Development of New Carceplexes and Hemicarceplexes and the Dynamic Combinatorial Library Study

by

Jianyu Sun

B. Sc. Nankai University, 1994
M. Sc. Nankai University, 1997

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(Department of Chemistry)

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

November, 2003

© Jianyu Sun, 2003
In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Chemistry

The University of British Columbia
Vancouver, Canada

Date December 04, 2003
Abstract

New disulfide linked [4]carceplex 54 guests was synthesized. This kind of carceplex has diastereotopic protons that can be used in the study of “twistomers” of the carceplex. The twisting process was studied by variable temperature NMR spectroscopy. The activation energy barrier $\Delta G^\ddagger$ of the twisting on 54 depends on the size of guest molecules. The smaller the guest molecule, the higher the $\Delta G^\ddagger$ is. We proposed an explanation for the observation. The location of guests inside the host molecule was also studied.

The reversibility of disulfide bond formation in this series of compounds and the self-assembly during the formation of carceplexes suggest 54 and the previously synthesized 32 are excellent candidates for the study of the first dynamic combinatorial libraries of carceplexes. Using the disulfide linked [4]carceplexes 54 and disulfide linked [5]carceplexes 32, we worked out reversible conditions to synthesize the corresponding carceplexes from dynamic combinatorial libraries. The yield of disulfide linked [5]carceplexes is improved significantly from 14% (kinetic condition) to 50% (thermodynamic condition). The result on disulfide linked [4]carceplexes is not as good as on disulfide linked [5]carceplexes. We believe the reason is that we haven’t found a good template for the disulfide [4]carceplex. Guest exchange was also observed in the library, which confirmed that this library is dynamic.
The preparation of [5]cavitand benzylthiol 31 was modified. Using the modified reaction condition, 31 can be synthesized in 40% yield from [5]cavitand, which is much higher than the yield with old reaction conditions (10%). This improvement made 32 a more useful template in the study of DCLs.

During the synthesis of disulfide linked [4]carceplexes, we synthesized a hemicarcerand, 58, with new trithiacarbonate linkers in very high yield (80%). An X-ray crystal structure was obtained. Based on template studies, we propose the template for the self-assembly of this hemicarcerand can be an inorganic salt, an unprecedented template for such a system.
Chapter 1: Introduction

1.1 Carceplexes and Hemicarceplexes ........................................1
  1.1.1 History ........................................................................1
  1.1.2 Self-Assembly and Template Effects ..............................7
  1.1.3 Elucidation of ‘Twistomers’ in Carceplexes ......................9
  1.1.4 Development of Bigger Carceplexes and Hemicarceplexes ...10
  1.1.5 Application of the Carceplexes and Hemicarceplexes ..........16

1.2 Dynamic Combinatorial Chemistry and Disulfide Bonds ..........17
  1.2.1 What are Dynamic Combinatorial Libraries? .................17
  1.2.2 Why Study Dynamic Combinatorial Libraries? ...............20
  1.2.3 What Kind of Reaction Can Be Used in Dynamic Combinatorial
       Libraries? ........................................................................22
  1.2.4 Disulfide Bonds and Dynamic Combinatorial Library .........28

1.3 Thesis Goals .......................................................................33
1.4 References .........................................................................34
Chapter 2: Synthesis and Study of Disulfide Linked Carceplexes 32, 54 and Trithiacarbonate Linked Hemicarcerand 58

2.1 Introduction .......................................................................................................................... 38
2.2 Results and Discussion ........................................................................................................ 40
  2.2.1 Synthesis and Characterization of Disulfide [4]carceplexes 54 ................................. 40
    2.2.1.1 Reaction Conditions Testing and Screening for Suitable Template Molecules ................................................................................................................. 40
    2.2.1.2 Characterization of Disulfide [4]Carceplex 54 ...................................................... 45
    2.2.1.3 Shielding Effects on Chemical Shifts of Bonded Guest Molecules ................................................................. 50
    2.2.1.4 Variable Temperature 'H NMR Behavior of Disulfide [4]Carceplex 54 ..................... 52
    2.2.1.5 Competition Experiments Between Successful Template Molecules ................................................................. 60
    2.2.2.1 Synthesis of 58 and 58•guest .................................................................................. 62
    2.2.2.2 Characterization of [4]hemicarcerand 58 and [4]hemicarceplexes 58•hexane .................. 64
    2.2.2.3 Shielding Effects on Chemical Shifts of Bonded Guest Molecules ................................................................. 67
    2.2.2.4 Complexation Study of 58•guest ............................................................................ 68
    2.2.2.5 Template Study of the Forming of Hemicarceplex 58 ............................................. 68
  2.2.3 Modification of Synthesis of Disulfide [5]carceplex 32 ............................................. 71
    2.2.3.1 Modification of the Reaction Conditions to Synthesize [5]Benzylthiol 31 .................. 72
    2.2.3.2 New Route to Synthesize [5]Benzylthiol 31 ......................................................... 75
    2.2.3.3 Synthesis of Disulfide [5]Carceplex 32 and the X-ray Crystal structure of 32•2DMA ................................................................. 76
2.2.4 Dynamic Combinatorial Chemistry Study Using Disulfide Carceplexes as Template

2.2.4.1 Proposal.................................................................78

2.2.4.2 Dynamic Combinatorial Chemistry Study Using [5]Cavitand Derivatives........................................80

2.2.4.2.1 Reduction of Disulfide [5]Carceplex 32●(DMF)2.............80

2.2.4.2.2 Synthesis and Study of 32●(DMF)2 in Redox Buffers........82

2.2.4.3 Dynamic Combinatorial Library Study Using [4]Cavitand Derivatives..................................................87

2.3 Summary and Outlook........................................................................87

2.4 Experimental......................................................................................89

2.4.1 General Experimental.....................................................................89

2.4.2 Synthesis of Disulfide [4]Carceplexes 54●guest.............................90

2.4.3 Competition Experiments of 54 with Different Guests...............92

2.4.4 Synthesis of Thiaiacarbonate Linked [4]Hemicarcerand 58........93

2.4.5 Synthesis of Thiaiacarbonate Linked [4]Hemicarceplexes 58●guests...93

2.4.6 Synthesis of Disulfide [5]Carceplexes 32●guests.......................95

2.4.6.1 Cavitand [5]benzylbromide 59..........................................96

2.4.6.2 Synthesis of Cavitand [5]Benzylthiol 31 from 59...............96

2.4.6.3 Synthesis of Cavitand [5]Benzylthioacetate 61 from 59.........97

2.4.6.4 Synthesis of Cavitand [5]Benzylthiol 31 from 61...............97

2.4.6.5 Synthesis of Disulfide [5]Carceplexes 32●guests...............97

2.4.7 Reversible Study by Disulfide [5]Carceplexes 32●guests............99

2.4.7.1 Reducing of 32●(DMF)2..............................................99

2.4.7.2 Synthesis of 32●guest in Redox Buffer..............................99

2.4.7.3 Guest Exchange Reaction.............................................100

2.4.7.4 Competition Experiment in Redox Buffer..........................100

2.4.8 Reversible Study by Disulfide [4]Carceplexes 54●guests...........101

2.4.8.1 Reducing of 54●ethyl sulfide........................................101

2.4.8.2 Synthesis of 54●guest in Redox Buffer.........................101

2.5 References....................................................................................102
List of Schemes

Scheme 1.1 Synthesis of the first carceplex 3.........................................................2
Scheme 1.2 Synthesis of the first full characterized carceplex 5..............................2
Scheme 1.3 Synthesis of hemicarceplex 7.................................................................3
Scheme 1.4 Synthesis of compound 18.................................................................6
Scheme 1.5 Synthesis of hemicarceplex 20..............................................................6
Scheme 1.6 Formation of disulfide-bridged carceplex 32•guest...............................14
Scheme 1.7 Synthesis of trimer carceplex 34...........................................................14
Scheme 1.8 Synthesis of bis-carceplex 34..............................................................15
Scheme 1.9 Synthesis of tris-carceplex 38..............................................................15
Scheme 2.1 Synthesis of 54•guest.............................................................................43
Scheme 2.2 Synthesis of Hemicarcerand 58............................................................63
Scheme 2.3 Synthesis of [5]benzylthiol 31..............................................................72
Scheme 2.4 New route of synthesis of [5]benzylthiol 31..........................................75
List of Figures

Figure 1.1 Tetrol based hemicarceplexes and hemicarcerands ........................................... 4
Figure 1.2 Schematic representation of the diastereomeric twistomers of carceplexes ......... 9
Figure 1.3 Schematical drawing of compound 21 ................................................................. 11
Figure 1.4 Schematical drawing of hemicarceplexes with wider cavitands .................... 12
Figure 1.5 Schematical drawing of [n] cavitands ................................................................. 13
Figure 1.6 Supercarceplex 39*(DMSO)7 ............................................................................. 16
Figure 1.7 Stabilizing of cyclobutadiene by hemicarceplex 7 .......................................... 16
Figure 1.8 (A) Dynamic combinatorial chemistry versus (B) traditional combinatorial chemistry .......................................................... 18
Figure 1.9 Diagrammatic representations of ‘casting’ and ‘molding’ in DCLs ................ 19
Figure 1.10 Formation of a DCL of imines from four amines and three aldehydes ...... 20
Figure 1.11 Free energy profile illustrate kinetic (A-C) versus thermodynamic (A-B) control of the product distribution .................................................. 21
Figure 1.12 A small dynamic combinatorial library, products ratio changed by the addition of template. Letter sizes are representative of product concentration .......................................................... 22
Figure 1.13 Reversible reactions that have been used to date for construction of dynamic combinatorial libraries .......................................................... 24
Figure 1.14 (a) Templating of a hydrazone library by a series of tetra alkyl ammonium salts; (b) templates .......................................................... 26
Figure 1.15 The preparation of hemicarcerand C2A4 and the first step in the proposed imine exchange mechanism .......................................................... 27
Figure 1.16 Structure of A-SS-A (47) ................................................................................. 28
Figure 1.17 Schematic representation of the scrambling process occurring in a mixture of disulfide-linked carbohydrate dimmers ................................................. 29
Figure 1.18 (a) Building blocks used in a macrocyclic disulfide DCL, (b) Templates used in a macrocyclic disulfide DCL, (c) Samples of disulfide macrocyclic compounds .......................................................... 30
Figure 1.19 PDI catalyzed disulfide exchange ..................................................................... 32
Figure 1.20  Reversible formation of cage-like compound 52

Figure 2.1 Schematic structure of disulfide [5]carseplex 32 • guest, disulfide

Figure 2.2 Mechanism for the formation of disulfide bonds

Figure 2.3  "H NMR spectrum of 54 • ethyl sulfide (CDCl3, 300 MHz, 300K), * = entrapped ethyl sulfide peaks.

Figure 2.4 Labeling for compounds 56, 57, 2, 54 • ethyl sulfide

Figure 2.5 Parts of the "H NMR spectra of [4]cavitand derivatives (CDCl3, 500 MHz, 300K). A) [4]cavitand 56; B) [4]benzylbromide 57; C) [4]benzylthiol 2; D) disulfide [4]carseplex 54 • ethyl sulfide

Figure 2.6 Mass spectrum (Maldi-TOF) of 54 • ethyl sulfide (theoretical mass: 2194; M + Ag+ = 2302)

Figure 2.7 Guest orientation of 54 • ethyl sulfide (hexyl feet are omitted)

Figure 2.8 Variable temperature behavior of 54 • ethyl sulfide. Parts of the "H NMR spectra are shown (CDCl3, 500 MHz): A) 325K; B) 300K; C) 280K; D) 270K; E) 230K. Labeling refers to Figure 2.5

Figure 2.9 Schematic representation of the diastereomeric twistmomers of 54 • guest

Figure 2.10 Free energy profile illustrating twisting process of carseplex 54

Figure 2.11 Schematic representation of the two conformations of 54 • ethylmethyl sulfide. (Side cut of 54 • ethylmethyl sulfide)

Figure 2.12 Schematic representation of the conformations of 54 • methyl sulfide. (Side cut of 54 • methyl sulfide)

Figure 2.13 Variable temperature behavior of 54 • methyl sulfide. Parts of the "H NMR spectra are shown (500 MHz): A) 370K, d5-nitrobenzene; B) 350K, d5-nitrobenzene; C) 325K, CDCl3; D) 190K, CD2Cl2; E) 180K, CD2Cl2. Labeling refers to Figure 2.5

Figure 2.14 Schematic representation of the guest orientation in 54 • guest. (A, 54 • methyl sulfide; B, 54 • ethylmethyl sulfide; C, 54 • ethylsulfide. Side cut of 54 • guest)
Figure 2.15 ¹H NMR spectra of [4]Hemicarcerand 58 (A) and [4]hemicarceplexes 58•n-hexane (B), (CDCl₃, 400 MHz, 300K), * = entrapped n-hexane; • = feet protons

Figure 2.16 Labeling for different resonances for [4]hemicarcerand 58 and [4]hemicarceplex 58•guest

Figure 2.17 Stereoviews of the X-ray crystal structure of hemicarceplex 58•n-hexane. (a) side view (hydrogen atoms are omitted for clarity), (b) top view (hydrogen atoms and feet pendants are omitted for clarity)

Figure 2.18 Maldi-MS of pentyl footed 58, (theoretical mass: 2168, M + Ag⁺ = 2276)

Figure 2.19 Labeling for compounds 59

Figure 2.20 Monitoring the bromination reaction of cavitand[5] by ¹H NMR spectra, crude [5]cavitand benzylbromide 59 without purification. A) bad bromination result, using old reaction conditions; B) good bromination result, using modified reaction conditions. (CDCl₃, 300 MHz, 300 K)

Figure 2.21 Stereoviews of the X-ray crystal structure of carceplex 32•2DMA (major conformation, hydrogen atoms are omitted for clarity). (a) side view, (b) top view

Figure 2.22 ORTEP plots of the X-ray crystal structure of 32•2DMA (only sulfurs and the entrapped DMA molecules are shown for clarity). (a) Major confirmation (63%). (b) Minor confirmation (37%)

Figure 2.23 Schematic proposal of dynamic combinatorial chemistry study with disulfide carceplex 32•2guests

Figure 2.24 Parts of the ¹H NMR spectra of A) 32•(DMF)₂; B) crude product mixture after reduced by ME₇red. (300K, 500 MHz, CDCl₃). # = entrapped DMF; * = CH₂-SH

Figure 2.25 Proposed mechanism for the reduction of 32•(DMF)₂ (guest molecules are omitted)

Figure 2.26 Parts of the ¹H NMR spectra of: A) the crude product in the synthesis of 32•(DMF)₂ in redox buffer; B) the crude product after the guest exchange from (DMF)₂ to (DMA)₂; C) blank reaction. (CDCl₃, 400 MHz, 300K)
Figure 2.27 Proposed mechanism of guest exchange of 32•guests.............................84

Figure 2.28 Upfield of ¹H NMR of 32•guests (CDCl₃, 400 MHz, 300K). A) crude product of guest competition experiment; B) 32•(DMA)₂; C) 32•(DMF)₂.................................................................85

Figure 2.29 Maldi-MS of crude product of guest competition experiment. (theoretical mass of 32•(DMF)₂: 1936; theoretical mass of 32•(DMA)₂: 1964; theoretical mass of 32•(DMF+DMA): 1950).................................................................86

Figure 2.30 Labeling for compounds 54•guest, 58 and 58•guest.................................90

Figure 2.31 Labeling for compounds 31, 32•guest, 59 and 61.................................95
List of Charts and Tables

Table 1.1 Selected template ratios for the formation of acetal-bridged carceplex 5•guest.................................................................8
Table 1.2 Disproportionation of A-SS-Ph in the presence of Ac(D)Pro(L)Val(D)-Val-PS.................................................................29
Table 2.1 Summary of chemical shifts of resonances for 54•guest, 2, 56, 57........49
Table 2.2 Mass spectroscopic data for the intermediates and final products.
(Significance retained before decimal point).................................50
Table 2.3 ¹H NMR chemical shifts for free and bound guests of carceplex 54•guest in CDCl₃ at ambient temperature.................................51
Table 2.4 Summary of activation barrier values for the interconversion of diastereotopic ArCH₂S protons in 54•guest and 32•guest........55
Table 2.5 Template ratios in the formation of 54•guest................................61
Table 2.6 Summary of chemical shifts of resonances for 58 and 58•guests.........65
Table 2.7 ¹H NMR chemical shifts for free and bound guests of Hemicarceplex 58•guest in CDCl₃ at ambient temperature.................................68
Table 2.8. Template study on the formation of hemicarcerand 58...............70
Chart 2.1 Unsuitable guests for the template formation of disulfide [4]carceplex 54•guest.................................................................44
Chart 2.2 Suitable guests for the template formation of disulfide [4]carceplex 54•guest.................................................................45
## List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIBN</td>
<td>2,2’-Azobisisobutyronitrile</td>
</tr>
<tr>
<td>BMC</td>
<td>(±)-trans-1,2-bis(mercaptoacetamido) cyclohexane</td>
</tr>
<tr>
<td>COSY</td>
<td>Correlation Spectroscopy</td>
</tr>
<tr>
<td>CPK</td>
<td>Cory-Pauling-Koltun (molecular models)</td>
</tr>
<tr>
<td>Cs₂CO₃</td>
<td>cesium carbonate</td>
</tr>
<tr>
<td>Δδ</td>
<td>change in chemical shift (in ppm)</td>
</tr>
<tr>
<td>δ</td>
<td>chemical shift (in ppm)</td>
</tr>
<tr>
<td>ΔG‡</td>
<td>free energy of activation</td>
</tr>
<tr>
<td>Δν</td>
<td>frequency difference in Hz</td>
</tr>
<tr>
<td>d</td>
<td>deuterium (i.e., nithobenzene-d5)</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-diazabicyclo[5.4.0]undec-7-ene</td>
</tr>
<tr>
<td>DMA</td>
<td>N,N-dimethylacetamide</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
</tr>
<tr>
<td>EXSY</td>
<td>Exchange Spectroscopy</td>
</tr>
<tr>
<td>equiv.</td>
<td>equivalents</td>
</tr>
<tr>
<td>h</td>
<td>hours</td>
</tr>
<tr>
<td>J</td>
<td>coupling constant</td>
</tr>
<tr>
<td>GDS</td>
<td>guest determining step</td>
</tr>
<tr>
<td>M</td>
<td>parent mass (mass spectra) or molar, moles per liter (concentration)</td>
</tr>
<tr>
<td>MALDI MS</td>
<td>matrix assisted laser desorption ionization mass spectrometry</td>
</tr>
<tr>
<td>ME&lt;sub&gt;red&lt;/sub&gt;</td>
<td>2-mercaptoethanol</td>
</tr>
<tr>
<td>ME&lt;sub&gt;ox&lt;/sub&gt;</td>
<td>2-hydroxyethyl disulfide</td>
</tr>
<tr>
<td>min</td>
<td>minute(s)</td>
</tr>
<tr>
<td>m/z</td>
<td>mass to charge ratio</td>
</tr>
<tr>
<td>NBS</td>
<td>N-bromosuccinimide</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>NMP</td>
<td>N-methylpyrrolidinone</td>
</tr>
<tr>
<td>NFP</td>
<td>N-formylpiperidine</td>
</tr>
<tr>
<td>PDI</td>
<td>protein disulfide isomerase</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>r.t.</td>
<td>room temperature</td>
</tr>
<tr>
<td>s</td>
<td>second(s)</td>
</tr>
<tr>
<td>TEA</td>
<td>triethylamine</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>$t_m$</td>
<td>mixing time</td>
</tr>
<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
</tr>
<tr>
<td>TMS</td>
<td>tetramethylsilane</td>
</tr>
<tr>
<td>1D</td>
<td>one-dimensional</td>
</tr>
<tr>
<td>2D</td>
<td>two-dimensional</td>
</tr>
</tbody>
</table>
Acknowledgments

I sincerely wish to thank Professor John Sherman, my research supervisor, for his guiding, encouragement and supporting during my study.

I would like to thank all the past and present members in Dr. Sherman’s group, for great discussion and helpful suggestion. Special thanks to Ayub Jasat, Emily Seo, Heidi Huttunen and Alfredo Franco for proof-reading my thesis and language instructions. I would also like to thank Christoph Naumann for his help at the beginning of this project.

This thesis would not have been possible without the assistance from the other members in the Chemistry Department, especially the mass spectrometry laboratory, NMR laboratory and X-ray crystallography laboratory. I would like to express my gratitude to them.

Finally, I wish to thank and dedicate this thesis to my wife, my parents, my brother and sister whose love and support are always there. Furthermore, I wish to thank my little daughter Michelle, who brought the totally new world to me.
Chapter 1: Introduction

1.1 Carceplexes and Hemicarceplexes

1.1.1 History

The idea of carceplex was proposed by Dr. Donald J. Cram in 1980s. Shortly after that, Cram’s group reported the first carceplex. Several years later, the first fully characterized carceplex was reported. Since then, laboratories worldwide joined the exploration of these compounds.

Carceplexes are closed surface molecules containing one or several molecules within their interior. Entrapped molecules are held in the interior of carceplexes in a non-covalent manner and guest escape can only occur upon rupture of covalent bonds. Hemicarceplexes differ from carceplexes in that the portals between the cavitand units are large enough for the guests to escape from the interior with sufficient heat treatment. Hemicarceplexes must be stable enough for isolation without loss of guest. When a temperature greater than 350 °C is required to liberate guests, the complex is referred to as a carceplex, and when temperature lower than 350 °C is required, the complex is a hemicarceplex. The distinction between a carceplex and a hemicarceplex depends on portal size, temperature and even the number of guests. Carcerands and hemicarcerands are those closed surface compounds when no guest is present.

The first carceplex reported has the structure of (Scheme 1.1). Synthetically, this was achieved by coupling two bowl-shaped cavitands (i.e., macrocyclic molecules with an enforced internal cavity, such as tetrabenzyl chloride bowl 1 and tetrabenzyl thiol bowl 2). The poor solubility of this carceplex in any solvent precluded full characterization.
The first soluble carceplex (5) was synthesized by bridging two molecules of tetrol 4 with four molecules of bromochloromethane in the presence of a suitable guest (Scheme 1.2).\(^3\) Phenethyls were chosen as the pendant groups for their lipophilicity, which are deemed necessary to increase the solubility of the compounds.\(^7\)

**Scheme 1.1** Synthesis of the first carceplex 3.

Compared to the development of the carceplex, that of hemicarceplex was much faster, because of its larger cavity and versatile utility. Many hemicarceplexes have been synthesized since the design and synthesis of the first hemicarceplex 7.\(^8\) The first hemicarceplex (7) was synthesized from triol 6, a side product in the synthesis of tetrol 4 (Scheme 1.3).\(^6\)\(^,\)\(^9\)

**Scheme 1.2** Synthesis of the first full characterized carceplex 5.
Scheme 1.3 Synthesis of hemicarceplex 7.

Hemicarceplex 7 has a cavity whose size is similar to that of carceplex 5, but it also contains a portal where carceplex 5 had a fourth acetal-bridge. The guests could be expelled from this portal by extensive heat treatment.

Expanding the distance between the two bowls by using longer linkers on a carceplex is an alternative and more widely adopted approach to hemicarceplexes. This approach essentially created a structure with four portals equally spaced throughout the longitudinal axis of the shell. The shape and size of the cavity are unique to each hemicarceplex depending on the bridging units. Tetrol 4 served as the basic building block for a large number of hemicarceplexes. Figure 1.1 summarizes some hemicarceplexes and hemicarcerands based on tetrol 4.
Figure 1.1 Tetrol based hemicarceplexes and hemicarcerands.

Hemicarceplexes with four threonide bridges, \((S,S)_4\cdot8a\cdot guest\) and \((S,S)_4\cdot9a\cdot guest\), were prepared by Cram et al. with DMSO, DMF, and DMA incorporated. These chiral center containing hosts showed unequal environment in their cavity.\(^\text{10}\)

Water solubility is particularly desired if hemicarcerands are candidates for drug delivery systems. Hydrolysis of the ester moieties of hemicarcerand \(10c\) provided the first water-soluble hemicarcerand \(11c\). Binding studies showed that it formed one-to-one stable hemicarceplexes with 14 guests in \(D_2O\) at pH 9.\(^\text{11}\)
Tetramethylene-bridged hemicarcerand 12 has so far proved to be the most versatile of this group.\textsuperscript{12,13,14,15} A large number of complexes have been reported with guests ranging from acyclic molecules to cyclic five-membered rings, and a series of mono, ortho-, meta-, and para-disubstituted and 1,2,3-trisubstituted benzene rings. X-ray structure data of free host 12 and several guest molecules complexes indicated that this host can adjust its cavity size by interbowl twisting to a certain angle to maximize the host-guest interactions. By successively increasing the length of the linker by one and two more methylenes, the pentamethylene and hexamethylene bridged hemicarcerands 13a and 14a were obtained in approximately 20\% yield. These hosts have even larger cavities and portals.\textsuperscript{16}

Hemicarcerand 15a and 15b can be obtained from oxidative coupling of tetraacetylenic ethers. It has a significantly large cavity with very large portals. However, the yield was very low (about 8 \%). The low yield obtained here maybe result from the lack of an appropriate template to direct the formation of the dimeric capsule.\textsuperscript{17}

Hemicarcerands 16b and 17b were synthesized in 72\% and 32\% yields separately.\textsuperscript{18} The unusually high yield for 16b in this reaction is probably due to templation by DMA, which is removed from the host during work-up, thus it can not be detected by NMR spectroscopy. We got a similar observation during the synthesis of hemicarceplex with four trithiacarbonate linkers, and the template effect was studied. This result will be discussed in section 2.2.2.

A great number of other tetrol based hemicarceplexes with different linkers also have been reported.\textsuperscript{8a}

Cavitand[4] benzylthiol was used to synthesize the first carceplex (3).\textsuperscript{2} Recently, Paek’s group treated cavitand[4] benzylthiol 2 with 1,2,4,5-tetrakis(bromomethyl) benzene and Cs$_2$CO$_3$ in DMA, furnishing compound 18 in 15\% yield (Scheme 1.4).\textsuperscript{19}
NOESY spectra indicated that this compound exists in a closed conformation, the two bridging aromatic rings that are being held parallel to each other within the cavity by weak $\pi-\pi$ interactions.

Scheme 1.4 Synthesis of compound 18.

Under high dilution conditions, the shell closing reaction between tetraiodide 19 and tetrathiol 2 in a mixture of guest/Cs$_2$CO$_3$ at 50 °C produced tetraoxatetrathia hemicarceplexes 20 in 10-13% yields.\textsuperscript{20} The successful guests include DMA, DMF, DMSO and NMP. This is a less symmetric host, whose carceroisomer\textsuperscript{21} and twistomer\textsuperscript{22} study was performed.\textsuperscript{23}

Scheme 1.5 Synthesis of hemicarceplex 20.
1.1.2 Self-Assembly and Template Effects

There have been many attempts in the chemical literature to define the term "self-assembly". The most recent definition is by Lehn, who proposed a general definition whereby self-assembly is "the evolution towards spatial confinement through spontaneous connection of a few/many components, resulting in the formation of discrete/extended entities at either the molecular, covalent or the supramolecular, non-covalent level".\textsuperscript{24} Gibb distilled and combined many definitions and proposed a generally accepted definition, that "self-assembly is the spontaneous assembly of sets of comparatively simple subunits (molecular or otherwise) into highly complex supramolecular or molecular species of defined structure".\textsuperscript{25} The formation of carcoplexes and hemicarceplexes is a good example of self-assembly. If we take a look at the synthesis of carcoplex 5, we will find that the yield can be achieved as high as 87%. This is remarkable for a reaction that joins seven molecules and makes eight new covalent bonds.\textsuperscript{26} Cram found when neat N-formylpiperidine (NFP), a molecule too large to fit the interior of the carcoplex, was used as a solvent, no carcoplex was formed. But when the NFP solvent was doped with 0.5 mol\% DMA, carcoplex 5•DMA was obtained in a 10% yield. Additionally, when the reaction was conducted in 1:1 mixture (v/v) of DMA and DMF, a 5:1 ratio of carcoplex 5•DMA to carcoplex 5•DMF was obtained.\textsuperscript{6} These results suggest that a template molecule is required for the self-assembly to form carcoplex 5•guest, and that the carcoplex can demonstrate selectivity toward one template over another. The different ability to act as a template is called template ratio. Further investigation in our group found that the template ratio to form carcoplexes 5•guest could be $10^6$-fold range from the best guest (pyrazine) to the worst (NMP) (Table 1.1).\textsuperscript{22,27}
Table 1.1 Selected template ratios for the formation of acetal-bridged carceplex 5•guest.²²

<table>
<thead>
<tr>
<th>Entry</th>
<th>Guest</th>
<th>5•guest</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pyrazine</td>
<td>1000000</td>
</tr>
<tr>
<td>2</td>
<td>Methyl acetate</td>
<td>470000</td>
</tr>
<tr>
<td>3</td>
<td>1,4-dioxane</td>
<td>290000</td>
</tr>
<tr>
<td>4</td>
<td>DMSO</td>
<td>70000</td>
</tr>
<tr>
<td>5</td>
<td>Acetone</td>
<td>6700</td>
</tr>
<tr>
<td>6</td>
<td>Thiophene</td>
<td>5800</td>
</tr>
<tr>
<td>7</td>
<td>±2-butanol</td>
<td>2800</td>
</tr>
<tr>
<td>8</td>
<td>Benzene</td>
<td>2400</td>
</tr>
<tr>
<td>9</td>
<td>Pyrrole</td>
<td>1000</td>
</tr>
<tr>
<td>10</td>
<td>1,3,5-trioxane</td>
<td>100</td>
</tr>
<tr>
<td>11</td>
<td>DMA</td>
<td>20</td>
</tr>
<tr>
<td>12</td>
<td>DMF</td>
<td>7</td>
</tr>
<tr>
<td>13</td>
<td>NMP</td>
<td>1</td>
</tr>
</tbody>
</table>

The driving force of the self-assembly of a carceplex is the intermolecular interaction between the cavitands and the template molecules. It was also demonstrated by our group that two molecules of tetrol formed a charged hydrogen-bonded complex with the presence of template, the weak forces of hydrogen-bond permit a guest exchange if other guest exists in the system until the second bridge is formed.²⁸ Recent studies in our group on the formation of hemicarceplex 12•guest found the template ratio can range 3600 times from the best p-xylene to the worst NFP. In addition, the transition state study showed that the GDS (Guest Determine Step: the step after which the exchange of guests is impossible) in the synthesis of 12•guest is the formation of the fourth bridge.²⁹
1.1.3 Elucidation of ‘Twistomers’ in Carceplexes

The crystal structures of the carceplexes 5•guest showed they are all chiral due to interbowl twists of 13-21°. It was suggested that this twist arises due to several favorable interactions: the increasing of ‘van der Waals’ contacts of host and guest, the conjugation of the acetal/phenol oxygens with the aromatic rings of the two bowls, and the minimization of steric repulsion between intra-bowl acetals of opposing bowls. If both the host and guest are highly symmetric, it is hard to observe the twisting on ¹H NMR spectroscopy, since the resulting racemic twistomers remain highly symmetric. Our group has studied the twistomer interconversion by dynamic NMR. For example, by using a chiral guest, and by making an asymmetric host.

![Figure 1.2 Schematic representation of the diastereomeric twistomers of carceplexes.](image)

When using a chiral molecule (R)-2-butanol as guest, the energy barrier for the interconversion of these twistomers is 12.6± 0.1 kcal/mol based on the coalescence temperature of five host and guest signals. When using an asymmetric host and DMSO as the guest, the energy barrier of twistomers was found to be 13.6± 0.2 kcal/mol. A second energy barrier was also detected by dynamic NMR, 12.7± 0.2 kcal/mol, which was caused by the rotation of DMSO about the host’s C₂ axis. The isomers caused by this rotation were named by Reinhoudt as carceroisomers.

For the same host, the twistomers’ energy barriers are different for different guest molecules. Smaller, better guests (eg. DMSO is a better template for the formation of carceplexes 5•guest than 2-butanol) should provide stronger host-guest
interactions in the ground state. The host’s cavity is larger in the transition state than in the ground state. So for the larger guest (e.g., 2-butanol), stereo factors would thus be expected to decrease the energy of the transition state and increase the energy of ground state, thus $\Delta G^\ddagger$ is lower for big guest molecules. We performed the twistomer study using disulfide [4]carceplexes in this thesis, which further supported this proposal. The details will be discussed in chapter 2.

1.1.4 Development of Bigger Carceplexes and Hemicarceplexes

A current trend in supramolecular chemistry is the creation of large hosts that can accommodate several guests or one large guest. Usually there are three ways to obtain those big hosts.

1) Using longer linkers to connect the two dimerized cavitands. This method yields larger cavity as well as bigger portals in the capsules.

2) Using bigger building blocks to form the dimerized capsules, which means synthesis of wider or deeper cavitands that can be used to build up capsules.

3) Instead of dimerizing two cavitands, build up the capsules by putting three, four or even more cavitands together to form larger cavity.

A series of hemicarceplexes with significantly large cavities has been synthesized by using longer linkers between the dimers, such as hemicarceplexes 12, 13, 14, 15, 16, 17 etc. in Figure 1.1. Even larger macrocyclic hosts based on the extended cavitands have been isolated, like compound 21 (Figure 1.3). Measured from the X-ray crystal structure, the cavity of 21 is approximately $19 \times 15Å$, while the portals are roughly $9.5 \times 11.5Å$. However, no guests have been successfully complexed within the host of 21 to date. This could be due to their extremely large portals. Actually, these compounds cannot be considered as true hemicarcerands.
Developing wider and deeper cavitands to build-up carceplexes and hemicarceplexes is another method to enlarge the cavity. We know [4]cavitand has been used widely in building carceplexes and hemicarceplexes. Most commonly, the upper rim of [4]cavitand was bridged by a methylene linkage, but ethylene and propylene have been used as well. In this way, the cavity shape and size have been altered, such as hemicarceplexes 22, 23, 24, 25, 26 (Figure 1.4).
Figure 1.4 Schematic drawing of hemicarceplexes with wider cavitands.

So far, most carceplexes and hemicarceplexes have been built with [4]cavitand derivatives. Cavitands composed of more than four aromatic subunits are also possible. A CPK model shows that the cavitand with five aromatic subunits possesses a much larger cavity than [4]cavitand. Christoph Naumann in our group has synthesized [n]cavitand with n subunits (n=5,6,7, Figure 1.5), which has paved the way for the creation of a new family of larger carceplexes from wider cavitands. [5]Cavitand adopts a rigid cone-like conformation, with $C_{5v}$ symmetry; [6]cavitand and [7]cavitand manifest lower symmetry. 1D EXSY showed that both [6]cavitand and [7]cavitand have some flexibility at room temperature, with interconversion between equivalent conformations.
The first [5]cavitand carceplex (32•guest) was synthesized by air oxidation of [5]cavitand benzylthiol 31 in DMF in the presence of C₅₂CO₃ (Scheme 1.6). It contains five disulfide linkers and has two DMF entrapped in it. ¹H NMR chemical shifts and NOEs between the host and guest protons suggest that the entrapped guests adopt an unexpected orientation within host 32.¹ The DMF guests are believed to lie in parallel planes perpendicular to the long C₅ axis of the host. This carceplex is also the first disulfide bond linked carceplex. The reversibility of the disulfide bond should allow for the study of the dynamic combinatorial library (DCL). In the next chapter, we synthesized disulfide [5]carceplexes 32•guest under thermodynamic conditions, and the guest exchange is performed successfully under the same conditions.
Attempts to make the carceplexes with [6]cavitand and [7]cavitand were unsuccessful, probably due to the flexibility of these two cavitands which prohibit the dimerization reaction.

Our group has recently used a new approach in synthesizing larger carceplexes, which is different from the conventional method developed by Cram for two-bowl carceplexes. Naveen Chopra of our group has synthesized the first trimer carceplex 34 from trimer 33 (Scheme 1.7). The cavity of this carceplex is roughly triple the size of previously reported dimer carceplexes. Three molecules of DMF were permanently entrapped into the carceplex. This provides an excellent opportunity to study how clusters of molecules can act as a single template. Now this trimer carceplex has been used widely in our group. Instead of three small DMF molecules, Darren Makeiff in our group carcerated one big molecule: 1,3,5-tris(ethynyl)benzene, into the trimer carceplex. The usage of this carceplex as a reaction chamber was successful. So far, the reactive intermediate acetophenone enol and phenyl ketene were stabilized by the inner phase of this trimer carceplex.

Scheme 1.7 Synthesis of trimer carceplex 34.
Since trimer 33 can form a trimer carceplex with large cavity, can tetramer 35 form an even larger carceplex? The results of this attempt showed that instead of forming one big cavity, it formed a bis-capsule carceplex 36 (Scheme 1.8), with two small molecule guests in each chamber. When put hexamer 37 in the same conditions, a tris-capsules carceplex 38 was formed (Scheme 1.9), with three small molecule guests in each of the three capsules.

Scheme 1.8 Synthesis of bis-carceplex 34.

Scheme 1.9 Synthesis of tris-carceplex 38.

Darren Makeiff of our group has built up a huge carceplex 39 (Figure 1.6), with six cavitand building blocks. This carceplex has a big single cavity, and mass spectra showed that seven DMSO were entrapped. NMR spectra showed that the portals on this carceplex were big enough for a water molecule going through, thus affecting the chemical shift of the entrapped DMSO. Carceplex 39 may be the biggest carceplex that has been synthesized from the conventional [4]cavitand.
1.1.5 Application of the Carceplexes and Hemicarceplexes

One ingenious application of carceplexes and hemicarceplexes is the stabilization of short-lived intermediates, protected from destruction by the constrictive binding of the surrounding hemicarcerand. Cram and co-workers first stabilized the highly reactive cyclobutadiene in solution at room temperature, which was called by Cram as the "Mona Lisa of organic chemistry" (Figure 1.7).\(^4^4\) Photolysis of the readily available hemicarceplex \(7 \cdot \alpha\)-pyrone in CDCl\(_3\) generated hemicarceplex \(7 \cdot \text{cyclobutadiene}\). In the absence of oxygen, \(7 \cdot \text{cyclobutadiene}\) can be stabilized up to 60 °C.

\[ \text{7 \cdot \alpha\)-pyrone} \xrightarrow{hv} \text{7 \cdot Cyclobutadiene} \]

Figure 1.7 Stabilizing of cyclobutadiene by hemicarceplex 7.

\(\alpha\)-Benzyne was generated photochemically from benzocyclobutenedione inside hemicarcerand 12. Photolysis above 400 nm gave incarcerated benzocyclopropene. This intermediate was previously studied in bulk solution only below -78 °C.
However, if protected by hemicarcerand 12, it is stable at room temperature. Further photolysis extruded CO and generated $12\cdot o$-benzyne.\textsuperscript{45} Hemicarceplex 12 can also stabilize 1,2,4,6-cycloheptatetraene and 5-methylcycloheptatetraene at room temperature.\textsuperscript{46}

Future potential uses of hemicarceplexes and carceplexes include: catalysis, drug and radiation delivery and release systems, separation science, guest-indicator systems, super- and semi-conducting polymers, memory storage devices, and scavenging impurities for water purification.

1.2 Dynamic Combinatorial Chemistry and Disulfide Bonds

1.2.1 What are Dynamic Combinatorial Libraries?

Dynamic combinatorial chemistry is a new but rapidly growing field. Many articles in this field have been published since the first publication in 1996.\textsuperscript{47}

Molecular recognition leading to the binding of a guest in a host involves a complex interplay of non-covalent interactions. Combinatorial chemistry rests on the constitution of combinatorial libraries (CLs), which consist of a large static population of different molecules prepared by connecting a set of units in various sequences. One application of the combinatorial chemistry is by using molecular recognition, the desired compounds are identified from the combinatorial libraries, and the interactions between the guest and acceptors can be further studied (Figure 1.8 (b)).
Dynamic combinatorial libraries (DCLs) is from conventional combinatorial libraries. But it goes one step further, library member in a DCL is assembled from building blocks connected through reversible bonds.\textsuperscript{47d,47g,47h} So there is always a continuous interchange of building blocks between the different members. The composition of the library is governed by thermodynamics rather than kinetics. When a guest is added into the DCL, the preferred receptor is not only selected by the guest but also amplified by the reassemble of the unselected compounds (Figure 1.8 (a)). This is the major advantage of dynamic combinatorial chemistry over traditional combinatorial chemistry. The yield of the desired composition will be improved significantly, and the yield of undesired products will be decreased.

The driving force for molecular evolution is the selection through ‘survival of the fittest’, often by a template effect. Templating of DCLs has been proposed by Lehn in two fashions as ‘casting’ and ‘molding’ (Figure 1.9).\textsuperscript{48}
"Casting consists of the receptor-induced assembly of a substrate that fits the receptor; molding consists of the substrate-induced assembly of a receptor that optimally binds/fits the substrate". Both processes involve a set of components, which was formed through reversible formation of bonds between building blocks. And the recognition directs the selection of one partner by the other.

*Casting* can be considered as the template effect that a cavity of an enzyme, protein or other macromolecule may have on a DCL. Huc and Lehn reported a good example of casting process in 1997,\textsuperscript{48} a DCL of imines whose composition is influenced by carbonic anhydrase II (CA), here CA is a well characterized Zn(II) metalloenzyme. A library was generated by the addition of three aldehydes and four amines in the system as precursor components. The resulting imines were analyzed by HPLC. The results showed that the concentration of the preferred imine 40 was amplified roughly two fold in the presence of CA, with the decreasing of some unfavorable imines (Figure 1.10). Thus the casting process was clearly observed.
Figure 1.10 Formation of a DCL of imines from four amines and three aldehydes.

The molding process is much more general. Most templating effects in macrocyclic chemistry and some other supramolecular chemistry are by molding. More examples will be given later in this chapter.

1.2.2 Why Study Dynamic Combinatorial Libraries?

In practice, most of the reactions used in organic synthesis are irreversible. The result of a given procedure is determined by the kinetics of the desired reaction as well as the kinetics of the undesired competing processes. Hence, yields and product distributions depend on the relative differences between the various transition-state Gibbs energies $\Delta G^\ddagger$. In those kinds of reactions, the preferred pathway is through the more favorable energetic transition state, rather than the more thermodynamically stable products. Figure 1.11 shows an example, under kinetic conditions, $A$ goes to $C$ rather than to $B$, because $\Delta G_C^\ddagger$ is smaller than $\Delta G_B^\ddagger$.\textsuperscript{49}
Figure 1.11 Free energy profile illustrate kinetic (A-C) versus thermodynamic (A-B) control of the product distribution.

The composition after an irreversible reaction is static, thus there is no way to change the ratio between different products after an irreversible reaction. Using a different catalyst is a common way to favor a product by changing the Gibbs energies of the initial or transition states. However, the catalysts available are still very limited, thus, improving kinetically controlled reactions has been and still remains a challenge.

For thermodynamically controlled reactions, the product distributions depend on only the relative Gibbs energies of the product, so the situation is much simpler. Let’s take a look at Figure 1.11 again. Under thermodynamic conditions, A gives B rather than C, because product B has a lower Gibbs energy than C; thus it is more stable than C. If we go one step further and actively manipulate the Gibbs energies of the products, the equilibria can be shifted in the desired direction to favor the product we want. In practice, this can be achieved by addition of a template that selectively recognizes, binds and stabilizes the desired compound.

Figure 1.12 shows how a DCL can be used in organic reactions. Without a template, the distributions of products depend only on the stabilities of the products themselves.
The addition of a template can selectively recognize one of the components in the equilibrium system, thus forming a more stable complex \( A + \text{template} \). If the Gibbs energy of this complex is low enough, it will dominate the mixture at the expense of undesired products. After switching off the exchange of building blocks, amplification should also allow for the isolation of product \( A \) as a stable species directly from the ‘frozen’ library.\(^{50}\)

\[
\begin{align*}
A & \rightleftharpoons B & C & \rightleftharpoons D & E \\
\uparrow & \text{Addition of template T} & \\
A \cdot T & \rightleftharpoons B & C & \rightleftharpoons D & E
\end{align*}
\]

**Figure 1.12** A small dynamic combinatorial library, products ratio changed by the addition of template. Letter sizes are representative of product concentration.

### 1.2.3 What Kind of Reaction Can Be Used in Dynamic Combinatorial Libraries?

Dynamic combinatorial chemistry can be a highly powerful technique in synthetic chemistry. However, this technique is still very limited in application. A major reason why the use of dynamic combinatorial chemistry is not more widespread is the rather limited number of reversible reactions available. For the DCL to be successful, the following requirements must be satisfied:

i) Ideally, a rapid reversible reaction is required, the exchange of library members must be rapid enough so that the template can ‘proof read’ all possible structures.

ii) The conditions of exchange should be mild enough to ensure ‘survival’ of the template and library members and to allow non-covalent recognition to operate.
iii) Moreover, isolation and handling of a library member in its pure form requires another set of conditions where the compound does not undergo any exchange reaction. Hence, it should also be possible to turn the exchange process on or off as required. The switching off process should not affect the characteristics of the members of the library. The isolated compound has to be stable under the conditions in which it is going to be used.

iv) One important constraint is that every single member of given a DCL must be sufficiently soluble. Sometimes the re-dissolving rate of the precipitated material is so slow that material becomes effectively trapped in the solid form, resulting in a shift of the equilibrium towards this kinetic trap, and affecting all members of the library.

All these constraints significantly limit the number of chemical reactions that can be used in DCLs.

The two types of reversible reactions commonly used in DCL study are non-covalent exchange and reversible covalent reactions. Non-covalent exchange processes have been used in DCLs involving hydrogen-bond and metal-ligand coordination. These processes are usually fast and proceed under mild conditions. One big shortcoming of this non-covalent interaction is removal of the template as well as isolation and re-use of the amplified molecules is difficult because of the labile connections between building blocks. Reversible covalent reactions do not have this problem. Building blocks are connected with covalent bonds, making them more stable to be isolated and analyzed. Figure 1.13 is an overview of the most versatile reversible covalent reactions that have been investigated in the context of DCLs.
Figure 1.13 Reversible reactions that have been used to date for construction of dynamic combinatorial libraries.

Trans-esterification (Figure 1.13 (a)) is not a very good candidate because it requires harsh conditions that may interfere with the weak interactions underlying recognition by a template. Pd(0)-catalyzed exchange of allyl esters (Figure 1.13 (b)) proceeds under mild conditions, and was used for the templated synthesis of cyclic porphyrin dimers under reversible conditions. Peptide-bond exchange has been used by Venton to form a dynamic library of peptides (Figure 1.13 (c)). Using an antibody as the molecular trap, some templating has been observed using this approach. With the development of efficient catalysts, olefin metathesis has become a promising reaction for the preparation of DCLs (Figure 1.13 (h)). The usefulness of this reaction, which
can be controlled through addition or removal of a catalyst, has been increasing rapidly.

Of all these reactions shown in Figure 1.13, the C-N double bond exchange and disulfide exchange have been the most popular.

Sanders et al. reported molecular amplification from a very simple dynamic mixture of hydrazone-based macrocyclic compounds, where a significant example of templating has been observed. A hydrazone-based dynamic system was prepared from a building block derived from L-proline 41 (Figure 1.14 (a)).\textsuperscript{52, 53, 54} Acid-catalyzed cyclization of this building block in chloroform initially yields a mixture of fifteen macrocycles. After a period of three days, the equilibrium system gave mainly cyclic dimer 42 (88%), which is the thermodynamic product. Trimer 43 is obtained around 11% yield and a little amount of other higher oligomers were obtained as well (Figure 1.14). Addition of a series of tetra alkyl ammonium salts (Figure 1.14) made the equilibrium shift significantly to the cyclic trimer, especially when acetylcholine (Ach) was added; it produced a 50-fold amplification of the cyclic trimer 43. It is believed that the observed amplification is due to the stabilization of the cyclic trimer via complexation with the ammonium salts. Sanders also found that Li\textsuperscript{+} was a good template to stabilize the cyclic trimer, which can amplify the trimer to 98% among the material in the library. Binding studies demonstrated that the selected trimer forms a 1:1 complex with Li\textsuperscript{+} ion with a binding constant of 4×10\textsuperscript{4} M\textsuperscript{-1}. 

25
Carceplex and hemicarceplex chemistry has attracted a considerable amount of interest in recent years. However, the constructions of these kinds of compounds have always been under kinetic conditions. Since these systems are very good examples of template effects, why not use dynamic conditions to template its formation? In the year 2000, Stoddart and co-workers first reported the dynamic synthesis of a larger
container-like molecule 45. This is the only report of using a hemicarceplex in a DCL study so far. Hemicarceplex 45 (C2A4) was formed by linking two tetraformyl cavitands 44 (C) with four 5-substituted m-phenylenediamines (linker A). The reversible nature of the imine bond makes it a dynamic system. This resulted in the near-quantitative assembly of the octamine hemicarceplex. Furthermore, the dynamic nature of the system was revealed upon addition of a m-phenylenediamine linker (linker B), which afforded an equilibrium mixture containing six different hemicarceplexes with different linkers (C2A4, C2A3B, C2A2B2 (two regioisomers), C2AB3, C2B4) (Figure 1.15)

![Diagram of molecular structures](image)

Figure 1.15 The preparation of hemicarcerand C2A4 and the first step in the proposed imine exchange mechanism.
1.2.4 Disulfide Bonds and Dynamic Combinatorial Library

Disulfide exchange is perhaps one of the most promising reaction for dynamic combinatorial chemistry. Extensive mechanistic studies on thiols and disulfides by Whitesides and others indicate that:\(^{56}\)

i) Disulfides tend to form readily from thiols in the presence of small amount of base and some oxidants such as oxygen, metal ions, organic oxides and halogen compounds.

ii) Disulfide exchange takes place efficiently under mild conditions in the presence of a catalytic amount of thiol.

iii) Disulfide exchange is negligible under acidic conditions; thus it can be switched off easily.

iv) Disulfides are stable toward many different functional groups.

All these properties make it a very suitable reaction for the DCLs.

Equilibration shift by reversible disulfide bond formation was demonstrated by Hioki and Still.\(^{57}\) First they designed a suitable disulfide-linked receptor A-SS-A 47 (Figure 1.16), which can bind tripeptide (D)Pro(L)Val(D)Val selectively.

![Figure 1.16 Structure of A-SS-A (47).](image)

Exposure of Ph-SS-A to thiophenol (HS-Ph) and triethyl amine provided an equilibrium distribution of 35% A-SS-Ph and 65% Ph-SS-Ph + A-SS-A. When an excess of the polymer bead-supported tripeptide was added into the system, the
composition shifted to 95% of A-SS-A and Ph-SS-Ph (Table 1.2), because the tripeptide worked as a template to favor the formation of the good peptide binder A-SS-A 47. And the 99.5% pure 47 was isolated by easily rinse the substrate-carrying beads with suitable solvent.

Table 1.2 Disproportionation of A-SS-Ph in the presence of Ac(D)Pro(L)Val(D)-Val-PS.

<table>
<thead>
<tr>
<th></th>
<th>A-SS-Ph</th>
<th>Ph-SS-Ph</th>
<th>A-SS-A (47)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absence of tripeptide-PS</td>
<td>35%</td>
<td>65%</td>
<td></td>
</tr>
<tr>
<td>Presence of tripeptide-PS</td>
<td>5%</td>
<td>95%</td>
<td>5%</td>
</tr>
<tr>
<td>Solution phase</td>
<td>5%</td>
<td>95%</td>
<td>5%</td>
</tr>
<tr>
<td>Resin phase</td>
<td>0</td>
<td>0%</td>
<td>90%</td>
</tr>
</tbody>
</table>

Ramström and Lehn have used disulfide exchange to develop a system closely related to casting. The dynamic carbohydrate libraries were generated from a small set (six compounds) of initial carbohydrate dimers through mild disulfide interchange. Lectin Concanavalin A (Con A) was used to amplify a carbohydrate guest from a pool of disulfide-linked sugar residues (Figure 1.17). Comparison of HPLC analysis of the solution of unbound species and a solution of hosts eluted from the beads showed a considerable amplification of the mannose-mannose dimer 48, which shows it is most efficiently bound to the Lectin.

![Figure 1.17](image)

**Figure 1.17** Schematic representation of the scrambling process occurring in a mixture of disulfide-linked carbohydrate dimers.
Sanders group has also studied DCLs composed of macrocyclic disulfides. The amplification of selected hosts was observed by the addition of some suitable templates. The DCL was made by mixing and oxidizing three building blocks A, B, C in a system (Figure 1.18(a)).\(^{59}\) The oxidation cannot be completed, as there will be some free thiol present. Thus the system is in a dynamic state.

(a)

\[ \text{A} \quad \text{B} \quad \text{C} \]

(b)

\[ \text{49} \quad \text{50} \]

(c)

\[ \text{B-C} \quad \text{A-B-C} \quad \text{A-A-B} \quad \text{A-A-A} \]

**Figure 1.18** (a) Building blocks used in a macrocyclic disulfide DCL, (b) Templates used in a macrocyclic disulfide DCL, (c) Samples of disulfide macrocyclic compounds.
If there is no template in the system, HPLC analysis showed that dimer B-C and trimer A-B-C are two major components in the DCL, together with at least 36 small peaks corresponding to other library members. When the DCL is exposed to 2-methylisoquinolinium iodide 49 (Figure 1.18 (b)), one minor component was amplified, which was a mixed trimer A-A-B, and most of the other library members were decreased. When the DCL was exposed to N-methylated morphine 50 (Figure 1.18 (b)), the trimer A-A-A was amplified significantly at the expense of other library members. Thus in this way, they could adjust the library composition to favor their formation.

Disulfide bridges represent important evolutionarily conserved structural motifs in many biologically important peptides and proteins. The formation of disulfide bridges is believed to play a major role in stabilizing the bioactive conformations as well as promoting entropic destabilization of the denatured state. Reduced, unfolded proteins containing multiple cysteine residues tend to oxidize rapidly and nonspecifically. The non-native disulfide bonds formed during oxidative folding must then be isomerized to form the native disulfide bonds, thus forming the most stable folded proteins. In practice, this isomerization can be achieved by introducing the unfolded proteins or wrong-folded proteins into a redox buffer, which is composed of mixtures of low molecular weight disulfides and thiols. The commonly used redox buffers are mixtures of oxidized and reduced glutathione, cysteine, cysteamine or 2-mercaptoethanol. A recent study has found that adding a little amount of catalyst such as protein disulfide isomerase (PDI) or (±)-trans-1,2-bis(mercaptoacetamido) cyclohexane (BMC) in the redox buffer can efficiently speed up the disulfide exchange process.
Figure 1.19 shows the mechanism for catalysis disulfide bond exchange by PDI. In basic conditions, the isomerization begins with a nucleophilic attack of a thiolate provided by PDI on a non-native disulfide bond. This attack results in an intermolecular disulfide and a substrate thiolate. Further disulfide rearrangements are then induced by this substrate thiolate. The driving force of this disulfide exchange is the refolding of protein to form the most stable native one.

In 1999, Tam-Chung etc. reported the dimerization of the trithiol 51 to form the small cage-like macrobicyclic tris(disulfide) 52 under equilibrium control (Figure 1.20). In the presence of reduced or oxidized 2-mercaptoethanol, an equilibrium is established between 51, 52 and the unidentified oligomeric material 53. \(^1\text{H} \text{NMR} \) spectrum study shows that 30% of the equilibrium mixture is composed of the dimeric cage 52. So an investigation on the effects upon addition of a guest that templates the formation of the macrobicyclic tris (disulfide) 52 would be interesting. Actually, in the next chapter of this thesis, we did find that upon addition of template molecules, the yield of the formation of disulfide [5]carceplex increased from 25%
(kinetic condition) to 50% (thermodynamic condition), which confirmed the disulfide rearrangement really drives the reaction to the most stable product.

Figure 1.20 Reversible formation of cage-like compound 52.

In conclusion, dynamic combinatorial libraries have potential application in organic synthesis. To date, research in dynamic combinatorial chemistry is still on the beginning step. And the suitable reactions for dynamic combinatorial chemistry are still limited. The identification of new dynamic covalent bonds and the development of catalysts which promote the fast exchange of covalent bonds are very important to further the study of dynamic combinatorial chemistry.

1.3 Thesis Goals
The major goals of this thesis are listed below:
1. Develop the new disulfide [4]carpeplex that can be used in dynamic combinatorial library study.
2. Modify the synthesis of intermediate [5]cavitand benzylthiol. This will make guest a more useful candidate for the study of DCLs.

3. Introduce disulfide-linked carceplexes into the DCL study. This will include the reversible synthesis of disulfide-linked carceplexes, guest exchange and guest competition in DCLs.

1.4 References


35. Naumann, C.; Román, E. Peinador, C.; Ren, T.; Patrick, B. O.; Kaifer, A. E.;
2003, 125, 9558.
44. Cram, D. J.; Tanner, M. E.; Thomas, R. hopra, N.; Sherman, J. C. Angew. Chem.,
46. (a) Warmuth, R.; Marvel, M. A. Angew. Chem., Int. Ed. Engl. 2000, 39, 1117. (b)
Kerdelhué, J.; Langenwalter, K. J.; Warmuth, R. J. Am. Chem. Soc. 2003, 125,
973.
47. (a) Brady, P. A.; Bonar-Law, R. P.; Rowan, S. J.; Suckling, C. J.; Sanders, J. K.
Miller, B. L. Trends Biotechnol. 1999, 17, 205. (e) Karan, C.; Miller, B. L. Drug
Chapter 2: Synthesis and Study of Disulfide Linked Carceplexes 32, 54 and Trithiacarbonate Linked Hemicarcerand 58

2.1 Introduction

As summarized in Chapter 1, a wide variety of carceplexes and hemicarceplexes have been synthesized. The most recent efforts in this field are in two directions: one is the enlargement of the cavity; the other is the development of the application of this kind of compound. One successful application of hemicarceplexes is as a reaction chamber. Several reaction intermediates have been stabilized in hemicarceplexes. In the year 2000, Stoddart reported another attractive application of hemicarceplexes. He introduced imine-linked hemicarceplexes into the study of dynamic combinatorial libraries (DCLs), due to the reversibility of imine bonds.¹

Christoph Naumman of our group synthesized disulfide [5]carceplexes 32 in 15-25% yield by oxidizing two cavitand[5] benzylthiols 31 in DMA or DMF solvent (they are also the incarcerated guests) with CS₂CO₃ as the base.² This carceplex not only has an enlarged cavity, but also provides a logical choice to study DCLs owing to its potential reversible linkage of two cavitand bowls. The questions we pose are: can we get a higher yield of disulfide [5]carceplex 32 if we synthesize it in a DCL? If we subject the formed 32 into a reversible system with another guest, can the guest exchange be observed? If we can get positive answers for these questions, it will provide us with a strong technique to efficiently screen the suitable templates.

Although disulfide [5]carceplex 32 provides us with a good system to study dynamic combinatorial libraries, it has two shortcomings: (1) the poor solubility of cavitand [5] benzylthiol 43 will hamper the exchange in a DCL; (2) it is very hard to make 31 from the commercially available starting material (overall yield 1.2% from 2-methyl resorcinol). Thus, we returned to the more common cavitand[4] derivatives. Cavitand [4]benzylthiol 2 (another name is tetrathiol) is soluble in many solvents, and it is easily synthesized from the commercially available starting materials (overall yield 30% from 2-methyl resorcinol). Now another question is posed: can the disulfide [4]carceplexes be synthesized? Previous members in our group have tried to synthesize disulfide [4]carceplex 54●guest, but were unsuccessful. Is there something intrinsically bad about 54●guest, or is it just because we haven’t figured out the suitable reaction conditions and suitable guests? In this thesis, we attempted the synthesis of disulfide [4]carceplex 54●guest and found this compound could be synthesized.
2.2 Results and Discussion

2.2.1 Synthesis and Characterization of Disulfide [4]carceplexes 54

2.2.1.1 Reaction Conditions Testing and Screening for Suitable Template Molecules

As I mentioned in the introduction of this chapter, before we got the product of disulfide [4]carceplex 54, we didn’t know whether the formation of this compound was possible. CPK model showed that there is no problem with the bond angle and bond length to form disulfide [4]carceplex; all of them are very similar to disulfide [5]carceplex 32. Even the height of cavities are similar, the cavity of 54 is little thinner than that of 32. So we believe the reason disulfide [4]carceplex has yet to be synthesized is that we haven’t figured out the suitable reaction conditions and suitable guest molecules. As we know, carceplex 54 was built up by connecting two cavitands by four disulfide bonds. A very common method to form disulfide bonds is by oxidizing thiols by air in basic conditions. Figure 2.2 shows the mechanistic path:

\[
\begin{align*}
\text{Ionization} & : RSH + B^- \rightarrow RS^- + BH \\
\text{Oxidation} & : RS^- + O_2 \rightarrow RS^- + O_2^{2-} \\
& \quad \rightarrow RS^- + O_2^{2-} \\
\text{Dimerization} & : 2RS^- \rightarrow RSSR \\
\text{Base Regeneration} & : O_2^{2-} + BH \rightarrow OH^- + B^- + 1/2 O_2
\end{align*}
\]

**Figure 2.2** Mechanism for the formation of disulfide bonds.

The formation of one mol of disulfide bonds only consumes a half mol of oxygen, so it is a very effective way to convert thiols into disulfide bonds. This method has been used successfully in the synthesis of disulfide [5]carceplex 32, and we planned to use the same method in the synthesis of disulfide [4]carceplex 54.
We planned to use DMF as our first guest candidate because of several reasons: (1) DMF is a guest for several carceplexes, such as carceplexes 5, 32, 55; (2) at the same time as a guest candidate, DMF can be used as a solvent, because DMF can dissolve Cs₂CO₃, a successful base in the synthesis of carceplex 32; 3) if DMF is not a good guest, we still can use it as a solvent to screen other guest molecules. So the initial reaction conditions for the synthesis of disulfide [4]carceplex 54 were as following: Cs₂CO₃ was mixed with DMF and stirred for several minutes, hexyl footed cavitand [4]benzylthiol 2 was added, the mixture was loosely covered and stirred at room temperature for six hours. After work-up, we just ran the ¹H NMR of the crude product to monitor the reaction, ¹H NMR spectrum showed there was no entrapped DMF in the crude product, which meant DMF is an inappropriate guest for 54. We went on to use DMF just as a solvent to screen other guests. After deciding the initial reaction conditions, the next thing is choosing the appropriate guest candidates. Comparing the CPK model of 54, 32 and thioether [4]carceplex 55, we found that the size of 54 is more similar to 55, only a little higher. Since Ayub Jasat of our group has synthesized a series of thioether [4]carceplexes 55 with different guest molecules, we planned to start our guest screening on the guests that were suitable to 55, as this will much simplify our project. All the guests tested are pre-fitted by building CPK models.

Our strategy was just using the initial reaction conditions and trying as many guest candidates as possible, until we get the signal of the first entrapped molecule. We could then go back to optimize the reaction conditions and try to improve the yield, and lastly use the optimized condition to screen more guest candidates. To make our guest screening more efficient, we put two guests in every screening system. If the signal of an entrapped guest was observed, we then would test them separately.

The first entrapped molecule observed was diethyl sulfide. The signals in the upfield region of the ¹H NMR spectrum indicated the formation of a carceplex (Figure 2.3). The product was purified and characterized by NMR and Maldi-MS (See section 2.2.1.2).
Figure 2.3 $^1$H NMR spectrum of 54•ethyl sulfide (CDCl$_3$, 300 MHz, 300K), * = entrapped ethyl sulfide peaks.

Since we have successfully synthesized the first disulfide [4]carceplex 54•ethylsulfide, the second thing was to optimize the reaction conditions and to improve the yield of the product. We planned to improve the reaction conditions in the following ways.

1) As we know, an obstacle to achieving a high yield in synthesis of carceplexes is the side reaction leading to polymer formation. A resolution to this problem is using a dilute reaction system. This will favor the intramolecular bond formation, which leads to a dimer carceplex, and hamper the intermolecular bond formation, which lead to oligomers.

2) Another condition to change is the reaction time. Is a longer or a shorter time better for the formation of this carceplex?

3) How about the temperature? Is higher temperature helpful? In most literature, the formation of a disulfide bond is under room temperature, because higher temperature may increase the possibility of over-oxidization.

4) Some guest candidates have very low boiling points. If the reaction is run in an open system, we can’t guarantee that the guests will not evaporate away. How about in a closed system?

We tried many reactions with varied conditions, and finally settled on: (Scheme 2.1)
Scheme 2.1 Synthesis of 54•guest

\[
\begin{align*}
\text{2} & \quad \text{Cs}_2\text{CO}_3 \\
& \quad \text{Guest} \\
& \quad \text{DMF} \\
& \quad \text{r.t.} \\
\text{2} & \quad \text{54•guest}
\end{align*}
\]

Cs\textsubscript{2}CO\textsubscript{3} (10 equiv. per tetrathiol) was dissolved in DMF and stirred for several minutes. Guest (2% mol to DMF) was added, then cavitand [4]benzylthiol 2 was added to the mixture (1 mg guest per mL of DMF), the system was closed and stirred at room temperature for two days. The oxygen existing in the system would complete the reaction. In this way, the yield of 54•ethyl sulfide was increased to 15%. For the detailed reaction procedure see the experimental section.

Since we knew the ethyl sulfide was a suitable guest, after studying the structure of ethyl sulfide we wanted to make a crude prediction that an appropriate guest for this disulfide carceplex might need a linear structure. We continued our guest screening with the modified reaction conditions, and most of the guest candidates we chose had a linear structure. Some of the previously tested guest candidates were also retested in the new reaction condition. A total of 42 guests were screened in separate reactions, and they are listed in Chart 2.1 and Chart 2.2. The guests chosen for screening were molecules that were commercially available, inexpensive, and requiring little purification prior to use. Potential guests screened also had to be inert under the standard set of reaction conditions. Finally, we found that some linear molecules can template the formation of disulfide [4]carcplex 54. Five of the obtained 54•guest were purified by column chromatography.
2,4-Pentandione was obtained in very low yield, thus we didn’t purify it. All the synthesized guest were characterized by NMR spectroscopy and Maldi-MS.


DMF  DMA  dimethyl carbonate  1,3-dimethylurea

N-methyl propionamide  3-pentanol  2-pentanol  ethylene carbonate

2,4-pentanediol  1,2-propanediol  1,4-butanediol  1-butanol

ethylacetate  methylacetate  diethyl carbonate  1-butyne-3-ol

methyl 2-furoate  methoxyacetaldehyde  methyl acetoacetate  2,3-butanedione
**Chart 2.1 Continued.**

1,3-dithiane 1,4-dithiane 1,4-thioxane ethylene trithiacarbonate

dimethyl sulfate tetrahydrothiophene thiophene 1,3-ditholane

CH$_3$CH$_2$OH CH$_3$OH
ethanol methanol

2-butanol acetone 2,3-pentanedione

**Chart 2.2 Suitable guests for the template formation of disulfide [4]carceplex 54**

methy sulfide methylethyl sulfide ethyl sulfide
diethyl ether 3-pentanone 2,4-pentandione

**2.2.1.2 Characterization of Disulfide [4]Carceplex 54**

Ethyl sulfide is the first guest that can template the formation of this carceplex. We observed the entrapped signals in the $^1$H NMR spectrum of the crude product. The crude product was then carefully purified by preparative TLC and was studied by $^1$H NMR
spectroscopy again (Figure 2.5D). The well resolved signals of the host protons suggested that a symmetric carceplex was formed. The strong triplet at -2.74 ppm might be the methyl groups of the entrapped ethyl sulfide. A 2D COSY showed the methylene group of this entrapped ethyl sulfide was at 0.87 ppm, which was covered by the strong signals of hexyl groups. A broad signal showed up at 4.0 ppm. If the structure of 54 is similar to 32, this broad peak should be ArCH₂S protons, which appeared as diastereotopic protons. This will be discussed in detail in Section 2.2.1.4.

Figure 2.4 Labeling for compounds 56, 57, 2, 54 ethyl sulfide.
Figure 2.5 Parts of the $^1$H NMR spectra of [4]cavitand derivatives (CDCl$_3$, 500 MHz, 300K). A) [4]cavitand 56; B) [4]benzylbromide 57; C) [4]benzylthiol 2; D) disulfide [4]carceplex 54•ethyl sulfide.

Before running Maldi-MS, a little silver acetate was added to a chloroform solution of this product to eliminate potential confusion from the Na$^+$ and K$^+$ adducts we usually observed. Maldi-MS spectrum showed a very clean and strong peak at 2302.2 m/z plus some other isotope peaks (Figure 2.6).
Figure 2.6 Mass spectrum (Maldi-TOF) of $54\cdot$ethyl sulfide (theoretical mass: 2194; M + Ag$^+$ = 2302)

The MS value is exactly that of the theoretical mass of the hexyl footed disulfide [4]carseplex $54$ plus entrapped ethyl sulfide plus a silver ion (theoretical mass 2104+90+108=2302). Two things became apparent: the unknown compound was disulfide [4]carseplex $54$ and only one ethyl sulfide was entrapped. Careful integration of the host and guest peaks confirmed that only one ethyl sulfide was incarcerated.

The starting materials, hexyl footed cavitand[4] $56$, intermediate cavitand[4] benzylbromide $57$, cavitand [4]benzylthiol $2$ and all the synthesized disulfide [4]carseplexes $54$$\cdot$guests were characterized by NMR and Maldi-MS. Figure 2.6 shows parts of the $^1$H NMR spectra of these compounds. Table 2.1 lists the chemical shifts of resonances for compound $56$, $57$, $2$, $54$$\cdot$guest, and Table 2.2 lists all the theoretical and experimental mass spectroscopy data of compound $56$, $57$, $2$, $54$$\cdot$guest.
Table 2.1 Summary of chemical shifts of resonances for 54•guest, 2, 56, 57.

<table>
<thead>
<tr>
<th>Compound</th>
<th>X</th>
<th>T/K</th>
<th>H₁</th>
<th>H₂</th>
<th>H₃</th>
<th>H₄</th>
<th>H₅</th>
<th>H₆</th>
<th>others</th>
</tr>
</thead>
<tbody>
<tr>
<td>56</td>
<td>H</td>
<td>300</td>
<td>6.95</td>
<td>5.86</td>
<td>4.24</td>
<td>4.74</td>
<td>2.17</td>
<td>1.95</td>
<td>--</td>
</tr>
<tr>
<td>57</td>
<td>Br</td>
<td>300</td>
<td>7.11</td>
<td>6.00</td>
<td>4.54</td>
<td>4.76</td>
<td>2.18</td>
<td>4.40</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>SH</td>
<td>300</td>
<td>7.04</td>
<td>5.94</td>
<td>4.45</td>
<td>4.73</td>
<td>2.17</td>
<td>3.56</td>
<td>SH: 1.87</td>
</tr>
<tr>
<td>54•ethyl sulfide</td>
<td>SS</td>
<td>325</td>
<td>7.03</td>
<td>5.85</td>
<td>4.35</td>
<td>4.69</td>
<td>2.15</td>
<td>4.08</td>
<td>Guest: 0.87, -2.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300</td>
<td>7.01</td>
<td>5.84</td>
<td>4.32</td>
<td>4.67</td>
<td>2.13</td>
<td>4.07</td>
<td>Guest: 0.87, -2.74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>230</td>
<td>6.96</td>
<td>5.83</td>
<td>4.23</td>
<td>4.58</td>
<td>2.09</td>
<td>4.30, 3.75</td>
<td>Guest: 0.83, -2.86</td>
</tr>
<tr>
<td>54•ethyl methyl sulfide</td>
<td>SS</td>
<td>320</td>
<td>7.06, 7.03</td>
<td>5.88, 5.83</td>
<td>4.61, 4.22</td>
<td>4.69</td>
<td>2.14</td>
<td>4.07</td>
<td>Guest: 1.28, -1.38, -1.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300</td>
<td>7.04, 7.01</td>
<td>5.87, 5.82</td>
<td>4.61, 4.19</td>
<td>4.66</td>
<td>2.13</td>
<td>3.55-4.50</td>
<td>Guest: 1.28, -1.37, -1.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>230</td>
<td>6.99, 6.95, 5.86, 5.81</td>
<td>4.58, 4.13</td>
<td>4.59</td>
<td>2.11</td>
<td>4.37,4.24, 3.79,3.73</td>
<td>Guest: 1.28, -1.36, -2.10</td>
<td></td>
</tr>
<tr>
<td>54•methyl sulfide</td>
<td>SS</td>
<td>370</td>
<td>7.61</td>
<td>6.11</td>
<td>4.79</td>
<td>4.97</td>
<td>2.47</td>
<td>4.33</td>
<td>Guest: -0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300</td>
<td>7.03</td>
<td>5.84</td>
<td>4.45</td>
<td>4.68</td>
<td>2.16</td>
<td>4.34-3.82</td>
<td>Guest: -0.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>220</td>
<td>6.97</td>
<td>5.82</td>
<td>4.39</td>
<td>4.59</td>
<td>2.11</td>
<td>4.33,4.75</td>
<td>Guest: -0.77</td>
</tr>
<tr>
<td>54•diethyl ether</td>
<td>SS</td>
<td>320</td>
<td>7.05</td>
<td>5.85</td>
<td>4.34</td>
<td>4.69</td>
<td>2.15</td>
<td>4.07</td>
<td>Guest: 1.79, -2.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300</td>
<td>7.04</td>
<td>5.84</td>
<td>4.32</td>
<td>4.66</td>
<td>2.14</td>
<td>3.75-4.60</td>
<td>Guest: 1.78, -2.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>230</td>
<td>6.98</td>
<td>5.83</td>
<td>4.25</td>
<td>4.58</td>
<td>2.10</td>
<td>4.31, 4.75</td>
<td>Guest: 1.79, -2.43</td>
</tr>
<tr>
<td>54•3-pentanone</td>
<td>SS</td>
<td>320</td>
<td>7.06</td>
<td>5.85</td>
<td>4.32</td>
<td>4.69</td>
<td>2.15</td>
<td>4.09</td>
<td>Guest: 0.86, -2.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300</td>
<td>7.03</td>
<td>5.84</td>
<td>4.32</td>
<td>4.66</td>
<td>2.12</td>
<td>3.75-4.33</td>
<td>Guest: 0.84, -2.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>230</td>
<td>6.99</td>
<td>5.83</td>
<td>4.20</td>
<td>4.58</td>
<td>2.10</td>
<td>4.30, 3.78</td>
<td>Guest: 0.82, -2.89</td>
</tr>
</tbody>
</table>
Table 2.2 Mass spectroscopic data for the intermediates and final products. (Significance retained before decimal point)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Theoretical mass</th>
<th>Mass of sample</th>
<th>Mass subtract of attached cation</th>
</tr>
</thead>
<tbody>
<tr>
<td>56</td>
<td>928</td>
<td>929 (M•H⁺)</td>
<td>928 (subtract H⁺)</td>
</tr>
<tr>
<td>57</td>
<td>1240</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>1056</td>
<td>1074 (M•NH₄⁺)</td>
<td>1056 (subtract NH₄⁺)</td>
</tr>
<tr>
<td>54•ethyl sulfide</td>
<td>2194</td>
<td>2302 (M•Ag⁺)</td>
<td>2194 (subtract Ag⁺)</td>
</tr>
<tr>
<td>54•ethylmethyl sulfide</td>
<td>2180</td>
<td>2288 (M•Ag⁺)</td>
<td>2180 (subtract Ag⁺)</td>
</tr>
<tr>
<td>54•diethyl ether</td>
<td>2178</td>
<td>2286 (M•Ag⁺)</td>
<td>2178 (subtract Ag⁺)</td>
</tr>
<tr>
<td>54•methyl sulfide</td>
<td>2166</td>
<td>2274 (M•Ag⁺)</td>
<td>2166 (subtract Ag⁺)</td>
</tr>
<tr>
<td>54•3-pentanone</td>
<td>2190</td>
<td>2298 (M•Ag⁺)</td>
<td>2190 (subtract Ag⁺)</td>
</tr>
</tbody>
</table>

The crystal of 54•diethyl ether was obtained, but the X-ray scanning was not very clear due to the effect of the long hexyl feet on the host. From the crude crystal structure data, we can see that two bowls are connected by disulfide bonds, and a diethyl ether molecule was incarcerated. The measurements and calculations of the bond lengths and bond angles were not successful.

2.2.1.3 Shielding Effects on Chemical Shifts of Bonded Guest Molecules

Guest molecules inside a cavity experience shielding effects that shift their ¹H NMR signals upfield compared to those signals free in solution (Table 2.3). ¹H NMR guest signals assignments were made for each of these carceplexes 54 with the help of 2D COSY experiments.
Table 2.3 $^1$H NMR chemical shifts for free and bound guests of carceplex 54•guest in CDCl$_3$ at ambient temperature.

<table>
<thead>
<tr>
<th>Guest Structure</th>
<th>Proton</th>
<th>$\delta_{\text{free}}$ ppm (multiplicity)</th>
<th>$\delta_{\text{bound}}$ ppm (multiplicity)</th>
<th>$\Delta\delta$ ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>ethyl sulfide</td>
<td>H$_a$</td>
<td>1.23 (t)</td>
<td>-2.74 (t)</td>
<td>3.97</td>
</tr>
<tr>
<td></td>
<td>H$_b$</td>
<td>2.52 (q)</td>
<td>0.87 (q)</td>
<td>1.65</td>
</tr>
<tr>
<td>ethylmethyl sulfide</td>
<td>H$_a$</td>
<td>1.21 (t)</td>
<td>-1.37 (t)</td>
<td>2.58</td>
</tr>
<tr>
<td></td>
<td>H$_b$</td>
<td>2.46 (q)</td>
<td>1.28 (q)</td>
<td>1.18</td>
</tr>
<tr>
<td></td>
<td>H$_c$</td>
<td>2.05 (s)</td>
<td>-1.90 (s)</td>
<td>3.95</td>
</tr>
<tr>
<td>methyl sulfide</td>
<td>H$_a$</td>
<td>2.08 (s)</td>
<td>-0.65 (s)</td>
<td>2.73</td>
</tr>
<tr>
<td>diethyl ether</td>
<td>H$_a$</td>
<td>1.16 (t)</td>
<td>-2.31 (t)</td>
<td>3.47</td>
</tr>
<tr>
<td></td>
<td>H$_b$</td>
<td>3.43 (q)</td>
<td>1.78 (q)</td>
<td>1.65</td>
</tr>
<tr>
<td>3-pentanone</td>
<td>H$_a$</td>
<td>1.02 (t)</td>
<td>-2.76 (t)</td>
<td>3.78</td>
</tr>
<tr>
<td></td>
<td>H$_b$</td>
<td>2.38 (q)</td>
<td>0.84 (q)</td>
<td>1.54</td>
</tr>
</tbody>
</table>

Integration of $^1$H NMR spectra showed that only one guest molecule was entrapped in the carceplex. It has been previously demonstrated that for carceplexes and hemicarceplexes, protons buried deep within the aryl lined hemispheres show large, positive $\Delta\delta$ values, while protons near the equatorial region show smaller, less positive $\Delta\delta$ values. From Table 2.3, we found that $\Delta\delta$ values for methyl protons are large (2.58-3.97 ppm), while the $\Delta\delta$ values for methylene protons are much smaller (1.54-1.65 ppm). Which means these linear guest molecules are always located averagely along the $C_4$ axis of the
carceplex, with both methyl groups buried deep into the two opposing hemispheres, and the methylene groups are closer to the equatorial region of the host (Figure 2.7).

![Figure 2.7 Guest orientation of 54•ethyl sulfide (hexyl feet are omitted).](image)

2.2.1.4 Variable Temperature $^1$H NMR Behavior of Disulfide [4]Carceplex 54

We studied the variable temperature behavior of 54•guest focusing on the ArCH$_2$S resonance H$_6$ (Figure 2.8). The $^1$H NMR spectrum of 54•ethyl sulfide at 300K yields a broad signal around 4.0 ppm, which we presumed to be diastereotopic proton H$_6$. At 325K (in CDCl$_3$ on 500 MHz), H$_6$ appears as a fairly sharp singlet, as the diastereotopic H$_6$ protons interconvert fast on the chemical shift timescale. Lowering the temperature first broadens the singlet (280K, in CDCl$_3$ on 500 MHz) due to the slower interconversion of diastereotopic protons H$_6$. At lower temperature two broad peaks appear (270K, in CDCl$_3$ on 500 MHz). At even lower temperature (230K), the two diastereotopic H$_6$ resonances were completely frozen, and thus appear as two doublets. The rate constant for interconversion of the diastereotopic H$_6$' and H$_6$'' resonance can be measured and calculated by dynamic NMR spectroscopy.
Figure 2.8 Variable temperature behavior of 54-ethyl sulfide. Parts of the $^1$H NMR spectra are shown (CDCl$_3$, 500 MHz): A) 325K; B) 300K; C) 280K; D) 270K; E) 230K. Labeling refers to Figure 2.5.
Several dynamic NMR techniques have been developed that evaluate rates or chemical fluxes associated with chemically exchanging systems. Most commonly used are the coalescence point, classic line-shape analysis and the more recent magnetization-transfer methods.

A well known method for obtaining information about free energy of activation by NMR is that using the coalescence temperature. The coalescence temperature $T_c$ is the temperature at which the two peaks of the doublet merge into one, that means the “valley” between the two separate peaks disappears.

For an exchange process between two nuclei A and B with a mutual coupling $J_{AB}$, the rate constant $k_c$ at coalescence temperature $T_c$ is then given by:

$$k_c = 2.22*(\Delta v^2+6J_{AB}^2)^{1/2}$$ (2-1)

Here $\Delta v$ is the separation in Hz between the two signals in the absence of exchange. It is determined experimentally from spectra recorded at temperature which is as far below the coalescence temperature as possible.

Once the exchange rate constant $k_c$ is determined by dynamic NMR, the free energy of activation $\Delta G^\ddagger$ at coalescent temperature $T_c$ can be calculated from the following equation (Eq. 2-3 and 2-4):

$$\Delta G^\ddagger = -RT_c \ln[k_c h/k_b T]$$ (2-2)

so $\Delta G^\ddagger = 4.58T_c(10.32 + \log[T_c/k_c])$ (2-3)

where $k_b$ is the Botzmann constant ($3.2995 \times 10^{-24}$ cal K$^{-1}$), $h$ is Plank’s constant ($1.5836 \times 10^{-34}$ cal s), $R$ is the gas constant ($1.9872$ cal K$^{-1}$mol$^{-1}$), and $T_c$ is the coalescence temperature.

We used the coalescence temperature method to measure and calculate the exchange rate constant between the diastereotopic $H_6^\prime$ and $H_6^\prime\prime$, and the free energy of activation $\Delta G^\ddagger$ for the exchange was also calculated. Table 2.4 summarizes the results.
Table 2.4 Summary of activation barrier values for the interconversion of diastereotopic ArCH$_2$S protons in 54•guest and 32•guest.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Guest</th>
<th>Coalescence temperature (K)</th>
<th>Rate constant $k_c$ at $T_c$ (s$^{-1}$)</th>
<th>$\Delta G^\ddagger$ (kcal mol$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>54</td>
<td>Methyl sulfide</td>
<td>322</td>
<td>641</td>
<td>14.8 ± 0.2</td>
</tr>
<tr>
<td>54</td>
<td>Ethylmethyl sulfide</td>
<td>296</td>
<td>614</td>
<td>13.6 ± 0.2</td>
</tr>
<tr>
<td>54</td>
<td>Ethyl sulfide</td>
<td>283</td>
<td>615</td>
<td>12.9 ± 0.2</td>
</tr>
<tr>
<td>54</td>
<td>Diethyl ether</td>
<td>295</td>
<td>638</td>
<td>13.5 ± 0.2</td>
</tr>
<tr>
<td>54</td>
<td>3-pentanone</td>
<td>268</td>
<td>576</td>
<td>12.3 ± 0.2</td>
</tr>
<tr>
<td>32</td>
<td>DMF</td>
<td>322</td>
<td>571</td>
<td>14.9 ± 0.2</td>
</tr>
<tr>
<td>32</td>
<td>DMA</td>
<td>303</td>
<td>496</td>
<td>14.0 ± 0.2</td>
</tr>
</tbody>
</table>

Recall that in Section 1.3, Robert Chapman and John Sherman have elucidated the "twistomers" in acetal-bridge carceplexes. Christopher Naumman also studied the disulfide bond torsional barrier of 32•guest. Here in disulfide [4]carceplexes 54, we think the diastereotopic property of proton H$_6$ is due to the twisting of top and bottom bowls with respect to one another (Figure 2.9).

Figure 2.9 Schematic representation of the diastereomeric twistomers of 54•guest.
In conformation A, H₆' points outside of the host, and shows up downfield in the ¹H NMR spectrum. H₆'' points to the inside of the host, as it is shielded by the benzyl ring units on the bowl. Thus its peak shows up at the upfield region of the ¹H NMR spectrum.

After twisting, as in conformation B, the position of H₆' and H₆'' exchanged, so their peaks on ¹H NMR spectrum will exchange as well. At high temperature (Figure 2.8, (a) 320K), the twisting process is very fast. The signal shows up in the ¹H NMR spectrum as the average of the signals of H₆' and H₆''. If the temperature is low enough, the exchange can be shut down. The H₆' and H₆'' signals now show up separately in the ¹H NMR spectrum (Figure 2.8, (e) 230K). The previous study of “twistomers” used unsymmetric acetal bridged hosts or symmetric hosts with chiral guests. Here in 54, because of the existence of diastereotopic proton H₆, we can use this symmetric host and symmetric guest to study the “twisting” property of carceplexes.

From Table 2.4 we can see, for the same disulfide [4]carceplex host 54, the activation energy barrier of twisting is different due to the difference of guests. Comparing ethyl sulfide, ethylmethyl sulfide, and methyl sulfide, AG‡ of twisting tends to be lower with the increasing length of the guest molecules. Comparing ethyl sulfide and diethyl ether, we found the same tendency: diethyl ether is smaller in size than ethyl sulfide due to the smaller atomic radius of oxygen than sulfur, and AG‡ of diethyl ether is higher than ethyl sulfide. Comparing disulfide [5]carceplexes 32•DMA and 32•DMF, we got the same result: the bigger the guest molecule, the lower the activation energy barrier of twisting. How do we explain these results?
Figure 2.10 Free energy profile illustrating twisting process of carceplex 54.

Figure 2.10 shows the different energies of the ground state and transition state in a twisting process. The activation energy barrier of twisting is different due to the difference of guests. $\Delta G^\ddagger$ of twisting tends to be higher with the increasing of the size of the guest molecules. The host’s cavity appears to be larger in the transition state $TS^\ddagger$ than in the ground state according to the unraveling of CPK model. Big guest molecules would fill the cavity of $TS^\ddagger$ better than small guests, thus the steric factor would be expected to lower the energy of $TS^\ddagger$; on the other hand, big guest will untwist the ground state of the host, and increase the energy of ground state (dotted curve). In this way, the activation energy barrier $\Delta G^\ddagger_{1}$ was smaller for big guest (dotted curve on the graph) than small guest $\Delta G^\ddagger_{2}$ (solid curve on the graph). Using this proposal, we can successfully explain the $\Delta G^\ddagger$ difference in Table 2.4.

We also studied the mobility of entrapped molecules by variable temperature NMR spectroscopy. Information regarding each guest’s preferred orientation with 54 can be determined from the $^1$H NMR $\Delta \delta$ in Table 2.3. For symmetric guests such as ethyl sulfide, methyl sulfide, diethyl ether and 3-pentanone, the end methyl groups are buried deep
within the bottom of the bowls, and the middle of the guests are located at the equator of the host (Figure 2.7), thus still showing symmetric signals on $^1$H NMR spectrum.

We studied the $^1$H NMR data of 54•ethylmethyl sulfide and found the $\Delta \delta$ of methyl protons connected directly to sulfur is larger than the $\Delta \delta$ of methyl protons connected to the methylene group, which means the methyl group attached to sulfur is closer to the bottom of the hemisphere, thus receiving a higher shielding effect (Table 2.3). This observation is different with that observed in acetal-bridged carcplex 5 and thioether carcplex 55. In 5•ethylmethyl sulfide, $\Delta \delta$ on H$_a$ is 4.44, $\Delta \delta$ on H$_c$ is 4.34, they are almost the same; in 55•ethylmethyl sulfide, $\Delta \delta$ on H$_a$ is 3.87, $\Delta \delta$ on H$_c$ is 4.28, $\Delta \delta$ are close too. Both methyl groups of ethylmethyl sulfide are located almost the same distance to the bottom of the bowl. How do we explain our observation? One explanation is, because the cavity of 54 is longer than the cavity of 5 and 55, ethylmethyl sulfide can move in the cavity from one end to the other end (Figure 2.11). The four disulfide bonds make the middle of the cavity very crowded and narrow, so the big sulfur atom on ethylmethyl sulfide can’t locate in the middle of the cavity, it preferred to reside at either end. Since the ethyl group has larger steric interactions than the methyl group, the favorable conformation is A (Figure 2.11), which makes H$_a$ become more shielded by the aromatic units on the bowl.

Figure 2.11 Schematic representation of the two conformations of 54•ethylmethyl sulfide. (side cut of 54•ethylmethyl sulfide)
If this proposal is true, we predict the entrapped dimethylsulfide will show two methyl proton signals at low temperature on the $^1\text{H}$ NMR spectrum. The possible conformations are shown in Figure 2.12.

![Figure 2.12 Schematic representation of the conformations of 54-methyl sulfide. (side cut of 54-methyl sulfide)](image)

**Figure 2.12** Schematic representation of the conformations of 54-methyl sulfide. (side cut of 54-methyl sulfide)

B is not a preferred conformation due to the steric effect between the disulfide bonds and the big sulfur atom on methylsulfide. At high temperature A and C will convert quickly through B, so only one single peak shows up on $^1\text{H}$ NMR. Lowering the temperature will make the conversion slow down, thus broaden the singlet. When the temperature is low enough, the conversion could be stopped, and the two methyl protons will appear as two singlets. We performed the variable temperature NMR (Figure 2.13); unfortunately, we couldn’t completely freeze the conversion. Because we couldn’t find a suitable deuterated solvent which has such a low boiling point. But the results we got so far are consistent with our prediction. We slowed down the conversion, and broadened the guest peak (Figure 2.13). This supported our proposal that since the cavity of 54 is longer than the guest molecules, the guests move quickly prefer to reside nearer to one bowl than the other.
Figure 2.13 Variable temperature behavior of $5_{4}\text{-methyl sulfide}$. Parts of the $^1H$ NMR spectra are shown (500 MHz): A) 370K, $d^5$-nitrobenzene; B) 350K, $d^5$-nitrobenzene; C) 325K, CDCl$_3$; D) 190K, CD$_2$Cl$_2$; E) 180K, CD$_2$Cl$_2$. Labeling refers to Figure 2.5.

2.2.1.5 Competition Experiments Between Successful Template Molecules

We performed competition experiments between successful template molecules. The template ratios were determined from the product ratios measured from each of a series of head-to-head competition reactions between different pairs of guests. The reaction
condition we chose was very similar to the condition we used to screen guest molecules, only that we added two different guest molecules with same molarity in one reaction system. Product ratios were determined by the integration of each set of guest signals in the $^1$H NMR spectra. The template ratios are summarized in Table 2.5.

**Table 2.5** Template ratios in the formation of 54•guest.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Guest</th>
<th>Template Ratio (54•guest)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methyl sulfide</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>Ethylmethyl sulfide</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>Ethyl sulfide</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>Diethyl ether</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>3-pentanone</td>
<td>1</td>
</tr>
</tbody>
</table>

From the data in Table 2.5, we found that the template ability of these guest are similar, it ranged 50 times from the best methyl sulfide to the worst 3-pentanone. The small difference of the template ability may be caused by many reasons such as desolvation ability, guest size, etc. 3-Pentanone is the worst guest. This maybe because the carbonyl group is too big, thus it cannot fit in the narrow cavity very well. Linear sulfides could be a series of suitable guests, the first three best guests are all linear sulfides. Among sulfide compounds, templating abilities increase with the decreasing of the molecule size (methyl sulfide > ethylmethyl sulfide > ethyl sulfide), this could be due to the steric effect between the host and guest (Figure 2.14).

**Figure 2.14** Schematic representation of the guest orientation in 54•guest. (A, 54•methyl sulfide; B, 54•ethymethyl sulfide; C, 54•ethylnsulfide. Side cut of 54•guest)
It has been demonstrated previously that the methyl group has a good CH-π interaction with [4]cavitand bowls. The best orientation for a methyl group to have a good interaction with [4]cavitand is pointing directly to the bottom of the bowl. If guests choose the orientation shown in Figure 2.14, ethylmethyl sulfide must have a bigger steric effect than methyl sulfide; and ethyl sulfide must have the biggest steric effect among these three guests. This could be one reason, which caused the different template ability of these sulfide compounds.


2.2.2.1 Synthesis of 58 and 58•guest

When we were using dimethyl trithiacarbonate (DMTC) as a guest candidate to do guest screening of disulfide [4]carceplexes 54•guest, a small singlet at -0.8 ppm of the ¹H NMR spectrum indicated the entrapment of a molecule. A product was isolated after column chromatography using CHCl₃/hexanes as eluent. The product was studied by ¹H NMR spectroscopy, and to my surprise, a new strong triplet appeared at -2.60 ppm. With the help of 2D COSY, we found the new peak is assigned to an entrapped n-hexane molecule, which could be going into the cavity of the host during the column purification when using CHCl₃/n-hexanes as eluent. We then used the crude product to test the complexation of hexane in an NMR tube using CDCl₃ as solvent. One week after the addition of n-hexane, the ¹H NMR spectrum showed a good entrapment of n-hexane. The host signals are symmetric. And integration of the host and guest peaks showed the yield of this complex is around 70% of the crude product. We then ran the reaction again with n-hexane as guest and without DMTC. No entrapped n-hexane was found after the reaction. When running the reaction with n-hexane as guest, together with DMTC, a very high yield of product with incarcerated n-hexane was formed. We clearly had a hemicarceplex, capable of complexing and retaining n-hexane on the minute timescale. We ran variable temperature NMR, but saw no diastereotopic ArCH₂S protons (these diastereotopic protons can be seen in variable temperature ¹H NMR of 32). The result
was disappointing, even at temperature as low as 180 K, we still couldn’t broaden any peak on the $^1$H NMR spectrum. As we know, disulfide bonds can be reduced to thiols in basic condition, and we have performed the reduction reaction successfully on disulfide [5]carceplex 32. To explore the existence of disulfide bonds on the product we had obtained, we used exactly the same reductive condition on this product, with 2-mercaptoethanol (ME$^{\text{red}}$) as reductant, and triethylamine (TEA) as base. The result showed this product is very resistant to the reducing conditions. Even with NaBH$_4$ as reductant, there is still no thiol formed. Inspection of the CPK model of 54 revealed that the portals on 54 are too small for an n-hexane molecule to go through. All these facts convinced us that we could not have obtained the disulfide [4]carceplex 54. Mass spectrum could be a useful way to determine the structure of this compound, however, all the Mass Spectra techniques that we can reach did not work well on this compound. We tried Maldi-MS, ESI-MS, APCI-MS, but none of them could get the parent peak clearly. We then synthesized a similar product with the pentyl group as feet, hoping the changing of the feet could give us more information on the mass spectrum. The peaks observed via ESI-MS were parallel to the one with hexyl feet, which meant no breaking of the feet on ESI, but the parent peak was still hard to get.

Finally, we grew a crystal of this compound successfully, and the X-ray crystal structure made everything clear. We had not synthesized disulfide [4]carceplex 54, but instead, we synthesized trithiacarbonate linked [4]hemicarceplex 58•hexane.

![Scheme 2.2 Synthesis of Hemicarcerand 58.](image-url)
2.2.2.2 Characterization of [4]hemicarcerand 58 and [4]hemicarceplexes 58•hexane

Before we got the X-ray crystal structure, the characterization of this compound was very difficult. NMR gave us some information, but it was not enough for us to decide the structure of this compound. Before complexing with $n$-hexane as guest, the $^1$H NMR spectrum showed that this hemicarcerand is not symmetric. The host peaks were not resolved very well, as all the peaks showed up as broad signals (Figure 2.15a). This is likely because the solvent molecules, such as CDCl$_3$, are trying to get into the empty host. But these solvents are not suitable guests, so they distorted the host's structure and tried to fit it, thus made the host a non-symmetric structure. Another explanation is the linker itself tends to fill the cavity, thus distorts the host. The third explanation is the spectrum showed up as a mixture of hemicarceplexes with different guests. After complexation with $n$-hexane, this suitable guest kicked out the solvent molecules, and made the host recover its symmetry, so the $^1$H NMR showed very good resolution (Figure 2.15b).

![Figure 2.15](image)

*Figure 2.15* $^1$H NMR spectra of [4]Hemicarcerand 58 (A) and [4]hemicarceplexes 58•$n$-hexane (B), (CDCl$_3$, 400 MHz, 300K), * = entrapped $n$-hexane; ■ = feet protons.
We also complexed \textit{n}-pentane and \textit{n}-heptane successfully within 58. The $^1$H NMR data are listed in Table 2.6. Figure 2.16 shows the labeling for different resonances for compound 58.

![Diagram of compound 58 with labels for H1, H2, H3, H4, H5, H6, and other chemical shifts.]

\textbf{Figure 2.16} Labeling for different resonances for [4]hemicarcerand 58 and [4]hemicarceplex 58\textit{\{guest.}

\begin{table}[h!]
\centering
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
\textbf{Compounds} & \textbf{H1} & \textbf{H2} & \textbf{H3} & \textbf{H4} & \textbf{H5} & \textbf{H6} & \textbf{Other} \\
\hline
58 & 7.09 & 5.80 & 4.12 & 4.76 & 2.17 & 4.40 & -- \\
58\textit{\{n-pentane} & 7.13 & 5.83 & 4.17 & 4.70 & 2.17 & 4.36 & Guest: 1.25, 0.72, -1.69 \\
58\textit{\{n-hexane} & 7.11 & 5.83 & 4.25 & 4.70 & 2.17 & 4.36 & Guest: 1.12, 0.31, -2.60 \\
58\textit{\{n-heptane} & 7.08 & 5.83 & 4.17 & 4.74 & 2.17 & 4.37 & Guest: 1.17, 0.87, -0.46-3.15 \\
\hline
\end{tabular}
\caption{Summary of chemical shifts of resonances for 58 and 58\textit{\{guests.}
\end{table}

What made the structure of this product clear was the X-ray crystal structure (Figure 2.17). We got a new hemicarceplex with four trithiacarbonate linkers. This is a new type of linker that had never been used before. The four thiocarbonyl groups are all pointing outside the cavity, thus making this compound have a big unoccupied cavity which can encapsulate guests such as \textit{n}-hexane, \textit{n}-pentane, \textit{n}-heptane etc. This also makes the H6
protons point to the outside of the cavity. Since the host is not twisted, these protons don’t show diastereotopic properties. The \( n \)-hexane guest was located along the \( C_4 \) axis of the host, with the two methyl groups buried in the bottom of the bowls.

Efforts to get a good mass spectrum was finally successful on Maldi-MS of penty1 footed hemicarcerand 58. We added a little silver acetate to a sample solution and incubated it for several hours. Then we ran the Maldi-MS with this sample. The power selection is very important in Maldi-MS. If the power is too high, it can break the molecule into fragments. Using this method, we finally got the peak corresponding to \( M + Ag^+ \) (2276), although the spectrum is still not very clean (Figure 2.18). We could only get the peaks of

**Figure 2.17** Stereoviews of the X-ray crystal structure of hemicarceplex 58•\( n \)-hexane. (a) side view (hydrogen atoms are omitted for clarity), (b) top view (ORTEP plots at 30% probability levels, hydrogen atoms and feet pendants are omitted for clarity).
the empty host. This may because the portals on the host are big enough for the \( n \)-hexane to escape in the MS instrument.

![Graph showing chemical shifts](image)

**Figure 2.18** Maldi-MS of pentyl footed 58, (theoretical mass: 2168, \( M + Ag^+ = 2276 \)).

### 2.2.2.3 Shielding Effects on Chemical Shifts of Bound Guest Molecules

The chemical shifts of the guests, free and entrapped are listed in Table 2.7. The \( \Delta \delta \) of the incarcerated guests showed that they were located symmetrically along the \( C_4 \) axis of the host. The location of \( n \)-hexane was also confirmed by the X-ray crystal structure. The methyl ends are pointing to the bottoms of the bowls, thus it has a larger \( \Delta \delta \) than the methylene protons. For methyl protons \( H_a \), \( \Delta \delta_{heptane} > \Delta \delta_{hexane} > \Delta \delta_{pentane} \). \( n \)-Hexane has one more methylene than \( n \)-pentane, making it longer, so the methyl groups are closer to the bottom of the bowls. \( n \)-Heptane has one more methylene than \( n \)-hexane, so the methyl groups are even closer to the bottom of the bowls.
Table 2.7 $^1$H NMR chemical shifts for free and bound guests of Hemicarceplex 58•guest in CDCl$_3$ at ambient temperature.

<table>
<thead>
<tr>
<th>Guest Structure</th>
<th>Proton</th>
<th>$\delta_{\text{free ppm}}$ (multiplicity)</th>
<th>$\Delta_{\text{bound ppm}}$ (multiplicity)</th>
<th>$\Delta \delta$ ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$-pentane</td>
<td>Ha</td>
<td>0.87 (t)</td>
<td>-1.69 (t)</td>
<td>2.56</td>
</tr>
<tr>
<td></td>
<td>Hb</td>
<td>1.25 (m)</td>
<td>0.72 (m)</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>Hc</td>
<td>1.31 (m)</td>
<td>1.25 (m)</td>
<td>0.06</td>
</tr>
<tr>
<td>$n$-hexane</td>
<td>Ha</td>
<td>0.87 (t)</td>
<td>-2.60 (t)</td>
<td>3.47</td>
</tr>
<tr>
<td></td>
<td>Hb</td>
<td>1.25 (m)</td>
<td>0.31 (m)</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>Hc</td>
<td>1.26 (m)</td>
<td>1.12 (m)</td>
<td>0.14</td>
</tr>
<tr>
<td>$n$-heptane</td>
<td>Ha</td>
<td>0.86 (t)</td>
<td>-3.15 (t)</td>
<td>4.01</td>
</tr>
<tr>
<td></td>
<td>Hb</td>
<td>1.25 (m)</td>
<td>-0.46 (m)</td>
<td>1.71</td>
</tr>
<tr>
<td></td>
<td>Hc</td>
<td>1.27 (m)</td>
<td>0.87 (m)</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>Hd</td>
<td>1.29 (m)</td>
<td>1.17 (m)</td>
<td>0.12</td>
</tr>
</tbody>
</table>

2.2.2.4 Complexation Study of 58•guest

We also tested the complexation ability of $n$-heptane, $n$-hexane and $n$-pentane by adding two of them in a CDCl$_3$ solution of hemicarcerand 58. After 7 days, a $^1$H NMR was run and the product ratios were determined by integrations of each set of guest signals. In this way, we found $n$-hexane is the best guest for 58, $n$-heptane is next, $n$-pentane is the worst one. The ratio of $n$-hexane : $n$-heptane : $n$-pentane is about 3 : 1.5 : 1.

2.2.2.5 Template Study of the Forming of Hemicarceand 58

As hemicarceand 58 was isolated free of guest, we wondered what templates its formation. There is precedent for solvent molecules acting as templates and being lost during the work-up. We therefore changed the solvent, expecting a dramatic change in the yield of 58. Complexation with $n$-hexane was performed on the crude products of each reaction. Since hemicarceand 58 can quantitatively complex with $n$-hexane to form
58•n-hexane, and hemicarceplex 58•n-hexane gives a definitive ¹H NMR spectrum (the spectrum of hemicarcerand 58 is not easily distinguished from oligomer), the yield of hemicarcerand 58 can be estimated from the formation of 58•n-hexane without isolation: the yields of 58•n-hexane were calculated from the relative integration of the guest signal at -2.60 ppm (representing 6H) to the total host signal at 5.83 ppm (H_{out}, representing 8H) (Figures 2.15). Use of N,N-dimethylacetamide (DMA) or N-formyipiperidine (NFP) as solvent instead of DMF, after complexation with n-hexane, gave 58•n-hexane in good yield. Since NFP in particular is rather large, it appears that the solvents are not acting as templates. We then ran the reaction in NFP, using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as base, with dimethyltrithiacarbonate as linker. After the reaction, the complexation test showed no 58•n-hexane had formed. This confirmed that NFP does not act as a template, and that dimethyltrithiacarbonate also does not act as a template for this reaction. Other reactions showed that DBU is a suitable base (vide infra). The only other possibility is the base. DBU is too big to be a template, but Cs₂CO₃ might be suitable, although this would be highly unusual in forming a hemicarcerand. We tested Na₂CO₃ and K₂CO₃, and found reactions to form 58 worked well in their presence (Table 2.8). Using DBU as base together with (NH₄)₂CO₃ in the reaction, followed by complexation of the crude product with n-hexane, 58•n-hexane was synthesized in very high yield (78%). Therefore, the template appears to be either a cation, an anion, or a mixture of ions (in cases where no suitable neutral template is present). We tested a series of salts, and report the results in Table 2.8.
<table>
<thead>
<tr>
<th>Template candidates</th>
<th>Base</th>
<th>Solvent</th>
<th>Yield (58•n-hexane)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cesium carbonate</td>
<td>Cs₂CO₃</td>
<td>DMF</td>
<td>62%</td>
</tr>
<tr>
<td>Cesium carbonate</td>
<td>Cs₂CO₃</td>
<td>DMA</td>
<td>64%</td>
</tr>
<tr>
<td>Cesium carbonate</td>
<td>Cs₂CO₃</td>
<td>NFP</td>
<td>72%</td>
</tr>
<tr>
<td></td>
<td>DBU</td>
<td>NFP</td>
<td>0%</td>
</tr>
<tr>
<td>DMF</td>
<td>DBU</td>
<td>DMF</td>
<td>78%</td>
</tr>
<tr>
<td>Potassium carbonate</td>
<td>K₂CO₃</td>
<td>NFP</td>
<td>67%</td>
</tr>
<tr>
<td>Sodium carbonate</td>
<td>Na₂CO₃</td>
<td>NFP</td>
<td>63%</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>NaHCO₃</td>
<td>NFP</td>
<td>57%</td>
</tr>
<tr>
<td>Ammonium carbonate</td>
<td>DBU</td>
<td>NFP</td>
<td>78%</td>
</tr>
<tr>
<td>Ammonium chloride</td>
<td>DBU</td>
<td>NFP</td>
<td>72%</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>DBU</td>
<td>NFP</td>
<td>0%</td>
</tr>
<tr>
<td>Tetraethylammonium chloride</td>
<td>DBU</td>
<td>NFP</td>
<td>0%</td>
</tr>
<tr>
<td>Ammonium acetate</td>
<td>DBU</td>
<td>NFP</td>
<td>56%</td>
</tr>
<tr>
<td>Ammonium benzoate</td>
<td>DBU</td>
<td>NFP</td>
<td>0%</td>
</tr>
<tr>
<td>Ammonium sulfate</td>
<td>DBU</td>
<td>NFP</td>
<td>0%</td>
</tr>
<tr>
<td>Barium carbonate</td>
<td>DBU</td>
<td>NFP</td>
<td>0%</td>
</tr>
<tr>
<td>Dimethylcarbonate</td>
<td>DBU</td>
<td>NFP</td>
<td>0%</td>
</tr>
<tr>
<td>Tetramethylammonium iodide</td>
<td>DBU</td>
<td>NFP</td>
<td>0%</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>DBU</td>
<td>NFP</td>
<td>0%</td>
</tr>
<tr>
<td>Potassium acetate</td>
<td>DBU</td>
<td>NFP</td>
<td>0%</td>
</tr>
<tr>
<td>Sodium benzoate</td>
<td>DBU</td>
<td>NFP</td>
<td>0%</td>
</tr>
</tbody>
</table>

*a Candidates in bold are successful templates, all other candidates are unsuccessful.

*b Yields were calculated by integration of guest signals (-2.60ppm) and host signals (5.83ppm) from ¹H NMR spectrum.
From the results in Table 2.8, it is evident that with NH₄Cl as template, 58•n-hexane forms in high yield, whereas with KCl as template, there is no 58•n-hexane formed. The template cannot be chloride alone. This hypothesis was confirmed by using tetraethylammonium chloride, the cation of which is too big to fit in the cavity of 58. In this case 58•n-hexane was not detected. Can the cation alone be the template? We attempted the reaction in the presence of ammonium acetate and ammonium benzoate. Ammonium acetate was found to be a good template, whereas ammonium benzoate was not. This suggests that the template is not the ammonium cation on its own, but instead, the whole salt (or multiple cations and anions) acts as a template in the formation of 58. The inability of ammonium benzoate to act as a template for this reaction may be attributed to the large size of the benzoate anion. We also tried several other potential templates, the results of which are summarized in Table 2.8. These results demonstrate that certain salts can template the formation of hemicarcerand 58. This differs from reports of cavitand-based coordination cages templated by anions such as CF₃COO⁻, BF₄⁻ and PF₆⁻. Here the whole ion pair of the salt (or higher order combination of cations and anions) templates the formation of a hemicarcerand with covalent linkers.

2.2.3 Modification of Synthesis of Disulfide [5]carceplex 32

Disulfide [5]carceplex 32 is the first carceplex which is linked by disulfide bonds. It provides a good candidate for us to study dynamic combinatorial libraries (DCLs). However, the synthesis of 32 is difficult: the yield of making [5]cavitand 28 is only 3.6%, the yield from [5]cavitand 28 to [5]benzylthiol 31 is 10%, and from 31 to the final product 32 is 16-25% yield depending on the guest. So the overall yield of 32 from commercially available 2-methyl resorcinol is only 1.2%. To make 32 more attractive in DCL studies, we must increase the yield of 28, 31 and final product 32. Unfortunately, efforts to improve the yield of starting material 28 were unsuccessful, maybe because of the instability of [5]cavitand 28 compared to [4]cavitand 27 and [6]cavitand 29. So we decided to modify the synthesis of the intermediate 31.
2.2.3.1 Modification of the Reaction Conditions to Synthesize [5]Benzylthiol 31

A former student of our group synthesized [5]benzylthiol 31 via a route similar to the method that had been used widely in the synthesis of [4]benzylthiol 2 (Scheme 2.3).\(^\text{10}\)

![Scheme 2.3 Synthesis of [5]benzylthiol 31](image)

The yield of 31 was significantly low, only 10% for the two steps reaction. When we used [4]cavitand 27 to do the same bromination and thiolation reactions, the overall yield of [4]benzylthiol is 80%. What makes them so different?

We found the reason for this difference is the different solubility of [4]cavitand and [5]cavitand derivatives in CCl₄. Both the [4]benzylbromide 60 and [5]benzylbromide 59 are not very soluble in CCl₄. After reacting for some time, the solution became cloudy. [5]Benzylbromide 59 is even less soluble than [4]benzylbromide 60, so after three or four of the five methyl groups on [5]cavitand were brominated, the cavitand might precipitate out from the solution, thus hampering the bromination on the rest methyl groups. The reaction was incomplete, which would lower the yield of 59, which in turn successively lowered the yield of [5]benzylthiol 31. To increase the solubility, we changed the reaction temperature, because most substances have a higher solubility at a higher temperature. So we performed the reaction at 60 °C instead of room temperature, hoping the higher temperature can make three or four brominated [5]cavitand more soluble in CCl₄, thus making the bromination complete.

The second modification was using a different initiator. We compared AIBN and benzoyl peroxide and found the latter is better in this reaction. The amount of the initiator could not be too small. Usually, for 300 mg of [5]cavitand, using at least 20 mg benzoate
peroxide yielded a good result. This maybe because using more initiator can speed up the bromination reaction, thus before precipitating out, all of the five methyl groups had been brominated completely.

Comparing the bromination reaction on different scales showed that this reaction could not be performed on a large scale, otherwise, the reaction would be incomplete. It is hard to monitor this reaction by TLC, due to the poor solubility of [5]benzylbromide 59 in any solvent. A good method to monitor it is $^1$H NMR. Although the [5]benzylbromide 59 is not very soluble in CDCl$_3$, the $^1$H NMR of the suspension can tell us whether the reaction is good or not (Figure 2.20).

![Figure 2.19 Labeling for compounds 59.](image)
Figure 2.20 Monitoring the bromination reaction of cavitand[5] by $^1$H NMR spectra, crude [5]cavitand benzylbromide 59 without purification. A) bad bromination result, using old reaction conditions; B) good bromination result, using modified reaction conditions. (CDCl$_3$, 300 MHz, 300 K).

If the reaction is gone to completion, the $^1$H NMR spectrum will show a symmetric compound structure, the peaks should be well resolved (Figure 2.20B). If the reaction is incomplete or overreacted, the $^1$H NMR spectrum will be messy. The [5]cavitand will lose its symmetry (Figure 2.20A).

The work-up of this reaction is very straightforward. Addition of ethanol in the crude product followed by filtration can get rid of the succinimide and leave [5]benzylbromide as a solid residue. This compound could be used directly in the thiolation reaction without any purification. The detailed procedure of the bromination reaction is explained in experimental section.
The second step of the synthesis of [5]benzylthiol 31 is using the crude [5]benzylbromide 59 and reacting it with thiourea. The key of this reaction is to keep it free of oxygen. We degassed the system by bubbling N₂ through the solution for a certain period of time. Then the reaction was run under the protection of N₂ all the time. After the reaction, the solution was transported to a 100 mL 1M NaOH aqueous solution and stirred for one hour. The solution was then transported to a 100 mL 2M HCl aqueous solution by multiple canula. [5]Benzylthiol 31 can be obtained with an overall yield as high as 40% for the two step reaction.

2.2.3.2 New Route to Synthesize [5]Benzylthiol 31

We also tried another route to synthesize [5]benzylthiol 31 (Scheme 2.4).

The bromination reaction was the same as in the first method, then the crude [5]benzylbromide 59 was reacted with thioacetic acid, and the resulting [5]thioacetate 61 was hydrolyzed to yield [5]benzylthiol 31. An advantage of this method is that the intermediate 61 is very soluble in chloroform. So it can be purified very easily by column chromatography. Then the pure 61 can be hydrolyzed quantitatively under basic conditions to yield pure [5]benzylthiol 31. It is well known that the hydrolysis of acetate can be achieved under acidic or basic conditions. We first tried acidic condition. After stirring a DMA solution of 61 in 2M HCl for several days, 61 was recovered. No evidence of the formation of [5]benzylthiol 31. It seems this thioacetate is very resistant to acidic conditions. We then tried basic condition. A THF solution of 61 was mixed with 2M NaOH aqueous solution. This mixture was stirred at room temperature overnight.
Then the pH was adjusted to acidic conditions by adding 2M HCl. After work-up, pure [5]benzylthiol 31 was obtained. In this method, the overall yield of the three steps reaction is about 30%.

Since the yield of this method is lower than the first method, and this route is one step longer than the first one, we used the first method to synthesize [5]benzylthiol 31 in our project.

2.2.3.3 Synthesis of Disulfide [5]Carceplex 32 and the X-ray Crystal structure of 32●(DMA)₂

We synthesized 32●(DMF)₂ and 32●(DMA)₂ successfully using the same method as Christoph Naumman used. The yields are 14.5% and 11.0% separately. The detailed experimental procedure was written in Christoph Naumman’s Ph. D thesis. We also synthesized these disulfide carceplexes under reversible conditions. The yields were much higher than kinetic conditions. This method will be discussed in the next section.

The crystal of 32●(DMA)₂ was grown up from a solvent mixture of hexanes/chloroform. The X-ray crystal structure shows that there are two conformations in a crystal unit. Each conformation has two DMA molecules entrapped inside the big cavity. The two DMA are located an equatorial position inside the cavity, with each DMA in one of the aryl lined hemispheres separately, and they are parallel to each other (Figure 2.21).
From the side view of the X-ray crystal structure, we can see that the five disulfide bonds are all twisting to one direction, thus making it a chiral molecule. This is consistent with the twistomer theory proposed before. Unlike its precursor cavitand [5], disulfide [5] carceplex 32 lost its $C_5$ symmetry. Figure 2.22 shows the exact position of the entrapped DMA molecules and the disulfide bonds. The connections between the sulfides cannot form an equal pentagon, so it is not $C_5$ symmetric. The asymmetry of the host constrains the location of guests. The top view of the X-ray crystal structure also shows that there are only two relative positions of guest molecules. The dipoles of the entrapped DMA are facing opposite directions (Figure 2.22).
2.2.4 Dynamic Combinatorial Chemistry Study Using Disulfide Carpeplexes as Template

2.2.4.1 Proposal

Recall that in Section 1.2.4 and Section 2.1, disulfide bonds have been used in the study of DCLs successfully, because disulfide bond rearrangement both in peptide refolding and capsule-like compounds formation have been achieved in redox buffers. Since disulfide [5]carpeplex 32 and disulfide [4]carpeplex 54 are disulfide bond linked, why not use these compounds in a DCL study and synthesize these capsule compounds in redox buffers?

Figure 2.22 ORTEP plots of the X-ray crystal structure of 32•2DMA (only sulfurs and the entrapped DMA molecules are shown for clarity). (a) Major conformation (63%). (b) Minor conformation (37%).

Figure 2.23 shows a schematic proposal of the equilibrium experiments to be done in this section. We used [5]cavitand derivatives as samples in this proposal. [4]Cavitand derivatives can be used in the same project as well.
Subjecting [5]benzylthiol 31 to redox conditions with guest DMF may lead to disulfide carceplex 32•(DMF)_2 and oligomeric disulfides, and thus will form a small DCL composed of [5]benzylthiol 31, carceplex 32•(DMF)_2 and oligomer disulfides. The ratio
of these library members depends on their own stability and the ratio of $\text{ME}^{\text{red}}/\text{ME}^{\text{ox}}$ in the redox buffer. Adjusting the ratio of $\text{ME}^{\text{red}}/\text{ME}^{\text{ox}}$ can move the equilibrium either to benzylthiol 31 or to the disulfide product. Since disulfide carceplex $32\bullet(\text{DMF})_2$ has a very good host-guest interaction, it will lower the free energy of this compound. We think it will be more stable than the oligomeric disulfides. So by choosing a proper redox buffer, we might get $32\bullet(\text{DMF})_2$ preferentially. In this way, disulfide carceplex $32\bullet(\text{DMF})_2$ might be synthesized in a higher yield than in kinetic conditions.

Subjecting disulfide carceplex $32\bullet(\text{DMF})_2$ to reducing conditions such as by adding much more $\text{ME}^{\text{red}}$ than $\text{ME}^{\text{ox}}$ might lead to the opening of the disulfide bonds and the release of guest. Guest exchange should be possible by subjecting $32\bullet(\text{DMF})_2$ to a suitable redox buffer with excess of guest DMA. If two different guests DMF and DMA exist in the same library, they will compete with each other in the formation of carceplex $32\bullet$ guests. The most stable one will be formed in higher yield. This could be $32\bullet(\text{DMF})_2$, $32\bullet(\text{DMA})_2$ or $32\bullet(\text{DMF+DMA})$. The template ratio determined this way gives the ratio under thermodynamic conditions. It will be interesting to see if we can compare the template ratios between thermodynamic conditions and kinetic conditions.

2.2.4.2 Dynamic Combinatorial Chemistry Study Using [5]Cavitand Derivatives

2.2.4.2.1 Reduction of Disulfide [5]Carceplex $32\bullet(\text{DMF})_2$

I started my experiments by reducing $32\bullet(\text{DMF})_2$ with $\text{ME}^{\text{red}}$. This is the prerequisite for the DCL study using disulfide carceplexes. The reduction of $32\bullet(\text{DMF})_2$ was performed by subjecting $32\bullet(\text{DMF})_2$ to a $\text{ME}^{\text{red}}$ solution, with DMA as solvent and triethylamine as base. After stirring the solution at room temperature for 24 hours under the protection of argon, 2M HCl aqueous solution was added to quench the reaction. Then the solution was extracted with CHCl$_3$, after removing the solvent, the crude product was monitored directly by NMR. The $^1\text{H}$ NMR spectrum showed that there was no $32\bullet(\text{DMF})_2$ left in the product, only benzylthiol 31 and some impurity (Figure 2.24).
This can be easily seen from Figure 2.24, because there is almost no entrapped DMF signals, and a doublet representing CH$_2$-SH proton showed up at 3.75 ppm. We propose the mechanism of this reduction to be based on the thiol-disulfide exchange (Figure 2.25):
In basic conditions, \( \text{ME}^{\text{red}} \) is deprotonated and form a thiolate. It attacks the disulfide bonds on \( 32\bullet(\text{DMF})_2 \) and forms the intermediate \( \textbf{62} \). This intermediate will be attacked again by other thiolate ions and will form the intermediate \( \textbf{63} \), and the stable small disulfide molecule \( \text{ME}^{\text{ox}} \). Since \( \text{ME}^{\text{red}} \) is in excess, the reaction will be pushed forward from left to right until all the disulfide bonds on \( 32\bullet(\text{DMF})_2 \) are reduced into benzylthiolate. Then the addition of a HCl solution will protonate the thiolates into stable benzylthiol \( \textbf{31} \).

2.2.4.2.2 Synthesis and Study of \( 32\bullet(\text{DMF})_2 \) in Redox Buffers

2-Mercaptoethanol redox buffer has been used extensively to study \textit{in vitro} oxidative protein folding. Here we planned to use this redox buffer to synthesize disulfide \([5]\)carceplex \( 32\bullet(\text{DMF})_2 \) reversibly from \([5]\)benzylthiol \( \textbf{31} \). As we know, the ratio of \( \text{ME}^{\text{ox}} \) to \( \text{ME}^{\text{red}} \) determines the redox property of this redox buffer. So working out the proper ratio of \( \text{ME}^{\text{ox}}/\text{ME}^{\text{red}} \) is the key step of this project. As I mentioned before, another important condition is that the experiment should be performed in an oxygen-free environment, otherwise the reaction will be irreversible. The oxygen-free environment can be monitored by blank experiments.

To figure out the proper redox buffer conditions, we varied the ratios of \( \text{ME}^{\text{ox}} \) to \( \text{ME}^{\text{red}} \) from 10:1 to 1:100 in different experiments. The ratios between \([5]\)benzylthiol \( \textbf{31} \) and the redox reagent was also varied. Because we could not perform this experiment in water solution as the peptides refolding experiment, we couldn’t use buffer solutions to adjust pH value in this system. To make the system in a basic environment, we tested different bases such as cesium carbonate, triethylamine and DBU. After working on it for long time, we finally got the exciting result, the redox buffer to synthesize \( 32\bullet(\text{DMF})_2 \) in reversible conditions was worked out. The proper ratios of \( \text{ME}^{\text{ox}}/\text{ME}^{\text{red}} \) are 1:1–1:3. The ratio between \([5]\)benzylthiol \( \textbf{31} \) to \( \text{ME}^{\text{ox}} \) is 1:10, which means 1 mol of function group on benzylthiol \( \textbf{31} \) to 2 mol of \( \text{ME}^{\text{ox}} \). TEA is a good base for the reaction. DMF was used as solvent and at the same time as guest. The system is stirred for 24 hours under argon.
atmosphere to make the reaction reach equilibrium. Then 2M HCl aqueous solution was used to quench the reaction, and CHCl₃ was added to extract the product from the suspension. After removing the solvent, ¹H NMR spectroscopy was used to determine the content of the crude product mixture.

Under the above conditions, 32•(DMF)₂ was synthesized successfully from 31. The yield was around 50%, which is much higher than the yield in the kinetic conditions (25%). Figure 2.26A is the ¹H NMR spectrum of the crude 32•(DMF)₂ product after the reaction, which is quite pure even without purification. We monitored the reaction system by ¹H NMR, and found that after 24 hours the yield of 32•(DMF)₂ would not increase anymore. So this system can reach equilibrium in 24 hours.

![Figure 2.26](image)

**Figure 2.26** Parts of the ¹H NMR spectra of: A) the crude product in the synthesis of 32•(DMF)₂ in redox buffer; B) the crude product after the guest exchange from (DMF)₂ to (DMA)₂; C) blank reaction. (CDCl₃, 400 MHz, 300K).
To make sure that there is no oxygen affecting the reaction, we performed a blank reaction as a control. In the control reaction, all the conditions were the same as above except that there was no ME\textsuperscript{0\textregistered} in the system, and the reaction was performed with exactly the same procedure as the above reaction. The \textsuperscript{1}H NMR spectrum showed \[5\]benzylthiol \textbf{31} was recovered after several days, and no oxidized product was found (Figure 2.26C).

We also performed the guest exchange experiment. We put \textbf{32}•(DMF)\textsubscript{2} in a redox buffer, DMA was used as solvent in this reaction. After 24 hours, all the entrapped DMF was replaced by DMA (Figure 2.26B). To account for this experimental observation, we proposed a mechanism that involves the thiol-disulfide exchange (Figure 2.27).

![Diagram of proposed mechanism](image)

**Figure 2.27** Proposed mechanism of guest exchange of \textbf{32}•guests
Since the amount of DMA is much more than DMF in this library, DMA was preferred as the guest in the carceplex 32. The guest exchange clearly showed that this system is in a dynamic situation. The changing of any conditions in the system will break the equilibrium, thus changing the content of this dynamic library. The control reaction was also performed, and there was no guest exchange was observed.

One more thing in our proposal was, if we put [4]benzylthiol 31 in a redox buffer containing two different guests, which guest will be entrapped preferentially? It could be two guest a, or two guest b, or one guest a and one guest b. Since we have worked out the redox buffer, we can now perform this experiment. We subjected 31 into a redox buffer, instead of using a single solvent, we used a 1:1 mixture of DMF/DMA as a solvent. When the reaction reached equilibrium, we determined the content of this library by $^1$H NMR spectrum (Figure 2.28) and Maldi-MS (Figure 2.29).

![Figure 2.28 Upfield of $^1$H NMR spectra of 32●guests (CDCl₃, 400 MHz, 300K). A) crude product of guest competition experiment; B) 32●(DMA)$_2$; C) 32●(DMF)$_2$.](image)
Figure 2.29 Maldi-MS of crude product of guest competition experiment. (theoretical mass of $32 \bullet (\text{DMF})_2$: 1936; theoretical mass of $32 \bullet (\text{DMA})_2$: 1964; theoretical mass of $32 \bullet (\text{DMF+DMA})$: 1950).

All the evidence showed that two major carceplexes were formed, one was $32 \bullet (\text{DMF})_2$, the other was $32 \bullet (\text{DMF+DMA})$. There was almost no $32 \bullet (\text{DMA})_2$ in the library. One explanation to this observation is, comparing (DMF)$_2$ and (DMF+DMA), (DMA)$_2$ is too big to be entrapped as a guest of 32. So it has the worst host-guest interaction, thus making it the least stable carceplex among the three. In this dynamic library, DMF and DMA are in equal amounts, the ratio of the products only depends on the stability. So the least stable $32 \bullet (\text{DMA})_2$ could not form in this library. In other words, the template ability of (DMA)$_2$ in thermodynamic conditions is worse than (DMF)$_2$ or (DMF+DMA). The thermodynamic template abilities of (DMF)$_2$ and (DMF+DMA) are almost the same. From $^1$H NMR spectrum, we can see (DMF)$_2$ is a little better template than (DMF+DMA) in the formation of $32 \bullet$ guests, but not so much.
2.2.4.3 Dynamic Combinatorial Library Study Using [4]Cavitand Derivatives

We also introduced the disulfide [4]carseplex 54•guest into the study of dynamic combinatorial libraries. The procedure and reaction conditions were similar to those used on disulfide [5]carseplexes. We first reduced 54•ethylsulfide by excess ME_red in a DMA solution. The disulfide carseplex was reduced quantitatively into [4] benzylthiol 2.

After we figured out the suitable redox buffer that was used on the DCL study of cavitand [5]derivatives, we introduced the cavitand [4]derivatives into the similar redox buffer. In such conditions, 54•guests were synthesized reversibly, but the yields were not as good as that of 32•guest. The reason for the low yields of 54•guests in thermodynamic conditions might be because we still haven't found the most suitable guest for host 54.

2.3 Summary and Outlook

Disulfide [4]carseplexes 54•guests have been synthesized from [4]cavitand benzylthiol. Similar to disulfide [5]carseplex 32, 54 has a diastereotopic proton that can be used in the study of “twistomers” of carseplex. With the help of variable temperature NMR, we concluded that the activation energy barrier ΔG‡ of the twisting on 54 depends on the size of guest molecules. The smaller the guest molecule, the higher the ΔG‡ is. Based on this conclusion, we proposed a possible explanation for the twisting process.

The location of guest molecule in the cavity of 54 was studied. 1H NMR spectra showed that the successful guest molecules were located along the C4 axis of 54, with end methyl groups buried deep into the aryl-lined hemispheres. Dynamic NMR also showed that the guest molecules were shifted from one end of the end to the other.

The synthesis method of [5]cavitand benzylthiol 31, a very important intermediate to synthesize disulfide [5]carseplex 32, was modified. Using the modified reaction condition, 31 can be synthesized in 40% yield from [5]cavitand. This improvement made 32 a more useful template in the study of DCLs.
A new hemicarcerand 58 with four novel trithiacarbonate linkers was synthesized. X-ray crystal analysis of 58•n-hexane showed the exact position of the host and guest molecules. A template study showed that the formation of 58 was templated by certain salts, which was very uncommon in the synthesis of hemicarcerands.

Disulfide carceplexes 32•guest and 54•guest were synthesized reversibly from DCLs. Especially for disulfide [5]carceplex 32•guest, the yield was much higher than that in kinetic conditions. This proved that 32•guest is more thermodynamically stable than those oligomer byproducts. The reversibility of disulfide bond make it possible for the disulfide bridges in the carceplex to open and close essentially one at a time, allowing the entrapped guest to escape and another suitable guest to enter. The guest exchange was observed on 32•guest in a redox buffer. All these observations demonstrate the power of dynamic chemistry, the stepwise breaking and remaking of dynamic covalent bonds. This significantly increased the yield of the more thermodynamically stable product (here is the disulfide linked carceplexes), and decreased the formation of less stable byproduct (the disulfide oligomers).

The yield of 54•guest from a DCL is not very good. This maybe because we still haven’t found the best guest molecules to lower the free energy of this carceplex. Another reason is maybe we haven’t got the suitable buffer to make this carceplex.

Future work includes the development of more suitable guests for carceplexes 32 and 54. Then we can compare the yields under kinetic conditions to those under thermodynamic conditions. This may help us find a better guest for 54, thus synthesize it from a DCL in higher yield. We can study the template effect of 32•guest or 54•guest both under kinetic conditions and thermodynamic conditions. It will be interesting if we can compare the template ratios between kinetic and thermodynamic conditions. Do they have the same tendency or reverse tendency? This may give us more information on the template effect in supramolecular chemistry. For the trithiacarbonate linked hemicarcerand 58, although we conclude that it is the inorganic salts that template the formation of this
hemicarcerand from the comparison reactions, we couldn’t get any information directly from NMR spectroscopy or X-ray crystallography. It would be interesting to see if we could characterize the structure of the entrapped salts, as they are largely unsolvated, which is highly unusual. Such structural information might also provide insight about why some salts are better templates than others, and why they do not remain intact upon work-up. In addition, the divergent thiacarbonate groups may find use in the formation of coordination networks of hemicarceplexes.

2.4 Experimental

2.4.1 General Experimental

All reagents were purchased from Aldrich Chemical Co., Inc. NFP, DMF, DMA were distilled and stored over 4 Å molecular sieves under an N₂ atmosphere. All other reagents were commercially available at >98% purity and were used without further purification. Silica gel (BDH, 230-430 mesh) was used for column chromatography. Silica gel thin layer chromatography was performed on glass-backed plates (Aldrich, silical gel 60, F254, 0.25 mm). ¹H NMR spectra were acquired using Bruker AV-300, AV-400, AMX-500 spectrometers at ambient temperature (300 K) using the residual CHCl₃ as a reference (δ = 7.24 ppm) unless noted otherwise. Matrix assisted laser desorption ionization (MALDI) mass spectra were recorded on a Bruker Biflex IV in reflectron mode. MALDI samples were prepared using 4-nitroaniline as the matrix. X-ray crystallography measurements were made on a Rigaku/ADSC CCD area detector with graphite monochromated Mo-Kα radiation machine and the data analysis was performed using teXsan crystallographic software package.
2.4.2 Synthesis of Disulfide [4]Carceplexes 54•guest.

\[ 54, \text{ } X = S^-, \text{ disulfide [4]carceplex} \]

\[ 58, \text{ } X = \text{trithiacarbonate linker} \]

**Figure 2.30** Labeling for compounds 54•guest, 58 and 58•guest.

**54•ethyl sulfide.** Cs\(_2\)CO\(_3\) (91.0 mg, 0.28 mmol, 10 equiv. per benzylthiol) was added to DMF (30 mL) and stirred for 2 min. Ethyl sulfide (0.90 mL, 2% mol of DMF) and 2 (30.0 mg, 0.028 mmol) were added to the mixture. The system was closed and stirred at room temperature for 48 hours, then poured into a 2M aqueous solution of HCl (50 mL). The resultant suspension was extracted with CHCl\(_3\) (3×10 ml). The CHCl\(_3\) extracts were combined and the solvent was removed *in vacuo*. The resulting white residue was dried under vacuum overnight, then purified through silica gel column, eluting with CHCl\(_3\)/hexanes (1:4). Precipitation of the product from CHCl\(_3\)/hexanes gave a white solid (4.8 mg, 15%).

(500 MHz, CDCl\(_3\)) \( \delta \) 7.01 (s, 8H, H\(_p\)); 5.84 (d, 8H, \( J = 7.6 \text{ Hz, H}_{\text{out}} \)); 4.67 (t, 8H, \( J = 8.0 \text{ Hz, H}_{\text{in}} \)); 4.32 (d, 8H, \( J = 7.6 \text{ Hz, H}_{\text{in}} \)); 4.07 (broad, 16H, CH\(_2\)-SS); 2.13 (m, 16H, CH-CH\(_2\)); 1.24-1.49 (m, 64H, feet (CH\(_2\))\(_4\)); 0.86 (t, 24H, \( J = 6.6 \text{ Hz, feet CH}_3 \)); 0.87 (overcovered, 4H, guest SCH\(_2\)CH\(_3\)); -2.74 (t, 6H, \( J = 7.5 \text{ Hz, SCH}_2CH_3 \)).

MALDI-MS (positive mode): 2302 (M + Ag\(^+\)); calcd. (M + Ag\(^+\)) 2302.
54·diethyl ether. Similar to the procedure of making 54·ethyl sulfide, except that diethyl ether was used instead of ethyl sulfide. 2 (30 mg, 0.028 mmol), Cs₂CO₃ (91 mg, 0.28 mmol), DMF (30 mL), diethyl ether (0.87 mL, 2% mol of DMF). 54·diethyl ether was obtained as a white solid (2.0 mg, 6%).

(500 MHz, CDCl₃) δ 7.04 (s, 8H, Hₚ); 5.84 (d, 8H, J = 7.6 Hz, H₀); 4.66 (t, 8H, J = 8.0 Hz, Hₘ); 4.32 (d, 8H, J = 7.6 Hz, Hₖ); 3.75-4.60 (broad, 16H, CH₂-SS); 2.14 (m, 16H, CH-CH₂); 1.78 (q, 4H, J = 7.1 Hz, OCH₂CH₃); 1.24-1.49 (m, 64H, feet (CH₂)₄); 0.86 (t, 24H, J = 6.6 Hz, feet CH₃); -2.33 (t, 6H, J = 7.1 Hz, OCH₂CH₃). MALDI-MS (positive mode): 2286 (M + Ag⁺); calcd. (M + Ag⁺) 2286.

54·3-pentanone. Similar to the procedure of making 54·ethyl sulfide, except that 3-pentanone was used instead of ethyl sulfide. 2 (30 mg, 0.028 mmol), Cs₂CO₃ (91 mg, 0.28 mmol), DMF (30 mL), 3-pentanone (0.87 mL, 2% mol of DMF). 54·3-pentanone was obtained as a white solid (1.0 mg, 3%).

(300 MHz, CDCl₃) δ 7.03 (s, 8H, Hₚ); 5.84 (d, 8H, J = 7.6 Hz, H₀); 4.66 (t, 8H, J = 8.0 Hz, Hₘ); 4.32 (d, 8H, J = 7.6 Hz, Hₖ); 3.75-4.33 (broad, 16H, CH₂-SS); 2.12 (m, 16H, CH-CH₂); 1.24-1.49 (m, 64H, feet (CH₂)₄); 0.86 (t, 24H, J = 6.6 Hz, feet CH₃); 0.84 (overcovered, 4H, guest COCH₂CH₃); -2.33 (t, 6H, J = 7.1 Hz, guest COCH₂CH₃). MALDI-MS (positive mode): 2298 (M + Ag⁺); calcd. (M + Ag⁺) 2298.

54·ethylmethyl sulfide. Similar to the procedure of making 54·ethyl sulfide, except that ethylmethyl sulfide was used instead of ethyl sulfide. 2 (30 mg, 0.028 mmol), Cs₂CO₃ (91 mg, 0.28 mmol), DMF (30 mL), ethylmethyl sulfide (0.75 mL, 2% mol of DMF). 54·ethylmethyl sulfide was obtained as a white solid (4.9 mg, 16%).

(500 MHz, CDCl₃) δ 7.04 (s, 4H, Hₚ); 7.01 (s, 4H, Hₚ'); 5.87 (d, 4H, J = 7.5 Hz, H₀); 5.82 (d, 4H, J = 7.6 Hz, H₀'); 4.66 (m, 8H, Hₘ); 4.61 (d, 4H, J = 7.6 Hz, Hₖ); 4.19 (d,
MALDI-MS (positive mode): 2288 (M + Ag⁺); calcd. (M + Ag⁺) 2288.

54•methyl sulfide. Similar to the procedure of making 54•ethyl sulfide, except that methyl sulfide was used instead of ethyl sulfide. 2 (30 mg, 0.028 mmol), Cs₂CO₃ (91 mg, 0.28 mmol), DMF (30 mL), methyl sulfide (0.75 mL, 2% mol of DMF). 54•methyl sulfide was obtained as a white solid (4.4 mg, 14%).

(300 MHz, CDCl₃) δ 7.03 (s, 8H, Hₚ); 5.84 (d, 8H, J = 7.5 Hz, Hₜₚ); 4.68 (t, 8H, J = 7.8 Hz, Hₚ); 4.45 (d, 8H, J = 7.5 Hz, Hₜₚ); 4.34 (broad, 8H, CH₂-SS); 3.82 (broad, 8H, CH₂-SS); 2.16 (m, 16H, CH-CH₂); 1.24-1.49 (m, 64H, feet (CH₂)₄); 0.86 (t, 24H, J = 6.6 Hz, feet CH₃); -0.65 (s, 6H, guest SCH₃).

MALDI-MS (positive mode): 2274 (M + Ag⁺); calcd. (M + Ag⁺) 2274.

2.4.3 Competition Experiments of 54 with Different Guests

Cs₂CO₃ (45.0 mg, 0.14 mmol, 10 equiv. per benzylthiol) was added to DMF (15 mL) and stirred for 2 min. Guest 1, guest 2 (both are 2% mol of DMF) and cavitand [4]benzylthiol 2 (15.0 mg, 0.014 mmol) were added to the mixture. The system was closed and stirred at room temperature for 48 hours, then poured into a 2M aqueous solution of HCl (25 mL). The resultant suspension was extracted with CHCl₃ (3×10 mL). The CHCl₃ extracts were combined and the solvent was removed in vacuo. The product mixture was then passed through a short silica gel column, eluting with CHCl₃/hexanes (1:2), to get rid of polymer byproducts. Product ratios were calculated from the ¹H NMR spectra by integration of each set of guest signals. The error in the integration is estimated to be ±10%.
2.4.4 Synthesis of Trithiacarbonate Linked [4]Hemicarcerand 58

Cs₂CO₃ (91.0 mg, 0.28 mmol, 10 equiv. per benzylthiol) was added to NFP (8 mL) and stirred at room temperature for 2 min. Dimethyltrithiacarbonate (0.35 mL, 2.8 mmol) and 2 (30.0 mg, 0.028 mmol) were added to the mixture. The system was stirred at room temperature for 2 hours under the protection of N₂, then poured into a 2M aqueous solution of HCl (25 mL). The resultant yellow suspension was extracted with CHCl₃ (3×10 mL). The CHCl₃ extracts were combined and the solvent was removed in vacuo. The resulting yellow solid was dried under vacuum overnight. Then purified through silica gel column, eluting with CHCl₃/cyclohexane (1:8). Precipitation of the product from CHCl₃/cyclohexanes gave a yellow solid. (22.3 mg, 70%).

(300 MHz, CDCl₃) δ 7.09 (s, 8H, Hₚ); 5.80 (s, 8H, H₀); 4.76 (s, 8H, Hₘ); 4.40 (s, 8H, CH₂-S); 4.05-4.18 (broad, 8H, Hₐ); 2.17 (m, 16H, CH-CH₂); 1.24-1.49 (m, 64H, feet (CH₂)₄); 0.86 (t, 24H, J = 6.6 Hz, feet CH₃).

MALDI-MS (positive mode): 2388 (M + Ag⁺); calcd. (M + Ag⁺) 2388.

2.4.5 Synthesis of Trithiacarbonate Linked [4]Hemicarceplexes 58•guests

58•n-hexane. Procedure A: Cs₂CO₃ (91.0 mg, 0.28 mmol, 10 equiv. per benzylthiol) was added to NFP (8 mL) and stirred at room temperature for 2 min. n-Hexane(1 mL), dimethyl trithiacarbonate (0.35 mL, 2.8 mmol) and 2 (30.0 mg, 0.028 mmol) were added to the mixture. The system was stirred at room temperature for 2 hours under the protection of N₂, then poured into a 2M aqueous solution of HCl (25 mL). The resultant yellow suspension was extracted with CHCl₃ (3×10 mL). The CHCl₃ extracts were combined and the solvent was removed in vacuo. The resulting yellow solid was dried under vacuum overnight. Then purified through silica gel column, eluting with CHCl₃/hexanes (1:8). Precipitation of the product from CHCl₃/hexanes gave a yellow solid. (21.9 mg, 66%).
Procedure B: Hemicarerrand 58 (20.0 mg, 0.0088 mmol) was dissolved in CHCl₃ (20 mL). n-Hexane (1 mL) was added. The mixture was incubated at room temperature for 7 days, after which the solvent was removed in vacuo. The crude product was purified by passing through a silica gel column, eluting with CHCl₃/hexanes (1:8). The product was precipitated from CHCl₃ by adding hexanes. (20.2 mg, 98%)

(400 MHz, CDCl₃) δ 7.11 (s, 8H, Hₚ); 5.83 (d, 8H, J = 7.5 Hz, Hₒᵤₑ); 4.70 (t, 8H, J = 7.9 Hz, Hₘ); 4.36 (s, 16H, CH₂-S); 4.25 (d, 8H, J = 7.5 Hz, Hₘᵢᵢᵢ); 2.17 (m, 16H, CH-CH₂); 1.24-1.49 (m, 64H, feet (CH₂₄)); 1.12 (m, 4H, guest CH₂CH₂CH₃); 0.86 (t, 24H, J = 6.6 Hz, feet CH₃); 0.31 (m, 4H, guest CH₂CH₂CH₃); -2.60 (t, 6H, J = 7.2 Hz, guest CH₂CH₂CH₃).

This structure was confirmed by X-ray crystallographic analysis:

<table>
<thead>
<tr>
<th>Empirical Formula</th>
<th>C₁₃₃H₁₆⁹O₁₆S₁₂Cl₉</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystal Color, Habit</td>
<td>Yellow, block</td>
</tr>
<tr>
<td>Crystal Dimensions</td>
<td>0.50 X 0.50 X 0.50 mm</td>
</tr>
<tr>
<td>Space Group</td>
<td>P1 (#2)</td>
</tr>
<tr>
<td>Lattice Parameters</td>
<td>a = 14.3703(4) Å</td>
</tr>
<tr>
<td></td>
<td>b = 23.5162(2) Å</td>
</tr>
<tr>
<td></td>
<td>c = 24.0682(6) Å</td>
</tr>
<tr>
<td></td>
<td>α = 75.911(4)°</td>
</tr>
<tr>
<td></td>
<td>β = 87.016(6)°</td>
</tr>
<tr>
<td></td>
<td>γ = 72.726(5)°</td>
</tr>
<tr>
<td></td>
<td>V = 7531.2(4) Å³</td>
</tr>
<tr>
<td>Z value</td>
<td>2</td>
</tr>
<tr>
<td>R</td>
<td>0.086</td>
</tr>
</tbody>
</table>
**58•n-pentane.** Similar to Procedure A and Procedure B of making 58•n-hexane, except that n-pentane was used instead of n-hexane.

(300 MHz, CDCl₃) δ 7.13 (s, 8H, Hₖ); 5.83 (d, 8H, J = 7.5 Hz, Hₜₖ); 4.70 (t, 8H, J = 7.9 Hz, Hₖ₌); 4.36 (s, 16H, CH₂-S); 4.17 (d, 8H, J = 7.5 Hz, Hₜₖ); 2.17 (m, 16H, CH-CH₂); 1.24-1.49 (m, 64H, feet (CH₂)₄); 1.25 (overcovered, 4H, guest CH₂CH₂CH₃); 0.86 (t, 24H, J = 6.6 Hz, feet CH₃); 0.72 (m, 4H, guest CH₂CH₂CH₃); -1.69 (t, 6H, J = 7.2 Hz, guest CH₂CH₂CH₂CH₃).

**58•n-heptane.** Similar to Procedure A and Procedure B of making 58•n-hexane, except that n-heptane was used instead of n-hexane.

(500 MHz, CDCl₃) δ 7.08 (s, 8H, Hₖ); 5.83 (d, 8H, J = 7.6 Hz, Hₜₖ); 4.74 (t, 8H, J = 8.1 Hz, Hₖ₌); 4.37 (s, 16H, CH₂-S); 4.17 (d, 8H, J = 7.6 Hz, Hₜₖ); 2.17 (m, 16H, CH-CH₂); 1.24-1.49 (m, 64H, feet (CH₂)₄); 1.17 (overcovered, 2H, guest CH₂CH₂CH₂CH₃); 0.87 (overcovered, 4H, CH₂CH₂CH₂CH₃); 0.86 (t, 24H, J = 6.6 Hz, feet CH₃); -0.55 (m, 4H, guest CH₂CH₂CH₂CH₃); -3.15 (t, 6H, J = 7.5 Hz, guest CH₂CH₂CH₂CH₃).

**2.4.6 Synthesis of Disulfide [5]Carcoplexes 32•guests.**

![Figure 2.31 Labeling for compounds 31, 32•guest, 59 and 61.](image_url)

31, X = SH, [5]benzylthiol
32, X = SS, disulfide [5]carceplex
59, X = Br, [5]benzylbromide
61, X = S-C(O)CH₃, [5]benzylthioacetate

Figure 2.31 Labeling for compounds 31, 32•guest, 59 and 61.
2.4.6.1 Cavitand [5]benzylbromide 59. Cavitand[5] 28 (148.0 mg, 0.2 mmol) was dissolved in CCl₄ (100 mL) and this solution was bubbled with N₂ for 2 hours. NBS (185.1 mg, 1.04 mmol, 5.2 equiv.) and benzoyl peroxide (50 mg) were added. The reaction mixture was stirred at 50 °C under a 100W tungsten filament lamp for 12 hours under the protection of N₂. Then the solvent was removed in vacuo. Ethanol (20 mL) was added, and the resulting suspension was sonicated for 5 min. Filtration of the suspension afforded a white solid, which was dried at 100 °C under vacuum for 24 hours. This crude product was used directly for the next reaction without purification.

(400 MHz, CDCl₃) δ 7.31 (s, 5H, Hₚ); 6.09 (d, 5H, J = 7.5 Hz, Hₒᵤ); 4.66 (d, 5H, J = 7.5 Hz, Hᵢₙ); 4.58 (s, 10H, CH₂-Br); 4.37 (d, 5H, J = 13.1 Hz, Hₒ); 3.39 (d, 5H, J = 13.1 Hz, Hᵦᵢ)

2.4.6.2 Synthesis of Cavitand [5]Benzylthiol 31 from 59. A solution of 59 (obtained from 103.6 mg cavitand[5] 28, 0.14 mmol) and thiourea (110.0 mg, 6.58 mmol) in DMF (20 mL) was bubbled by N₂ for 2 hours. Then stirred at room temperature for 18 hours under the protection of N₂. The reaction mixture was then transferred into a 2M aqueous solution of degassed NaOH (30 mL) through a canula, and stirred for an additional 1 hour. The reaction mixture was then poured into a 2M aqueous solution of HCl (100 mL). The resultant gelatinous suspension was extracted with CHCl₃ (3×30 mL). The combined organic extracts were washed with water (30 mL). After removing the solvent in vacuo, a pale yellow residue was obtained. The latter material was passed through a short silica gel column (CHCl₃ eluent) to yield a white solid, which upon recrystallization from CHCl₃/ethanol afforded 31 as a white crystal (54.2 mg, 43% yield for the two step reaction from cavitand[5] 28).

(300 MHz, CDCl₃) δ 7.25 (s, 5H, Hₚ); 6.03 (d, 5H, J = 7.3 Hz, Hₒᵤ); 4.56 (d, 5H, J = 7.3 Hz, Hᵢₙ); 4.37 (d, 5H, J = 13.0 Hz, Hₒ); 3.75 (d, 10H, J = 7.1 Hz, CH₂-S); 3.39 (d, 5H, J = 13.0 Hz, Hᵦᵢ); 1.55 (t, 5H, J = 7.1 Hz, SH).

MALDI-MS (positive mode): 901 (M + H⁺); calcd. (M + H⁺) 901.
2.4.6.3 Synthesis of Cavitand [5]Benzylthioacetate 61 from 59. A mixture of crude 59 (obtained from 111.0 mg cavitand[5] 28, 0.15 mmol) and DMA (30 mL) was cooled to 0°C. Thioacetic acid (146 μL, 2.0 mmol) was added to the stirred solution, followed by the addition of triethylamine (278 μL, 2 mmol). The reaction mixture was slowly warmed to room temperature and left stirring for 15 hours. The reaction mixture was poured into 100 mL of aqueous 2M HCl, and extracted with chloroform (3×10 mL). The organic layers were combined, and the solvent was removed under reduced pressure. The remaining residue was subjected to column chromatography using chloroform as the eluent to yield 61 as a white solid (63.0 mg, 38%).

(400 MHz, CDCl₃) δ 7.22 (s, 5H, Hₚ); 5.97 (d, 5H, J = 7.5 Hz, Hₚ); 4.38 (d, 5H, J = 7.5 Hz, Hₚ); 4.34 (d, 5H, J = 12.9 Hz, Hₖ); 4.16 (s, 10H, CH₂-S); 3.35 (d, 5H, J = 12.9 Hz, Hₖ); 2.22 (s, 15H, COCH₃).

MALDI-MS (positive mode): 1133 (M + Na⁺); 1149 (M + K⁺); calcd. (M + Na⁺) 1133; calcd. (M + K⁺) 1149.

2.4.6.4 Synthesis of Cavitand [5]Benzylthiol 31 from 61. Cavitand [5]benzylthioacetate 61 (47.3 mg, 0.043 mmol) was dissolved in a THF + H₂O mixture solvent (THF/H₂O = 5/1, 10 mL). This solution was degassed by bubbling through N₂ for an hour, then a completely degassed 2M aqueous NaOH solution (7 mL) was added. The mixture was stirred at 50°C for 12 hours under the protection of N₂, then poured into 5% aqueous acetic acid solution (30 mL) and extracted with CHCl₃ (3×10 mL). The organic layers were combined and the solvent was removed in vacuo. The obtained crude product was performed on silica gel column, CHCl₃ as eluent and gave a white product (353.0 mg, 85%).
2.4.6.5 Synthesis of Disulfide [5]Carceplexes 32•guests.

32•(DMF)₂. Excess Cs₂CO₃ (91.0 mg, 0.28 mmol) was added to DMF (15 mL) and stirred for 2 min. A solution of 31 (15 mg, 0.017 mmol) in DMF (15 mL) was added by pump syringe during 2 hours. The system was loosely covered and stirred at room temperature for 24 hours. The mixture was poured into a 2M aqueous solution of HCl (50 mL). The resultant suspension was extracted with CHCl₃ (3×10 mL), and the CHCl₃ extracts were combined and the solvent was removed in vacuo. The resulting white residue was dried under vacuum overnight, then purified through silica gel column, eluting with CHCl₃. Precipitation of the product from CHCl₃/hexanes gave a white solid (2.4 mg, 14%).

(500 MHz, CDCl₃) δ 7.14 (s, 10H, Hₚ); 5.94 (d, 10H, J = 7.5 Hz, H₀ut); 5.32 (s, 2H, guest OCH); 4.57 (d, 10H, J = 7.5 Hz, Hᵢn); 4.52-3.75 (broad, 20H, CH₂-S); 4.52 (d, 10H, J = 13.0 Hz, H₀b); 3.29 (d, 10H, J = 13.0 Hz, Hᵢb); 0.28 (s, 6H, N-CH₃); 0.20 (s, 6H, N-CH₃).

MALDI-MS (positive mode): 1937 (M + H⁺); 1960 (M + Na⁺); calcd. (M + H⁺) 1937; calcd. (M + Na⁺) 1960.

32•(DMA)₂. Similar to the procedure of making 32•(DMF)₂, except that DMA was used as solvent and guest instead of DMF (1.8 mg, 11% yield).

(400 MHz, CDCl₃) δ 7.10 (s, 10H, Hₚ); 5.89 (d, 10H, J = 7.6 Hz, H₀ut); 4.70 (d, 10H, J = 7.6 Hz, Hᵢn); 4.50-3.80 (broad, 20H, CH₂-S); 4.28 (d, 10H, J = 12.9 Hz, H₀b); 3.29 (d, 10H, J = 12.9 Hz, Hᵢb); 0.39 (s, 6H, N-CH₃); 0.35 (s, 6H, N-CH₃); -0.37 (s, 6H, COCH₃).

MALDI-MS (positive mode): 1965 (M + H⁺); 1987 (M + Na⁺); calcd. (M + H⁺) 1965; calcd. (M + Na⁺) 1987.
This structure was confirmed by X-ray crystallographic analysis:

<table>
<thead>
<tr>
<th>Empirical Formula</th>
<th>C_{99}H_{89}O_{22}S_{10}Cl_{3}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystal Color, Habit</td>
<td>clear, platelet</td>
</tr>
<tr>
<td>Crystal Dimensions</td>
<td>0.50 X 0.20 X 0.20 mm</td>
</tr>
<tr>
<td>Space Group</td>
<td>P2_1/a (#14)</td>
</tr>
<tr>
<td>Lattice Parameters</td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>21.665(4) Å</td>
</tr>
<tr>
<td>b</td>
<td>21.915(5) Å</td>
</tr>
<tr>
<td>c</td>
<td>22.869(5) Å</td>
</tr>
<tr>
<td>β</td>
<td>104.35(1)°</td>
</tr>
<tr>
<td>V</td>
<td>12439(2) Å^3</td>
</tr>
<tr>
<td>Z value</td>
<td>4</td>
</tr>
<tr>
<td>R</td>
<td>0.120</td>
</tr>
</tbody>
</table>

2.4.7 Reversible Study by Disulfide [5]Carceplexes 32•guests

2.4.7.1 Reducing of 32•(DMF)_2. 32•(DMF)_2 (2 mg), DMF (1 mL) and triethylamine (50 μL) were mixed and degassed completely by bubbling through argon for 2 hours. Degassed ME\textsuperscript{red} (0.1 mL, excess) was added. The mixture was stirred at room temperature for 20 hours under the protection of argon, then 2M HCl (10 ml) was added to quench the reaction. The suspension was then extracted with CHCl\textsubscript{3} (3 x 5 mL). The combined organic extracts were washed with water, condensed \textit{in vacuo}. The obtained crude product was dissolved in CDCl\textsubscript{3} and performed on the \textsuperscript{1}H NMR directly to monitor the result of the reaction.

2.4.7.2 Synthesis of 32•guest in Redox Buffer.

32•(DMF)_2. Cavitand [5]benzylthiol 31 (10 mg, 0.011 mmol), ME\textsuperscript{red} (25.7 mg, 0.22 mmol, 20 equiv.) and DMF (7.5 mL) were mixed and degassed completely by bubbling argon for 2 hours. This formed solution (A). ME\textsuperscript{ox} (17.0 mg, 0.11 mmol, 10 equiv.),
triethylamine (700 µL, excess) and DMF (7.5 mL) were mixed and degassed completely by bubbling argon for 2 hours. This formed solution (B). Solution (A) and solution (B) were combined and mixed through a canula. The final buffer system composed of 31 (0.73 mM), ME$^{\text{red}}$ (14.7 mM, 20 equiv.), ME$^{\text{ox}}$ (7.3 mM, 10 equiv.), triethylamine (excess) and DMF (15 mL). The mixture was stirred at room temperature for 24 hours under the protection of argon, then 2M HCl (50 mL) were added to quench the reaction. The suspension was then extracted with CHCl$_3$ (3 × 10 mL). The combined organic extracts were washed with water, condensed in vacuo. The obtained crude product was dissolved in CDCl$_3$ and performed on $^1$H NMR. The yield was calculated from the integration of the host and guest peaks (estimated yield 50%).

32•(DMA)$_2$. Similar to the procedure of making 32•(DMF)$_2$, except that DMA was used as solvent and guest instead of DMF. (yield calculated from $^1$H NMR spectrum, 42%).

2.4.7.3 Guest Exchange Reaction. ME$^{\text{red}}$ (15.4 mg, 0.2 mmol, 40 equiv.) and DMA (7.5 mL) were mixed and degassed completely by bubbling argon for 2 hours. This formed solution (A). 32•(DMF)$_2$ (10 mg, 0.005 mmol, crude product from section 2.4.7.4), ME$^{\text{ox}}$ (15.4 mg, 0.1 mmol, 20 equiv.), triethylamine (700 µL, excess) and DMA (7.5 mL) were mixed and degassed completely by bubbling argon for 2 hours. This formed solution (B). Solution (A) and solution (B) were combined and mixed through a canula. The final buffer system composed of 32•(DMF)$_2$ (0.33 mM), ME$^{\text{red}}$ (13.3 mM, 40 equiv.), ME$^{\text{ox}}$ (6.7 mM, 20 equiv.), triethylamine (excess) and DMA (15 mL). The mixture was stirred at room temperature for 24 hours under the protection of argon, then 2M HCl (50 mL) were added to quench the reaction. The suspension was then extracted with CHCl$_3$ (3 × 10 mL). The combined organic extracts were washed with water, and condensed in vacuo. The obtained crude product was dissolved in CDCl$_3$ and performed on $^1$H NMR directly to monitor the result of the reaction.

2.4.7.4 Competition Experiment in Redox Buffer. Similar to the procedure of making 32•(DMF)$_2$ in redox buffer, except that a mixed solvent of DMA/DMF = 1/1 was used as
solvent and guest instead of only DMF. The obtained crude product was dissolved in CDCl₃ and performed on ¹H NMR directly to monitor the result of the reaction.

2.4.8 Reversible Study by Disulfide [4]Carceplexes 5₄•guests

2.4.8.1 Reducing of 5₄•ethyl sulfide. 5₄•ethylsulfide (2 mg), DMF (1 mL) and triethylamine (50 µL) were mixed and degassed completely by bubbling argon for 2 hours. Degassed ME<sup>red</sup> (0.1 mL, excess) were added. The mixture was stirred at room temperature for 20 hours under the protection of argon, then 2M HCl (10 mL) were added to quench to reaction. The suspension was then extracted with CHCl₃ (3 × 5 mL). The combined organic extracts were washed with water, and condensed in vacuo. The obtained crude product was dissolved in CDCl₃ and performed on ¹H NMR directly to monitor the result of the reaction.

2.4.8.2 Synthesis of 5₄•guest in Redox Buffer.

5₄•ethyl sulfide. Cavitand [4]benzyithiol 2 (12.7 mg, 0.012 mmol), ME<sup>red</sup> (15.0 mg, 0.19 mmol, 16 equiv.) and DMF (7.5 mL) were mixed and degassed completely by bubbling argon for 2 hours. This formed solution (A). ME<sup>ox</sup> (14.8 mg, 0.096 mmol, 8 equiv.), triethylamine (700 µL, excess), ethyl sulfide (0.5 mL) and DMF (7.5 mL) were mixed and degassed completely by bubbling argon for 2 hours. This formed solution (B). Solution (A) and solution (B) were combined and mixed through a canula. The final buffer system composed of 2 (0.80 mM), ME<sup>red</sup> (12.7 mM, 16 equiv.), ME<sup>ox</sup> (6.4 mM, 8 equiv.), triethylamine (excess), ethyl sulfide (0.5 mL) and DMF (15 mL). The mixture was stirred at room temperature for 24 hours under the protection of argon, then 2M HCl (50 mL) were added to quench the reaction. The suspension was then extracted with CHCl₃ (3 × 10 mL). The combined organic extracts were washed with water, and condensed in vacuo. The obtained crude product was dissolved in CDCl₃ and performed on ¹H NMR, the yield was calculated from the integration of the host and guest peaks (estimated yield 8%).
54-ethylmethyl sulfide. Similar to the procedure of making 54-ethyl sulfide, except that ethylmethyl sulfide (0.5 mL) was used as guest instead of ethyl sulfide. (yield calculated from ${}^1$H NMR spectrum, 2%).

2.5 References