>
</tr>
<tr>
<td>Licensed content date</td>
<td>Aug 29, 2003</td>
</tr>
<tr>
<td>Volume number</td>
<td>301</td>
</tr>
<tr>
<td>Issue number</td>
<td>5637</td>
</tr>
<tr>
<td>Type of Use</td>
<td>Thesis / Dissertation</td>
</tr>
<tr>
<td>Requestor type</td>
<td>Scientist/Individual at a research institution</td>
</tr>
<tr>
<td>Format</td>
<td>Print and electronic</td>
</tr>
<tr>
<td>Portion</td>
<td>Figure</td>
</tr>
<tr>
<td>Number of figures/tables</td>
<td>1</td>
</tr>
<tr>
<td>Order reference number</td>
<td>None</td>
</tr>
<tr>
<td>Title of your thesis / dissertation</td>
<td>Water-Soluble Deep-Cavity Cavatands: Synthesis, Molecular Recognition, and Interactions with Phospholipid Membranes</td>
</tr>
<tr>
<td>Expected completion date</td>
<td>Dec 2012</td>
</tr>
<tr>
<td>Estimated size/pages</td>
<td>143</td>
</tr>
</tbody>
</table>

Total: 0.00 USD
This is a License Agreement between Sarah Lop ("You") and Elsevier ("Elsevier"). The license consists of your order details, the terms and conditions provided by Elsevier, and the payment terms and conditions.

License Details

License Number: 301141504775
License date: Oct 17, 2012
Licensed content publisher: Elsevier
Licensed content publication: Tetrahedron
Licensed content title: Recent synthetic ion channels and pores
Licensed content author: Stefan Mattle, Abhijyan Som, Nathalie Sordé
Licensed content date: 26 July 2004
Licensed content volume number: 60
Licensed content issue number: 31
Number of pages: 31
Type of Use: reuse in a thesis/dissertation
Portion: figures/tables/illustrations
Format: both print and electronic
Are you the author of this Elsevier article? No
Will you be translating? No
Order reference number: None
Title of your thesis/dissertation: Water-Soluble Deep-Cavity Cavatands: Synthesis, Molecular Recognition, and Interactions with Phospholipid Membranes
Expected completion date: Dec 2012
Estimated size (number of pages): 140
Elsevier VAT number: GB 494 6272 12
Permissions price: 0.00 USD
VAT/Local Sales Tax: 0.00 USD

Total: 0.00 USD

Back
PERMISSION/LICENSE IS GRANTED FOR YOUR ORDER AT NO CHARGE

This type of permission/license, instead of the standard Terms & Conditions, is sent to you because no fee is being charged for your order. Please note the following:

- Permission is granted for your request in both print and electronic formats, and translations.
- If figures and/or tables were requested, they may be adapted or used in part.
- Please print this page for your records and send a copy of it to your publisher/graduate school.
- Appropriate credit for the requested material should be given as follows: "Reprinted (adapted) with permission from (COMPLETE REFERENCE CITATION). Copyright (YEAR) American Chemical Society." Insert appropriate information in place of the capitalized words.
- One-time permission is granted only for the use specified in your request. No additional uses are granted (such as derivative works or other editions). For any other uses, please submit a new request.

If credit is given to another source for the material you requested, permission must be obtained from that source.
This is a License Agreement between Sarah loup ("You") and Nature Publishing Group ("Nature Publishing Group"). The license consists of your order details, the terms and conditions provided by Nature Publishing Group, and the [payment terms and conditions](https://s100.copyright.com/MyAccount/viewLicenseDetails?licenseKeyId=2012101_1350493253617).

<table>
<thead>
<tr>
<th>License Details</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>License Number</td>
<td>3011450413617</td>
</tr>
<tr>
<td>License date</td>
<td>Oct 17, 2012</td>
</tr>
<tr>
<td>Licensed content publisher</td>
<td>Nature Publishing Group</td>
</tr>
<tr>
<td>Licensed content publication</td>
<td>Nature</td>
</tr>
<tr>
<td>Licensed content title</td>
<td>Antimicrobial peptides of multicellular organisms</td>
</tr>
<tr>
<td>Licensed content author</td>
<td>Michael Zasloff</td>
</tr>
<tr>
<td>Licensed content date</td>
<td>Jan 24, 2002</td>
</tr>
<tr>
<td>Type of Use</td>
<td>reuse in a thesis/dissertation</td>
</tr>
<tr>
<td>Volume number</td>
<td>415</td>
</tr>
<tr>
<td>Issue number</td>
<td>6870</td>
</tr>
<tr>
<td>Requestor type</td>
<td>academic/educational</td>
</tr>
<tr>
<td>Format</td>
<td>print and electronic figures/tables/illustrations</td>
</tr>
<tr>
<td>Portion</td>
<td>1</td>
</tr>
<tr>
<td>Number of figures/tables/illustrations</td>
<td>no</td>
</tr>
<tr>
<td>High-res required</td>
<td>no</td>
</tr>
<tr>
<td>Figures</td>
<td>Figure 2</td>
</tr>
<tr>
<td>Author of this NPG article</td>
<td>no</td>
</tr>
<tr>
<td>Your reference number</td>
<td>None</td>
</tr>
<tr>
<td>Title of your thesis / dissertation</td>
<td>Water-Soluble Deep-Cavity Cavitands: Synthesis, Molecular Recognition, and Interactions with Phospholipid Membranes</td>
</tr>
<tr>
<td>Expected completion date</td>
<td>Dec 2012</td>
</tr>
<tr>
<td>Estimated size (number of pages)</td>
<td>140</td>
</tr>
<tr>
<td>Total</td>
<td>0.00 USD</td>
</tr>
</tbody>
</table>
Biomolecular Engineering by Combinatorial Design and High-Throughput Screening: Small, Soluble Peptides That Permeabilize Membranes

Author: Ramesh Rathinakumar and William C. Wimley

Publication: Journal of the American Chemical Society

Date: Jul 1, 2008

Copyright © 2008, American Chemical Society

PERMISSION/LICENSE IS GRANTED FOR YOUR ORDER AT NO CHARGE

This type of permission/license, instead of the standard Terms & Conditions, is sent to you because no fee is being charged for your order. Please note the following:

- Permission is granted for your request in both print and electronic formats, and translations.
- If figures and/or tables were requested, they may be adapted or used in part.
- Please print this page for your records and send a copy of it to your publisher/graduate school.
- Appropriate credit for the requested material should be given as follows: "Reprinted (adapted) with permission from (COMPLETE REFERENCE CITATION). Copyright (YEAR) American Chemical Society." Insert appropriate information in place of the capitalized words.
- One-time permission is granted only for the use specified in your request. No additional uses are granted (such as derivative works or other editions). For any other uses, please submit a new request.

If credit is given to another source for the material you requested, permission must be obtained from that source.
Vita

Sarah Ioup was born in Manchester, England and grew up in Norcross, Georgia. She earned her B.S. in chemistry from the University of Chicago in 2003 and subsequently worked for Sigma Coatings in Harvey, Louisiana as a coatings chemist for two years. She enrolled in the graduate program at the University of New Orleans in 2006, where she joined the research group of Professor Bruce C. Gibb and studied organic supramolecular chemistry.