SYNTHESIS AND PROPERTY INVESTIGATION OF SCHIFF-BASE POLYCAVITAND NANOCAPSULES

by

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ABSTRACT OF THE DISSERTATION

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Disclosed are studies on the design and synthesis of polycavitand nanocapsules and their encapsulation properties. In Chapter 1, a general overview about capsular molecules is presented. In Chapter 2, the synthesis, characterization and chiral guest recognition of chiral tetracavitand nanocapsules and chiral hemicarcerands are described. For tetracavitand nanocapsule 41a, a two-step enantiomerization process is discussed. In Chapter 3, the rational design and synthesis of giant nanocapsules, including octahedral and rhombicuboctahedral nanocapsules is discussed. These nanocapsules formed quantitatively from condensation reactions between deep cavitands and linear aromatic linkers. In Chapter 4, effective molarities (EMs) of a series of hemicarcerands and nanocapsules were measured. The results show that solvents and the linker rigidity play an important role in the formation of hemicarcerands and EMs of nanocapsules 23, 65a, 67a and 69 are mainly controlled by entropy. Furthermore, stepwise EMs of hemicarcerands 20b, 20d and 20g are discussed, too. In Chapter 5, the synthesis of hemicarcerands through liquid-assisted grinding (LAG) is described. Furthermore, encapsulation inside hemicarcerands 20b, 20d and 20g by grinding is discussed. In Chapter 6, water-soluble acylhydrazone rhombicuboctahedral nanocapsules were synthesized. Preliminary results of protein encapsulation are also presented.
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DEDICATION

To my wife

To my parents and brother
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Chapter 1

General Introduction

Molecular container compounds are hollow, spherical host molecules with cavities that are large enough to bind one or multiple guests. In the 1985, D. J. Cram and co-workers synthesized the first closed shell molecular container 1, termed carcerand, which permanently entrapped one small guest molecule. In the following years, a wide variety of hemicarcerands were reported. Different from carcerands, hemicarcerands form stable complexes (hemicarcerplexes) at ambient temperature, yet release guests at elevated temperatures. These hemicarcerplexes are stabilized by constrictive and intrinsic binding (Fig. 1-2). Intrinsic binding is the Gibbs free energy of complexation, which arises from non-covalent interactions between the guest and the host. Constrictive binding is the activation energy for complexation. It is a physical barrier, by which portals of the hemicarcerands confine guests with larger cross sections inside the cavities. If portals are too small, constrictive binding will be very high, preventing the guest from escaping without breaking bonds. K. N. Houk proposed, that constrictive binding is controlled by two gating mechanisms, namely “French door” and “Sliding door” mechanisms (Fig. 1-3). The ‘French door’ mechanism involves the conformational change of spanners (-O-CH$_2$-O- groups of the cavitand) as shown in Fig. 1-3C and an increased separation between cavitands leads to a ‘sliding door’.
Figure 1-1: Carceplexes 1⊙G and guests.

Figure 1-2: Constrictive and intrinsic binding for encapsulation.
One distinctive feature of hemicarceplexes is confinement of the guest within a small space that is insulated by the host from the outside environment. This may enhance or reduce reactivity of the guest and prevents dimerization of guests or their reaction with bulk phase components, as long as they cannot enter the cavity. Therefore, they provide a simple and efficient way to stabilize otherwise fleeting species. One impressive example is the observation of \( o \)-benzyne 5 inside hemicarcerand 2 (Fig.1-4). \( o \)-Benzyne 5 is highly unstable and believed to be an intermediate in nucleophilic aromatic substitutions. By encapsulation, it was directly observed. At 77 K, photolysis of hemicarceplex 2⊙3 yielded 2⊙4, which is stable at room temperature. Without protection by 2, free benzocyclopropenone 4 could only be observed at -80 °C. Further photolysis generated the incarcerated \( o \)-benzyne 2⊙5 which could be characterized by \(^1\)H-NMR and \(^{13}\)C-NMR spectroscopy at -75 and -98 °C, respectively.
Although a very high yield was achieved for certain hemicarceplexes with appropriate template guests\textsuperscript{6}, generally, the synthesis is still challenging\textsuperscript{7}. Especially for larger poly-cavitand capsules, the yield is even lower. For example, a covalently bonded hexa-cavitands nanocapsule was reported by J. C. Sherman and coworkers in only 0.8% yield from the starting tetrol-cavitand\textsuperscript{8}. Therefore, especially for the synthesis of nanometer-sized multi-cavitand capsules, it is highly desirable to find alternative ways to efficiently synthesize container molecules.

Molecular capsules built by self-assembly of simple modular molecules are easily accessed and display a lot of remarkable properties. They can form through either non-covalent interactions or dynamic covalent chemistry (DCC), which typically yields the most thermodynamically stable product by taking advantage of proof
reading and error correction. So far, metal-coordination, hydrogen-bonding, hydrophobic interactions and electrostatic interactions (salt-bridges) have been utilized as the non-covalent interactions in self-assembly capsule formation.

Metal-coordination interactions have been used successfully to build hundreds of complicated two and three-dimensional structures, such as polygons, polyhedrons, platonic solids and other sophisticated architectures. This approach has been pioneered by the groups of M. Fujita, P. J. Stang and K. N. Raymond. A striking example is octahedral cage synthesized by the Fujita group. It forms quantitatively by simply mixing ethylenediamine capped PdII ion and 1,3,5-tris(4-pyridine)triazine in a 6:4 ratio (Fig. 1-5). The highly charged molecular cage is water-soluble and coplanar triazine units provide a large hydrophobic surface, which makes it an extraordinary candidate for molecule encapsulation in aqueous solutions. Cage has been used as catalyst or as reaction vessel, in which reactions yield unusual products, and which stabilizes reactive species.

Another elegant design is tetrahedral cage reported by the Nitschke group. Combing dynamic imine bonds with metal-coordination interactions, they successfully assemble from twelve 2-formylpyridine, six 4,4-diaminobiphenyl-2,2-disulfonic acid and four Fe(II) ions in basic aqueous solution (Fig. 1-6). Similar to 8, the hydrophobic cavity of 11 exhibits a high binding affinity towards hydrophobic guests. For example, white phosphorus, a nonpolar, flammable tetrahedral molecule, was encapsulated in the cage and became very stable in aerated water and O2-insensitive.
Hydrogen-bonding interactions, essential for the functionality and structure of many biomolecules, have also been widely used in artificial self-assemblies. The research on hydrogen-bonded capsules was pioneered by J. Rebek Jr., V. Böhmer, J. L. Atwood and D. Reinhoudt. With well-defined glycoluril subunits, J. Rebek Jr. and co-workers were the first to successfully assemble hydrogen-bonded spherical
capsules, which they coined ‘soft balls’\textsuperscript{16}, ‘tennis balls’\textsuperscript{17} and ‘jelly donuts’\textsuperscript{18}, as well as cylindrical capsules made from resorcinarene units\textsuperscript{11b}. Unlike coordination cages, these capsules are much more labile due to the weaker H-bonding interactions. Typically, they form in nonpolar solvents, since polar protic solvents may disrupt hydrogen bonds and further disassemble these capsules. However, through careful design, some hydrogen-bonded cages are stable in aqueous solution\textsuperscript{19}. Among non-polar solvents, mesitylene is frequently used by the Rebek group, as it is too large to enter the cavity and only guests are selectively encapsulated.

**Figure 1-7:** Self-assembly of hydrogen bonded hexa-resorcinarene capsule 13.

Impressively, L. R. MacGillivray and J. L. Atwood assembled hexa-resorcinarene capsule 13 in wet chloroform. This giant snub cube is linked by 60 hydrogen bonds between the six resorcinarenes 12c that occupy six square faces of the snub cube and eight water molecules sitting on the eight triangular faces. It has an estimated volume of about 1400 Å\textsuperscript{3} (Fig. 1-7)\textsuperscript{20}. Since water molecules are subcomponents of 13, dry solvents won’t yield the capsule. It is believed that 13 could potentially encapsulate large guest molecules, like fullerenes. However, only small guests were reported to be captured to date\textsuperscript{11c,21}.
The hydrophobic effect drives the assembly of the hydrophobic parts of molecules in water by minimizing the total amount of hydrated hydrophobic surface of the system. The larger the hydrophobic surface, the more water molecules will be expelled upon aggregation and the stronger will be the interactions\textsuperscript{22}. In most cases, assemblies driven by hydrophobic forces do not have a mono-dispersed architecture, but exist as poly-dispersed aggregation, like micelles, that are in a rapid, dynamic equilibrium. However, through precise design, discrete assemblies can be made. For example, utilizing hydrophobic interactions, B. C. Gibb and co-workers were able to assemble dimeric, tetrameric and hexameric capsules in aqueous solution in the presence of suitable guests (Fig. 1-8). With small guests or without guests, octaacid-cavitand \textbf{14} exists as monomer\textsuperscript{12b}. Elongated guests, however, lead to a dimeric capsule and tetra-ammonium salts give either a tetrameric capsule or a hexameric capsule depending on the alkyl chain length of the ammonium salts\textsuperscript{23}. Therefore, morphologies of final products are programmed by both the hydrophobic effect and the template effect. Meanwhile, M. Shionoya and coworkers reported a box-shaped and a tetrahedral capsule, which were assembled from gear-shaped amphiphile subunits \textbf{15} (Fig. 1-9)\textsuperscript{24}. Unlike Gibb’s hosts, the box-shaped capsule could form even without adding any guests and remained intact up to 353K. The authors attribute the high stability to the hydrophobic effect and electrostatic forces.
First introduced by Jean-Marie Lehn\textsuperscript{25}, dynamic covalent chemistry (DCC) relies on thermodynamically controlled reversible reactions and allows synthesis of many complex molecules from multiple components in one pot reactions\textsuperscript{26}, which is difficult or impossible to achieve by traditional multi-step synthetic methods. Among reversible reactions, imine, hydrazone, disulfide, boronic ester formation and metathesis reactions have been employed intensively in constructing capsules (Scheme 1-1). In this thesis, imine and acyl hydrazone formation were used for the thermodynamic synthesis of hemicarcerands and cavitand-based nanocapsules. The reason why we choose Schiff-bases is as follows. Firstly, transamination in Brønsted...
or Lewis-acid catalyzed Schiff-base reactions is very fast\textsuperscript{27}; Secondly, Schiff-bases can be easily reduced to non-dynamic secondary amines and hydrazides and if needed, further modified\textsuperscript{28}. Thirdly, reaction conditions are mild, typically not requiring dry solvents and an inert atmosphere, so that many functional groups are compatible with the chemistry.

1) Imine Formation:

\[
\begin{align*}
R_1 - &\text{H} + R_2 - \text{NH}_2 &\xrightleftharpoons{\text{Cat. H}^+}\text{Catalyst}\quad R_1 - \text{N}_-\text{H} + R_2 - \text{H}_2\text{O}
\end{align*}
\]

2) Hydrazone Formation:

\[
\begin{align*}
R_1 - &\text{H} + R_2 - \text{HN}_-\text{NH}_2 &\xrightleftharpoons{\text{Cat. H}^+}\text{Catalyst}\quad R_1 - \text{N}_-\text{O} + R_2 - \text{H}_2\text{O}
\end{align*}
\]

3) Disulfide Formation:

\[
R_1 - \text{SH} + R_2 - \text{SH} \xrightarrow{[O]} [R] \xrightarrow{[R]} R_1 - \text{S}_-\text{S}_-R_2
\]

4) Boronic Ester Formation:

\[
R_1 - \text{B} &\text{-OH} + R_2 - \text{OH} + R_3 - \text{OH} \xrightleftharpoons{\text{2H}_2\text{O}} R_1 - \text{B}_-\text{O}_-\text{O}_-R_2 + 2\text{H}_2\text{O}
\]

5) Metathesis:

\[
R_1 - \text{\(\rightleftharpoons\)} + R_2 - \text{\(\rightleftharpoons\)} \xrightarrow{\text{Grubbs cat.}} R_1 - \text{\(\rightleftharpoons\)}R_2
\]

Scheme 1-1: Reversible bond formations.

In 1991, D. J. Cram and coworkers reported the first octaamine hemicarcerand 18g\textsuperscript{29}. In pyridine, two equivalents of tetraformylcavitand 16 and four equivalents of \textit{m}-phenylenediamine 17g were stirred for 4 days at 65 °C, yielding \textasciitilde40\% 18g after column chromatography. Since the reaction is un-catalyzed, the transamination is very slow and it is not regarded as DCC. In 2000, J. F. Stoddart, D. J. Cram and coworkers improved the synthesis by adding catalytic amounts of TFA, which gave the same hemicarcerand 18g almost quantitatively within 1 hour (Fig. 1-10)\textsuperscript{27b}. The TFA-catalyzed reversible imine bond formation is under thermodynamic control and
thermodynamically less stable intermediates eventually break down and lead ultimately to the most thermodynamically favored product.

**Figure 1-10:** Synthesis of octaimine hemicarcerand 18g.

Inspired by above research, our group generalized the idea by demonstrating that tetraformylcavitand 19 can form hemicarcerands 20b-h quantitatively with various diamines 17b-h and further applied it for the synthesis of larger nanocapsules (Fig. 1-11). X. Liu et. al. discovered that in chloroform, the condensation of tetraformylcavitand 19 and ethylenediamine 17a yielded 82% octahedral nanocapsule 23\(^{30}\). Considering that 24 imine bonds formed in one pot, the yield for each bond formation must be higher than 99%, which demonstrates the superiority of DCC. Because of the dynamic nature of imine bonds, the product distribution is solvent dependent\(^{31}\). For example, instead of octahedral nanocapsule 23, tetrahedral 22 and square antiprismatic nanocapsule 21 are the major product in THF (35%) and CH\(_2\)Cl\(_2\) (65%), respectively.
Figure 1-11: Thermodynamically controlled synthesis of nanocapsules 21-23 and hemicarcerands 20b-h.

Another example from our group is the quantitative formation of chiral cubic cage 25, which collects 20 building blocks in one pot and represents the state of the art with respect to the number of components and yield. $C_3$-trialkoxy-triformylcyclobenzylene (CTB) 24 is a tritopic molecule with nearly $90^\circ$ angle between the planes of the aryl units, which makes it a suitable building block for 25 (Fig. 1-12). In chloroform containing catalytic TFA, 24 and $p$-phenylenediamine 17h (ratio of 8:12) assembled quantitatively into 25$^{32}$. Again, the yield is benefited from the dynamic nature of the imine bond.
The synthesis of imine-based capsule is also explored by several other groups. For example, the tetrahedral cage 27 reported by the Cooper group and the adamantoid cage 30 from Mastalerz group show excellent gas adsorption and selective uptake properties (Fig. 1-13). Recently, by employing cage 27, A. I. Cooper and coworkers could successfully separate mesitylene and its isomers 4-isotoluenes. The adamantoid cage 30, on the other hand, has the highest surface area compared with other discrete organic compounds and can selectively adsorb CO$_2$ over methane, probably due to the inside phenol groups, which may form hydrogen bonds with CO$_2$. 

**Figure 1-12**: Edge-directed synthesis of cubic nanocapsule 25.
Figure 1-13: Synthesis of tetrahedral cage 27 and adamantoid cage 30.

In chapter 2, I will discuss the synthesis and characterization of chiral tetra-cavitand nanocapsules and chiral hemicarcerands. In chapter 3, the rational design of octahedral and rhombicuboctahedral nanocapsules will be described. In chapter 4, the mechanism, solvent/template effects and cooperativity in the assembly of hemicarcerand and poly-cavitand nanocapsules will be investigated. In chapter 5, I will explore hemicarcerand formation and guest encapsulation through vortex grinding. In chapter 6, the synthesis of water-soluble acylhydrazone nanocapsules and the attempt to encapsulate cytochrome c will be detailed.
References


Chapter 2

Thermodynamically Controlled Synthesis of Chiral Tetra-cavitand Nanocapsules and Chiral Hemicarcerands

(The work was partially done by James Bennett)

2.1 Introduction

Asymmetric synthesis and chiral recognition induced by chiral hosts have gained a lot of interest in the past two decades. Although a large number of synthetic hosts have been developed in order to mimic enzymes, most of them only achieve modest enantiomeric excesses (ees) in chiral recognition studies. Therefore, it is necessary to develop new generations of chiral capsules. Chiral capsules can be roughly divided into three classes. In the first class, the chiral capsule is built up from at least one chiral building block (class I). In the second class, it is built up from achiral building blocks and rendered chiral by postmodification (class II). In the third class (class III), the capsule is constructed from achiral building blocks but is asymmetrically folded or assembled. For class I and II, the chiral information is transferred to the capsule space remotely, which often leads to a low chiral discrimination. For example, the Cram group reported two chiral hemicarcerands 31 and 32 that belong to class I (Fig. 2-1). Each hemicarcerand contains one chiral bridge, which produces two new chiral portals (portals A). The other two portals (portals B) are also chiral, induced by twisting, but are flanked by achiral units. Therefore, portals B won’t give good discrimination. Additionally, since portals A are larger than portals B in 31, it is expected that guests enter the cavity through portals A preferentially. However, for 32, portals A are more rigid than portals B due to the five-member ring of the chiral bridge. Thus, the size of portal A is smaller and guests are likely to pass through portals B. By using these two...
hemicarcerands, various racemic guests were tested either by thermally driving guests into the cavity or by a seal-in method, in which guests were introduced into the cavity during the chiral bridge close-up process. As expected, 31 gave higher selectivity than 32 and thermal encapsulation was better than the seal-in method. However, for both, the chiral selectivity is not very high.

Figure 2-1: Chiral hemicarcerands 31 and 32 used for the investigation of chiral recognition.

Chiral hosts belonging to class II were recently reported by J. Rebek Jr. and coworkers\textsuperscript{2c}. Achiral hydrogen bonded cylindrical capsule 33 is able to accommodate two medium-sized guest molecules (Fig. 2-2)\textsuperscript{4}. If one (R)-mandelic acid 34 is first encapsulated inside the capsule, the remaining space in the cavity will be chiral and can potentially recognize chiral guests. The hand direction shows the possible orientations of the second chiral guest. In the cylindrical complex 33⊙34, two orientations can be adopted, which results in three combinations, A-C. Among them, A is much more effective than B and C in chiral recognition. However, since the guest tumbles freely within the capsule, the overall selectivity is low. In fact, the highest selectivity was only 1.6:1.
**Figure 2-2**: The orientations of (R)-mandelic acid 34 and a second chiral guest in cylindrical capsule 33 (reproduced with permission from reference\textsuperscript{2c}).
Figure 2-3: Enantioselective catalytic cycle of azo-Cope rearrangement catalyzed with tetrahedral cage 35 (reprinted with permission from reference\textsuperscript{2f}).

Unlike the other two classes, the cavity space of capsules in class III is chiral. An example is the tetrahedral cage [Ga₄L₆]\textsuperscript{12-} 35, which was developed by K. N. Raymond and coworkers. It was assembled from six biscatecholamide ligands and four metal ions. Three bidentate catecholates coordinate to each gallium ion, which results in two enantiomeric forms: \textit{ΛΛΛΛ-35} and \textit{ΔΔΔΔ-35}. They can be resolved by
addition of (-) N’-methylnicotinium iodide. The enantiomeric pure 35 has been recently used to promote an asymmetric azo-Cope rearrangement. Fig. 2-3 shows the catalytic cycle. In the first step, the substrate (S) displaces the ammonium salt, giving the substrate complex 35⊙S. Within the confined chiral space, the rearrangement was accelerated and one enantiomer formed preferentially. Presumably, the transition state (TS) of this enantiomer fits better in the chiral host and thus, the activation energy is lower than that for the other enantiomer. Since the product is further hydrolyzed in aqueous solution to the neutral aldehyde, which has little affinity towards the host, and the negatively charged 35 binds preferentially cationic guests, the neutral aldehyde is exchanged by the ammonium salt, finishing the cycle. In this study, a much higher selectivity (78% ee) was observed.

In this chapter, the synthesis of chiral tetra-cavitand nanocapsules and chiral hemicarcerands with chiral space using DCC and their chiral recognition studies will be presented and discussed.

2.2 Thermodynamically controlled synthesis of chiral tetra-cavitand nanocapsules

Chiral tetra-cavitand nanocapsules 41a, b are assembled from four equivalents of cavitands 40a, b and eight equivalents of ethylenediamine 17a through the formation of 16 imine bonds, which was discovered by the former group member, James Bennett. They formed quantitatively, if these components are reacted in CHCl_3 in the presence of catalytic amounts of TFA (Scheme 2-1). The connectivity in capsule 41a, b is identical to that in tetrahedral capsule 22 (see Chapter 1 and Fig. 2-5). However, they are asymmetrically folded as confirmed by X-ray crystallography and NMR.
spectroscopy and the enantiomerization barrier is approximately 20 kcal/mol (vide infra).

Scheme 2-1: Synthesis of tetra-cavitand nanocapsules 41a, b.

2.2.1 Synthesis of cavitands 40a, b

The synthesis of cavitands 40a, b is outlined in Scheme 2-2. In the presence of concentrated HCl, the condensation of resorcinol 36 and aldehyde 37a, b yielded resorcin[4]arene octols 12a, b. Without purification, bromination of 12a, b with NBS gave tetrabromooctols 38a, b. Subsequently, tetrabromocavitands 39a, b were obtained by reacting crude 38a, b with 1-bromo-3-chloropropane under basic conditions. After purification by silica gel column chromatography, they are treated with iPrMgCl·LiCl, which allows the exchange of the bromide to MgCl. This was followed by addition of electrophile DMF and subsequent quench with diluted acid. Pure 40a, b were obtained after column chromatography in overall 23% yield based on resorcinol.
Scheme 2-2: Synthesis of cavitands 40a, b.

Tetraformylcavitands 40a, b are more flexible as compared to their analogue 16 due to their longer spanners (Fig. 2-5). As a result, $C_2$- and $C_r$-symmetric conformations are possible for them. In the $C_r$-symmetric conformation, the cone angle is $\sim 94^\circ$. However, the crystal structure of methyl-footed tetrabromocavitand 39 reveals that the $C_2$-symmetric conformation is favored in the solid state7. Therefore, cavitand 40a, b are likely to adopt the $C_2$-symmetric conformation, which is consistent with the outcome of the condensation reaction with 17a, which will be discussed in the next section.

2.2.2 Characterization of chiral tetra-cavitand nanocapsules 41a, b

In order to determine the structure and confirm connectivity of 41a, b, X-ray quality crystals of 41a were grown by slow diffusion of methanol into its chloroform solution over one week. The same procedure only yielded small crystals for 41b, which were not suitable for X-ray analysis. Since 41a and 41b differ only by their feet, the connectivity and arrangement of cavitand core and linkers are likely identical in both.
Figure 2-4: Stereo view of X-ray structure of chiral tetra-cavitand nanocapsule 41a along (A) and perpendicular (B) to the pseudo $C_2$-symmetry axis.

The X-ray structure of 41a shows that the cavitand adopts $C_2$- rather than $C_4$-symmetric conformations as expected and that the nanocapsule is asymmetrically twisted. 41a exists as a pair of enantiomers in the unit cell and each enantiomer has a pseudo $C_2$-symmetry axis. Viewed along the axis, the top two cavitands in the same color are related by symmetry and so are the bottom two (Fig. 2-4A). Viewed in the direction perpendicular to the symmetry axis, two linkers in red are forced to fold inside, which divides the nanocapsule interior into two channel like cavities (Fig. 2-4B). Two chloroform molecules accommodate the right channel and one chloroform molecule and two methanol molecules occupy the space of the left channel. The folded structure is stabilized by CH-π and van der Waals interactions between the -O(CH$_2$)$_3$O- spanners and aryl units. Interactions between solvent guests
and aryl units also partially contribute to the twisted structure.

Further inspection of the crystal structure shows that two symmetry related cavitands are connected with each other through a single linker and each one is singly and doubly linked to the other two cavitands. The connectivity in nanocapsule 41a is the same as that of nanocapsule 22 produced from cavitand 19 (Fig. 2-5). This implies that the two enantiomers of 41a are interchangeable via a TS similar to 22.

The structure of 41a, b was also analyzed by NMR spectroscopy, MALDI-TOF MS and GPC. Fig. 2-6 shows the partial 1H NMR spectrum at -30 °C, MALDI-TOF MS and GPC trace of 41a. Although the 1H NMR spectrum at low temperature is very complicated with some signals overlapping, the signals of the imine and aryl protons of 41a are well resolved. Eight imine signals and eight aryl protons in a ratio of 2:2:2:2:2:2:2:2, respectively, are observed. The NMR signal pattern is consistent with the low symmetry chiral structure observed by X-ray crystallography. The single peak in the GPC trace indicates that the nanocapsule formed quantitatively. MALDI-TOF shows the isotopic cluster at the expected mass-to-charge ratio $m/z = 4358.72$ (4358.93 calculated for [M + H]+). The diffusion constant of nanocapsule 41a in CDCl$_3$ was measured by DOSY NMR spectroscopy and is $D = 3.42 \times 10^{-6}$ cm$^2$/s at 25 °C. Assuming that 41a is roughly spherical in CDCl$_3$, its solvodynamic radius is calculated to be 11.8 Å according to the ‘Stokes-Einstein’ equation. As compared to tetrahedral nanocapsule 22, the value is smaller, which indicates that the solution structure is very compact and again consistent with the X-ray structure.
In order to determine the stability of the asymmetrical fold of 41a and to find out under which conditions 41a can be resolved, it is very important to know the enantiomerization barrier of 41a.
2.2.3 Mechanism and energetics of enantiomerization

The mechanism and barrier of enantiomerization of 41a was investigated by VT NMR spectroscopy. These experiments uncovered that enantiomerization is a two-step process as illustrated in Fig. 2-7. The first step takes place at low to medium temperature (process I). In this process, the pair of symmetry related cavitands in orange rotate simultaneously and give transient, chiral intermediate (R)-I. Then, the
other pair of cavitands in blue further rotates in the same direction, which converts \((R)\)-I back into \((R)\)-41 and so does the other enantiomer, \((S)\)-41a. Thus, the time averaged structure of \((R)\)-41a, which is observed when process I is fast on the NMR time scale, has a \(D_2\)-symmetry. The second step is observed in the medium to high temperature range (process II). It corresponds to the enantiomerization of transient \((R)\)-I to \((S)\)-I via an achiral intermediate I. Then, \((S)\)-I refolds into \((S)\)-41a. The time averaged structure of \((R)\)-41a in this temperature range has \(D_{2h}\)-symmetry.

**Figure 2-7**: Overall dynamic process of chiral tetra-cavitand nanocapsule 41a.
Figure 2-8: Left: Partial $^1$H NMR spectra (400 MHz, CD$_2$Cl$_2$) of 41a at different temperatures: T = -30 °C (1); -20 °C (2); -10 °C (3); 0 °C (4); 10 °C (4); 20 °C (6); 30 °C (7); 40 °C (9); 50 °C (10); 60 °C (11). Right: $^1$H NMR spectra of signals f and j simulated by DNMR3 at different exchange rates: $k_{ex} = 6000, 2500$ and $1000$ s$^{-1}$. 
Figure 2-9: EXSY NMR spectrum (400 MHz, CD$_2$Cl$_2$, -20 °C, d1 = 2s, mix = 700 ms, nt = 8, ni = 256) of 41a. For the analysis of the kinetics of process I, EXSY signals between exchanging aryl protons, (f, j), for example, were analyzed, since they are far enough separated in the X-ray structure of 41a that simultaneous NOE signals are not possible.

Figure 2-10: Left: Partial $^1$H NMR spectra (400 MHz, CDCl$_2$CDCl$_2$) of 41a at T = 80 °C, 90 °C, 100 °C and 110 °C (top to bottom) showing singlets assigned to the imine protons of 41a. Right: $^1$H NMR spectra of inner two imine signals simulated by DNMR3 at different exchange rates: $k_{ex} =$ 6000, 2500 and 1600 s$^{-1}$.

The low temperature NMR spectrum of 41a at -30 °C in CD$_2$Cl$_2$ is displayed in Fig. 2-8 (spectrum 1), which shows the imine and aryl protons. The number of signals is
consistent with the $C_2$-symmetry of 41a and the X-ray structure. As the temperature is increased to 60 °C (spectrum 11), the imine and aryl signals coalesce into four signals with an integration of 4:4:4:4. Thus, at this temperature, the average structure of 41a has $D_2$-symmetry consistent with process I. According to 2D-EXSY NMR spectrum (Fig. 2-9), it was found that proton pairs (b, c), (a, e), (d, g), (f, j), (i, k), (h, l), (m, p) and (n, o) exchange with each other. The exchange rate constants at different temperatures were estimated from cross peak intensities in the 2D-EXSY or line shape analysis. Results are summarized in Table 2-1. Based on these data, the activation enthalpy and entropy can be calculated ($\Delta H^\ne = 16.5\pm0.2$ kcal/mol and $\Delta S^\ne = 6.4\pm0.7$ cal/mol/K) by using an Eyring plot (Eq. 2-1). At r.t, the activation free energy of the dynamic process was calculated to be $\Delta G^\ne$ (298.15K) = 14.6±0.4 kcal/mol.

$$\ln \frac{k}{T} = \frac{\Delta H^\ne}{R} \cdot \frac{1}{T} + \ln \frac{k_B}{h} + \frac{\Delta S^\ne}{R}$$  \hspace{1cm} (2-1)

**Table 2-1**: Exchange rate constant $k_{ex}$ and rate constants $k^+$ and $k^-$ of process I of 41a at different temperatures.

<table>
<thead>
<tr>
<th>T (K) $^a$</th>
<th>$k_{ex}$ (s$^{-1}$)</th>
<th>$k^+$, $k^-$ (s$^{-1}$)</th>
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<tr>
<td>241.72</td>
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<td>0.18</td>
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<td>252.15</td>
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<td>500</td>
</tr>
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<tr>
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<td>6000</td>
<td>3000</td>
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$^a$The first two data were measured by 2D-EXSY and the last three were obtained from line shape analysis.
Table 2-2: Exchange rate constant $k_{ex}$ and rate constants $k^+$ and $k^-$ of process II of 41a at different temperatures.

<table>
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<th>$k^+$, $k^-$ (s$^{-1}$)</th>
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<td>384.47</td>
<td>50</td>
<td>25</td>
</tr>
</tbody>
</table>

$^a$The first three data were measured by 2D-EXSY and the last three were obtained from line shape analysis.

The second dynamic process corresponds to the racemization at high temperature. High temperature NMR experiments were performed in CDCl$_2$CDCl$_2$. Warming the sample to 110 °C led to the coalescence of one pair of imine signals (Fig. 2-10). Likely, if the temperature was high enough, the other pair of imine would also coalesce to one signal. At this temperature, racemization is very fast and the average structure of 41a has the same symmetry as nanocapsule 22. Again, 2D-EXSY and line shape analysis were used to measure the rate constants at different temperatures (Table 2-2). The activation free energy of process II is $\Delta G^\neq (298.15K) = 21.5 \pm 0.7$ kcal/mol ($\Delta H^\neq = 25.9 \pm 0.4$ kcal/mol and $\Delta S^\neq = 14.8 \pm 1.1$ cal/mol/K).

2.2.4 Solvent effect on enantiomerization barrier

According to above kinetic results, the enantiomerization barrier is too low for the resolution of the racemic mixture at room temperature. However, the barrier height is affected by solvents and the cavitand feet. Therefore, it is possible to increase the barrier by optimizing the structure and choosing the right solvent. Data in Table 2-3 show that for each nanocapsule, the free activation energy decreases as the van der Waals radius of the solvent increases. As discussed in Section 2.2.3, the structure is partially stabilized by CH-$\pi$ interactions between solvent molecules and aryl groups.
of the host. The small solvent molecules better fit into the host cavities, which maximizes the interaction and hence increases the barrier. On the other hand, for the same solvent, the pentyl-footed capsule 41a has a higher barrier than phenethyl-footed capsule 41b. An explanation is that small feet allow cages to adopt more compact structures, which may increase the interaction between spanners and aryl units. As a result, the barrier is higher with 41a.

Table 2-3: Enantiomerization barrier $\Delta G_{323}^\neq$ of nanocapsules 41a, b.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>$\Delta G_{323}^\neq$ (41a)/kcal.mol$^{-1}$</th>
<th>$\Delta G_{323}^\neq$ (41b)/kcal.mol$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD$_2$Cl$_2$</td>
<td>n.d.</td>
<td>21.1</td>
</tr>
<tr>
<td>CDCl$_3$</td>
<td>21.7</td>
<td>21</td>
</tr>
<tr>
<td>CDCl$_2$CDCl$_2$</td>
<td>21.1</td>
<td>20.6</td>
</tr>
</tbody>
</table>

2.2.5 Chiral recognition

Chiral recognition of 41a in solution was studied by NMR titrations at different temperatures. Different optically pure chiral guests were added to the solution of 41a. Guests, that bind the host strongly, may induce splitting of host signals due to the formation of diastereomeric complexes. Among chiral guests tested (Chart 2-1), only (-)-(S)-phenylethanol 47 induced signal splitting. Presumably, other guests cannot bind the host efficiently or don’t induce different complexation induced shifts (CIS) in both diastereomeric complexes. At -30 °C, 47 was added to the host solution in CD$_2$Cl$_2$ in portions (Fig. 2-11). At 1.3%, one of the imine peaks split. As the guest concentration was increased, other imines also showed splitting. As a control experiment, racemic 47 was added under the same conditions, and didn’t give splitting, confirming that the signal splitting induced by optically pure 47 is due to chiral recognition by 41a. NMR titrations performed at room temperature also showed signal splitting. However, addition of much more chiral guest was required to induce
splitting as compared to -30 °C, indicating a low enthalpy-driven binding affinity.

Chart 2-1: Chiral guests used for host guest recognition.
**Figure 2-11:** Partial $^1$H NMR spectra (400 MHz, CD$_2$Cl$_2$, -30 °C) of 41a in the presence of increasing amounts of (S)-47 (left) and (S,R)-47 (right). From bottom to top, the volume percent of enantiomerically pure /racemic co-solvent is 0%, 0.7%, 1.3%, 2.0% and 2.7%. Imine signals that start splitting upon addition of the enantiomerically pure co-solvent are indicated with arrows.

### 2.3 Synthesis of chiral hemicarcerands

Apart from ethylenediamine 17a, reactions of 40a with other linkers were also explored. Most of them resulted in complex mixtures (Chart 2-2). However, for $m$-phenylenediamine 17g, hemicarcerand 53 formed (Scheme 2-3). As shown in Fig. 2-12, the TFA-catalyzed condensation of two equivalents of 40a and four equivalents of 17g was monitored by GPC and yielded a single species after 1 hour. Its molecular mass is consistent with 53. After longer time, high molecular weight products slowly formed in the GPC. Eventually, 53 nearly disappeared. The mixture after 1 hour was analyzed by MALDI-TOF MS. The major isotopic cluster corresponds to a condensation product of two cavitands and three 17g with two unreacted aldehyde
groups (see appendix). Presumably, hemicarcerand 53 is a kinetic product and over time, free aldehydes reacted with the excess diamine to form larger structures. 53 was isolated by the addition of triethylamine (TEA) or reduction. To avoid polymerization, the reaction of cavitand 52, which has only three aldehyde groups, was investigated. Indeed, the reaction of two equivalents of 52 with three equivalents of 17g leads to the quantitative formation of hemicarcerand 54 (Scheme 2-3).

**Scheme 2-3**: Synthesis of chiral hemicarcerands 54 and 55 and diastereomer 57.
Chart 2-2: Linkers tested to react with cavitand 40a.

Figure 2-12: GPC trace of reaction mixture after 1 (A), 5 (B), 18 (C), 28 hours (D) and 1 week (E).
Fig. 2-13 shows the partial $^1$H NMR spectra for hemicarcerands 53 and 54. Both have three imine, three H$_2$, two H$_1$ and two H$_3$ signals, which is consistent with the symmetry of the chiral structure. For 53, the unreacted –CHO proton resonates at 10.3 ppm and 54 gives an additional H$_a$ signal at 6.5 ppm. The similar splitting pattern between 53 and 54 also confirms that they have the same connectivity.

![Partial $^1$H NMR spectrum (400 MHz, CDCl$_3$, 25 °C) of hemicarcerands 53 (bottom) and 54 (top).](image)

**Figure 2-13**: Partial $^1$H NMR spectrum (400 MHz, CDCl$_3$, 25 °C) of hemicarcerands 53 (bottom) and 54 (top).

In order to further prove that 53 and 54 are chiral, NMR titrations of 53 with (S)-1-phenylethanol (S)-47 were performed in CDCl$_3$ (Fig. 2-14). The addition of (S)-47 induced upfield shifts and splitting of imine and aldehyde signals. As a control, (S,R)-47 only lead to upfield shifts but no splitting was observed. Thus, 53 and 54 are chiral molecules. Compared to 41a, the enantiomerization of 53 and 54 requires breaking imine bonds. Thus, the resolution of both enantiomers should be possible. In order to obtain an enantiomerically pure hemicarcerand, several approaches were investigated.
Figure 2-14: Partial $^1$H NMR spectra (300 MHz, CDCl$_3$, 25 °C) of 53 in the presence of increasing amounts of (S,R)-47 (left) and (S)-47 (right). From A-D, the volume percent of enantiomerically pure /racemic co-solvent is 0%, 0.7%, 1.3%, and 2.7%. Signals that start splitting upon addition of the enantiomerically pure co-solvent are indicated with dashed lines.

A chiral column was first used to directly resolve the mixture. Unfortunately, no separation was achieved under different conditions, possibly due to the weak binding affinity of 53 towards the stationary phase of the column. In another attempt, an enantiomerically pure ligand 56 was chemically attached to racemic 55 making it a mixture of diastereomers 57, which could be potentially separated by column chromatography, which, unfortunately, failed again.

Since 53 and 54 are dynamic in the presence of catalytic amounts of TFA, one of the enantiomers may be enriched by the addition of optically pure chiral guests. The experiment was performed in CDCl$_3$ and monitored with NMR spectroscopy. Several enantiomerically pure guests were tested and the maximum “de” of 20% was achieved in the presence of 10 % (S)-47. The low ‘de’ value is partially attributed to weak binding affinity towards chiral guests in CDCl$_3$, which competes with (S)-47.
Water as solvent would increase guest affinity, since water molecules won’t compete with guests for binding sites\(^1\). Thus, assembly of a water-soluble chiral hemicarcerand was attempted with water-soluble cavitand \(40e\). The synthesis of \(40e\) is outlined in scheme 2-4. Tetrabromocavitand \(39c\) was obtained by bridging the adjacent phenol groups of \(38c\) with propylene di(p-toluenesulfonate). Then, protection of hydroxide groups with TBDPSCl yielded \(39d\), which was followed by adding \(^i\)PrMgCl·LiCl and DMF to convert the bromide to an aldehyde. Then, \(40d\) was treated with TBAF to retrieve the hydroxide groups, giving \(40c\). Finally, the cavitand was rendered water-soluble by converting the –OH groups to sulfate ester groups. Unfortunately, in the presence or absence of guests, \(40e\) and ethylenediamine \(17a\) didn’t form capsules in aqueous solution.
2.4 Conclusions

In this chapter, chiral tetra-cavitand nanocapsules and chiral hemicarcerands were synthesized. The tetra-cavitand nanocapsules are asymmetrically folded and the enantiomerization is a two-step process, whose mechanism and potential energy surface was investigated by VT-NMR and 2D-EXSY NMR studies. It was found that the enantiomerization barrier is ‘cavitand feet’ and solvent dependent. For chiral hemicarcerands, the chirality is permanent without breaking imine bonds. Several resolution approaches have been investigated to resolve the mixture and most of them failed. However, enantiomeric enrichment induced by adding enantiomerically pure guests was achieved.

2.5 Experimental section

2.5.1 General procedures

Reagents and chromatography solvents were purchased from Aldrich and used without further purification except that chloroform was passed through K₂CO₃ prior to use. THF was dried over Na/benzophenone and distilled under argon. ¹H NMR spectra recorded in CDCl₃, CD₂Cl₂ and CDCl₂CDCl₂ were referenced to residual CHCl₃, CHDCl₂ and CHCl₂CDCl₂ at 7.26, 5.30 and 6.00 ppm. ¹³C NMR spectra recorded in CDCl₃ were referenced to ¹³CDCl₃ at 77.5 ppm. Sample temperature in VT NMR studies was calibrated using methanol (-50 - 20 °C) and ethylene glycol (30 - 110 °C) as standards and calibration curves implemented in the Varian NMR software. Mass spectra were recorded on an Applied Biosystems Voyager DE-Pro mass spectrometer (MALDI-TOF). 2,4,6-Trihydroxylacetophenone (THAP) was used as the matrix. Gel permeation chromatography (GPC) was performed on a Ranin Varian HPLC system equipped with dual wavelength UV/Vis detector (280 nm),
Eppendorf CH-30 column heater and two Jordi GPC columns (cross linked DVB; 10 Å pore size; MW cutoff ~ 25,000; 7.8mm × 30cm) with CH₂Cl₂/1% NEt₃ as mobile phase at a flow of 1 mL/min. Approximate molecular weights of analytes were determined from a semi-logarithmic calibration plot.

### 2.5.2 Synthesis of 39c

Tetrabromooctol 38c (1.05 g, 0.96 µmol), TsO(CH₂)₃OTs (1.8 g, 4.62 µmol) and DBU (1.15 mL, 7.69 µmol) were dissolved in 100 mL anhydrous DMA. Under argon, the solution was heated at 50 °C for 48 hours. Then, slightly pink precipitate formed upon addition of a large amount of distilled water. The solid was filtered, dried and purified by column chromatography (MeOH-CH₂Cl₂ = 1:9). 270 mg 39c (22%) was obtained as reddish solid.

![Structure of 39c](image)

¹H NMR spectrum (300 MHz, CDCl₃, 25 °C): δₓ = 7.11 (s, 4H, Hₐ), 4.95 (t, J = 8.1 Hz, 4H, Hₗ), 4.63 (m, 8H, Hₐ), 3.96 (m, 8H, H₁), 3.60 (t, J = 6.2 Hz, 8H, H₄), 2.45-2.13 (m, 8H, H₀₁ and H₁₀), 2.03 (m, 8H, H₁), 1.78-1.50 (m, 12H, H₃ and –OH, overlap with H₂O), 1.27 (m, 8H, H₂). ¹³C NMR spectrum (125 MHz, CDCl₃, 25 °C): δₓ = 154.19, 134.83, 123.77, 112.30, 71.12, 63.04, 35.66, 35.52, 32.63, 29.84, 24.52.

### 2.5.3 Synthesis of 39d

Tetrabromocavitand 39c (800 mg, 0.64 mmol) and imidazole (348 mg, 5.12 mmol)
were dissolved in 50 mL anhydrous THF, which gave a deep red solution. Under argon, TBDPSCl (1 mL, 3.84 mmol) was added to the solution. After a few minutes, precipitate formed, which indicates the start of the reaction. After stirring overnight at room temperature, the precipitate was filtered off and the red filtrate was collected. THF was removed in vacuum leaving a viscous oil. It was purified by silica gel column chromatography (Hexane-CH₂Cl₂ = 1:1) and 1.2 g 39d (89%) was obtained as a white solid.

\[
\begin{align*}
\text{Br} & \quad \text{H₄₁} \\
\text{O} & \quad \text{H₄₅} \\
\text{H₅₂} & \quad \text{H₆₄} \\
\text{H₅₃} & \quad \text{H₇₄} \\
\text{H₃₄} & \quad \text{H₇₃} \\
\text{H₄₁} & \quad \text{H₆₅} \\
\text{H₃₃} & \quad \text{H₇₅} \\
\text{H₃₂} & \quad \text{H₇₆}
\end{align*}
\]

\(^1\text{H NMR spectrum (400 MHz, CDCl}_3\text{, 25 °C)}: \delta_H = 7.61 \text{ (d, } J = 7.6 \text{ Hz } 16\text{H, H}_{a₄}), 7.38-7.27 \text{ (m, } 24\text{H, H}_{a₂ \text{ and H}_{a₃}}), 7.02 \text{ (s, } 4\text{H, H}_{a₁}), 4.95 \text{ (t, } J = 7.9 \text{ Hz, } 4\text{H, H}_{m}), 4.62 \text{ (m, } 8\text{H, H}_{o}), 3.95 \text{ (m, } 8\text{H, H}_{i}), 3.56 \text{ (t, } J = 6.1 \text{ Hz, } 8\text{H, H}_{₄}), 2.27 \text{ (m, } 4\text{H, H}_{o₁}), 2.01 \text{ (m, } 4\text{H, H}_{i₁}), 1.93 \text{ (m, } 8\text{H, H}_{i}), 1.57 \text{ (m, } 8\text{H, H₃}, \text{ overlap with H₂O}), 1.23 \text{ (m, } 8\text{H, H}_{₂}), 0.99 \text{ (s, } 36\text{H, H₃}). \text{ } ^{13}\text{C NMR spectrum (125 MHz, CDCl}_3\text{, 25 °C): } \delta_C = 154.23, 135.75, 134.80, 134.24, 129.66, 127.76, 123.72, 112.34, 70.84, 64.06, 35.33, 35.06, 32.60, 29.90, 27.09, 23.91, 19.40. \text{ MS (MALDI-TOF) } m/z: 2292.6145 \text{ (M + Na}^+, 100%); \text{ Caled for C}_{120}\text{H}_{140}\text{O}_{12}\text{Si}_4\text{Br}_4 + \text{Na}^+: 2292.6040. \]
2.5.4 Synthesis of 40c

Tetrabromocavitand 39d (200 mg, 96 µmol) was dried in vacuum overnight at 100 °C and then dissolved in 30 mL THF under argon. 10 mL THF was added to a second dried flask, which was charged with iPrMgCl solution (0.8 mL, 2 M) and anhydrous LiCl (65 mg, 1.54 mmol). The suspension was stirred under argon until all LiCl was dissolved. Then, the resulting solution was cannulated to the solution of 39d at -78 °C. After Br/MgCl exchange was complete, which was monitored by $^1$H NMR spectroscopy, anhydrous DMF (0.12 mL, 1.54 mmol) was added to the solution. After 1 hour, the reaction was quenched with HCl solution (5 mL, 1 M). The product was extracted with ethyl acetate (3 × 30 mL). The combined organic solutions were washed with sat. NaCl solution dried over anhydrous MgSO$_4$ and concentrated under vacuum. Without purification, the product was dissolved in 10 mL anhydrous THF, followed by addition of TBAF (301 mg, 1.15 mmol). After 3 hours, THF was removed and the obtained solid was washed with water, filtered and dried. The crude mixture was purified by silica gel column chromatography (MeOH-CH$_2$Cl$_2$ = 5:95) and 47 mg 40c (45% starting from 39d) was obtained as a yellowish solid.

$^1$H NMR spectrum (500 MHz, CDCl$_3$ + DMSO-d6, 25 °C): $\delta_H$ = 10.20 (s, 4H, -CHO), 7.39 (s, 4H, $H_a$), 4.97 (t, $J = 7.7$ Hz, 4H, $H_m$), 4.60 (m, 8H, $H_o$), 3.91 (m, 8H, $H_l$), 3.52 (t, $J = 6.0$ Hz, 8H, $H_i$), 2.45 (s, 4H, -OH, overlap with water), 2.13-1.87 (m, 16H, $H_{o1}$, $H_{i1}$ and $H_l$), 1.57 (m, 8H, $H_3$), 1.24 (m, 8H, $H_2$).
2.5.5 Synthesis of 40e

The synthesis of 40e followed a reported procedure\textsuperscript{1b}. Tetraformylcavitand 40c (47 mg, 47.8 µmol) was dissolved in 1 mL anhydrous DMSO, which gave a yellow solution. PySO\textsubscript{3} (92 mg, 0.76 mmol) was added to the solution under argon and it was stirred at room temperature for 1 hour. Then, the reaction was quenched with 1 mL water and basified with Na\textsubscript{2}CO\textsubscript{3} until pH = 8. The solvent was removed in vacuum and the resulting solid was suspended and sonicated in 2 mL DMSO. The insoluble solid was filtered off. The collected filtrate was concentrated and precipitated by CH\textsubscript{3}CN. The white solid of 40e (20 mg, 30%) was obtained after filtration.

\[
\begin{align*}
\text{CHO} & \quad \text{H}_1, \text{H} \subscript{f1}O \\
\text{O} & \quad \text{CHO} \\
\text{H} & \quad \text{H}_1 \\
\text{H} & \quad \text{H}_1 \\
\text{NaO}_3\text{SO} & \quad \text{NaO}_3\text{SO}
\end{align*}
\]

\textsuperscript{1}H NMR spectrum (300 MHz, D\textsubscript{2}O, 25 °C): \(\delta_H = 10.12\) (s, 4H, -CHO), 7.74 (br, 4H, H\textsubscript{a}), 4.92 (br, 4H, H\textsubscript{m}, overlap with HOD), 4.62 (br, 8H, H\textsubscript{o}, overlap with HOD), 3.97 (br, 8H, H\textsubscript{a}), 3.52 (br, 8H, H\textsubscript{i}), 2.18 (br, 8H, H\textsubscript{i}), 1.90 (m, 8H, H\textsubscript{ol} and H\textsubscript{i1}), 1.56 (br, 8H, H\textsubscript{3}), 1.19 (br, 8H, H\textsubscript{2}).

2.5.6 Synthesis of 40a (Procedure 1)

Tetrabromocavitand 39a (324.28 mg, 0.26 mmol) was dried in vacuum overnight at 100 °C and then dissolved in 40 mL THF under argon. 10 mL THF was added to a second dried flask, which was charged with 2.1 mL iPrMgCl solution (2 M) and anhydrous LiCl (175.2 mg, 4.17 mmol). The suspension was stirred under argon until all LiCl was dissolved. Then, the resulting solution was cannulated to the solution of
39a at -78°C. After Br/MgCl exchange was complete, which was monitored by \(^1\)H NMR spectroscopy, anhydrous DMF (0.32 mL, 4.17 mmol) was added to the solution. After 1 hour, the reaction was quenched with HCl solution (10 mL, 1 M). The product was extracted with ethyl acetate (3 × 30 mL). The combined organic solutions were washed with sat. NaCl solution, dried over anhydrous MgSO\(_4\) and concentrated under vacuum. The product was further purified by silica column (EtOAc-CH\(_2\)Cl\(_2\) = 4:35). 160 mg (60%) of a white solid was obtained.

\(^1\)H NMR spectrum (400 MHz, CDCl\(_3\), 25 °C): \(\delta_H = 10.26\) (s, 4H, -CHO), 7.36 (s, 4H, H\(_a\)), 5.01 (t, \(J = 8.0\) Hz, 4H, H\(_m\)), 4.64 (m, 8H, H\(_0\)), 3.94 (m, 8H, H\(_i\)), 2.32 (m, 4H, H\(_{o1}\)), 2.08-1.88 (m, 12H, H\(_1\) and H\(_{i1}\)), 1.42-1.12 (m, 24H, H\(_2\), H\(_3\) and H\(_4\)), 0.86 (t, \(J = 7.4\) Hz, 12H, H\(_5\)). \(^13\)C NMR spectrum (100 MHz, CDCl\(_3\), 25 °C): \(\delta_C = 189.7, 158.5, 134.4, 130.8, 128.7, 122.7, 74.0, 35.2, 33.5, 32.2, 30.2, 27.5, 22.8, 14.4\). MS (MALDI-TOF) \(m/z\): 1041.5546 (M + H\(^+\), 100%); Calcd for C\(_{64}\)H\(_{80}\)O\(_{12}\) + H\(^+\): 1041.5723.

2.5.7 Synthesis of 40b

Prepared by procedure 1 from tetrabromocavitand 39b (615 mg, 0.44 mmol), \(^1\)PrMgCl solution (3.55 mL, 7.11 mmol), anhydrous LiCl (300 g, 7.11 mmol) and DMF (0.55 mL, 7.11 mmol), 446.4 mg of 40b (85%) was obtained as white solid.
H NMR spectrum (300 MHz, CDCl₃, 25 °C): δ_H = 10.28 (s, 4H, -CHO), 7.37 (s, 4H, H₄), 7.17 (m, 12H, H₃ and H₅), 7.09 (m, 8H, H₆), 5.09 (t, J = 7.5 Hz, 4H, H_m), 4.60 (m, 8H, H_0), 3.95 (m, 8H, H_i), 2.53 (m, 8H, H_i1), 2.29 (m, 12H, H_2 and H_i1), 2.00 (m, 4H, H_o1). ¹³C NMR spectrum (100 MHz, CDCl₃, 25 °C): δ_C = 189.6, 158.6, 141.6, 134.1, 130.4, 128.7, 126.2, 122.8, 73.8, 37.4, 34.3, 33.5, 30.1. MS (MALDI-TOF) m/z: 1177.5410 (M + H⁺, 100%); Calcd for C₇₆H₇₂O₁₂+ H⁺: 1177.5102.

2.5.8 Synthesis of 41a (Procedure 2)

A solution of tetraformylcavitand 40a (56.68 mg, 54.50 µmol), ethylenediamine 17a (6.54 mg, 109.00 µmol) and TFA (0.6 µL, 9.6 µmol) in CHCl₃ (6.3 mL) was stirred at room temperature overnight. Then, the solvent was removed and 41a formed quantitatively as a yellowish solid.

H NMR spectrum (500 MHz, CDCl₃, 60 °C): δ_H = 8.68 (s, 4H, H_im), 8.60 (s, 4H, H_im), 8.51 (s, 4H, H_im), 8.08 (s, 4H, H_im), 7.61 (s, 4H, H_a), 7.53 (s, 4H, H_a), 6.99 (s,
$^1$H NMR spectrum (500 MHz, CDCl$_3$, 25 °C): $\delta_{H}$ = 8.71 (s, 4H, H$_{im}$), 8.64 (s, 4H, H$_{im}$), 8.53 (s, 4H, H$_{im}$), 8.14 (s, 4H, H$_{im}$), 7.62 (s, 4H, Aryl-H), 7.58 (s, 4H, Aryl-H), 7.23-6.90 (m, 88H, aryl-H), 5.25 (m, 8H, H$_m$), 5.06 (s, 4H, H$_m$), 4.96 (d, $J = 9.2$ Hz, 4H, H$_o$), 4.86 (s, 4H, H$_m$), 4.68 (d, $J = 11.7$ Hz, 4H, H$_o$), 4.52 (m, 8H, H$_o$), 4.29-3.21

2.5.9 Synthesis of 41b

Prepared by procedure 2 from tetraformylcavitand 40b (309.64 mg, 0.26 mmol), ethylenediamine 17a (31.6 mg, 0.53 mmol) and TFA (3.1 µL, 41.4 µmol) in CHCl$_3$ (32 mL), 41b formed quantitatively as a yellowish solid.

\[
\begin{align*}
\text{N} & \quad \text{H}_{im} \\
\text{O} & \quad \text{H}_{im} \quad \text{H}_{im} \\
\text{H} & \quad \text{H} \quad \text{H} \\
1 & \quad 2 & \quad 3
\end{align*}
\]

$^{13}$C NMR spectrum (100 MHz, CDCl$_3$, 25 °C): $\delta_C$ = 158.93, 158.77, 158.49, 157.94, 157.88, 156.41, 154.86, 154.17, 154.11, 135.09, 134.26, 134.14, 133.85, 133.50, 128.31, 127.85, 126.54, 125.91, 124.92, 123.40, 121.83, 120.92, 73.31, 69.05, 68.01, 64.58, 64.04, 62.22, 37.07, 35.41, 34.03, 33.95, 33.81, 33.54, 32.43, 32.39, 32.19, 30.77, 29.93, 27.82, 27.66, 22.89, 22.78, 14.36, 14.32.

DOSY NMR spectrum (500 MHz, CDCl$_3$, 25 °C): $D = 3.42 \times 10^{-6}$ cm$^2$/s. MS (MALDI-TOF) $m/z$: 4358.7154 (M + H$^+$_, 100%); Calcd for C$_{272}$H$_{352}$O$_{32}$N$_{16}$ + H$^+$: 4358.9306.
(m, 80H, H_{o}, H_{i} and H_{3}), 2.71-1.59 (m, 96H, H_{o1}, H_{i1} and H_{1} and H_{2}). ¹³C NMR spectrum (125 MHz, CDCl₃, 25 °C): δ_{C} = 158.85, 158.72, 158.48, 158.34, 157.84, 156.91, 156.39, 155.97, 155.04, 154.37, 154.35, 142.18, 142.06, 141.89, 141.75, 134.82, 134.08, 133.95, 133.71, 133.38, 128.78, 128.66, 128.61, 128.60, 128.57, 128.04, 127.83, 126.19, 126.04, 125.97, 125.92, 125.87, 124.74, 123.73, 122.03, 121.32, 76.03, 75.74, 73.50, 73.07, 71.41, 69.44, 68.40, 64.83, 64.62, 64.05, 62.44, 39.35, 37.86, 37.70, 34.97, 34.65, 34.44, 34.20, 33.97, 33.75, 30.81, 30.02, 29.95.

DOSY NMR spectrum (500 MHz, CDCl₃, 25 °C): D = 3.66 × 10⁻⁶ cm²/s. MS (MALDI-TOF): MS (MALDI-TOF) m/z: 4902.3142 (M + H⁺, 100%); Caled for C_{320}H_{320}O_{32}N_{16} + H⁺: 4902.4084.

2.5.10 Synthesis of 52

The white solid 52 (9%) was obtained as the byproduct of the synthesis of 40a according to procedure 1.

1H NMR spectrum (300 MHz, CDCl₃, 25 °C): δ_{H} = 10.26 (s, 1H, -CHO), 10.23 (s, 2H, -CHO), 7.64 (s, 2H, H_{a1}), 7.52 (s, 1H, H_{a1}), 7.22 (s, 1H, H_{a1}), 6.53 (s, 1H, H_{a2}), 5.23-5.07 (m, 4H, H_{m}), 4.84-4.62 (m, 6H, H_{o}), 4.60-4.48 (m, 2H, H_{o}), 4.05-3.70 (m, 8H, H_{i}), 2.31-1.71 (m, 16H, H_{o1}, H_{i1} and H_{i}), 1.40-1.13 (m, 24H, H_{2}-H_{4}), 0.91-0.80 (m, 12H, H_{5}). ¹³C NMR spectrum (100 MHz, CDCl₃, 25 °C): δ_{C} = 189.47, 189.25, 158.69, 158.49, 158.23, 158.95, 135.56, 134.40, 133.86, 131.27, 130.96, 124.02,
122.25, 108.69, 76.51, 75.84, 75.21, 72.97, 35.68, 35.27, 33.03, 32.97, 32.18, 32.09, 30.11, 29.95, 27.80, 27.62, 22.83, 22.78, 14.30, 14.29. MS (MALDI-TOF) m/z: 1035.5217 (M + H⁺, 100%); Calcd for C₆₃H₈₀O₁₁ + H⁺: 1035.5592.

2.5.11 Synthesis of 53 (Procedure 3)

Tetraformylcavitand 40a (6.88 mg, 6.61 µmol) and m-phenylenediamine 17g (1.07 mg, 9.92 µmol) were loaded into an NMR tube and dissolved in CDCl₃ (500 µL) followed by addition of a solution of 1% TFA in CDCl₃ (7 µL) and several beads of molecular sieve. After 2 hours, 53 formed in 86% determined by ¹H NMR spectroscopy.

¹H NMR spectrum (300 MHz, CDCl₃, 25 °C): δHH = 10.34 (s, 2H, -CHO), 8.72 (s, 2H, Hₘ₁), 8.66 (s, 2H, Hₘ₃), 8.52 (s, 2H, Hₘ₄), 7.60 (s, 2H, Hₐ₁), 7.58 (s, 2H, Hₐ₁), 7.39 (t, 2H, J = 8.5 Hz, Hₐ₄), 7.35 (t, 1H, J = 8.0 Hz, Hₐ₄), 7.20 (d, 2H, J = 7.6, Hₐ₃), 7.12 (d, 2H, J = 8.2 Hz, Hₐ₃), 6.98 (s, 2H, Hₐ₁), 6.94 (dd, 2H, J = 8.2 Hz, Hₐ₃), 6.80 (s, 2H, Hₐ₁), 6.71 (t, 1H, J = 2.1 Hz, Hₐ₂), 6.54 (t, 1H, J = 2.0 Hz, Hₐ₂), 5.05 (t, 2H, J = 8.0 Hz, Hₘ), 5.00 (t, 2H, J = 7.3 Hz, Hₘ), 4.95-4.83 (m, 6H, Hₘ and Hₖ), 4.66 (m, 2H, Hₖ), 4.50 (m, 2H, Hₖ), 4.41-4.29 (m, 4H, Hₖ), 4.28-4.13 (m, 6H, Hₖ), 4.09-3.83 (m, 12H, Hₖ), 3.77 (m, 2H, Hₙ), 3.63 (m, 2H, Hₙ), 2.41-1.76 (m, 32H, Hₙ₁, Hₙ₁ and Hₙ), 1.47-1.11
(m, 48H, H2-Ha), 0.99-0.77 (m, 24H, H5). MS (MALDI-TOF) m/z: 2299.3077 (M + H+, 100%); Caled for C146H172O18N6 + H+: 2299.2828.

2.5.12 Synthesis of 54

Prepared by procedure 3 from triformylcavitand 52b (76.68 mg, 75.77 µmol), m-phenylenediamine 17g (12.27 mg, 113.66 µmol) and TFA (77 µL, 1% v/v), the yellow solid 54 was obtained quantitatively after removing solvent in vacuum.

\[ \text{1H NMR spectrum (300 MHz, CDCl}_3, 25 ^\circ\text{C): } \delta_{H} = 8.65 \text{ (s, 2H, H}_{im}, 8.63 \text{ (s, 2H, H}_{im}), 8.50 \text{ (s, 2H, H}_{im}), 7.52 \text{ (br, 4H, H}_{a4}), 7.33 \text{ (t, 2H, J = 8.0 Hz, H}_{a1}), 7.29 \text{ (t, 1H, J = 7.7 Hz, H}_{a4}), 7.13 \text{ (d, 2H, J = 8.4, H}_{a3}), 7.09 \text{ (d, 2H, J = 7.7 Hz, H}_{a3}), 6.94 \text{ (dd, 2H, J}_1=7.8 \text{ Hz, J}_2=2.1 \text{ Hz, H}_{a3}), 6.77 \text{ (s, 2H, H}_{a1}), 6.69 \text{ (t, 1H, J = 2.0 Hz, H}_{a2}), 6.65 \text{ (s, 2H, H}_{a1}), 6.49 \text{ (s, 2H, H}_{a3}), 6.48 \text{ (t, 2H, J = 2.0 Hz, H}_{a2}), 4.98 \text{ (t, 2H, J = 8.3Hz, H}_{m}), 4.95 \text{ (t, 2H, J = 8.0 Hz, H}_{m}), 4.81 \text{ (t, 2H, J = 7.7 Hz, H}_{m}), 4.78 \text{ (t, 2H, J = 8.6 Hz, H}_{m}), 4.65-4.40 \text{ (m, 8H, H}_{o}), 4.32 \text{ (m, 4H, H}_{o}), 4.15 \text{ (m, 4H, H}_{o}), 4.04-3.77 \text{ (m, 12H, H}_{i}), 3.55 \text{ (m, 4H, H}_{i}), 2.33-1.63 \text{ (m, 32H, H}_{o1}, H}_{i1} \text{ and H}_{i1}, 1.37-1.10 \text{ (m, 48H, H}_{2-Ha}), 0.88-0.70 \text{ (m, 24H, H}_{5}). \] \[ \text{13C NMR spectrum (125 MHz, CDCl}_3, 25 ^\circ\text{C): } \delta_{C} = 158.87, 158.20, 158.05, 157.59, 157.12, 157.08, 156.38, 155.85, 155.72, 155.11, 154.96, 154.65, 154.33, 154.03, 135.64, 135.25, 134.87, 134.09, 133.31, 131.29, 129.98, \]
129.64, 129.52, 126.53, 125.96, 125.11, 124.75, 123.89, 122.41, 120.46, 119.96, 117.81, 114.18, 109.74, 108.67, 72.92, 72.97, 71.84, 71.23, 70.66, 68.76, 68.41, 67.57, 35.21, 34.82, 34.34, 34.28, 33.82, 33.53, 32.45, 32.34, 32.32, 32.26, 30.29, 30.08, 29.93, 29.69, 29.37, 27.82, 27.56, 22.90, 22.89, 22.85, 22.82, 14.37, 14.35. MS (MALDI-TOF) m/z: 2243.2259 (M + H\(^+\), 100%); Calcd for C\(_{144}\)H\(_{172}\)O\(_{16}\)N\(_{6}\) + H\(^+\): 2243.2908.

2.5.13 Synthesis of 55

Prepared by procedure 3 from tetraformylcavitand 40a (1.025 g, 0.99 mmol), m-phenylenediamine 17g (159.7 mg, 1.47 mmol) and 5 µL neat TFA, the obtained solid was dissolved in 10 mL anhydrous THF. Under argon, Ni(OAc)\(_2\) (0.81 g, 3.27 mmol) and NaBH\(_3\)CN (0.93 g, 14.8 mmol) was added to the above solution. After 2 days, the reaction was complete as determined by \(^1\)H NMR spectroscopy and THF was removed in vacuum. The solid was sonicated in 5 mL NH\(_4\)OH for 10 minutes, filtered and washed with water. Then, the crude product was subjected to column chromatography (EtOAc-CH\(_2\)Cl\(_2\) = 1:99) and 169 mg of 55 (15%) was obtained as yellowish solid.

\(^1\)H NMR spectrum (400 MHz, CDCl\(_3\), 25 °C): \(\delta\) = 7.52 (s, 2H, H\(_{a1}\)), 7.49 (s, 2H, H\(_{a1}\)),
7.18 (t, 2H, J = 8.5 Hz, H\textsubscript{a4}), 6.94 (t, 1H, J = 7.9 Hz, H\textsubscript{a4}), 6.78 (s, 2H, H\textsubscript{a1}), 6.68 (s, 2H, H\textsubscript{a1}), 6.22 (dd, 2H, J\textsubscript{3} = 8.2 Hz, J\textsubscript{4} = 1.9 Hz, H\textsubscript{a3}), 6.20 (dd, 2H, J\textsubscript{3} = 8.5 Hz, J\textsubscript{4} = 2.1 Hz, H\textsubscript{a3}), 6.11 (t, 1H, J = 2.3 Hz, H\textsubscript{a2}), 6.06 (dd, 2H, J\textsubscript{3} = 8.2 Hz, J\textsubscript{4} = 1.9 Hz, H\textsubscript{a3}), 6.20 (dd, 2H, J\textsubscript{3} = 8.5 Hz, J\textsubscript{4} = 2.1 Hz, H\textsubscript{a3}), 5.85 (t, 2H, J = 2.0 Hz, H\textsubscript{a2}), 4.97 (t, 2H, J = 8.0 Hz, H\textsubscript{m}), 4.91 (t, 2H, J = 7.6 Hz, H\textsubscript{m}), 4.86 (t, 2H, J = 7.4 Hz, H\textsubscript{m}), 4.84 (d, 4H, J = 5.5 Hz, H\textsubscript{2}), 4.80 (t, 2H, J = 7.4 Hz, H\textsubscript{m}), 4.75-4.65 (m, 4H, H\textsubscript{o}), 4.51 (m, 2H, H\textsubscript{o}), 4.41-4.30 (m, 10H, H\textsubscript{6} and H\textsubscript{o}), 4.26-4.10 (m, 10H, H\textsubscript{6} and H\textsubscript{o}), 4.09-4.03 (m, 2H, -NH\textsubscript{2}), 3.99-3.77 (m, 14H, H\textsubscript{1} and –NH\textsubscript{2}), 3.66 (m, 2H, H\textsubscript{i}), 3.52-3.36 (m, 4H, H\textsubscript{i} and –NH\textsubscript{2}), 3.25 (t, 2H, J = 5.6 Hz, -OH), 2.35-1.80 (m, 32H, H\textsubscript{o1}, H\textsubscript{i1} and H\textsubscript{i}), 1.41-1.17 (m, 48H, H\textsubscript{2}-H\textsubscript{4}), 0.95-0.77 (m, 24H, H\textsubscript{5}). 13C NMR spectrum (125 MHz, CDCl\textsubscript{3}, 25 °C): δ\textsubscript{C} = 157.04, 156.98, 155.49, 155.27, 155.20, 154.63, 154.01, 153.33, 150.53, 149.78, 148.83, 134.62, 134.46, 134.34, 134.25, 133.86, 133.78, 133.64, 133.56, 131.02, 130.02, 131.02, 130.02, 126.45, 125.73, 125.63, 125.36, 125.05, 124.26, 123.72, 123.68, 105.02, 101.87, 100.80, 99.87, 85.95, 73.60, 73.49, 72.42, 72.13, 69.69, 69.47, 67.95, 67.17, 58.77, 39.96, 39.21, 38.54, 36.50, 35.91, 35.66, 35.52, 34.65, 34.51, 34.49, 32.36, 32.31, 32.25, 30.74, 30.51, 30.39, 30.01, 29.94, 27.62, 27.61, 27.59, 22.83, 14.39. MS (MALDI-TOF) m/z: 2353.3647 (M + K\textsuperscript{+}, 100%); Calcd for C\textsubscript{146}H\textsubscript{188}O\textsubscript{18}N\textsubscript{6} + K\textsuperscript{+} 2353.3674.

### 2.5.14 Synthesis of 57

Hemicarcerand 55 (55 mg, 23.7 µmol), Fmoc-Ala 56 (59 mg, 190 µmol), DCC (43 mg, 209 µmol) and DMAP (2.3 mg, 19 µmol) were dissolved in 10 mL HPLC grade CH\textsubscript{2}Cl\textsubscript{2}. After a few minutes, cloudiness formed. Anhydrous DMF was added until the solution was transparent. After 24 hours, the solvent was removed and the solid was subject to column chromatography (MeOH-CH\textsubscript{2}Cl\textsubscript{2} = 1:9). 15 mg of 57 (24%)
was obtained as a yellowish solid.

\[ ^1H \text{NMR spectrum (500 MHz, CDCl}_3, 25 ^\circ \text{C): } \delta_H = 7.75 \text{ (br, 2H, H}_{a8}, 7.60 \text{ (br, 2H, H}_{a5}), 7.52 \text{ (s, 2H, H}_{a1}), 7.50 \text{ (s, 2H, H}_{a1}), 7.39 \text{ (br, 2H, H}_{a7}), 7.31 \text{ (br, 2H, H}_{a6}), 7.18 \text{ (t, 2H, J = 7.6 Hz, H}_{a4}), 6.94 \text{ (t, 1H, J = 8.0 Hz, H}_{a4}), 6.78 \text{ (s, 2H, H}_{a1}), 6.77 \text{ (s, 1H, H}_{a1}), 6.69 \text{ (s, 1H, H}_{a1}), 6.32-6.18 \text{ (m, 2H, H}_{a3}), 6.12 \text{ (br, 1H, H}_{a2}), 6.06 \text{ (m, 1H, H}_{a3}), 5.86 \text{ (br, 2H, J = 2.0 Hz, H}_{a2}), 5.41-5.28 \text{ (m, 2H, H}_{m}), 5.09-4.90 \text{ (m, 4H, H}_{m}), 4.90-4.78 \text{ (m, 6H, H}_{m}, H_7 \text{ and H}_{12}), 4.70 \text{ (m, 2H, H}_{o}), 4.62-4.48 \text{ (m, 4H, H}_{o} \text{ and H}_{6}), 4.48-4.29 \text{ (br, 14H, H}_{o} \text{ and H}_{6}), 4.29-4.11 \text{ (m, 12H, H}_{o}, H_6 H_8, H_{10} \text{ and H}_{11}), 4.10-4.02 \text{ (m, 2H, -NH}_2), 4.01-3.62 \text{ (m, 16H, H}_{i} \text{ and -NH}_2), 3.58-3.37 \text{ (m, 4H, H}_{i} \text{ and -NH}_2), 3.26 \text{ (t, 2H, J = 5.8 Hz, -OH)}, 2.37-1.86 \text{ (m, 32H, H}_{o1}, H_{i1} \text{ and H}_{i}), 1.51-1.00 \text{ (m, 51H, H}_2 \text{-H}_4 \text{ and H}_0), 0.96-0.81 \text{ (m, 24H, H}_3). \]

\[ ^13C \text{NMR spectrum (125 MHz, CDCl}_3, 25 ^\circ \text{C): } \delta_C = 173.54, 157.05, 157.00, 155.93, 155.86, 155.52, 155.29, 155.24, 154.70, 154.70, 154.07, 153.46, 153.41, 153.38, 150.58, 149.81, 148.87, 144.09, 141.60, 134.64, 134.52, 134.32, 134.28, 134.23, 133.89, 133.80, 133.71, 133.68, 133.66, 133.61, 131.04, 130.02, 127.99, 127.34, 126.47, 125.88, 125.78, 125.66, 125.38, 125.08, 124.27, 124.15, 123.76, 123.71, 120.24, 105.08, 102.03, 101.94, 100.82, 99.90, 96.10, 73.63, 73.52, 72.45, 72.20, 69.69, 69.44, 69.29, 68.01, 67.25, 61.00, 58.79, 50.04,\]
47.39, 39.99, 39.24, 38.59, 36.53, 35.94, 35.66, 35.55, 34.67, 34.54, 32.38, 32.34, 32.27, 30.77, 30.50, 30.42, 30.13, 30.04, 27.62, 22.85, 14.40.

MS (MALDI-TOF) m/z: 2610.5155 (M + H⁺, 100%); Calcd for C₁₆₄H₂₀₅O₂₁N₇ + H⁺: 2610.5290.

2.6 References


Chapter 3

Rational Design and Synthesis of Nanocapsules

3.1 Introduction

The synthesis of discrete nanocapsules has gained much attention in the past years due to their potential applications in material sciences\(^1\) and biomedical research\(^2\). Among them, nanocapsules assembled through metal-coordination\(^3\) and hydrogen-bonding interactions\(^4\) have been studied in great detail. Technically, most of them were synthesized through directional bonding approaches, which include the edge-directed approach and face-directed approach\(^5\). In the edge-directed approach, the building blocks define the edges of the assembly and are connected at the vertices. An example illustrating the idea is the tetrahedral capsule 11 introduced in chapter 1, in which ligands can be viewed as the six edges of the tetrahedron and are connected with four metal ions that occupy the vertices\(^6\). In the face-directed approach, some or all faces of the assembly are occupied by planar building blocks and are connected with one another or with other building blocks to form the assembly. The synthesis of Fujita’s octahedral capsule 8 (see Chapter 1) is an example of this approach\(^3a\). Four out of eight faces in capsule 8 are defined by planar 2,4,6-tri(pyridin-4-yl)-1,3,5-triazine units and metals on the vertices connect them together.

Covalent nanocapsules are rare due to the difficulty in synthesis. For example, the most well-known covalent nanocapsules, fullerenes, form in very low yields under extreme conditions\(^7\). Many other covalent capsules suffer from the same problem owing to the fact that most covalent bonds are non-dynamic and the product
formation is under kinetic control. As a result, a low yield is very often obtained in multi-step covalent nanocapsule synthesis. Therefore, it is desirable to assembly nanocapsules through dynamic covalent bonds under thermodynamic control.

![Diagram of nanocapsules](image)

**Figure 3-1**: Several representative *edge-directed approaches* for designing nanocapsules (reprinted with permission from reference\(^5\)).

DCC, introduced in the first chapter, combines the dynamic features of a reversible bond formation and high stability of a covalent bond, which provides a promising way to assemble nanocapsules, that have high stability, in high yield. Among DCC
reactions, we intensively studied Schiff-base reactions and most nanocapsules assembled from Schiff-base reactions give quantitative yields. Since imine bonds are directional, *edge-directed* and *face-directed approaches* can be applied to design imine-based nanocapsules. Several representative *edge-directed approaches* are shown in Fig. 3-1. For example, tetrahedral capsules can be assembled from six linear building blocks and four tritopic building blocks, for which a cone angle close to 60° is required (A). Increasing the cone angle to 90° leads to a cubic capsule (C) and further increasing it to 109.5° results in an adamantoid capsule when reacting with six 109.5° bent building blocks (B). Six tetratopic building blocks with a cone angle of 60° react with twelve linear building blocks to produce an octahedral nanocapsule.

The *face-directed approach* has been intensively employed in metal-coordination nanocapsules. In the field of dynamic covalent nanocapsules, it was first utilized in 2007 by our group. A rhombicuboctahedral nanocapsule 75 was synthesized from six cavitands 19 and eight 1,3,5-tris(4-aminophenyl)benzene 17s (Fig. 3-2). 75 is

**Figure 3-2:** The formation of rhombicuboctahedral nanocapsule 75 through the *face-directed approach* (reprinted with permission from reference ⁵).
composed of eight triangular and eighteen square faces. The triangular faces in green are defined by six planar 17s, which connect to eight square faces in red defined by 19 through 24 imine bonds.

Although many nanocapsules have been successfully synthesized by the above two approaches, it remains a challenge to rationally design a nanocapsule from building blocks in terms of shape, size and the number of components. Unexpected architectures are often produced, mainly because building blocks are flexible to some extend and may result in deviations from predictions, which assume that all building blocks are very rigid. For example, the Nischke group reported five different discrete metal-organic assemblies formed through the same building blocks by varying templates. Therefore, it is very useful to find out some guidelines for designing certain nanocapsules.

In this chapter, the rational design of octahedral nanocapsules and the improved synthesis of rhombicuboctahedral nanocapsules are presented.

3.2 Design and synthesis of octahedral nanocapsules

3.2.1 Background

As introduced in chapter 1, our group reported the synthesis of octahedral nanocapsule 23 in ~80% in chloroform from six equivalents of cavitand 19 and twelve equivalents of ethylenediamines 17a. The cone angle of cavitand 19 is ~65° and smaller than the ideal angle of 90°. As a result, attempts to prepare other octahedral nanocapsules by reacting 19 with linear diamines, such as 1, 4-phenylenediamine 17i or benzidine 17j, failed but formed tetrahedral nanocapsules.
instead. Increasing the cone angle of the cavitand by simply increasing the length of the spanners (-O(CH\(_2\)_3O-) results in flexible cavitand 40a, which gives either a tetrahedral nanocapsule 41a with ethylenediamine 17a or a complex mixture with 17i. Therefore, in order to synthesize octahedral nanocapsules, two criterions have to be fulfilled. Firstly, the building blocks should be rigid. Secondly, the cone angle of cavitands should be close to 90°. Deep cavitand 63 was found to meet above two criterions and can quantitatively form octahedral nanocapsules with rigid linear linkers 17i and 17j.

3.2.2 Deep cavitands 62-64

3.2.2.1 Synthesis

The synthesis of deep cavitands is outlined in Scheme 3-1. Tetraiodocavitands 59-61 were synthesized according to the literature\(^\text{11}\). Suzuki coupling on 59-61 gave deep cavitands 62-64, respectively, in 60-70% after purification by silica gel column chromatography.

**Scheme 3-1**: Synthesis of deep cavitands 62-64.
3.2.2.2 Conformational analysis

![Chart 3-1](image)

**Chart 3-1**: Cone angles of 62-64 and their parent cavitands 19, 58 and 40a in $C_\tau$-symmetric conformation.

Based on CPK models, deep cavitand 62 is very rigid and has $C_\tau$-symmetry. It has the same cone angle as its parent cavitand 19 and thus, is expected to give tetrahedral nanocapsules with rigid linear linkers (see Chapter 2, Fig. 2-5). By contrast, $C_\tau$-symmetric deep cavitands 63 and 64 have cone angles of 85.4° and 93.7°, respectively, and are ideal for assembling octahedral nanocapsules (Fig. 3-1). However, they are relatively flexible due to the longer spanners, which is similar to cavitands 58 and 40a. As a result, they could also potentially adopt $C_2$-symmetric conformations. Indeed, gas phase calculations indicate that their $C_2$-symmetric conformations are more favored. Modeling studies also showed, that in the $C_2$-symmetric conformation, two of the inserted phenyl rings of 63 are in close contacted (Fig. 3-3D) and create a pocket underneath in the cavitand. The volume of the pocket is estimated to be 30-40Å³, which is too small to accommodate a solvent molecule. Therefore, we postulated that the $C_2$-symmetric conformation is
destabilized in solution and that the $C_r$-symmetric conformation is more favored (Fig. 3-3E, F). In the case of 64, the opening between the two inserted phenyl rings in the $C_2$-symmetric conformation is still large enough to hold a single solvent molecule due to its longer spanners (Fig. 3-2A), so that the $C_2$-symmetric conformation is favored in solution as well as in the gas phase. Based on the above hypothesis, only 63 can yield octahedral nanocapsules. As a control experiment, cavitand 58 without extended phenyl rings should adopt $C_2$-symmetry and would not give octahedral nanocapsules.

**Figure 3-3:** Modeling of deep cavitands 63 (A-C) and 64 (D-E) in $C_2$- and $C_r$-symmetric conformations (reprinted with permission from reference 12).

### 3.2.3 Synthesis and characterization of octahedral nanocapsules

To test this hypothesis, six equivalents of 63 and twelve equivalents of rigid linear linkers 17i and 17j were mixed in CDCl$_3$ under argon in the presence of catalytic amounts of TFA (Scheme 3-2A) and the reaction was monitored by $^1$H NMR spectroscopy. The expected octahedral nanocapsules 65a and 65b completely formed within 1 hour. Both are stable under argon, but yellowish precipitate formed slowly in
air. Another linear linker 17\text{p}, which is flexible due to the ethylene group between the two benzene rings, yielded a hemicarcerand 66 instead of an octahedral nanocapsule (Scheme 3-2B). The results indicate that high rigidity of linkers is required for the formation of octahedral nanocapsules. The reactions between 58 and 17i, j were also performed and gave complex mixtures. This further supports the importance of introducing the second phenyl rings.

Scheme 3-2: Synthesis of octahedral nanocapsules 65a, b (A) and hemicarcerand 66 (B).

The formation of octahedral nanocapsules was confirmed by NMR spectroscopy, MALDI-TOF MS and GPC (Fig. 3-4). The $^1$H NMR spectrum of 65a shows one multiplet each for the imine protons, H$_{im}$, the aryl protons, H$_{a1}$, H$_{a2}$, H$_{a3}$ and H$_{a4}$, the benzylic protons, H$_m$, and the spanner protons, H$_o$ and H$_i$, in a ratio 1:1:1:1:1:1:1:1:1, which is consistent with the symmetry of the octahedral nanocapsule. MALDI-TOF MS shows an isotopic cluster at the expected mass-to-charge ratio at $m/z = 8603.10$ (8603.36 calculated for [M + H]$^+\)$. The GPC trace shows a single peak at 13.88
minutes, which indicates the quantitative formation of 65a.

![Figure 3-4: 1H NMR spectrum (A), MALDI-TOF MS (B) and GPC (C) of octahedral nanocapsule 65a.](image)

3.2.4 Synthesis and characterization of tetrahedral nanocapsules

Capsule formation for deep cavitands 62 and 64 were also investigated. In the case of cavitand 64, the condensation with linkers 17i-j, p gave complicated mixtures. As mentioned earlier, the \( C_2 \)-symmetric conformation of 64 is more stable than the \( C_4 \)-symmetric conformation in solution, which perfectly explains the outcome. For 62, tetrahedral nanocapsules 67a-c formed with 17i-j, p similar to 19\(^4\). However, unlike 19, which gives quantitative yields, different amount of octahedral nanocapsules 68a-c were produced along with 67a-c when 62 was used (Scheme 3-3). Again, the results are consistent with our hypothesis.
Scheme 3-3: Synthesis of tetrahedral 67a-c and octahedral nanocapsules 68a-c.

Fig. 3-5 shows the $^1$H NMR spectrum, MALDI-TOF MS and GPC of the condensation products of four equivalents of 62 and eight equivalents of 17i in CDCl$_3$. Based on GPC, the product mixture is composed of only tetrahedral nanocapsule 67a (retention time at 14.77 minutes) and octahedral nanocapsule 68a (retention time at 14.02 minutes) and their ratio is around 67a: 68a = 9:1. Tetrahedral nanocapsule 67a was captured on MALDI-TOF MS, which gives an isotopic cluster at the expected mass-to-charge ratio at $m/z = 5511.63$ (5511.65 calculated for [M + H]$^+$). Unfortunately, octahedral nanocapsule 68a was not observed by MS probably due to its low yield.
Figure 3-5: $^1$H NMR spectrum (A), MALDI-TOF MS (B) and GPC trace (C) of tetrahedral nanocapsule 67a and octahedral nanocapsule 68a. Signals belonging to 67a are labeled with black arrows and those belonging to 68a are labeled with red arrows.

In the $^1$H NMR spectrum, signals of 67a are labeled with black arrows. Two sets of multiplets are observed for imine protons ($H_{im}$) and aryl protons ($H_1$), consistent with the reported $^1$H NMR spectrum of tetrahedral nanocapsules 22$^{13}$. Signals of 68a are
labeled with red arrows and show only one multiplet each for imine and aryl protons.

**Figure 3-6:** Partial $^1$H NMR spectra (400 MHz, CDCl$_3$, 25 °C) of a mixture of 67a and 68a after different times. Signals belonging to 68a are labeled with arrows.

The equilibration between 67a and 68a is slow and was followed by $^1$H NMR spectroscopy (Fig. 3-6). It showed that nanocapsules completely formed within 5 minutes and are composed of 10% of 68a and 90% 67a based on the integration of imine protons. The ratio stayed almost constant for the next 3 hours. After 3 days, 68a had increased to ~20% and the reaction mixture reached an equilibrium after 7 days, and is composed of ~30% of 68a and ~70% of 67a. Impressively, the reaction is very fast, which indicates that building blocks are well preorganized and simply ‘snap’ into position. However, the long equilibration time shows that transamination is slow under the condition.
3.2.5 Improved synthesis and characterization of rhombicuboctahedral nanocapsules

Yong Liu et al. found that in the formation of 75, 20s formed as the byproduct and 75 was isolated in only 60% after 2 days reaction (Fig. 3-2). Without quenching the reaction, 75 eventually converts into 20s after about 5 days, since 20s precipitates from the reaction solution. In order to accelerate transamination, which could suppress the formation of 20s, a high concentration of TFA was added to the solution, resulting in significant acetal cleavage, which further lowered the yield.

Since deep cavitands 62 and 63 are very stable towards TFA and both have $C_4$-symmetric conformations, they are expected to give much higher yields of rhombicuboctahedral nanocapsules. In fact, rhombicuboctahedral nanocapsules 69 and 70 formed quantitatively in both cases without acetal cleavage and hemicarcerand byproduct formation by simply mixing six equivalents of 62 or 63 and eight equivalents of 17s in the presence of catalytic amounts of TFA (Scheme 3-4). Remarkably, assembly of these nanocapsules requires only 1 hour to complete, which is much shorter than the formation of 75. Presumably, the formyl groups of deep
cavitands 62 and 63 are less sterically hindered than those of cavitand 19, making the extended version more reactive.

![Scheme 3-4: Synthesis of rhombicuboctahedral nanocapsules 69 and 70.](image)

Again, the formation of 69 and 70 is supported by NMR spectroscopy, GPC and MALDI-TOF MS (Fig. 3-7). $^1H$ NMR spectra of 69 and 70 show one multiplet each for imine protons (H$_{im}$), aryl protons (H$_{a1}$-H$_{a6}$), spanner protons (H$_o$ and H$_i$) and benzylic protons (H$_m$) at a ratio of 1:1:1:1:1:1:1:1, which is consistent with the symmetry of the rhombicuboctahedral nanocapsules. MALDI-TOF MS (linear mode) of 69 displays a major peak at mass-to-charge ratio at m/z = 9779.8018 Da. The observed mass-to-charge ratio at m/z = 10117.4707 of 70 was assigned to [70 + H]$^+$. 
Figure 3-7: $^1$H NMR spectra and MALDI-TOF MS of 69 and 70.

Table 3-1: TFA-catalyzed condensation products of deep cavitands 62-64 and linkers 17i-j, p. s.

<table>
<thead>
<tr>
<th>Reactants</th>
<th>62</th>
<th>63</th>
<th>64</th>
</tr>
</thead>
<tbody>
<tr>
<td>17s</td>
<td>69 (Quant.)</td>
<td>70 (Quant.)</td>
<td>Mix</td>
</tr>
<tr>
<td>17i</td>
<td>67a (90%) + 68a (10%)$^a$</td>
<td>65a (quant.)</td>
<td>Mix</td>
</tr>
<tr>
<td>17j</td>
<td>67b (80%) + 68b (20%)</td>
<td>65b (quant.)</td>
<td>Mix</td>
</tr>
<tr>
<td>17p</td>
<td>67c (80%) + 68c (20%)</td>
<td>66 (95%)</td>
<td>ppt$^b$</td>
</tr>
</tbody>
</table>

$^a$The mixture is a kinetic ratio. After equilibration, the ratio is $67a/68a = 7:3$.

$^b$Precipitate formed upon mixing the building blocks.
The condensation products of deep cavitands are summarized in Table 3-1. From these data, one can conclude that the rigidity of both cavitands and linkers and their geometry are important for the formation of octahedral nanocapsules.

3.2.6 Size of nanocapsules

As discussed in chapter 2, the diffusion rate of nanocapsules, including tetrahedral, octahedral and rhombicuboctahedral nanocapsules, can be measured by DOSY NMR spectroscopy\textsuperscript{14} and their sizes are estimated with the ‘Stokes-Einstein’ equation by treating them as spheres in solution. DOSY NMR spectra of 65a, 65b and 70 are shown in Fig. 3-8. The x-axis is the chemical shift of protons and y-axis is the diffusion rate constant. Signals belonging to the same species have the same diffusion rate and larger species will give lower diffusion rates. Signals highlighted by dash lines, for example, have the same diffusion rate and are all from the same nanocapsule. Signals in these spectra that have higher diffusion rates are either from solvent, water or from impurities. Radii of nanocapsules 65a, 65b and 70 are 2.16 nm, 2.34 nm and 1.92 nm, respectively.
Figure 3-8: DOSY NMR spectra of 65a, 65b and 70.
Table 3-2. Diffusion rates and radii of poly-imine nanocapsules.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound No.</th>
<th>D ($\times 10^{-10}$ m$^2$/s)</th>
<th>r (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65a</td>
<td>2.11</td>
<td>1.92</td>
</tr>
<tr>
<td>2</td>
<td>65b</td>
<td>1.73</td>
<td>2.34</td>
</tr>
<tr>
<td>3</td>
<td>67a</td>
<td>2.50</td>
<td>1.61</td>
</tr>
<tr>
<td>4</td>
<td>67b</td>
<td>2.14</td>
<td>1.88</td>
</tr>
<tr>
<td>5</td>
<td>67c</td>
<td>2.06</td>
<td>1.96</td>
</tr>
<tr>
<td>6</td>
<td>69</td>
<td>1.85</td>
<td>2.18</td>
</tr>
<tr>
<td>7</td>
<td>70</td>
<td>1.80</td>
<td>2.24</td>
</tr>
</tbody>
</table>

The diffusion rate and estimated radii of major nanocapsules are summarized in Table 3-2. Among all nanocapsules, 65b has the largest radius and is also the largest poly-imine nanocapsule reported so far. Rhombicuboctahedral nanocapsules, 56 and 57 are a little bit smaller, but CPK modeling studies show that all these nanocapsules are large enough to hold one small protein, such as insulin and cytochrome c. Therefore, they can be potentially used to encapsulate large biomolecules.

3.3 Synthesis of octahedral nanocapsule 74 from tetraaminocavitand 72

3.3.1 Background

From above discussion, deep cavitands are very versatile and can be used to assemble both octahedral and rhombicuboctahedral nanocapsules. However, p-xyleneidineamine 17i and benzidine 17j are air sensitive and tend to form radicals, followed by polymerization. Therefore, nanocapsules containing these building blocks have to be stored under argon. Upon exposure to air, yellowish precipitates form slowly, which could be the polymerization products. Both diamines are also potentially carcinogenic, so that special attention should be paid when handling them. In order to avoid using 17i and 17j, another strategy was explored, in which the functional groups on the cavitand and linker are reversed. To test this approach, tetraaminocavitand 72, which has the same symmetry as its analogue 63, and terephthalaldehyde 73 are used to
assemble the new octahedral nanocapsule 74.

3.3.2 Synthesis of tetraaminocavitand 72

The synthesis of tetraaminocavitand 72 is outlined in Scheme 3-5. The direct coupling of 60 and 4-aminophenylboronic acid pinacol ester was first employed (Route 1). However, the desired product was not obtained. The major products are mono- and di-substituted species. This is attributed to either the low solubility of 72 and/or intermediates in THF. Another route employed involves the coupling of 60 and 4-nitrophenylboronic acid, which gave a tetrinitrocavitand 71 (Route 2). Subsequent reduction using Pd/C and NaBH₄ yielded tetraaminocavitand 72. MeOH is a key component in the reaction mixture, which supplies the proton source for the reduction and increases the solubility of the product. Without MeOH, tetrinitrocavitand was
barely reduced even after several days. The overall yield is ~40%.

3.3.3 Synthesis of nanocapsule 74

As anticipated, octahedral nanocapsule 74 quantitatively formed from six equivalents of cavitand 72 and twelve equivalents of terephthalaldehyde 73 within 1 hour (Scheme 3-6). And also, as predicted, nanocapsule 74 is very robust and air insensitive. Monitored by $^1$H NMR spectroscopy, 74 stayed intact after several weeks in air and no precipitate formed. Based on its robustness, 74 is more suitable for applications in molecular recognition or material sciences, than 65a, b.

![Scheme 3-6: Synthesis of octahedral nanocapsule 74.](image)

Again, the structure of 74 is supported by NMR spectroscopy and MALDI-TOF MS (Fig. 3-9). The top $^1$H NMR spectrum in Fig. 3-10 displays signals of cavitand 72. Upon mixing with terephthalaldehyde 73 in the presence of catalytic amounts of TFA, H2 and H3 shift downfield tremendously. The remaining aryl protons (H4), benzylic protons (Hm) and spanner protons (H6 and H7) shift downfield slightly. The newly formed imine signal and the disappearance of the amine signal also indicate the formation of the nanocapsule. Furthermore, the signals of the nanocapsule are broader than those of the cavitand consistent with the formation of a large structure and the
number of multiplets is consistent with its symmetry. The MALDI-TOF MS (linear mode) shows one major peak, which has the expected mass-to-charge ratio at m/z = 8603.05 (8603.38).

Figure 3-9: $^1$H NMR spectrum and MALDI-TOF MS of 74.

### 3.4 Conclusions

In this chapter, an edge-directed approach and face-directed approach for the assembly of octahedral and rhombicuboctahedral nanocapsules are developed, respectively. In the edge-directed approach, six tetraformyl deep cavitands 63 reacted with twelve linear aromatic diamines 17i, j to quantitatively form octahedral
nanocapsules 65a, b. It was found that the rigidity and geometry of cavitands and linkers are important for the quantitative formation of octahedral nanocapsules. Later, by using the same approach, six tetraamine deep cavitands 72 and twelve terephthaldehydes 73 quantitatively yielded octahedral nanocapsule 75, which is less toxic and more stable towards air than 65a, b. In the face-directed approach, two rhombicuboctahedral nanocapsules 69 and 70 formed quantitatively within 1 hour. As compared to a previously synthesized rhombicuboctahedral nanocapsule 75, the formation of 69 and 70 is more efficient.

3.5 Experimental section

3.5.1 General procedures

Reagents and chromatography solvents were purchased from Aldrich and used without further purification except that chloroform was passed through K₂CO₃ prior to use. THF was dried over Na/benzophenone and distilled under argon. ¹H NMR spectra recorded in CDCl₃ were referenced to residual CHCl₃ at δ_H = 7.26. ¹³C NMR spectra recorded in CDCl₃ were referenced to ¹³CDCl₃ at δ_C = 77.5 ppm. Mass spectra were recorded on an Applied Biosystems Voyager DE-Pro mass spectrometer (MALDI-TOF). External standards were used for calibration and 2,4,6-trihydroxylacetophenone (THAP) as matrix. Gel permeation chromatography (GPC) was performed on a Thermo SpectraSYSTEM HPLC system equipped with dual wavelength UV/Vis detector (280 nm), Eppendorf CH-30 column heater and two Jordi GPC columns (cross linked DVB; 103 Å pore size; MW cutoff ~ 25,000; 7.8 mm × 30 cm) with CH₂Cl₂/1% NEt₃ as mobile phase at a flow of 1 mL/min. Approximate molecular weights of analytes were determined from a semilogarithmic calibration plot.
3.5.2 Synthesis of 58

Tetrabromocavitand 167 (520 mg, 0.53 mmol) was dried under vacuum overnight at 100 °C and then dissolved in 20 mL THF under argon. THF (10 mL) was added to a second dried flask, which was charged with iPrMgCl/THF solution (2.6 mL, 2 M) and anhydrous LiCl (216 mg, 5.10 mmol). The suspension was stirred under argon until all LiCl was dissolved. Then, the resulting solution was cannulated to the solution of 167 at -78 °C. After the Br/MgCl exchange was complete, which was monitored by 1H NMR spectroscopy, anhydrous DMF (0.43 mL, 6.33 mmol) was injected to the solution. After 1 hour, the reaction was quenched with aqueous HCl solution (10 mL, 1M). The product was extracted with ethyl acetate (3×30 mL). The combined organic solutions were washed with sat. NaCl solution, dried over anhydrous Na2SO4 and concentrated under vacuum. The product was further purified by silica gel column chromatography (THF-CH2Cl2 = 2:98). A white solid was obtained in 25% yield.

![Chemical Structure](image)

1H NMR spectrum (400 MHz, CDCl3, 25 °C): δH = 10.35 (s, 4H, -CHO), 7.56 (s, 4H, H_a), 5.35 (t, J = 8.1 Hz, 4H, H_m), 4.51 (m, 8H, H_o), 3.67 (m, 8H, H_i), 2.10 (m, 8H, H_1), 1.42-1.18 (m, 24H, H_2-H_4), 0.88 (t, J = 7.1 Hz, 12H, H_5). 13C NMR spectrum (100 MHz, CDCl3, 25 °C): δC = 190.24, 155.85, 137.37, 130.17, 125.13, 74.42, 34.50, 33.32, 32.28, 27.87, 23.07, 14.53. MS (MALDI-TOF) m/z: 1007.4911 (M + Na+, 100%); Calcd for C_60H_72O_12 + Na+: 1007.4916.
3.5.3 Synthesis of 60

Tetrabromocavitand 167\textsuperscript{13} (530 mg, 0.54 mmol) was dried overnight under vacuum at 100 °C and then dissolved in 30 mL THF under argon. At -78°C, BuLi/Hexane (1.6 mL, 2.5 M) was added into the above solution. After 3 hours, I\textsubscript{2} (1.3 g, 5.12 mmol) was added to the solution. The reaction was quenched with water. The product was extracted with ethyl acetate (3 × 30 mL). The combined organic solutions were washed with Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3} solution (20 mL, 10%), water and sat. NaCl solution, dried over anhydrous Na\textsubscript{2}SO\textsubscript{4} and concentrated under vacuum. The product was further purified by silica gel column chromatography (CH\textsubscript{2}Cl\textsubscript{2}-Hexane = 1:1). A slightly yellow solid was obtained in 81% yield.

\[\text{1H NMR spectrum (500 MHz, CDCl\textsubscript{3}, 25 °C ): } \delta_{\text{H}} = 7.29 (s, 4H, H\textsubscript{a}), 5.27 (t, J = 8.2 Hz, 4H, H\textsubscript{m}), 4.46 (m, 8H, H\textsubscript{o}), 3.76 (m, 8H, H\textsubscript{i}), 2.04 (m, 8H, H\textsubscript{1}), 1.38-1.15 (m, 24H, H\textsubscript{2}, H\textsubscript{3} and H\textsubscript{4}), 0.86 (t, J = 7.2 Hz, 12H, H\textsubscript{5}).\text{13C NMR spectrum (125 MHz, CDCl\textsubscript{3}, 25 °C): } \delta_{\text{C}} = 154.88, 136.98, 125.25, 90.36, 70.89, 36.05, 35.24, 32.31, 27.87, 23.05, 14.54.\text{ MS (MALDI-TOF) m/z: 1399.1543 (M + Na\textsuperscript{+}, 100%); Calcd for C\textsubscript{56}H\textsubscript{68}O\textsubscript{8}I\textsubscript{4} + Na\textsuperscript{+}: 1399.0984.}\]

3.5.4 Synthesis of 61

Tetrabromocavitand 39\textsuperscript{a}\textsuperscript{14} (1.03 g, 0.99 mmol) was dried overnight under vacuum at 100 °C and then dissolved in 40 mL THF under argon. THF (10 mL) was added to a
second dried flask, which was charged with ¹PrMgCl/THF solution (5 mL, 2 M) and anhydrous LiCl (420 mg, 9.91 mmol). The suspension was stirring under argon until all LiCl was dissolved. The resulting solution was cannulated to the solution of 39a at -78 °C. After the Br/MgCl exchange was complete, which was monitored by ¹H NMR spectroscopy, I₂ (2.52 g, 9.92 mmol) was added at the same temperature. The reaction was quenched with water. The product was extracted with ethyl acetate (3 × 50 mL). The combined organic solutions were washed with Na₂S₂O₃ solution (50 mL, 10%), water and sat. NaCl solution, dried over anhydrous Na₂SO₄ and concentrated under vacuum. The product was further purified by silica column chromatography (Hexane-EtOAc = 1:4). A slightly yellow product was obtained in 70%.

¹H NMR spectrum (400 MHz, CDCl₃, 25 °C): δH = 7.04 (s, 4H, H₄), 4.91 (t, J = 7.5 Hz, 4H, H₅), 4.67 (m, 8H, H₆), 3.94 (m, 8H, H₇), 2.32 (m, 4H, H₈), 2.08 (m, 4H, H₉), 1.93 (m, 8H, H₁₀), 1.36-1.14 (m, 24H, H₁₁, H₁₂, H₁₃, H₁₄, H₁₅, H₁₆, H₁₇, H₁₈, H₁₉, H₂₀, H₂₁), 0.86 (t, J = 6.9 Hz, 12H, H₂₂) ¹³C NMR spectrum (100 MHz, CDCl₃, 25 °C): δC = 157.18, 134.89, 125.78, 90.23, 70.29, 36.46, 36.22, 34.45, 29.85, 27.74, 23.03, 14.59. MS (MALDI-TOF) m/z: 1455.1230 (M + Na⁺, 100%); Calcd for C₆₀H₇₆O₈I₄ + Na⁺: 1455.1611.

3.5.5 Synthesis of 63 (Procedure 1)

A Schlenk flask charged with tetraiodocavitand 60 (176.5 mg, 177.4 µmol), potassium (4-formylphenyl)trifluoroborate (225.6 mg, 1.06 mmol), Pd(OAc)₂ (17.3
mg, 71 µmol), PPh₃ (37.2 mg, 142 µmol), and K₂CO₃ (294 mg, 2.13 mmol) was evacuated and refilled with argon three times. After adding THF (10 mL) and deionized water (1 mL), the flask was sealed and kept stirring at 100 °C overnight. The solution was acidified with aqueous HCl (3 mL, 1 M) and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with sat. NaCl solution, dried over anhydrous Na₂SO₄ and concentrated under vacuum. The product was obtained as a white solid in 65.4% yield after purification by silica gel column chromatography (EtOAc-CH₂Cl₂ = 5:95).

¹H NMR spectrum (300 MHz, CDCl₃, 25 °C): \( \delta_{HH} = 10.03 \) (s, 4H, -CHO), 7.89 (d, \( J = 8.0 \) Hz, 8H, \( Ha1 \)), 7.63 (s, 4H, \( Ha3 \)), 7.34 (d, \( J = 8.0 \) Hz, 8H, \( Ha2 \)), 5.26 (t, \( J = 8.4 \) Hz, 4H, \( Hm \)), 3.84 (m, 8H, \( Ho \)), 3.41 (m, 8H, \( Hi \)), 2.23 (m, 8H, \( H1 \)), 1.47-1.18 (m, 24H, \( H2 \), \( H3 \) and \( H4 \)), 0.91 (t, \( J = 6.8 \) Hz, 12H, \( H3 \)). ¹³C NMR spectrum (125 MHz, CDCl₃, 25 °C): \( \delta_C = 192.18, 151.88, 142.38, 136.83, 136.02, 131.04, 130.58, 129.76, 125.16, 72.76, 35.22, 34.41, 32.37, 28.09, 23.16, 14.60 \). MS (MALDI-TOF) m/z: 1311.6413 (M + Na⁺, 100%); Calcd for C₈₄H₈₈O₁₂ + Na⁺: 1311.6167.

3.5.6 Synthesis of 64

Prepared by procedure 1 from tetraiodocavitand 61 (103.15 mg, 72 µmol), potassium (4-formylphenyl)trifluoroborate (92 mg, 432.2 µmol), Pd(OAc)₂ (3.5 mg, 14.4 µmol),
PPh₃ (7.5 mg, 28.8 µmol), and K₂CO₃ (119.3 mg, 864.4 µmol), 64 was obtained as a white solid in 71% yield after purification by silica column chromatography (EtOAc-CH₂Cl₂ = 1:35).

![Chemical Structure]

¹H NMR spectrum (300 MHz, CDCl₃, 25 °C): δ_H = 10.04 (s, 4H, -CHO), 7.88 (d, J = 8.0 Hz, 8H, Hₐ₁), 7.54 (d, J = 8.0 Hz, 8H, Hₐ₂), 7.46 (s, 4H, Hₐ₃), 5.01 (t, J = 8.0 Hz, 4H, Hₗ), 3.79 (m, 8H, Hₒ₁), 3.52 (m, 8H, Hᵢ₁), 2.12 (m, 8H, H₁), 1.87 (m, 4H, Hₒ₁), 1.54 (m, 4H, Hᵢ₁, overlap with water), 1.44-1.20 (m, 24H, H₂ and H₃ and H₄), 0.90 (t, J = 6.8 Hz, 12H, H₅). ¹³C NMR spectrum (100 MHz, CDCl₃, 25 °C): δ_C = 192.54, 154.37, 143.57, 135.54, 134.38, 132.15, 129.50, 128.39, 125.72, 71.79, 36.53, 34.68, 32.49, 30.32, 27.99, 23.18, 14.62. MS (MALDI-TOF) m/z: 1367.6341 (M + Na⁺, 100%); Calcd for C₈₈H₉₆O₁₂ + Na⁺: 1367.6799.

3.5.7 Synthesis of nanocapsules (procedure 2)

Stock solutions of tetraformylcavitand 63 (10 mg/mL), diamines 17i, j, p or triamine 17s (10 mg/mL) and TFA (1% v/v) in CDCl₃ were prepared. Then, the cavitand and linker stock solutions were mixed in stoichiometric ratio followed by the addition of catalytic amounts of TFA solution. Several molecular sieves were added to this solution. After 1-2 hours, the solution was diluted to 0.5 ml with CDCl₃, if necessary, and transferred into a NMR tube. The reaction was monitored by ¹H NMR
spectroscopy. After completion, 10 µL triethylamine (TEA) was added to quench TFA and the product was precipitated by adding methanol.

**Synthesis of 65a:** Prepared by procedure 2 from tetraformylcavitand 63 (2.32 mg, 1.80 µmol), 1,4-phenylenediamine 17i (0.40 mg, 3.6 µmol) and TFA (2.5 µL, 1%v/v in CDCl₃), 65a formed quantitatively within 1 hour determined by $^1$H NMR spectroscopy.

$^1$H NMR spectrum (300 MHz, CDCl₃, 25 °C): $\delta_H = 8.53$ (s, 24H, $H_m$), 7.93 (d, $J = 8.3$ Hz, 48H, $H_a1$), 7.67 (s, 24H, $H_a3$), 7.31 (d, $J = 8.3$ Hz, 48H, $H_a1$), 7.26 (s, 48H, $H_a4$), 5.30 (t, $J = 7.2$ Hz, 24H, $H_m$), 3.89 (m, 48H, $H_o$), 3.46 (m, 48H, $H_i$), 2.26 (m, 48H, $H_1$), 1.52-1.22 (m, 144H, $H_2$, $H_3$ and $H_4$), 0.92 (t, $J = 6.4$ Hz, 72H, $H_5$). $^{13}$C NMR spectrum (125 MHz, CDCl₃, 25 °C): $\delta_C = 159.81$, 152.10, 150.45, 139.22, 136.68, 135.88, 130.82, 130.74, 128.88, 124.81, 122.32, 72.78, 35.20, 34.50, 32.47, 28.22, 23.19, 14.63. DOSY NMR (500 MHz, CDCl₃, 25 °C): $D = 2.11 \times 10^{-6}$ cm²/s. MS (MALDI-TOF) m/z: 8603.1035 (M + H⁺, 100%); Calcd for C₅₇₆H₅₇₆O₄₈N₂₄ + H⁺: 8603.3647.
Synthesis of 65b: Prepared by procedure 2 from tetraformylcavitand 63 (2.07 mg, 1.61 µmol), benzidine 17j (0.59 mg, 3.22 µmol) and TFA (2 µL, 1% v/v in CDCl₃), 65b formed quantitatively within 1 hour determined by ¹H NMR spectroscopy.

¹H NMR spectrum (300 MHz, CDCl₃, 25 °C): δ_H = 8.55 (s, 24H, H_m), 7.94 (d, J = 7.8 Hz, 48H, H_a1), 7.75-7.60 (m, 72H, H_a3 and H_a2), 7.41-7.25 (m, 96H, H_a4 and H_a5), 5.31 (t, J = 8.1 Hz, 24H, H_m), 3.91 (m, 48H, H_o), 3.48 (m, 48H, H_i), 2.26 (m, 48H, H_1), 1.49-1.28 (m, 144H, H_2, H_3 and H_4), 0.92 (t, J = 6.8 Hz, 72H, H_5). ¹³C NMR spectrum (125 MHz, CDCl₃, 25 °C): δ_C = 160.09, 152.10, 151.51, 139.22, 138.86, 136.69, 135.87, 130.82, 128.88, 128.11, 124.82, 121.89, 72.82, 35.24, 34.45, 32.48, 28.22, 23.20, 14.64. DOSY NMR spectrum (500 MHz, CDCl₃, 25 °C): D = 1.73 × 10⁻⁶ cm²/s. MS (MALDI-TOF) m/z: 9516.8574 (M + H⁺, 100%); Calcd for C₆₄H₆₂O₄₈N₂₄ + H⁺: 9516.7676.

Synthesis of 66: Prepared by procedure 2 from tetraformylcavitand 63 (2.12 mg, 1.65 µmol), 1,2-ethylenedianiline 17p (0.70 mg, 3.30 µmol) and TFA (2 µL, 1% v/v in CDCl₃), 66 formed in 95% within 1 hour determined by ¹H NMR spectroscopy.
\(^1\)H NMR spectrum (500 MHz, CDCl\(_3\), 25 °C): \(\delta_{\text{H}} = 8.44\) (s, 8H, \(H_{\text{im}}\)), 7.89 (d, \(J = 7.7\) Hz, 16H, \(H_{a2}\)), 7.69 (s, 8H, \(H_{a3}\)), 7.29 (d, \(J = 7.7\) Hz, 48H, \(H_{a2}\)), 7.04 (d, \(J = 8.3\) Hz, 16H, \(H_{a4}\)), 6.96 (d, \(J = 8.3\) Hz, 16H, \(H_{a5}\)), 5.31 (t, \(J = 8.3\) Hz, 8H, \(H_{m}\)), 3.90 (m, 16H, \(H_{o}\)), 3.45 (m, 16H, \(H_{i}\)), 2.98 (s, 16H, \(H_{6}\)), 2.25 (m, 16H, \(H_{1}\)), 1.46-0.90 (t, \(J = 7.1\) Hz, 24H, \(H_{5}\)). \(^{13}\)C NMR spectrum (125 MHz, CDCl\(_3\), 25 °C): \(\delta_{\text{C}} = 161.86, 151.89, 137.93, 136.31, 135.75, 130.63, 130.08, 128.23, 124.37, 72.53, 62.95, 34.95, 34.14, 32.19, 27.94, 22.92\). MS (MALDI-TOF) m/z: 3284.6484 (M \(+\) H\(^+\), 100%); Calcd for C\(_{224}\)H\(_{224}\)O\(_{16}\)N\(_{8}\) \(+\) H\(^+\): 3284.7094

**Synthesis of 67a and 68a:** Prepared by procedure 2 from tetraformylcavitand 62\(^\text{15}\) (3.58 mg, 2.91 µmol), 1,4-phenylenediamine 17i (0.63 mg, 5.81 µmol) and TFA (4 µL, 1% v/v in CDCl\(_3\)), 90% 67a and 10% 68a formed within 1 hour determined by GPC.
H NMR spectrum (500 MHz, CDCl$_3$, 25 °C) of 67a: $\delta_H = 8.53$ (s, 8H, H$_{im}$), 8.51 (s, 8H, H$_{im}$), 7.94 (d, $J = 7.5$ Hz, 16H, H$_{a1}$), 7.83 (d, $J = 7.5$ Hz, 16H, H$_{a1}$), 7.38 (s, 8H, H$_{a3}$), 7.35 (s, 8H, H$_{a3}$), 7.29 (s, 16H, H$_{a4}$), 7.27 (d, $J = 8.6$ Hz, 16H, H$_{a2}$), 7.24 (s, 16H, H$_{a4}$), 7.08 (d, $J = 8.6$ Hz, 16H, H$_{a2}$), 5.48-5.30 (m, 12H, H$_o$), 4.97-4.78 (m, 16H, H$_m$), 4.64 (m, 4H, H$_i$), 4.51-4.30 (m, 12H, H$_i$), 3.56 (m, 4H, H$_o$), 2.47-2.2 (m, 32H, H$_1$), 1.57-1.34 (m, 96H, H$_2$, H$_3$ and H$_4$), 1.04-0.86 (m, 48H, H$_5$). $^{13}$C NMR spectrum (125 MHz, CDCl$_3$, 25 °C) of 67a: $\delta_C = 159.92$, 159.08, 153.33, 153.08, 152.86, 152.77, 150.68, 149.78, 139.06, 138.99, 138.92, 138.77, 137.74, 137.69, 135.78, 135.70, 131.03, 130.76, 129.64, 129.17, 128.81, 122.57, 122.33, 122.26, 120.81, 120.67, 101.57, 101.41, 99.43, 37.70, 37.61, 32.59, 32.58, 32.55, 31.09, 30.89, 30.43, 28.23, 28.20, 28.16, 23.24, 23.21, 23.18, 14.64, 14.63. DOSY NMR (500 MHz, CDCl$_3$, 25 °C) of 67a: D = $2.50 \times 10^{-6}$ cm$^2$/s. MS (MALDI-TOF) of 67a m/z: 5511.6259 (M + H$^+$, 100%); Caled for C$_{368}$H$_{352}$O$_{32}$N$_{16}$ + H$^+$: 5511.6503.

**Synthesis of 67b and 68b:** Prepared by procedure 2 from tetraformylcavitand 62 (2.33 mg, 1.88 µmol), benzidine 17j (0.69 mg, 3.77 µmol) and TFA (2 µL, 1% v/v in
CDCl$_3$), 80% $67b$ and 20% $68b$ within 1 hour formed determined by GPC.

$^1$H NMR spectrum (300 MHz, CDCl$_3$, 25 °C) of $67b$: $\delta_H = 8.54$ (s, 8H, H$_{im}$), 8.51 (s, 8H, H$_{im}$), 7.94 (d, $J = 8.6$ Hz, 16H, H$_{a1}$), 7.85 (d, $J = 8.6$ Hz, 16H, H$_{a1}$), 7.66 (d, $J = 8.6$ Hz, 16H, H$_{a3}$), 7.60 (d, $J = 8.6$ Hz, 16H, H$_{a5}$), 7.38 (s, 8H, H$_{a3}$), 7.36 (s, 8H, H$_{a3}$), 7.31 (d, $J = 8.4$ Hz, 16H, H$_{a4}$), 7.26 (d, $J = 8.4$ Hz, 16H, H$_{a4}$), 7.20 (d, $J = 7.8$ Hz, 16H, H$_{a2}$), 7.10 (d, $J = 7.8$ Hz, 16H, H$_{a2}$), 5.41-5.34 (m, 12H, H$_o$), 4.91-4.85 (m, 16H, H$_m$), 4.76 (m, 4H, H$_i$), 4.43-4.35 (m, 12H, H$_i$), 3.71 (m, 4H, H$_o$), 2.43-2.30 (m, 32H, H$_1$), 1.55-1.38 (m, 96H, H$_2$, H$_3$ and H$_4$), 1.11-0.92 (m, 48H, H$_5$). $^{13}$C NMR spectrum (125 MHz, CDCl$_3$, 25 °C) of $67b$: $\delta_C = 160.39$, 160.27, 159.56, 153.30, 153.07, 152.95, 152.82, 151.82, 151.70, 151.01, 139.02, 138.95, 138.80, 137.86, 137.79, 137.63, 135.75, 135.70, 135.62, 131.00, 130.77, 129.65, 129.23, 129.14, 128.87, 128.16, 128.09, 127.77, 122.04, 121.92, 121.89, 120.82, 120.71, 115.91, 101.44, 101.16, 99.77, 37.69, 37.64, 32.59, 31.06, 30.98, 30.53, 28.19, 23.22, 14.65. DOSY NMR (500 MHz, CDCl$_3$, 25 °C) of $67b$: D = 2.14 × 10$^{-6}$ cm$^2$/s. MS (MALDI-TOF) of $67b$ m/z: 6119.9712 (M+ H$^+$, 100%); Caled for C$_{416}$H$_{384}$O$_{32}$N$_{16}$+ H$: 6119.9100.
Synthesis of 67c and 68c: Prepared by procedure 2 from tetraformylcavitand 62 (2.35 mg, 1.90 µmol), 1,4-ethylenediamine 17p (0.81 mg, 3.81 µmol) and TFA (2 µL, 1% v/v in CDCl₃), 80% 67c and 20% 68c formed within 1 hour determined by GPC.

1H NMR spectrum (500 MHz, CDCl₃, 25 °C) of 67c: δ_H = 8.50 (s, 8H, H_m), 8.47 (s, 8H, H_m), 7.92 (d, J = 7.7 Hz, 16H, H_a1), 7.85 (d, J = 7.7 Hz, 16H, H_a1), 7.37 (s, 8H, H_a3), 7.36 (s, 8H, H_a3), 7.34 (d, J = 8.2 Hz, 16H, H_a5), 7.29 (d, J = 8.2 Hz, 16H, H_a5), 7.24-7.16 (m, 48H, H_a4 and H_a2), 7.12 (d, J = 7.6 Hz, 16H, H_a2), 5.41-5.27 (m, 12H, H_o), 4.95-4.86 (m, 16H, H_m and H_i), 4.42-4.29 (m, 12H, H_i), 3.88 (m, 4H, H_o), 2.94 (s, 16H, H_6), 2.91 (s, 16H, H_6), 2.46-2.27 (m, 32H, H_i), 1.61-1.36 (m, 96H, H_2, H_3 and H_4), 1.03-0.92 (m, 48H, H_3). 13C NMR spectrum (125 MHz, CDCl₃, 25 °C) of 67c: δ_C = 159.88, 159.52, 153.22, 153.06, 152.98, 152.87, 150.57, 150.24, 140.70, 140.52, 139.04, 139.00, 138.97, 138.81, 137.74, 137.67, 135.76, 135.73, 130.91, 130.71, 129.98, 129.72, 129.64, 129.45, 129.40, 129.30, 128.77, 121.59, 121.52, 120.77, 120.66, 115.70, 101.37, 100.16, 38.46, 38.43, 37.67, 37.62, 32.59, 31.01, 30.60, 28.19, 23.24, 23.21, 14.64. DOSY NMR spectrum (500 MHz, CDCl₃, 25 °C) of 67c: D = 2.06 × 10⁻⁶ cm²/s. MS (MALDI-TOF) of 67c m/z: 6345.1584 (M + H⁺, 100%); Caled for C₄₃₂H₄₁₆O₃₂N₁₆⁺: 6345.1495.
**Synthesis of 69:** Prepared by procedure 2 from tetraformylcavitand 62 (2.41 mg, 1.90 µmol), 1,3,5-tris(4-aminophenyl)benzene 17s (0.91 mg, 2.61 µmol) and TFA (2 µL, 1% v/v in CDCl₃), 69 formed quantitatively within 1 hour determined by ¹H NMR spectroscopy.

![Chemical structure of 69]

¹H NMR spectrum (300 MHz, CDCl₃, 25 °C): δ_H = 8.53 (s, 24H, H_im), 7.92 (d, J = 8.3 Hz, 48H, H_a1), 7.82 (s, 24H, H_a3), 7.76 (d, J = 8.3 Hz, 48H, H_a5), 7.39 (s, 24H, H_a6), 7.32 (d, J = 8.3 Hz, 48H, H_a4), 7.19 (d, J = 8.3 Hz, 48H, H_a2), 5.24 (m, 24H, H_o), 4.89 (t, J = 7.8 Hz, 24H, H_m), 4.25 (m, 24H, H_i), 2.39 (m, 48H, H_1), 1.58-1.35 (m, 144H, H_2, H_3 and H_4), 0.98 (t, J = 6.7 Hz, 72H, H_5). ¹³C NMR spectrum (125 MHz, CDCl₃, 25 °C): δ_C = 160.48, 153.03, 152.10, 142.30, 139.21, 138.94, 138.14, 135.58, 130.68, 129.53, 128.90, 124.54, 125.02, 121.85, 120.72, 101.05, 37.59, 32.59, 30.84, 28.20, 23.21. DODY NMR (500 MHz, CDCl₃, 25 °C): D = 1.85 × 10⁻⁶ cm²/s. MS (MALDI-TOF) m/z: 9779.8018 (M + H⁺, 100%); Calcd for C₆₇₂H₆₀₀O₄₈N₂₄ + H⁺: 9780.5500
**Synthesis of 70**: Prepared by procedure 2 from tetraformylcavitand 63 (1.88 mg, 1.46 µmol), 1,3,5-tris(4-aminophenyl)benzene 17s (0.85 mg, 2.43 µmol) and TFA (2 µL, 1% v/v in CDCl₃), 70 formed quantitatively within 1 hour determined by ¹H NMR spectroscopy.

**¹H NMR spectrum** (300 MHz, CDCl₃, 25 °C): δ_H = 8.52 (s, 24H, H_m), 7.95 (d, J = 8.2 Hz, 48H, H_a1), 7.79 (s, 24H, H_a3), 7.74 (d, J = 8.2 Hz, H_a2), 7.67 (s, 24H, H_a6), 7.38-7.27 (m, 96H, H_a4 and H_a5), 5.32 (t, J = 7.5 Hz, 24H, H_m), 3.87 (m, 48H, H_o), 3.68 (m, 48H, H_i), 2.28 (m, 48H, H_j), 1.54-1.31 (m, 144H, H_2, H_3 and H_4), 0.94 (t, J = 6.8 Hz, 72H, H_5). **¹³C NMR spectrum** (125 MHz, CDCl₃, 25 °C): δ_C = 160.37, 152.11, 151.98, 142.50, 139.45, 139.29, 136.72, 135.82, 131.03, 130.85, 128.90, 128.65, 125.23, 124.76, 121.87, 72.65, 35.12, 34.56, 32.51, 28.26, 23.20, 14.84. **DOSY NMR spectrum** (500 MHz, CDCl₃, 25 °C): D = 1.80 × 10⁻⁶ cm²/s. **MS (MALDI-TOF) m/z**: 10117.4707 (M + H⁺, 100%); Calcd for C₆₉₆H₆₄₈O₄₈N₂₄ + H⁺: 10117.4104.
3.5.8 Synthesis of 71

Tetraiodocavitand 60 (317 mg, 0.23 mmol), (4-nitrophenyl)boronic acid (254 mg, 1.84 mmol), Pd(OAc)$_2$ (20 mg, 0.092 mmol), PPh$_3$ (48 mg, 0.18 mmol) and K$_2$CO$_3$ (152.5 mg, 1.10 mmol) and a stirring bar were added into a Schlenk flask. The flask was flushed with argon. Under argon, THF (10 mL) and H$_2$O (1 mL) were added. The solution was stirred at room temperature for 10 min. The flask was sealed and heated to 100 °C overnight with stirring. The resulting mixture was acidified with 10 mL HCl solution (1 M) and extracted with ethyl acetate (3 × 30 mL). The combined organic solutions were dried over MgSO$_4$. The salt was filtered off and the solvent was removed in vacuum. The crude product was purified by silica gel column chromatography (Hexane-CH$_2$Cl$_2$ = 1:4) to yield 71 as white solid (220 mg, 71%).

1H NMR spectrum (500 MHz, CDCl$_3$, 25 °C): $\delta_H$ = 8.21 (d, $J = 9.0$ Hz, 8H, H$_{a3}$), 7.39 (s, 4H, H$_{a1}$), 7.21 (d, $J = 9.0$ Hz, 8H, H$_{a2}$), 5.26 (d, $J = 7.3$ Hz, 4H, H$_o$), 4.85 (t, $J = 7.6$ Hz, 4H, H$_m$), 4.22 (d, $J = 7.3$ Hz, 4H, H$_i$), 2.36 (m, 8H, H$_1$), 1.52-1.37 (m, 24H, H$_2$-H$_4$), 0.96 (t, $J = 7.2$ Hz, 12H, H$_5$). $^{13}$C NMR spectrum (125 MHz, CDCl$_3$, 25 °C): $\delta_C$ = 152.54, 147.33, 140.92, 138.92, 131.18, 127.75, 123.50, 121.22, 100.90, 37.33, 32.24, 30.53, 27.85, 22.93, 14.36. MS (MALDI-TOF) m/z: 1355.5612 (M - H$^+$, 100%); Calcd for C$_{80}$H$_{83}$O$_{16}$N$_4$ - H$^+$: 1355.5798.
3.5.9 Synthesis of 72

Pd/C (10%, 20 mg) and NaBH₄ (37 mg, 0.97 mmol) were added to a round bottom flask, which was charged with 71 (160 mg, 0.12 mmol) in THF (5 mL). After stirring for 10 minutes, methanol (1 mL) was added. After stirring overnight, the resulting suspension was filtered through celite and the filtrate was concentrated under vacuum. The crude mixture was subjected to column chromatography (MeOH-CH₂Cl₂ = 2:98) yielding 72 as a white solid (65 mg, 45%).

\[ \text{NH}_2 \]
\[ \text{O} \]
\[ \text{H}_5 \]
\[ \text{H}_6 \]
\[ \text{H}_1 \]
\[ \text{H}_2 \]
\[ \text{H}_3 \]
\[ \text{H}_4 \]

\(^1\)H NMR spectrum (500 MHz, CDCl₃, 25 °C): \( \delta_H = 7.52 \) (s, 4H, Hₐ₁), 6.93 (d, \( J = 8.1 \) Hz, 8H, Hₐ₂), 6.65 (d, \( J = 8.1 \) Hz, 8H, Hₐ₃), 5.26 (t, \( J = 8.0 \) Hz, 4H, Hₘ), 3.85 (m, 8H, Hₐ), 3.65 (br, 8H, -NH₂), 3.45 (m, 8H, H₁), 2.18 (m, 8H, H₄), 1.41-1.21 (m, 24H, H₂-H₄), 0.88 (t, \( J = 7.2 \) Hz, 12H, H₅). \(^13\)C NMR spectrum (125 MHz, CDCl₃, 25 °C): \( \delta_C = 152.21, 145.62, 136.23, 131.21, 130.85, 125.53, 123.80, 114.85, 72.41, 35.03, 24.24, 32.26, 27.94, 22.95, 14.37. \) MS (MALDI-TOF): m/z (M + Na⁺, 100%), Calcd for C₈₀H₉₂O₈N₄⁺Na⁺: 1259.6807; found: 1259.6774.

3.5.10 Synthesis of 74

Prepared by procedure 2 from tetraaminocavitand 72 (10.69 mg, 8.65 µmol), terephthalaldehyde 73 (2.32 mg, 17.3 µmol) and TFA (10 µL, 1% v/v in CDCl₃), 74 formed quantitatively after about 7 hours determined by \(^1\)H NMR spectroscopy.
$^1$H NMR spectrum (500 MHz, CDCl$_3$, 25 °C): $\delta_H = 8.55$ (s, 24H, H$_{im}$), 8.00 (s, 48H, H$_{a4}$), 7.66 (s, 24H, H$_{a1}$), 7.32-7.18 (m, 96H, H$_{a2}$ and H$_{a3}$), 5.32 (t, $J = 7.7$ Hz, 24H, H$_{im}$), 3.91 (m, 48H, H$_{o}$), 3.49 (m, 48H, H$_{i}$), 2.26 (m, 48H, H$_{1}$), 1.49-1.27 (m, 144H, H$_{2}$-H$_{4}$), 0.92 (t, $J = 7.1$ Hz, 72H, H$_{5}$).

$^{13}$C NMR spectrum (125 MHz, CDCl$_3$, 25 °C): $\delta_C = 159.71$, 152.01, 150.84, 138.81, 136.32, 133.67, 130.72, 130.60, 129.38, 124.26, 120.87, 72.50, 34.92, 34.24, 27.96, 22.94, 14.34. MS (MALDI-TOF): m/z (M + H$^+$, 100%), Calcd for C$_{576}$H$_{576}$O$_{48}$N$_{24}$+H$^+$: 8603.3823, found: 8603.0572.

3.6 References


Chapter 4
Mechanism, Template Effects and Cooperativity in the Thermodynamically Controlled Assembly of Octaimine Hemicarcerands and Polyimine Nanocapsules

4.1 Introduction
Self-assembly is the central theme in supramolecular chemistry and describes a process, in which a well-defined discrete supramolecular architecture spontaneously forms from a given set of components under thermodynamic control\(^1\). As discussed in previous chapters, assemblies are typically held together through non-covalent interactions or dynamic covalent bonds\(^2\). It is believed that cooperativity plays an important role in the self-assembly process\(^3\). Cooperativity is a scientific term and defined as interaction between structural units within a molecule or between molecules in an assemblage that enable the system to respond more sharply to an external change than would isolated units according to Collins English Dictionary\(^4\). Cooperativity is central for many biological systems and can be positive or negative depending on whether a particular interaction is enhanced or weakened by another interaction. Therefore, to better understand self-assembly processes, it would be helpful to device methods to assess the magnitude of cooperativity. However, especially for complex systems, this is particularly difficult. On the other hand, in the self-assembly of closed systems, such as macrocycles and capsules, effective molarity (EM), which is an empirical parameter to assess the efficiency of an intramolecular reaction versus a related intermolecular reaction, can be used to quantify the magnitude of cooperativity\(^3\).

By definition, EM can be classified into thermodynamic effective molarity, EM\(_T\) and
kinetic effective molarity, EMₖ. For thermodynamic controlled processes, EMₜ is defined as the ratio of intramolecular equilibrium constant, Kᵢₙtra, over intermolecular equilibrium constant, Kᵢₙter (Eq. 4-1). Since Kᵢₙtra is dimensionless and Kᵢₙter has a unit [1/M], the unit of EMₜ is [M]. Another way to express EM is by using Gibbs free energy of both processes, which can be further divided into enthalpy and entropy contributions (Eq. 4-2). This allows EMₜ to be separated into an enthalpy part (EMₜₕ) and entropy part (EMₜₛ). In this chapter, EMₜ of Schiff-base hemicarcerand and nanocapsule assembly processes will be mainly discussed. Similarly, for kinetic controlled processes, EMₖ is the ratio of rate constants of intra- over intermolecular reactions (Eq. 4-3) and is related to the activation free energy (Eq. 4-4).

\[
EMₜ = \frac{Kᵢₙtra}{Kᵢₙter} \quad (4-1)
\]

\[
EMₜ = \exp\left[\frac{(ΔGᵢₙtra - ΔGᵢₙter)}{RT}\right] = \exp\left[-\frac{(ΔHᵢₙtra - ΔHᵢₙter)}{RT}\right] \times \exp\left[-\frac{(ΔSᵢₙtra - ΔSᵢₙter)}{RT}\right] = EMₜₕ \times EMₜₛ \quad (4-2)
\]

\[
EMₖ = \frac{kᵢₙtra}{kᵢₙter} \quad (4-3)
\]

\[
EMₖ = \exp\left[\frac{(ΔGₑᵢₙtra - ΔGₑᵢₙter)}{RT}\right] = \exp\left[-\frac{(ΔHₑᵢₙtra - ΔHₑᵢₙter)}{RT}\right] \times \exp\left[-\frac{(ΔSₑᵢₙtra - ΔSₑᵢₙter)}{RT}\right] \quad (4-4)
\]

EMₖ has been thoroughly studied in cyclization reactions⁶. Fig. 4-1 shows a cyclization process and its reference intermolecular reaction⁷. Molecules A and B form a new chemical bond via a bimolecular reaction. By linking A and B together through a covalent or non-covalent tether, the intermolecular reaction becomes an intramolecular reaction, which is accompanied by the loss of 30 e.u. in translational and rotational entropy. As a result, EMₖ can be as high as 10⁶.⁶ M given that the
enthalpy penalty is zero \((E_{M_H} = 1)^8\). However, if an enthalpy penalty is involved \((E_{M_H} < 1)\), which may be caused by torsions in the transition state (TS), \(E_{M_K}\) will decrease accordingly. On the contrary, if strain is relieved upon formation of the new bond, the enthalpy will decrease \((E_{M_H} > 1)\), resulting in an even higher EM. Furthermore, in the near attack conformation (NAC) of the TS, which is required for the reaction to proceed, several rotatable bonds may be frozen leading to loss of conformational entropy accordingly. Previous studies have shown that, for each frozen bond, the entropy loss is around 4 e.u. on average\(^9\).

Figure 4-1: Enthalpy and entropy contribution to EM (reproduced with permission from reference\(^7\)).

By using above estimation, Eq. 4-5 can be obtained, in which \(r\) is denoted as the number of rotors\(^8\). Studies show, that in cyclization reactions of small rings, typically less than seven members, entropy estimated by Eq. 4-5 fits very well with
experimental results. However, for larger rings, predicted values are normally smaller than experimental results, which arises from the fact that some bonds are not fully frozen in the cyclization TS.

\[ \Delta S^*_{\text{intra}} - \Delta S^*_{\text{inter}} = 30 - 4r \]  

(4-5)

For thermodynamically controlled self-assembly processes, EM\(T\) is mostly used and has been intensively studied by G. Ercolani and C. A. Hunter. They proposed several models to calculate EM\(T\) for macrocyclization and self-assembly process\(^{1c,10}\) and for the assessment of the cooperativity in self-assembly processes\(^{3,11}\). These models can help researchers to better understand self-assembly processes and also answer some fundamental questions. For example, what is the concentration range, in which self-assembly is favored over polymerization processes? What is the driving force for self-assembly processes? Why are certain architectures favored? In this chapter, we utilized the model developed by G. Ercolani to measure EMs of a series of hemicarcerands \(20a-f\), trisbridged hemicarcerands \(98b, d, g\) and \(99b,d\), macrocyclic biscavitanid hosts \(100-102b, d, g\) and nanocapsules \(23, 65a, 67a\) and \(69\) (Chart 4-2). Hosts \(98-102\) are models for intermediates in the assembly of hemicarcerands \(20\). Based on these data, the mechanism, template effects and cooperativity of these assembly processes are discussed.
Chart 4-1: Cavitands 19, 77, 79, 80, 82, 83, 85-90; deep cavitands 62, 63, 91-97; diamines 17a-g; triamine 17s and acetamines 76a-g, i, s.
Chart 4-2: Octaimine hemicarcerands 23a-g; tris-bridged hexaimine hemicarcerands 98b, d, g and 99b, d; tetraimine macrocycles 100-102b, d, g; tetraamine macrocycles 103/104b, d and polyimine tetrahedral nanocapsule 67a, octahedral nanocapsules 23 and 65a and rhombicuboctahedral nanocapsule 69.
4.2 Results

4.2.1 Synthesis of cavitands

Scheme 4-1: Synthesis of mono- 80, di- 79 and 85, tri- 82 and tetraformylcavitand 19.

The synthesis of cavitands functionalized with one, two, three and four formyl groups is outlined in Scheme 4-1. These cavitands are needed to prepare hemicarcerands and macrocycles. Starting from tetrabromocavitand 77, six to ten equivalents of BuLi per cavitand were used to fully exchange bromide to lithium. Mono- (80, 5%), di- (79, 15%), tri- (82, 31%) and tetraformylcavitand 19\textsuperscript{12} were obtained by subsequent addition of two, three and six equivalents of DMF, respectively. Surprisingly, through this synthetic route, only 1,2-diformylcavitand 79 was obtained but not
1,4-diformylcavitand 85. In order to make 85, an alternative route was employed. It was found that 1,4-isomer 83 was the only detectable di-substituted product, when only two equivalents of BuLi were added to tetrabromocavitand 77, followed by quenching with dilute HCl solution. After treating 83 with exactly two equivalents of BuLi or a little less, the addition of DMF gave 1,4-diformylcavitand 85.

Scheme 4-2: Synthesis of tetraformyl deep cavitands 62 and 63 and monoformyl deep cavitands 91 and 92.

The syntheses of deep cavitands 62 and 63 are described in Chapter 3 (Scheme 4-2). The mono substituted cavitands 91 and 92 formed as byproducts in these syntheses and were isolated from these reaction mixtures. They were used in this chapter as reference compounds to determine imine exchange equilibrium constants.

4.2.2 Synthesis of hemicarcerands 20b-g and nanocapsules 23

Hemicarcerands 20b-g formed quantitatively after 1 hour upon reacting cavitand 19 with two equivalents of diamines 17b-g in chloroform in the presence of catalytic amounts of TFA. The synthesis of octahedral nanocapsule 23 has been described in the literature13, but the purity was reported to be only 82% (Scheme 4-3). Here, a modified procedure was developed that gave pure nanocapsule. We found that the addition of p-xylene as co-solvent accelerated the formation of 23 and also increased
its purity. Presumably, \( p \)-xylene temporarily templates the formation of hemicarcerand \( 20a \), which can readily transform into nanocapsule \( 23 \). Although \( 23 \) did not form quantitatively with this method, the higher purity allowed purification by crystallization. Fig. 4-4 shows the \(^1\text{H} \) NMR spectrum of \( 23 \) in different solvent mixtures. From top to bottom, the concentration of \( p \)-xylene increases from 0 to 30\%. Maximum purity of \( 23 \) is reached at 20\% \( p \)-xylene. The lower purity at 30\% \( p \)-xylene is possibly due to the decreased solubility of the product (spectrum 5). The \(^1\text{H} \) NMR spectrum of pure \( 23 \) is shown on the bottom (spectrum 6).

\[
\begin{align*}
\text{Scheme 4-3: Synthesis of octaimine hemicarcerands } & \ 20b-g \text{ and polyimine octahedral nanocapsule } 23. \\
&
\end{align*}
\]
Figure 4-4: Partial $^1$H NMR spectra (400 MHz, CDCl$_3$, 25 °C) of products obtained from 19 and 17a in different solvents. 1), CHCl$_3$; 2), CHCl$_3$ + 5% p-xylene; 3), CHCl$_3$ + 10% p-xylene; 4), CHCl$_3$ + 20% p-xylene; 5), CHCl$_3$ + 30% p-xylene; 6), crystal of 23 in CDCl$_3$.

4.2.3 Synthesis of bis- and tris-bridged hosts 98-102

The tris-bridged isomers 98/99b, d were produced by mixing two equivalents of cavitand 82 and three equivalents of diamines 17b, d (Scheme 4-4). The isomers 98b, d have a high symmetry and the splitting in their $^1$H NMR spectra is consistent with their structures. The other isomers 99b, d have a lower symmetry leading to a more complex splitting pattern in their $^1$H NMR spectra, similar to that for 54 (see Chapter 2), and thus, are assigned to chiral isomers. To further confirm the assignment, the mixtures were reduced and the resulting hexaamine hemicarcerands 103/104b, d were separated by column chromatography. For $m$-phenylenediamine 17g, only hemicarcerand 98g was obtained.
Scheme 4-4: Synthesis of hexaimine tris-bridged hemicarcerands 98b, d, g and 99b, d and hexaamine tris-bridged hemicarcerands 103/104b, d.

Reactions of 1,4-diformylcavitand 85 with two equivalents of diamines 17b, d, g gave quantitatively macrocycles 100b, d, g. However, the same reaction with 1,2-diformylcavitand 79 produced two types of macrocycles 101/102b, d, g (Scheme 4-5). They have the same MW and similar chemical shifts and splitting patterns in their $^1$H NMR spectra. The assignment of each isomer was achieved by using HMQC and DOSY NMR spectroscopy. Take 101/102b for example. Macrocycle 101b has $C_{2h}$ symmetry, whereas 102b has $C_{2v}$ symmetry. Thus, the two protons (H$_1$ and H$_2$) on C* of 101b are nonequivalent and could be differentiated in the HMQC NMR spectrum, whereas those of 102b are equivalent. In addition, the diffusivity of 101b, which was measured by DOSY, is smaller than that of 102b and again is consistent with the assignment, since 101b has a more compact structure. Furthermore,
H₃ of 101b is high field shifted as compared to 102b, indicating that the proton of 101b is more shielded, which is also consistent with the assignment (see experimental section for details).

Scheme 4-5: Synthesis of tetraimine macrocycles 100-102b, d, g.
Besides nanocapsule 23, EM of three other rigid nanocapsules 67a, 69 and 65a were also measured in this chapter (Chart 4-2). Their synthesis was described in Chapter 3.

4.3 Theory

4.3.1 Formation of a single assembly

For the study of our hemicarcerands and nanocapsules, we slightly modified Ercolani’s model\textsuperscript{1c}. Eq. 4-6 shows the formation of hemicarcerands, which is used as an example to illustrate the model. \( m \) Equivalents of cavitand \( C \) bearing \( c \) aldehyde groups react with \( n \) equivalents of linker \( L \) bearing \( l \) amino groups to form a hemicarcerand \( H \) plus \( B \) equivalents of water. In this assembly, the total number of building blocks is \( N \), which equals to \((m + n)\). \( B \) is the number of imine bonds. Therefore, the number of intermolecular reactions is \((N - 1)\) and that of intramolecular reactions is \((B - N + 1)\). Assuming that \( K_{\text{inter}} \) is identical for each intermolecular step of the self-assembly process, the stability constant, \( K_H \), can be expressed by Eq. 4-9. \( K_\sigma \) is a statistic factor accounting for configurational entropy and is defined by Eq. 4-10, in which \( \sigma_C \), \( \sigma_L \), \( \sigma_H \) and \( \sigma_{H2O} \) are symmetry numbers of cavitand \( C \), linker \( L \), hemicarcerand \( H \) and water, respectively\textsuperscript{14}.
Scheme 4-6. Hemicarcerand H formation without (Eq. 4-6) and in the presence of a denaturation agent R-NH₂ (Eq. 4-7) and the reference intermolecular reaction with equilibrium constant \( K_{\text{inter}} \) (Eq. 4-8).

\[
K_H^i = \frac{[H][H_2O]^B}{[L]^m[C]^n} = K_{\sigma} K_{\text{inter}}^{(N+1)} \prod_{i=1}^{B-N+1} K_{\text{intra}}(i)
\]

\[
K_{\sigma} = \frac{\alpha_{C}^m \times \alpha_{L}^n}{\alpha_{H} \times \alpha_{H2O}^B}
\]

Inserting effective molarity into Eq. 4-9 gives Eq. 4-11 and Eq. 4-12, in which the product of the individual EM\(_i\) are replaced with EM, the geometric mean of EM\(_i\).
Since hemicarcerands are very stable in non-aqueous solvents, free cavitand and linker cannot be detected by $^1$H NMR spectroscopy and it was impossible to measure the stability constant directly from Eq. 4-9. In order to decrease the stability of hemicarcerands, we used a denaturation approach\textsuperscript{15}. Hemicarcerands are titrated with monoamine, RNH$_2$, in the presence of catalytic amounts of TFA, which gives denatured products, cavitand, C' and linker, L (Eq. 4-7). Under these conditions, the equilibrium constant, $K_s$, is defined by Eq. 4-13. The intermolecular equilibrium constant $K'_\text{inter}$ can be measured through the reference reaction (Eq. 4-8), in which monofunctionalized cavitand $C''$ and monofunctionalized linker $L'$ react with each other to form cavitand $C'''$ and monoamine. Thus, $K'_\text{inter}$ is expressed in Eq. 4-14.

\[
K_H = K_0 K_{\text{inter}} \prod_{i=1}^{B-N+1} EM_i 
\]  

(4-11)

\[
K_H = K_0 K_{\text{inter}} B EM^{B-N+1} 
\]  

(4-12)

Furthermore, we define the monoamine concentration dependent equilibrium constants $K''_{\text{inter}}$ and $K'_S$ by Eq. 4-15 and Eq. 4-16.

\[
K''_{\text{inter}} = \frac{K_{\text{inter}}}{[\text{RNH}_2]} = \frac{[C''']}{[C''] \times [L']} 
\]  

(4-15)

\[
K'_S = \frac{K_S}{[\text{RNH}_2]^B} = \frac{[H]}{[L]^m \times [C'^n]} = K_0 K'_\text{inter} B EM^{B-N+1} 
\]  

(4-16)
According to G. Ercolani, Eq. 4-17 holds, in which \([C']_0\) and \([L]_0\) are the initial concentrations of building blocks and \(x\) is the fraction of reacted functional groups in the assembly. At equilibrium, the concentrations of \(C'\) and \(L\) are given by Eq. 4-18.

\[
[C']_0 + [L]_0 = N[H] + \frac{N}{B} \frac{1}{K_{\text{inter}}} \frac{x}{(1-x)^2} \tag{4-17}
\]

From Eq. 4-18 and Eq. 4-16, Eq. 4-19 can be obtained.

\[
[H] = \frac{K_{\alpha}}{c_{\text{int}} K_{\text{inter}}} K^{(B-N)EM(B-N+1)x^N} \tag{4-19}
\]

Inserting Eq. 4-19 into Eq. 4-17 gives Eq. 4-20.

\[
[C']_0 + [L]_0 = \frac{1}{K_{\text{inter}}} \left( N K_{\alpha} K^{(B-N+1)EM(B-N+1)x^N} \frac{N}{B} \frac{x}{(1-x)^2} + \frac{N}{B} \frac{x}{(1-x)^2} \right) \tag{4-20}
\]

With the amount of hemicarcerands \([H]\) and the initial concentration of building blocks \(([C']_0 + [L]_0)\), the percentage of hemicarcerands at equilibrium as function of \(K_{\text{inter}}\), \(EM\) and \(x\) is given by Eq. 4-21.

\[
\%[H] = 100/(1+(1/(K_{\alpha} c_{\text{int}})) B K^{(B-N+1)EM(B-N+1)(1-x)^2x^{N-1}})) \tag{4-21}
\]

Since initial concentration of building blocks \(([C']_0 + [L]_0)\), the percentage of hemicarcerands \(H\%\) and intermolecular equilibrium constant \(K_{\text{inter}}\) can be obtained experimentally, a plot of \(H\%\) against \(([C']_0 + [L]_0)\) can be constructed for a given \(EM\).
value by varying x. These plots are refined iteratively by varying EM until the curve fits the experimental H% at the experimental ([C’]_0 + [L]_0). Using this procedure, the geometric EM was determined for each hemicarcerand and nanocapsule studied in this chapter.

4.3.2 Formation of two competing assemblies from one set of reactants

Ercolani’s model only discusses the situation of formation of a single assembly. However, in our study, some tris-bridged hemicarcerands (98/99b, d) and macrocycles (101/102b, d, g) formed as a mixture from a set of cavitand and linker reactants. Therefore, the mass balance has to be modified in these cases. In all such cases reported in this thesis, both assemblies, H_1 and H_2, have identical statistic factor (K_α), number of building blocks (N), imine bonds (B) and equilibrium constants (K'' inter). Under these conditions, the new mass balance is given by Eq. 4-22, Eq. 4-23 and Eq. 4-24.

\[
[C’]_0 + [L]_0 = N[H_1] + N[H_1] + \frac{N}{B} \frac{K''_\text{inter}}{1-x} \frac{x}{(1-x)^2} \quad (4-22)
\]

\[
[H_1] = \frac{K_\alpha}{c^{n\beta} \rho m} K'' \text{inter}^{(B-N)EM_1(B-N+1)} x^N \quad (4-23)
\]

\[
[H_2] = \frac{K_\alpha}{c^{n\beta} \rho m} K'' \text{inter}^{(B-N)EM_2(B-N+1)} x^N \quad (4-24)
\]

Given that \%((H_1 + H_2) = N([H_1] + [H_2])/([C’]_0 + [L]_0) × 100\%, Eq. 4-25, Eq. 4-26 and Eq. 4-27 can be obtained.

\[
[C’]_0 + [L]_0 = \frac{NK_\alpha}{c^{n\beta} \rho m} K'' \text{inter}^{(B-N)(EM_1(B-N+1)+EM_2(B-N+1))} x^N + \frac{N}{B} \frac{1}{K\text{inter}} \frac{x}{(1-x)^2} \quad (4-25)
\]
Eq. 4-27 shows that the ratio of the two assemblies ‘η’ is concentration independent. Therefore, Eq. 4-28 and Eq. 4-29 hold.

\[
\frac{[H_1]}{[H_2]} = (EM_1/EM_2)_{(B-N+1)} = \eta
\]  

(4-27)

\[
%([H_1] + [H_2]) = 100/(1+(1/((K_{eq}/c_{eq})B K_{inter}^{(B-N+1)} EM_1^{(B-N+1)} + EM_2^{(B-N+1)}(1-\eta)^2(2x(N-1)))}})
\]

(4-26)

\[
%([H_1] + [H_2]) = 100/(1+(1/((K_{eq}/c_{eq})B K_{inter}^{(B-N+1)}(1+\eta) EM_2^{(B-N+1)}(1-\eta)^2(2x(N-1))))}
\]

(4-29)

Eq. 4-28 and Eq. 4-29 have the same form as Eq. 4-20 and Eq. 4-21. Thus, EM_1 and EM_2 can be calculated for each assembly process.

4.4 Effective molarity

4.4.1 EM of hemicarcerand 20b

The determination of EM for hemicarcerand 20b is presented in this section to illustrate the above method. EM values for the other hemicarcerands and nanocapsules were obtained similarly.

The denaturation equilibrium for 20b using butylamine as the denaturant is shown in Scheme 4-7. Hemicarcerand 20b (3.2 mg, 1.59 μmol) in 1 mL CDCl₃ and 3 μL TFA solution in CDCl₃ (1%v/v) were placed in a Young NMR tube. 24 hours after the addition of BuNH₂ solution in CDCl₃ (10 mg/mL), the ^1H NMR spectrum was recorded (Fig 4-5). After addition of BuNH₂, a new imine signal appeared, that grew with increasing concentration of BuNH₂ on the expense of the hemicarcerand imine.
signal. We assign the new imine signal to the imine protons of cavitand 86. The assignment is supported by MALDI-TOF analysis of denaturation mixtures, which shows that at equilibrium, only 20b and cavitands are present. Hemicarcerand intermediates composed of two cavitands that are linked by one, two or three diamines 17b could not be detected. This supports the idea that the assembly of octaimine hemicarcerands are ‘all-or-nothing’ processes (vice infra). Since BuNH₂ less than ten equivalents barely denatured the hemicarcerand, ¹H NMR spectra at 10, 12, 14, 16 and 18 equivalents were used for the determination of EM.

Scheme 4-7: Denaturation of hemicarcerand 20b with BuNH₂.

Scheme 4-8 shows the reference reaction used to determine $K^{''}_{\text{inter}}$ for 20b. The CDCl₃ solutions of cavitand 87, BuNH₂ and NH₂(CH₂)₃NHa 76b were mixed in a NMR tube. After 24 hours, which allows the reaction to fully equilibrate, the ¹H NMR spectrum was recorded and analyzed (Fig. 4-4). The equilibrium constant, $K_{\text{obs}}$, can be measured directly from the integration of the imine protons and the integration of the α-CH₂ protons of BuNH₂ and 76b according to Eq. 4-30. The intrinsic equilibrium constant ($K^{'}_{\text{inter}}$) is obtained from $K_{\text{obs}}$ by taking into account the statistic factors of all components (Eq. 4-31). The monoamine concentration dependent equilibrium constant, which is used to calculate EM, is given in Eq. 4-32.
Scheme 4-8: Intermolecular equilibrium constant determination between 87 and 90b.

Figure 4-5: $^1$H NMR spectra (500 MHz, CDCl$_3$, 25 °C) of equilibrated mixture of 20b, 17b, 76b and BuNH$_2$ after titration. They are pure hemicarcerand 20b and the denatured mixture after addition of 2, 4, 6, 8, 10, 12, 14, 16, 18 equivalents of BuNH$_2$ (top to bottom). Imine (H) is the imine proton of 20b, imine (F) is the imine proton of denatured hemicarcerand and –CH$_2$N- are the α-protons of BuNH$_2$. 
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Figure 4-6: $^1$H NMR spectrum (400 MHz, CDCl$_3$, 25 °C) of equilibrated mixture of 76b, 87, 90b and BuNH$_2$.

$K_{\text{obs}} = K_{\alpha} \times K'_{\text{inter}} = \frac{I(90b)}{I(87)} \times \frac{I(\text{BuNH}_2)}{I(76b)}$  \hspace{1cm} (4-30)

$K_{\alpha} = \frac{\alpha(87)}{\alpha(90b)} \times \frac{\alpha(76b)}{\alpha(\text{BuNH}_2)} = 1$  \hspace{1cm} (4-31)

$K''_{\text{inter}} = K'_{\text{inter}} / [\text{BuNH}_2]$  \hspace{1cm} (4-32)

In the above example, $K_{\text{obs}} = 0.46$ and $K'_{\text{inter}} = 0.46$. Assuming that the integration error is 5%, the error for $K'_{\text{inter}}$ is $\Delta K'_{\text{inter}} / K'_{\text{inter}} = \frac{2}{\sqrt{4 \times (5\%)^2}} = 10\%$.

The values for $K''_{\text{inter}}, \text{H}\%$ and $([C']_0 + [L]_0)$, that are needed to determine EM for each denaturation mixture in Fig. 4-3, were determined as follows. $K''_{\text{inter}}$ was calculated by Eq. 4-32 from $K'_{\text{inter}}$ and [BuNH$_2$], which was calculated from the integration of the $\alpha$-CH$_2$ protons of butylamine (Fig. 4-6). \text{H}\% was calculated from the integration of the host imine signal, I(imine-H), and the cavatand imine signals, I(imine-F) as $\text{H}\% = \frac{I(\text{imine-H})}{I(\text{imine-H}) + I(\text{imine-F})}$. Finally, $([C']_0 + [L]_0)$ is six times the concentration of the hemicarcerand 20b used in the experiment (Table 4-1).

Using $K''_{\text{inter}}, ([C']_0 + [L]_0), \text{H}\%$ and $c = 4, l = 2, p = 1, N = 6, B = 8$ and $K\sigma =$
\( \sigma(76)^2 \sigma(17b)^4 \sigma(20b) \sigma(BuNH_2)^8 = 32 \), allowed determination of EM for spectra with 10-18 equivalents of butylamine in Fig. 4-3. Data are listed in Table 4-1. The average of these five titrations EM = 1.7 M is used for further discussion.

**Table 4-1:** Parameters and EMs for the last five titrations in Fig. 4-5.

<table>
<thead>
<tr>
<th>( K_{\text{inter}} )</th>
<th>( [C^+]_0 + [L]_0 ) (mM)</th>
<th>( H% )</th>
<th>equivalents of ( BuNH_2 )</th>
<th>EM (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>26.431</td>
<td>7.712</td>
<td>48.78</td>
<td>18</td>
<td>1.54</td>
</tr>
<tr>
<td>28.556</td>
<td>7.861</td>
<td>53.17</td>
<td>16</td>
<td>1.55</td>
</tr>
<tr>
<td>30.819</td>
<td>8.015</td>
<td>60.63</td>
<td>14</td>
<td>1.80</td>
</tr>
<tr>
<td>35.297</td>
<td>8.176</td>
<td>67.25</td>
<td>12</td>
<td>1.78</td>
</tr>
<tr>
<td>41.736</td>
<td>8.344</td>
<td>73.62</td>
<td>10</td>
<td>1.76</td>
</tr>
</tbody>
</table>

**4.4.2 Equilibrium constants (\( K_{\text{inter}} \))**

\[ \begin{align*}
R' \quad & \quad NHAc \\
& \quad AcHN \\
& \quad R' = 95a \quad 96a \\
95a, 96a & + 76g \quad \xrightleftharpoons[K_{\text{inter}}]{(4-38)} \quad 95b, 96b \\
& + Ph-NH_2 \\
95b, 96b & + 17a \quad \xrightleftharpoons[K_{\text{inter}}]{(4-39)} \quad 97 \\
& + Ph-NH_2
\end{align*} \]

**Scheme 4-9:** Transamination equilibrium that serve as reference systems.
Equilibrium constants $K_{\text{chem}}$ and $K'_{\text{inter}}$ for the intermolecular transamination equilibriums 4-33 - 4-39 are summarized in Table 4-2. For aliphatic linkers, $K'_{\text{inter}}$ increases as the length increases (entry 2, 4, 6, 8, 10 and 12). This trend is reasonable, because the acetyl group is electron withdrawing, which decreases the reactivity of the amino group and thus, lowers the equilibrium constant.

**Table 4-2:** $K'_{\text{inter}}$ in the assembly of hemicarcerands and nanocapsules.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Cavitand</th>
<th>Mono-amine</th>
<th>$K_{\text{obs}}$</th>
<th>$K_o$</th>
<th>$K_{\text{inter}}(K_{\text{chem}})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1$^a$</td>
<td>87</td>
<td>BzlnH$_2$</td>
<td>0.46</td>
<td>1</td>
<td>$K_{\text{chem}} = 0.46 \pm 0.046$</td>
</tr>
<tr>
<td>2$^a$</td>
<td>87</td>
<td>76a</td>
<td>0.26</td>
<td>1</td>
<td>$0.26 \pm 0.026$</td>
</tr>
<tr>
<td>3$^b$</td>
<td>88</td>
<td>76a</td>
<td>0.57</td>
<td>1</td>
<td>$0.57 \pm 0.081$</td>
</tr>
<tr>
<td>4$^a$</td>
<td>87</td>
<td>76b</td>
<td>0.46</td>
<td>1</td>
<td>$0.46 \pm 0.046$</td>
</tr>
<tr>
<td>5$^b$</td>
<td>88</td>
<td>76b</td>
<td>1</td>
<td>1</td>
<td>$1 \pm 0.14$</td>
</tr>
<tr>
<td>6$^a$</td>
<td>87</td>
<td>76c</td>
<td>0.83</td>
<td>1</td>
<td>$0.83 \pm 0.083$</td>
</tr>
<tr>
<td>7$^b$</td>
<td>88</td>
<td>76c</td>
<td>1.81</td>
<td>1</td>
<td>$1.810 \pm 0.25$</td>
</tr>
<tr>
<td>8$^a$</td>
<td>87</td>
<td>76d</td>
<td>0.86</td>
<td>1</td>
<td>$0.86 \pm 0.086$</td>
</tr>
<tr>
<td>9$^b$</td>
<td>88</td>
<td>76d</td>
<td>1.88</td>
<td>1</td>
<td>$1.88 \pm 0.47$</td>
</tr>
<tr>
<td>10$^a$</td>
<td>87</td>
<td>76e</td>
<td>0.91</td>
<td>1</td>
<td>$0.91 \pm 0.091$</td>
</tr>
<tr>
<td>11$^b$</td>
<td>88</td>
<td>76e</td>
<td>1.97</td>
<td>1</td>
<td>$1.97 \pm 0.28$</td>
</tr>
<tr>
<td>12$^a$</td>
<td>87</td>
<td>76f</td>
<td>0.94</td>
<td>1</td>
<td>$0.94 \pm 0.094$</td>
</tr>
<tr>
<td>13$^b$</td>
<td>88</td>
<td>76f</td>
<td>2.04</td>
<td>1</td>
<td>$2.04 \pm 0.29$</td>
</tr>
<tr>
<td>14$^a$</td>
<td>95a</td>
<td>76g</td>
<td>0.54</td>
<td>1</td>
<td>$0.54 \pm 0.054$</td>
</tr>
<tr>
<td>15$^a$</td>
<td>95a</td>
<td>76h</td>
<td>1.61</td>
<td>1</td>
<td>$1.61 \pm 0.16$</td>
</tr>
<tr>
<td>16$^a$</td>
<td>95a</td>
<td>76s</td>
<td>0.76</td>
<td>1</td>
<td>$0.76 \pm 0.076$</td>
</tr>
<tr>
<td>17$^a$</td>
<td>96a</td>
<td>76h</td>
<td>1.81</td>
<td>1</td>
<td>$1.81 \pm 0.18$</td>
</tr>
</tbody>
</table>

a) The error is estimated based on a 5% integration error so that error propagation results in an overall 10% standard error.

b) The equilibrium constant is calculated as the ratio of the corresponding $K_{\text{inter}}$ of equilibrium 4-34 and $K_{\text{chem}}$ and has a 14% standard error.

### 4.4.3 EM of hemicarcerands and nanocapsules

EMs of hemicarcerands 20a-g and nanocapsules 23, 65a, 67a and 69 are listed in Table 4-3. Since hemicarcerand 20a was not observed either by GPC or $^1$H NMR spectroscopy, EM of 20a could not be measured experimentally, but was estimated from EM of nanocapsule 23 by assuming that the maximum amount of 20a formed in the reaction between of 19 and 17a is 5%. The reported value is an upper limit and the
real EM value could be much smaller.

**Table 4-3**: EM of hemicarcerands and nanocapsules in CDCl₃.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Hemicarcerand or nanocapsule</th>
<th>Mono-amine</th>
<th>EM/mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20a</td>
<td>---</td>
<td>b4.8</td>
</tr>
<tr>
<td>2</td>
<td>20b</td>
<td>BuNH₂</td>
<td>(1.7±0.13) × 10³</td>
</tr>
<tr>
<td>3</td>
<td>20c</td>
<td>BuNH₂</td>
<td>70.8±0.96</td>
</tr>
<tr>
<td>4</td>
<td>20d</td>
<td>BuNH₂</td>
<td>(3.7±0.1) × 10³</td>
</tr>
<tr>
<td>5</td>
<td>20e</td>
<td>BuNH₂</td>
<td>(3.4±0.21) × 10²</td>
</tr>
<tr>
<td>6</td>
<td>20f</td>
<td>BuNH₂</td>
<td>(1.1±0.081) × 10²</td>
</tr>
<tr>
<td>7</td>
<td>20g</td>
<td>PhNH₂</td>
<td>(6.7±0.39) × 10⁴</td>
</tr>
<tr>
<td>8</td>
<td>67a</td>
<td>PhNH₂</td>
<td>(5.5±0.4) × 10²</td>
</tr>
<tr>
<td>9</td>
<td>23</td>
<td>BzlNH₂</td>
<td>23.5±1.01</td>
</tr>
<tr>
<td>10</td>
<td>69</td>
<td>PhNH₂</td>
<td>(1.2±0.1) × 10⁴</td>
</tr>
<tr>
<td>11</td>
<td>65a</td>
<td>PhNH₂</td>
<td>(1.5±0.1) × 10²</td>
</tr>
</tbody>
</table>

a) The error is reported as the standard error of different titrations.
b) EM was estimated (see text).

A plot of Lg(EM) against the average number of rotors per intramolecular reaction for the hemicarcerands (A) and the nanocapsules (B) is shown in Fig. 4-5. It shows that EM varies significantly by four orders of magnitude among the different hemicarcerands and nanocapsules. Among the hemicarcerands with aliphatic linkers (20a-f), EM alternates in magnitude as the number of rotors is increased. Hemicarcerands assembled from linkers with even numbers of carbon atoms have lower EMs than those with odd numbers of carbon atoms. Hemicarcerand 20d, which has 1, 5-diaminopentane as the linker, has the highest EM among aliphatic linkers. The trend is likely a result of torsional strain in the different linkers. To form a hemicarcerand, linkers with even number of carbon atoms have at least one gauche conformation, which increases torsional strain in the linker relative to linkers with odd numbers of carbon atoms and thus, leads to a low EM. Linkers with odd numbers of carbon atoms can adopt *anti* conformations, which are free of torsional strain.
Another general trend among the EMs for hemicarcerands with aliphatic linkers is that EM initially tends to increase with increasing linker length until 20d (five carbons), after which it starts to decrease. This trend is explained with an interplay between enthalpy and entropy contributions to EM. As the length of linker increases, torsional strain decreases but entropy decreases. For shorter linkers, the enthalpy penalty is dominant, and an increase in EM is observed as the linker length increases. For longer linkers, however, the entropy penalty becomes the major factor affecting EM and thus, EM decreases further as the length of the linker increases and more bonds are frozen during the assembly. Additionally, solvent and/or template effects also have a profound influence on EM, which will be discussed in Section 4.4.6.

Compared to aliphatic linkers, 20g, which has rigid 1,3-phenylenediamines linkers, has a substantially higher EM, which in part is a result of the lower number of rotors that are frozen in the assembly process. Among nanocapsules, EM of octahedral nanocapsule 23 assembled from cavitand 19 and ethylenediamine 17a is much lower than those of other more rigid nanocapsules 65a, 67a and 69. Presumably, many bonds of flexible linkers are frozen upon formation of nanocapsule 23, which decreases entropy and leads to a much lower EM. The aromatic linkers, however, are better pre-organized and fewer bonds are frozen. Therefore, the entropy penalty is smaller and typically results in higher EMs (Fig. 4-7A). Interestingly, a curved relationship of Lg(EM) against the number of frozen rotors per intramolecular bond formation is observed (Fig. 4-7B), which resembles the relationship between lgEMs against number of ring members for macrocyclisation reactions18, and indicates, that EMs of these nanocapsules are mainly modulated by entropy. Indeed, the results of MM3 calculation also demonstrate that these nanocapsules are nearly free of strain.
Figure 4-7: Plot of Lg(EM) against the number of frozen rotors in each intramolecular reaction in hemicarcerands (A) and nanocapsules (B) in CDCl$_3$. 
4.4.4 EMs of tris-bridged hemicarcerands and macrocycles and stepwise effective molarities EM$_i$ (i = 1-3)

A proposed mechanism for the formation of octaimine hemicarcerands H is outlined in Scheme 4-10. The first step involves the reaction of cavitand C with one linker L. Intermediate A1 formed with the equilibrium constant of K$_1$ = K$_{\text{inter}}$. It further reacts with another cavitand C or linker L to produce A2-A4. The latter can either proceed forward by ring closure to form assemblies M1-M3 or by polymerization to form larger aggregates Ai. The result of the reactions between diformylicavitands 79 and 85 and diamines suggests that macrocycle M1-M3 are intermediates. Other macrocycles could be also present in very low concentrations, but were neglected, since we have no experimental support for them. EM$_1$, EM$_1$' and EM$_1''$ are effective molarities for M1-M3, respectively. Upon reacting with one additional linker, M1-M3 yield tris-bridged hemicarcerands H1 and H2. The formation of H1 is possible from M1 or from M2. Since EM is path dependent, two effective molarities, EM$_2$ and EM$_2'$ are involved. H2 can only form from M2 with EM$_2''$. H2 is a kinetic product on the way towards hemicarcerand H. On the other hand, H1 can react with one additional linker to give hemicarcerand H. Within this assembly process, every transformation is under equilibrium.

EM values in CDCl$_3$ for tris-bridged hemicarcerands 98b, d, g and 99b, d and macrocycles 100-102b, d, g, which are models for intermediates H1-H2 and M1-M3, respectively, are tabulated in Table 4-4.
Table 4-4: EM of tris-bridged hemicarcerands and macrocycles in CDCl$_3$.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Hemicarcerand</th>
<th>Mono-amine</th>
<th>EM/mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100b</td>
<td>BzlNH$_2$</td>
<td>14.5±0.71</td>
</tr>
<tr>
<td>2</td>
<td>100d</td>
<td>BzlNH$_2$</td>
<td>45.3±3.2</td>
</tr>
<tr>
<td>3</td>
<td>100g</td>
<td>PhNH$_2$</td>
<td>45.5±2.12</td>
</tr>
<tr>
<td>4</td>
<td>101b (25%)</td>
<td>BzlNH$_2$</td>
<td>61.5±1.4</td>
</tr>
<tr>
<td>5</td>
<td>102b (75%)</td>
<td>BzlNH$_2$</td>
<td>(1.9±0.04)×10$^2$</td>
</tr>
<tr>
<td>6</td>
<td>101d (50%)</td>
<td>BzlNH$_2$</td>
<td>45.3±1.5</td>
</tr>
<tr>
<td>7</td>
<td>102d (50%)</td>
<td>BzlNH$_2$</td>
<td>45.3±1.5</td>
</tr>
<tr>
<td>8</td>
<td>101g (71%)</td>
<td>PhNH$_2$</td>
<td>(2.5±0.2)×10$^2$</td>
</tr>
<tr>
<td>9</td>
<td>102g (29%)</td>
<td>PhNH$_2$</td>
<td>(1.0±0.07)×10$^2$</td>
</tr>
<tr>
<td>10</td>
<td>98b (87%)</td>
<td>BzlNH$_2$</td>
<td>(1.0±0.3)×10$^2$</td>
</tr>
<tr>
<td>11</td>
<td>99b (13%)</td>
<td>BzlNH$_2$</td>
<td>40.5±11.3</td>
</tr>
<tr>
<td>12</td>
<td>98d (50%)</td>
<td>BzlNH$_2$</td>
<td>(4.2±0.4)×10$^2$</td>
</tr>
<tr>
<td>13</td>
<td>99d (50%)</td>
<td>BzlNH$_2$</td>
<td>(4.2±0.4)×10$^2$</td>
</tr>
<tr>
<td>14</td>
<td>98g</td>
<td>PhNH$_2$</td>
<td>(5.9±0.5)×10$^3$</td>
</tr>
</tbody>
</table>

a) The error is reported as the standard error of multiple titrations.

4.4.5 Stepwise EM$_i$ (i = 1-3)

Knowledge of the EM for all intermediates H$_1$-H$_2$ and M$_1$-M$_3$ (Table 4-3 and Table 4-4) allows calculation of EM$_i$ for each ring closure according to Eq. 4-12. Table 4-5 lists all stepwise EM$_i$ (i = 1-3) for assembly of hemicarcerands 20b, 20d and 20g.
EM\textsubscript{1}s of the first ring closure are all very small consistent with the linker length and flexibility. Possibly, oligomers A1-A4 are very flexible due to the presence of many rotational bonds. Upon formation of macrocycles, most rotational bonds are frozen. This significant entropy loss leads to the low EM\textsubscript{1}s. For the second ring closure, higher EM\textsubscript{2}s are observed in most cases. This is reasonable considering that, compared to oligomers, macrocycles are more pre-organized and thus, fewer bonds are frozen in the formation of the second ring. The only exception is EM\textsubscript{2}’’ (20b), which is lower than EM\textsubscript{1}’ (20b). It is likely that additional torsional strain is introduced in the formation of H2 (20b) due to its twisted structure, which makes it less favored. For all hemicarcerands, very high EM\textsubscript{3}s are measured for closing of the final ring. Again, fewer bonds are frozen, which is less entropy disfavored. In addition, template effects induced by the encapsulation of solvents can enhance EM\textsubscript{3}. Template effect will be discussed in Section 4.4.5.

<table>
<thead>
<tr>
<th>Table 4-5. Stepwise EM for the formation of hemicarcerands.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>EM\textsubscript{1}</td>
</tr>
<tr>
<td>EM\textsubscript{1}’</td>
</tr>
<tr>
<td>EM\textsubscript{1}’’</td>
</tr>
<tr>
<td>EM\textsubscript{2}</td>
</tr>
<tr>
<td>EM\textsubscript{2}’</td>
</tr>
<tr>
<td>EM\textsubscript{2}’’</td>
</tr>
<tr>
<td>EM\textsubscript{3}</td>
</tr>
</tbody>
</table>

Semi-logarithmic plots of stepwise EM\textsubscript{i}s for different paths are shown in Fig. 4-8. These diagrams support that the formation of the three hemicarcerands is driven by positive cooperativity in CDCl\textsubscript{3}, and formation of an intramolecular imine bond facilitates subsequent intramolecular steps.
Figure 4-8: Stepwise EMs of 20b, 20d and 20g (top to bottom).
4.4.6 Solvent effects

Solvent plays an important role in the formation of hemicarcerands and nanocapsules by properly solubilizing intermediates, thermodynamically stabilizing one assembly over another one through solvation effects and/or templating capsule formation. With respect to solubility, all intermediates leading to the formation of assemblies should be soluble in the solvent. Otherwise, the equilibrium may shut down and the thermodynamic product may not form or only lower amounts are obtained. Template effects are especially important for the formation of hemicarcerands, which have small cavity sizes comparable to the sizes of solvent molecules. Solvent may act as template and thermodynamically or kinetically favor a particular assembly. In order to address the question of which effect is dominant in the formation of hemicarcerands, EMs of hemicarcerands in different solvents were measured (Table 4-6). Due to the low solubility of 20b, 20d and 20g in aromatic solvents, only chlorinated solvents were used. On the other hand, 20c is good soluble in aromatic solvents, which allowed EM measurements in three aromatic and three chlorinated solvents. The results for 20c show that, CD₂Cl₂ and p-xylene-d₁₀ give the highest EM. In CDCl₂CDCl₂ and benzene-d₆, EM is lowest. EM roughly correlates with the number of possible CD-π interactions between guest and host. According to Rebek’s 55% rule and CPK modeling, two CDCl₃ or CD₂Cl₂ can fit in the cavity of 20c. Thus, CDCl₃ can form two CD-π interactions, but CD₂Cl₂ can form four CH-π interactions. Although one CDCl₂CDCl₂ can enter the cavity, its rigid structure only allows one end to interact with one hemisphere. Therefore, it can at most form one CD-π interaction. As for aromatic solvents, p-xylene fits the cavity perfectly. It can form three strong CD-π interactions with one hemisphere and possibly, three weak CD-π interactions with the other one. Overall, more than three CD-π interactions are present.
Toluene bearing one methyl group can only form three CD-π interactions and no CH-π interaction is present between benzene and the host.

Since EM is highly solvent-dependent, we hypothesize that solvent likely acts as template. To confirm this hypothesis, two control experiments were performed. In the first experiment, 5% $p$-xylene-d10 was added to CDCl$_3$. Since CDCl$_3$ is still in majority in the solvent mixture, EM in the mixture and pure CDCl$_3$ should be very similar, if EM is mainly governed by solvation effects. However, the difference is huge and EM in the presence of 5% $p$-xylene is almost the same as that in pure $p$-xylene. The same behavior was also observed when 5% $p$-xylene was added to benzene. These results indicate that the template effect is the key factor controlling the formation of hemicarcerands.

EMs of the other hemicarcerands 20b, d, g are also solvent dependent, especially those for hemicarcerands assembled from aliphatic diamines. This could be due to the fact that aliphatic linkers are flexible. Thus, the cavity size and shape can change slightly in order to complex the template more efficiently. By contrast, rigid hemicarcerand 20g and the corresponding intermediates M1-M3 and H1-H2 are well pre-organized and ready to assemble into the hemicarcerand, so that assembly is less affected by the solvent.
Table 4-6: EMs of hemicarcerands in different solvents.

<table>
<thead>
<tr>
<th>Hemicarcerands</th>
<th>Solvent</th>
<th>Mono-amine</th>
<th>EM/mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>20b</td>
<td>CDCl$_3$</td>
<td>BuNH$_2$</td>
<td>(1.7±0.13) × 10$^3$</td>
</tr>
<tr>
<td>20b</td>
<td>CD$_2$Cl$_2$</td>
<td>BuNH$_2$</td>
<td>(1.8±0.19) × 10$^3$</td>
</tr>
<tr>
<td>20b</td>
<td>CDCl$_2$CDCl$_2$</td>
<td>BzlNH$_2$</td>
<td>13.5±1.73</td>
</tr>
<tr>
<td>20c</td>
<td>CDCl$_3$</td>
<td>BuNH$_2$</td>
<td>70.8±0.96</td>
</tr>
<tr>
<td>20c</td>
<td>CD$_2$Cl$_2$</td>
<td>BuNH$_2$</td>
<td>(3.1±0.059) × 10$^2$</td>
</tr>
<tr>
<td>20c</td>
<td>CDCl$_2$CDCl$_2$</td>
<td>BzlNH$_2$</td>
<td>4.3±0.25</td>
</tr>
<tr>
<td>20c</td>
<td>Benzene-d6</td>
<td>BzlNH$_2$</td>
<td>3.6±0.15</td>
</tr>
<tr>
<td>20c</td>
<td>Toluene-d8</td>
<td>BuNH$_2$</td>
<td>88.9±6.04</td>
</tr>
<tr>
<td>20c</td>
<td>p-Xylene-d10</td>
<td>BuNH$_2$</td>
<td>(4.5±0.23) × 10$^2$</td>
</tr>
<tr>
<td>20c</td>
<td>p-Xylene-d10</td>
<td>BuNH$_2$</td>
<td>(3.9±0.21) × 10$^2$</td>
</tr>
<tr>
<td>20c</td>
<td>Benzene-d6 + 5% p-Xylene-d10</td>
<td>BuNH$_2$</td>
<td>(4.2±0.18) × 10$^2$</td>
</tr>
<tr>
<td>20d</td>
<td>CDCl$_3$</td>
<td>BuNH$_2$</td>
<td>(3.7±0.1) × 10$^3$</td>
</tr>
<tr>
<td>20d</td>
<td>CD$_2$Cl$_2$</td>
<td>BuNH$_2$</td>
<td>(3.3±0.3) × 10$^3$</td>
</tr>
<tr>
<td>20d</td>
<td>CDCl$_2$CDCl$_2$</td>
<td>BuNH$_2$</td>
<td>(6.0±0.8) × 10$^2$</td>
</tr>
<tr>
<td>20g</td>
<td>CDCl$_3$</td>
<td>PhNH$_2$</td>
<td>(6.7±0.39) × 10$^4$</td>
</tr>
<tr>
<td>20g</td>
<td>CD$_2$Cl$_2$</td>
<td>PhNH$_2$</td>
<td>(1.2±0.096) × 10$^5$</td>
</tr>
<tr>
<td>20g</td>
<td>CDCl$_2$CDCl$_2$</td>
<td>PhNH$_2$</td>
<td>(9.6±0.60) × 10$^3$</td>
</tr>
</tbody>
</table>

An excellent demonstration of the importance of the template is the unexpected formation of ~10% and ~35% of 20a in HMPA containing p-xylene or p-dimethoxybenzene as template, respectively (Fig. 4-9). In CDCl$_3$, CD$_2$Cl$_2$ or THF, the same condensation reaction does not yield 20a but leads to larger tetra-, hexa- or octa-cavitand nanocapsules$^{12,13}$. Even if p-xylene or p-dimethoxybenzene is added to the reaction mixture in CDCl$_3$, 20a still doesn’t form (see Section 4.5.2). Thus, HMPA must enhance the template effect of p-xylene or p-dimethoxybenzene. The rigid, bulky structure of HMPA makes it a poor solvent for solvating the inner surface of cavitand 19 in contrast to CDCl$_3$, p-xylene and p-dimethoxybenzene.

In CDCl$_3$, the hemicarceplex 20a⊙p-xylene slowly disappeared, because linkers can be exchanged against CDCl$_3$ due to the dynamics of the imine bonds. The formation of the strained hemicarcerand 20a not only supports the importance of template
effects but also may explain why nanocapsule 23 forms more efficiently with addition of \( p \)-xylene.

![NMR spectrum](image)

**Figure 4-9:** \(^1\)H NMR spectrum (500 MHz, CDCl\textsubscript{3}, 25 °C) of the products formed in the reaction between one 19 and two 17a in HMPA containing \( p \)-dimethoxybenzene, which include \( \sim 35\% \) 20a⊙\( p \)-dimethoxybenzene. Signals assigned to 20a and \( p \)-dimethoxybenzene are labeled with black arrows and open circles, respectively.

Aside from octaimine hemicarcerands, solvent effects on EMs of tris-bridged hemicarcerands 98b and 99b and macrocycles 100-102b were studied in CD\textsubscript{2}Cl\textsubscript{2}, CDCl\textsubscript{3}, CDCl\textsubscript{2}CDCl\textsubscript{2}, too (Table 4-7). In CDCl\textsubscript{3} and CDCl\textsubscript{2}CDCl\textsubscript{2}, EMs of 100b are similar to each other and slightly smaller than that in CD\textsubscript{2}Cl\textsubscript{2}. 101b and 102b show higher EMs. Interestingly, in CDCl\textsubscript{3} and CD\textsubscript{2}Cl\textsubscript{2}, 102b is the major and 101b is minor product. Changing the solvent to CDCl\textsubscript{2}CDCl\textsubscript{2} inverts the product ratio. It is likely that 102b is more compressed than 101b. Thus, in the smaller solvents, 102b is favored. CDCl\textsubscript{2}CDCl\textsubscript{2}, which is larger than the other two solvents, better templates 101b. Although 99b is more compressed than 98b, the enthalpy penalty could make it unflavored.
**Table 4-7:** EMs of tris-bridged hemicarcerands and macrocycles in different solvents.

<table>
<thead>
<tr>
<th>entry</th>
<th>Hemicarcerand</th>
<th>Solvent</th>
<th>Mono-amine</th>
<th>EM/mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100b</td>
<td>CDCl₃</td>
<td>BzINH₂</td>
<td>14.5±0.71</td>
</tr>
<tr>
<td>2</td>
<td>100b</td>
<td>CD₂Cl₂</td>
<td>BzINH₂</td>
<td>57.3±9.6</td>
</tr>
<tr>
<td>3</td>
<td>100b</td>
<td>CDCl₃CDCl₂</td>
<td>BzINH₂</td>
<td>11.4±2.3</td>
</tr>
<tr>
<td>4</td>
<td>101b (25%)</td>
<td>CDCl₃</td>
<td>BzINH₂</td>
<td>61.5±1.4</td>
</tr>
<tr>
<td>5</td>
<td>102b (75%)</td>
<td>CDCl₃</td>
<td>BzINH₂</td>
<td>(1.9±0.04)×10²</td>
</tr>
<tr>
<td>6</td>
<td>101b (25%)</td>
<td>CD₂Cl₂</td>
<td>BzINH₂</td>
<td>86.8±3.3</td>
</tr>
<tr>
<td>7</td>
<td>102b (75%)</td>
<td>CD₂Cl₂</td>
<td>BzINH₂</td>
<td>(2.6±0.09)×10²</td>
</tr>
<tr>
<td>8</td>
<td>101b (71%)</td>
<td>CDCl₂CDCl₂</td>
<td>BzINH₂</td>
<td>(2.1±0.14)×10²</td>
</tr>
<tr>
<td>9</td>
<td>102b (29%)</td>
<td>CDCl₂CDCl₂</td>
<td>BzINH₂</td>
<td>85.8±5.6</td>
</tr>
<tr>
<td>10</td>
<td>98b (87%)</td>
<td>CDCl₃</td>
<td>BzINH₂</td>
<td>(1.0±0.3)×10²</td>
</tr>
<tr>
<td>11</td>
<td>99b (13%)</td>
<td>CDCl₃</td>
<td>BzINH₂</td>
<td>40.5±11.3</td>
</tr>
<tr>
<td>12</td>
<td>98b (92%)</td>
<td>CD₂Cl₂</td>
<td>BzINH₂</td>
<td>82.1±9.4</td>
</tr>
<tr>
<td>13</td>
<td>99b (8%)</td>
<td>CD₂Cl₂</td>
<td>BzINH₂</td>
<td>23.7±2.7</td>
</tr>
<tr>
<td>14</td>
<td>98b (49%)</td>
<td>CDCl₂CDCl₂</td>
<td>BzINH₂</td>
<td>21.9±1.5</td>
</tr>
<tr>
<td>15</td>
<td>99b (51%)</td>
<td>CDCl₂CDCl₂</td>
<td>BzINH₂</td>
<td>22.4±1.6</td>
</tr>
</tbody>
</table>

### 4.4.7 Octaimine hemicarcerand assembly is an ‘all-or-nothing’ process

One unique feature of self-assembly processes, which exhibit positive cooperativity, is that they are ‘all-or-nothing’ processes. This means that only the final products are populated and all intermediates leading to the products are at sub detection level. Since hemicarcerands 20b, 20d and 20g show positive cooperativity in CDCl₃, it is expected that they exhibit ‘all-or-nothing’ behavior. In other words, only 19; 17b, d, g; 20b; 20d and 20g are populated at equilibrium, and A1-Ai, M1-M3 and H1-H2 (Scheme 4-10) are only present in extremely small amounts, if at all. To confirm this, equilibrium constants of all species were calculated and the distribution of each species at different reactant concentration plotted using chemEQL (water concentration was set to be 50 mM). Fig. 4-10 shows a speciation plot for 20b over a wide concentration range. At very low concentration (<10⁻⁸ M), neither 20b nor intermediates are populated and free cavitand 19 and propylenediamine 17b are the major species. At concentrations between 10⁻⁸ M and 10⁻⁵ M, the concentration of 20b...
increases dramatically but intermediates are still not populated. Above $10^{-5}$ M, only 20b is present. This speciation plot confirms that hemicarcerand assemblies are ‘all-or-nothing’ processes.

The ‘all-or-nothing’ behavior is also supported by MALDI-TOF MS analysis of denaturation products of 20d (Fig. 4-11). Intermediates composed of two cavitands (e.g. macrocycles or tris-bridged hemicarcerands) are not observed, since they would have MW > 1922 (two cavitands 19 linked by one linker 17d).

![Speciation plot for the assembly of hemicarcerand 20b.](image)
Figure 4-11: MALDI-TOF MS of denatured products of 20d.

4.5 Conclusions

In this chapter, EMs of a series of hemicarcerands were measured using a capsule denaturation approach and Ercolani’s capsule self-assembly model. The rigidity and conformation of linkers and the solvent have a profound influence on the EM of the assemblies. It was proved that solvent mainly acts as the template during the formation of hemicarcerands. Furthermore, the major intermediates involved in the formation of hemicarcerands were synthesized for the first time and a stepwise formation of hemicarcerands was proposed. Geometric mean EMs of all intermediates and stepwise EMs (i = 1-3) of assembly processes were also determined, which were found to be solvent-dependent. In CDCl₃, a positive cooperativity for 20b, d, g was observed based on EMs, which is accompanied with a ‘all-or-nothing’ process. Additionally, EMs of nanocapsules were measured, showing that the rigidity of linker is essential for the formation of nanocapsules, and that EM correlates with the average number of rotors that are frozen in intramolecular steps. This suggests that EM in nanocapsules is mainly controlled by entropy.
4.6 Experimental section

4.6.1 General procedures

Reagents and chromatography solvents were purchased and used without further purification except for chloroform, which was passed through K$_2$CO$_3$ prior to use. THF was dried over Na/benzophenone and distilled under argon. $^1$H NMR spectra recorded in C$_6$D$_6$, C$_6$D$_5$CD$_3$, CD$_3$C$_6$D$_3$CD$_3$, CDCl$_3$, CD$_2$Cl$_2$ and CDCl$_2$CDCl$_2$ were referenced to residual C$_6$HD$_5$, C$_6$D$_5$CHD$_2$, CD$_3$C$_6$D$_4$CHD$_2$, CHCl$_3$, CHDCl$_2$ and CHCl$_2$CDCl$_2$ at $\delta_H = 7.16$, 2.09, 2.30, 7.26, 5.30 and 6.00 ppm, respectively. $^{13}$C NMR spectra recorded in CDCl$_3$ were referenced to $^{13}$CDCl$_3$ at $\delta_C = 77.25$ ppm. NMR spectra were recorded on VARIAN 500, 400 and 300 MHz NMR instruments. Mass spectra were recorded with an Applied Biosystems Voyager DE-Pro mass spectrometer (MALDI-TOF) with 2,4,6-trihydroxyacetophenone (THAP) as matrix. Positive molecular ions were usually detected as proton, sodium or potassium adducts for the compounds reported herein.

4.6.2 Synthesis of 79 (Procedure I)

Tetrabromocavitand 77 (2.16 g, 1.91 mmol) was dried overnight in a round bottom flask under vacuum at 120 °C. After it cooled to room temperature, the flask was purged with argon and anhydrous THF (50 mL) was added. The solution was cooled to -78 °C and BuLi in hexane (7.7 mL, 2.5 M, 19.3 mmol) was slowly added. After 3 hours, anhydrous DMF (0.17 mL, 2.3 mmol) was added at the same temperature. After stirring for 1 hour, hydrochloric acid (20 mL, 1 M) was added to quench the reaction and the resulting mixture was partitioned between EtOAc and water. The organic layer was separated and the aqueous layer was extracted with EtOAc (2 $\times$ 50 mL). The combined organic layers were dried over MgCl$_2$. After removing the solvent,
a pale yellow solid was obtained, which was purified by silica gel column chromatography (EtOAc-CH₂Cl₂ = 5:95). 0.25 g of 17 (15%) was obtained as a white solid.

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\begin{align*}
\text{1H NMR spectrum (400 MHz, CDCl₃, 25 °C): } & \delta_H = 10.25 \text{ (s, 2H, } -\text{CHO}), 7.31 \text{ (s, 2H, } H_{a1}), 7.07 \text{ (s, 2H, } H_{a2}), 6.51 \text{ (s, 2H, } H_1), 5.89 \text{ (d, 1H, } J = 7.3 \text{ Hz, } H_o), 5.81 \text{ (d, 2H, } J = 7.3 \text{ Hz, } H_o), 5.73 \text{ (d, 1H, } J = 7.3 \text{ Hz, } H_o), 4.89 \text{ (t, 1H, } J = 8.3 \text{ Hz, } H_m), 4.80 \text{ (t, 2H, } J = 8.3 \text{ Hz, } H_m), 4.71 \text{ (t, 1H, } J = 8.3 \text{ Hz, } H_m), 4.49-4.38 \text{ (m, 4H, } H_i), 2.28-2.16 \text{ (m, 8H, } H_2), 1.47-1.30 \text{ (m, 24H, } H_3-H_5), 0.95-0.88 \text{ (m, 12H, } H_6). \end{align*}
\]

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\text{13C NMR spectrum (100 MHz, CDCl₃, 25 °C): } \delta_C = 190.73, 155.42, 155.14, 154.97, 154.65, 140.07, 139.18, 138.99, 138.14, 125.43, 124.43, 120.63, 117.13, 100.49, 100.16, 99.76, 36.60, 36.40, 36.21, 32.29, 32.23, 32.17, 30.12, 29.94, 29.77, 27.79, 22.99, 14.39. \]

MS (MALDI-TOF): m/z (M + H⁺, 100%), Calcd for C₅₄H₆₅O₁₀ + H⁺: 873.4578; found: 873.4630.

4.6.3 Synthesis of 80

Prepared by procedure 1 from tetraiodocavitand 59 (5 g, 4.43 mmol), BuLi in hexane (8.5 mL, 2.5 M, 21.3 mmol) and anhydrous DMF (0.4 mL, 5.3 mmol), 184 mg of 80 (5%) was obtained as white solid.
\( ^1H \) NMR spectrum (400 MHz, CDCl\(_3\), 25 °C): \( \delta_H = 10.27 \) (s, 1H, -CHO), 7.34 (s, 1H, H\(_{a1}\)), 7.10 (s, 3H, H\(_{a2}\) and H\(_{a3}\)), 6.52 (s, 2H, H\(_1\)), 6.49 (s, 1H, H\(_2\)), 5.82 (d, 2H, \( J = 7.3 \) Hz, H\(_o\)), 5.74 (d, 2H, \( J = 7.3 \) Hz, H\(_o\)), 4.81 (t, 2H, \( J = 8.2 \) Hz, H\(_m\)), 4.72 (t, 2H, \( J = 8.2 \) Hz, H\(_m\)), 4.44 (d, 2H, \( J = 7.3 \) Hz, H\(_i\)), 4.43 (d, 2H, \( J = 7.3 \) Hz, H\(_i\)), 2.23 (m, 8H, H\(_3\)), 1.47-1.32 (m, 24H, H\(_4\)-H\(_6\)), 0.95-0.90 (m, 12H, H\(_7\)). \(^{13}\)C NMR spectrum (125 MHz, CDCl\(_3\), 25 °C): \( \delta_C = 191.08, 155.60, 155.35, 155.15, 155.10, 139.89, 139.30, 138.82, 138.44, 125.82, 124.44, 121.03, 120.91, 117.18, 117.08, 100.39, 99.88, 36.64, 36.63, 32.50, 32.43, 30.33, 30.15, 28.03, 28.01, 23.17, 14.58, 14.57. MS (MALDI-TOF): m/z (M + Na\(^{+}\), 100%), Calcd for C\(_{53}\)H\(_{64}\)O\(_9\) + Na\(^{+}\): 867.4442; found: 867.4403.

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4.6.4 Synthesis of 82

Prepared by procedure 1 from tetrabromocavitand 79 (2.18 g, 1.93 mmol), BuLi in hexane (7.7 mL, 2.5 M, 19.3 mmol) and anhydrous DMF (0.45 mL, 5.8 mmol), 0.54 g of 82 (31%) was obtained as a white solid.
\(^1\)H NMR spectrum (500 MHz, CDCl\(_3\), 25 °C): \(\delta_H = 10.26\) (s, 1H, -CHO), \(10.25\) (s, 2H, -CHO), \(7.29\) (s, 3H, \(H_{a1}\) and \(H_{a3}\)), \(7.06\) (s, 1H, \(H_{a2}\)), \(6.55\) (s, 1H, \(H_1\)), \(5.89\) (d, 2H, \(J = 7.7\) Hz, \(H_o\)), \(5.81\) (d, 2H, \(J = 7.2\) Hz, \(H_o\)), \(4.90\) (t, 2H, \(J = 8.2\) Hz, \(H_m\)), \(4.81\) (t, 2H, \(J = 8.2\) Hz, \(H_m\)), \(4.46\) (d, 2H, \(J = 7.4\) Hz, \(H_i\)), \(4.44\) (d, 2H, \(J = 7.4\) Hz, \(H_i\)), \(2.28\) - \(2.16\) (m, 8H, \(H_2\)), \(1.47\) - \(1.32\) (m, 24H, \(H_3\) - \(H_5\)), \(0.95\) - \(0.90\) (m, 12H, \(H_6\)). \(^{13}\)C NMR spectrum (125 MHz, CDCl\(_3\), 25 °C): \(\delta_C = 190.44, 190.24, 155.21, 155.18, 154.82, 154.68, 139.89, 139.49, 139.01, 138.43, 134.43, 125.19, 125.14, 124.68, 124.33, 120.51, 117.28, 100.38, 100.05, 77.49, 77.23, 76.98, 36.34, 36.15, 32.14, 32.08, 29.90, 29.73, 27.69, 22.88, 14.29. MS (MALDI-TOF): m/z (M + Na\(^+\), 100%), Calcd for C\(_{55}\)H\(_{64}\)O\(_{11}\) + Na\(^+\): 923.4341; found: 923.4370.

### 4.6.5 Synthesis of 85

In a round bottom flask, tetrabromocavitand 79 (1.5 g, 1.33 mmol) was dried under vacuum at 120 °C overnight, after which it was cooled down to room temperature and flushed with argon. Under argon, anhydrous THF (30 mL) was added into the flask. At -78 °C, BuLi in hexane (1.06 mL, 2.5 M, 2.65 mmol) was slowly added into the solution. After 1 hour, hydrochloric acid (20 mL, 1 M) was added and the resulting mixture was extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with sat. Na\(_2\)CO\(_3\) solution and brine and dried over MgSO\(_4\). After removing
the solvent, the pale yellow solid was purified by silica column chromatography (CH$_2$Cl$_2$) and 83 (0.29 g, 0.3 mmol) was obtained. It was dried and dissolved in anhydrous THF (30 mL). At -78°C, BuLi in hexane (0.5 mL, 2.5 M, 1.25 mmol) was slowly added into the solution. After stirring for 1 h, anhydrous DMF (0.1 mL, 1.35 mmol) was added. After another 1 h, hydrochloric acid (1 M, 20 mL) was added to quench the reaction. The mixture was extracted with EtOAc (3 × 30 mL) and the combined organic layers were washed with sat. Na$_2$CO$_3$ solution and brine and dried over MgSO$_4$. After evaporating the solvent, the residual solid was subjected to silica column chromatography (EtOAc-CH$_2$Cl$_2$, 5:95). 249 mg of 85 (22%) was obtained as a white solid.

1H NMR spectrum (400 MHz, CDCl$_3$, 25°C) $\delta_H$ = 10.25 (s, 2H, -CHO), 7.31 (s, 2H, H$_{a2}$), 7.07 (s, 2H, H$_{a1}$), 6.54 (s, 2H, H$_1$), 5.81 (d, 4H, J = 7.6 Hz, H$_o$), 4.81 (d, 4H, J = 8.3 Hz, H$_m$), 4.43 (d, 4H, J = 7.6 Hz, H$_i$), 2.21 (m, 8H, H$_2$), 1.48-1.30 (m, 24H, H$_3$-H$_5$), 0.92 (t, 12H, J = 7.0 Hz, H$_6$). 13C NMR spectrum (100 MHz, CDCl$_3$, 25°C): $\delta_C$ = 190.54, 155.24, 154.99, 139.58, 138.60, 125.45, 120.77, 117.21, 100.14, 36.40, 32.21, 29.95, 27.78, 22.95, 14.35. MS (MALDI-TOF): m/z (M + H$^+$, 100%), Calcd for C$_{54}$H$_{65}$O$_{10}$ + H$^+$: 873.4578; found: 873.4500.

4.6.6 Synthesis of 91 (Procedure 2)

Tetraiodocavitand 59 (351 mg, 0.27 mmol), potassium 4-formylphenyltrifluoroborate
(67.6 mg, 0.32 mmol), Pd(OAc)$_2$ (6 mg, 0.027 mmol), PPh$_3$ (14 mg, 0.054 mmol) and K$_2$CO$_3$ (73 mg, 0.54 mmol) were placed in a Schlenk flask. The flask was flushed with argon. Under argon, 10 mL THF and 1 mL H$_2$O were added. The solution was stirred at room temperature for 10 minutes. Then the flask was sealed and heated to 100 °C overnight. The resulting mixture was diluted with ethyl acetate and washed with 0.1 M HCl solution. The organic layer was separated and dried over MgSO$_4$. The salt was filtered off and the solvent was removed in vacuum. The yellowish solid obtained was further purified by silica gel column chromatography (Hexane-CH$_2$Cl$_2$ = 1:4) and 111.3 mg of 91 (34%) was obtained as a yellowish solid.

$^1$H NMR spectrum (500 MHz, CDCl$_3$, 25 °C): $\delta_H =$ 10.06 (s, 1H, -CHO), 7.92 (d, 2H, $J = 8.5$ Hz, H$_2$), 7.28 (s, 1H, H$_{a1}$), 7.16 (d, 2H, $J = 8.5$ Hz, H$_1$), 7.26 (s, 1H, H$_{a3}$, overlap with CHCl$_3$), 7.12 (s, 2H, H$_{a2}$), 6.00 (d, 2H, $J = 7.4$ Hz, H$_6$), 5.50 (d, 2H, $J = 7.4$ Hz, H$_o$), 4.88 (t, 2H, $J = 8.1$ Hz, H$_m$), 4.82 (t, 2H, $J = 8.1$ Hz, H$_{n_m}$), 4.29 (m, 4H, H$_i$), 2.23 (m, 8H, H$_3$), 1.47-1.32 (m, 24H, H$_4$-H$_6$), 0.95-0.90 (m, 12H, H$_7$). $^{13}$C NMR spectrum (125 MHz, CDCl$_3$, 25 °C): $\delta_C =$ 192.08, 155.37, 155.09, 155.04, 152.39, 141.13, 139.28, 139.03, 138.83, 138.64, 135.57, 130.68, 128.67, 129.57, 121.27, 120.93, 120.43, 99.38, 99.13, 93.37, 92.48, 38.26, 37.70, 32.16, 32.12, 30.39, 30.30, 27.74, 27.68, 22.89, 14.31, 14.30. MS (MALDI-TOF): m/z (M + Na$^+$, 100%), Calcd for C$_{59}$H$_{65}$O$_9$I$_3$ + Na$^+$: 1321.1654; found: 1321.1539.
4.6.7 Synthesis of 93

Prepared by procedure 2 from tetraiodocavitand 60 (269 mg, 0.20 mmol), potassium 4-formylphenyltrifluoroborate (50 mg, 0.24 mmol), Pd(OAc)$_2$ (4.4 mg, 0.020 mmol), PPh$_3$ (10.2 mg, 0.039 mmol) and K$_2$CO$_3$ (54 mg, 0.39 mmol), 52 mg 93 (21%) was obtained as a yellowish solid after column chromatography (Hexane-CH$_2$Cl$_2$ = 1:9).

$^1$H NMR spectrum (500 MHz, CDCl$_3$, 25 °C): $\delta_H = 10.05$ (s, 1H, -CHO), 7.89 (d, 2H, $J = 7.2$ Hz, H$_2$), 7.47 (s, 1H, H$_{a1}$), 7.45 (s, 1H, H$_{a3}$), 7.39 (d, 2H, $J = 7.2$ Hz, H$_1$), 7.26 (s, 2H, H$_{a2}$, overlap with CHCl$_3$), 5.28 (t, 2H, $J = 7.7$ Hz, H$_m$), 5.24 (t, 2H, $J = 7.7$ Hz, H$_m$), 4.53 (m, 2H, H$_o$), 4.42 (m, 2H, H$_o$), 4.33 (m, 2H, H$_o$), 4.15 (m, 2H, H$_o$), 4.04 (m, 2H, H$_i$), 3.64 (m, 2H, H$_i$), 3.38 (m, 2H, H$_i$), 3.28 (m, 2H, H$_i$), 2.23 (m, 8H, H$_3$), 1.47-1.32 (m, 24H, H$_4$-H$_{6}$), 0.95-0.90 (m, 12H, H$_7$). $^{13}$C NMR spectrum (125 MHz, CDCl$_3$, 25 °C): $\delta_C = 192.09, 155.97, 153.32, 153.12, 153.02, 142.42, 137.47, 137.01, 136.57, 135.82, 135.71, 130.84, 130.57, 129.39, 125.81, 124.57, 123.63, 90.09, 89.39, 72.01, 70.96, 70.72, 70.54, 35.85, 35.03, 34.99, 34.95, 32.09, 32.06, 27.68, 27.62, 22.81, 22.79, 14.30, 14.29. MS (MALDI-TOF): m/z (M + Na$^+$, 100%), Calcd for C$_{63}$H$_{73}$O$_9$I$_3$ + Na$^+$: 1377.2280; found: 1377.2178.

4.6.8 Synthesis of 98b, 99b, 103b and 104b (Procedure 3)
Solutions of propylene-1,3-diamine 17b in CHCl₃ (8.11 mg, 109.5 µmol, 10 mg/mL) and TFA in CHCl₃ (66 µL, 1% v/v) were added to a solution of cavitand 82 in CHCl₃ (65.72 mg, 73.0 µmol, 10 mg/mL). After stirring under argon for 3 days, the solvent was removed and a yellow solid was obtained in 90%, which includes a mixture of 98b and 99b (ratio, 6.7:1). Pure 98b was obtained by recrystallization from CH₂Cl₂/methanol. Excess NaBH₄ was added to the solution of both isomers in CH₂Cl₂-MeOH = 1:1. After stirring overnight, the solvent was removed in vacuum. The white residual solid was suspended in water and sonicated for 10 minutes. The precipitate was filtered, collected and dried in vacuum and purified by preparative TLC (isopropanol-CH₂Cl₂ = 3:7). Two reduced isomers 103b and 104b were obtained as white powder.

Spectroscopic data for 98b: ¹H NMR spectrum (500 MHz, CDCl₃, 25 °C): δ_H = 8.38 (s, 4H, -CHN-), 8.37 (s, 2H, -CHN-), 7.13 (s, 2H, H₃), 7.11 (s, 4H, H₄), 7.05 (s, 2H, H₂), 6.50 (s, 2H, H₁), 5.68 (d, 4H, J = 7.8 Hz, H₆), 5.64 (d, 4H, J = 7.8 Hz, H₇), 4.85 (t, 4H, J = 8.3 Hz, H₉), 4.79 (t, 4H, J = 8.3 Hz, H₈), 4.49 (d, 4H, J = 7.3 Hz, H₅), 4.40 (d, 4H, J = 7.9 Hz, H₆), 3.67-3.43 (m, 12H, H₇), 2.28-2.16 (m, 16H, H₈), 1.47-1.28 (m, 54H, H₃-H₅ and H₆). ¹³C NMR spectrum (100 MHz, CDCl₃, 25 °C): δ_C = 156.14, 155.66, 155.12, 153.50, 153.37, 138.83, 138.52, 138.31, 124.34,
124.24, 121.61, 121.32, 120.69, 117.32, 100.70, 100.19, 62.21, 61.89, 36.64, 36.58, 33.80, 33.59, 32.20, 32.16, 30.04, 29.92, 27.77, 22.91, 14.31. MS (MALDI-TOF): m/z (M + H\(^+\), 76.3%), Calcd for C\(_{119}\)H\(_{146}\)O\(_{16}\)N\(_6\) + H\(^+\): 1916.0867; found: 1916.0825.

Spectroscopic data for 103b: \(^1\)H NMR spectrum (500 MHz, CDCl\(_3\), 25 °C): \(\delta_H = 7.07\) (s, 2H, H\(_{a3}\)), 7.05 (s, 4H, H\(_{a1}\)), 7.03 (s, 2H, H\(_{a2}\)), 6.37 (s, 2H, H\(_1\)), 5.82 (d, 4H, J = 7.2 Hz, H\(_o\)), 5.75 (d, 4H, J = 7.2 Hz, H\(_o\)), 4.72 (m, 8H, H\(_m\)), 4.47 (d, 4H, J = 7.2 Hz, H\(_i\)), 4.34 (d, 4H, J = 7.2 Hz, H\(_i\)), 3.64 (s, 8H, H\(_{10}\)), 3.53 (s, 4H, H\(_o\)), 2.72-2.59 (m, 12H, H\(_7\)), 2.26-2.11 (m, 18H, -NH- and H\(_2\)), 1.48-1.27 (m, 54H, H\(_3\)-H\(_5\) and H\(_8\)), 0.94-0.85 (m, 24H, H\(_6\)). \(^1\)C NMR spectrum (125 MHz, CDCl\(_3\), 25 °C): \(\delta_C = 154.71, 154.38, 155.14, 153.56, 138.50, 138.21, 138.14, 137.72, 126.92, 125.84, 120.88, 119.65, 119.29, 117.35, 101.05, 99.62, 50.30, 49.15, 43.83, 37.16, 36.93, 32.24, 30.52, 30.19, 29.91, 27.83, 27.80, 22.89, 14.31. MS (MALDI-TOF): m/z (M + H\(^+\), 100%), Calcd for C\(_{119}\)H\(_{159}\)O\(_{16}\)N\(_6\) + H\(^+\): 1929.1891; found: 1929.2055.

Spectroscopic data for 104b: \(^1\)H NMR spectrum (500 MHz, CDCl\(_3\), 25 °C): \(\delta_H = 7.07\) (s, 2H, H\(_{a1}\)), 7.03 (s, 2H, H\(_{a1}\)), 7.02 (s, 4H, H\(_{a2}\)), 6.44 (s, 2H, H\(_1\)), 5.92 (d, 2H, J = 7.2 Hz, H\(_o\)), 5.79 (m, 4H, H\(_o\)), 5.59 (d, 2H, J = 7.2 Hz, H\(_o\)), 4.72 (m, 8H, H\(_m\)), 4.49 (d, 2H,
$J = 7.2$ Hz, Hi), 4.39 (m, 4H, H), 4.29 (d, 2H, $J = 7.2$ Hz, H), 3.76 (d, 2H, $J = 12.4$, H), 3.67 (d, 2H, $J = 10.7$, H), 3.61 (d, 2H, $J = 10.7$, H), 3.52 (d, 2H, $J = 11.7$, H), 3.43 (d, 2H, $J = 11.7$, H), 3.18 (d, 2H, $J = 12.4$, H), 2.95-2.47 (m, 12H, H), 2.27-2.08 (m, 22H, -NH- and H), 1.46-1.27 (m, 54H, H-H and H), 0.94-0.85 (m, 24H, H). $^{13}$C NMR spectrum (125 MHz, CDCl$_3$, 25 °C): $\delta C = 154.85$, 154.83, 154.46, 154.18, 154.08, 154.05, 153.78, 153.45, 153.16, 138.53, 138.51, 138.34, 138.30, 138.13, 138.08, 138.03, 128.35, 127.50, 125.51, 119.65, 120.91, 119.66, 119.13, 117.19, 100.51, 100.26, 99.69, 99.52, 53.61, 48.64, 48.41, 43.55, 43.40, 42.68, 37.19, 37.15, 37.02, 36.97, 32.29, 30.39, 30.35, 30.20, 27.88, 27.85, 22.94, 14.35. MS (MALDI-TOF): m/z (M + H, 100%), Calcd for C$_{119}$H$_{159}$O$_{16}$N$_{6}$ + H: 1929.1891; found: 1919.2026.

4.6.9 Synthesis of 98d, 99d, 103d and 104d

Prepared by procedure 3 from pentylene-1,5-diamine 17d in CHCl$_3$ (6.82 mg, 66.9 µmol, 10 mg/mL), TFA in CHCl$_3$ (40 µL, 1% v/v) and cavitand 82 in CHCl$_3$ (40.14 mg, 44.6 µmol, 10 mg/mL), a mixture of 98d and 99d (ratio, 1:1) formed. Pure 98d was obtained by recrystallization from CH$_2$Cl$_2$/hexane. The reduction of the mixture yielded 103d and 104d as a white powder.
Spectroscopic data for 98d: $^1$H NMR spectrum (400 MHz, CDCl$_3$, 25 °C): $\delta_H = 8.34$ (s, 4H, -CHN-), 8.33 (s, 2H, -CHN-), 7.13 (s, 2H, H$_{a3}$), 7.11 (s, 4H, H$_{a1}$), 7.05 (s, 2H, H$_{a2}$), 6.47 (s, 2H, H$_1$), 5.63 (d, 4H, $J = 7.5$ Hz, H$_o$), 5.58 (d, 4H, $J = 7.7$ Hz, H$_o$), 4.85 (t, 4H, $J = 8.2$ Hz, H$_m$), 4.77 (t, 4H, $J = 8.2$ Hz, H$_m$), 4.44 (d, 4H, $J = 7.5$ Hz, H$_i$), 4.37 (d, 4H, $J = 7.7$ Hz, H$_i$), 3.53-3.39 (m, 12H, H$_7$), 2.30-2.13 (m, 16H, H$_2$), 1.47-1.28 (m, 66H, H$_3$-H$_5$, H$_8$ and H$_9$), 0.95-0.77 (m, 24H, H$_6$). $^{13}$C NMR spectrum (125 MHz, CDCl$_3$, 25 °C): $\delta_C = 155.87, 155.79, 155.13, 153.58, 153.56, 153.49, 138.84, 138.81, 138.77, 138.45, 124.43, 124.40, 121.58, 121.24, 120.85, 117.36, 100.80, 100.09, 63.11, 62.97, 36.68, 32.29, 32.23, 31.57, 30.91, 30.15, 30.07, 27.86, 22.98, 14.38. MS (MALDI-TOF): m/z (M + H$^+$, 100%), Calcd for C$_{125}$H$_{159}$O$_{16}$N$_6$ + H$^+$: 2001.1853; found: 2001.2066.

Spectroscopic data for 103d: $^1$H NMR spectrum (400 MHz, MeOD + 0.4% TFA-D, 25 °C) $\delta_H = 7.50$ (s, 2H, H$_{a3}$), 7.49 (s, 4H, H$_{a1}$), 7.34 (s, 2H, H$_{a2}$), 6.51 (s, 2H, H$_1$), 6.10 (d, 4H, $J = 7.4$ Hz, H$_o$), 5.90 (d, 4H, $J = 7.4$ Hz, H$_o$), 4.85 (t, 4H, $J = 8.4$ Hz, H$_m$), 4.78 (t, 4H, $J = 7.9$ Hz, H$_m$), 4.46 (d, 4H, $J = 7.4$ Hz, H$_i$), 4.34 (d, 4H, $J = 7.4$ Hz, H$_i$), 3.99 (s, 4H, H$_{10}$), 3.97 (s, 8H, H$_{10}$), 3.65 (s, 6H, -NH-), 3.21-3.06 (m, 12H, H$_7$), 2.47-2.23 (m, 16H, H$_2$), 1.94-1.77 (m, 12H, H$_8$), 1.49-1.28 (m, 60H, H$_3$-H$_5$ and H$_9$), 0.96-0.77 (m, 24H, H$_6$). $^{13}$C NMR spectrum (100 MHz, MeOD + 0.4% TFA-D, 25
Spectroscopic data for 104d: $^1$H NMR spectrum (300 MHz, MeOD + 0.4% TFA-D, 25 °C): $\delta_H = 7.51$ (s, 4H, $H_a$), 7.50 (s, 2H, $H_a$), 7.36 (s, 2H, $H_a$), 6.59 (s, 2H, $H_1$), 6.24 (d, 2H, $J = 7.6$ Hz, $H_o$), 6.11 (d, 2H, $J = 7.6$ Hz, $H_o$), 5.93 (m, 4H, $H_o$), 4.86-4.73 (m, 8H, $H_m$), 4.57 (d, 2H, $J = 7.6$ Hz, $H_i$), 4.50-4.38 (m, 6H, $H_i$), 4.27-3.99 (m, 12H, $H_{10}$), 3.64 (s, 6H, -NH$^-$), 3.26-3.04 (m, 12H, $H_7$), 2.48-2.24 (m, 16H, $H_2$), 1.93-1.67 (m, 12H, $H_8$), 1.59-1.25 (m, 60H, $H_3$ and $H_9$), 0.97-0.88 (m, 24H, $H_6$). $^{13}$C NMR spectrum (125 MHz, MeOD + 0.4% TFA-D, 25 °C): $\delta_C = 155.55$, 154.44, 154.19, 154.05, 153.77, 153.70, 139.30, 139.17, 138.85, 138.70, 138.43, 138.24, 138.18, 123.29, 123.23, 123.00, 121.55, 119.56, 119.07, 118.97, 116.57, 100.27, 100.10, 70.38, 41.63, 40.94, 40.78, 37.13, 36.92, 36.85, 31.89, 31.70, 31.66, 31.62, 29.53, 29.30, 27.70, 24.81, 24.61, 24.37, 23.20, 22.66, 13.26, 13.24, 13.22. MS (MALDI-TOF): $m/z$ (M + $H^+$, 100%), Calcd for $C_{125}H_{171}O_{16}N_6 + H^+$: 2013.2783; found: 2013.2614.

4.6.10 Synthesis of 98g

Prepared by procedure 3 from solutions of 1,3-phenylenediamine 17g in CHCl$_3$ (5.41 mg, 50.1 µmol, 10 mg/mL), TFA in CHCl$_3$ (30 µL, 1% v/v) and cavitand 82 in CHCl$_3$ (30.06 mg, 33.4 µmol, 10 mg/mL), 8g formed quantitatively.
$^1$H NMR spectrum (400 MHz, CDCl$_3$, 25 °C): $\delta_H = 8.46$ (s, 4H, -CH$_2$N-), 8.43 (s, 2H, -CHN-), 7.37 (t, 2H, $J = 7.92$ Hz, H$_9$), 7.34 (t, 1H, $J = 7.92$ Hz, H$_7$), 7.24 (s, 2H, H$_{a3}$, overlap with CHCl$_3$), 7.23 (s, 4H, H$_{a1}$), 7.09 (s, 2H, H$_{a2}$), 6.80 (dd, 4H, $^3J = 7.92$ Hz, H$_8$), 6.77 (dd, 2H, $^3J = 7.92$ Hz, $^4J = 2.05$ Hz, H$_8$), 6.62 (t, 2H, $J = 2.05$ Hz, H$_9$), 6.56 (s, 2H, H$_1$), 6.47 (t, 1H, $J = 2.05$ Hz, H$_7$), 5.71 (d, 4H, $J = 7.51$ Hz, H$_o$), 5.65 (d, 4H, $J = 7.72$ Hz, H$_o$), 4.92 (t, 4H, $J = 8.06$ Hz, H$_m$), 4.84 (t, 4H, $J = 4.86$ Hz, H$_m$), 4.64 (d, 4H, $J = 7.51$ Hz, H$_i$), 4.56 (d, 4H, $J = 7.72$ Hz, H$_i$), 2.33-2.17 (m, 16H, H$_2$), 1.51-1.31 (m, 48H, H$_3$-H$_5$), 0.96-0.81 (m, 24H, H$_6$). $^{13}$C NMR spectrum (100 MHz, CDCl$_3$, 25 °C): $\delta_C = 157.14$, 156.97, 155.33, 154.06, 153.82, 153.75, 153.59, 153.20, 139.33, 139.21, 138.73, 138.51, 130.86, 124.44, 124.37, 122.72, 122.47, 120.75, 118.65, 117.51, 115.65, 100.91, 100.51, 36.73, 32.28, 32.23, 30.13, 29.93, 27.87, 23.00, 14.39. MS (MALDI-TOF): m/z (M + H$^+$, 100%), Calcd for C$_{128}$H$_{141}$O$_{16}$N$_6$ + H$: 2019.0426; found: 2019.0283.

4.6.11 Synthesis of 100b

Prepared by procedure 3 from solutions of propylene-1,3-diamine 17b in CHCl$_3$ (0.13 mg, 1.83 µmol, 10 mg/mL), TFA in CHCl$_3$ (1.6 µL, 1% v/v) and cavitand 85 in
CHCl₃ (1.80 mg, 1.83 µmol, 10 mg/mL), 100b formed quantitatively after reacting overnight.

1H NMR spectrum (300 MHz, CDCl₃, 25 °C): δ_H = 8.40 (s, 4H, -Cl/N-), 7.12 (s, 4H, H₂), 7.04 (s, 4H, H₃), 6.43 (s, 4H, H₁), 5.65 (d, 8H, J = 7.3 Hz, H₉), 4.77 (t, 8H, J = 8.1 Hz, H₈), 4.45 (d, 8H, J = 7.3 Hz, H₇), 3.61 (t, 8H, J = 7.4 Hz, H₆), 2.31-2.12 (m, 16H, H₂), 1.45-1.30 (m, 52H, H₃-H₅ and H₆), 0.91 (t, 24H, J = 6.9 Hz, H₆). 13C NMR spectrum (100 MHz, CDCl₃, 25 °C): δ_C = 156.68, 154.98, 153.33, 138.88, 138.54, 124.79, 121.16, 120.94, 117.45, 100.07, 61.64, 36.67, 33.75, 32.28, 30.04, 27.86, 22.99, 14.40. MS (MALDI-TOF): m/z (M + H⁺, 100%), Calcd for C₁₁₄H₁₄₁O₁₆N₄ + H⁺: 1823.0371; found: 1823.0197.

4.6.12 Synthesis of 100d

Prepared by procedure 3 from solutions of pentylene-1,5-diamine 17d in CHCl₃ (0.25 mg, 2.49 µmol, 10 mg/mL), TFA in CHCl₃ (2.2 µL, 1% v/v) and cavitand 85 in CHCl₃ (2.17 mg, 2.49 µmol, 10 mg/mL), 100d formed quantitatively after reacting overnight.
\(^1\)H NMR spectrum (300 MHz, CDCl\(_3\), 25 °C): \(\delta_H = 8.36\) (s, 4H, -\(\text{CH}_2\text{N-}\)), 7.12 (s, 4H, H\(_{a2}\)), 7.06 (s, 4H, H\(_{a1}\)), 6.43 (s, 4H, H\(_1\)), 5.63 (d, 8H, \(J = 7.3\) Hz, H\(_6\)), 4.76 (t, 8H, \(J = 8.1\) Hz, H\(_m\)), 4.41 (d, 8H, \(J = 7.3\) Hz, H\(_i\)), 3.51 (t, 8H, \(J = 6.6\) Hz, H\(_7\)), 2.28-2.15 (m, 16H, H\(_2\)), 1.46-1.29 (m, 60H, H\(_3\)-H\(_5\), H\(_8\) and H\(_9\)), 0.91 (t, 24H, \(J = 6.9\) Hz, H\(_6\)). \(^{13}\)C NMR spectrum (100 MHz, CDCl\(_3\), 25 °C): \(\delta_C = 156.74, 155.00, 153.16, 138.84, 138.53, 125.14, 120.94, 120.88, 117.36, 100.09, 62.64, 36.63, 32.30, 30.81, 30.05, 27.87, 25.07, 22.99, 14.40\). MS (MALDI-TOF): m/z (M + H\(^+\), 100%), Calcd for C\(_{118}\)H\(_{149}\)O\(_{16}\)N\(_4\) + H\(^+\): 1879.1007; found: 1879.1164.

**4.6.13 Synthesis of 100g**

Prepared by procedure 3 from solutions of \(m\)-phenylenediamine 17g in CHCl\(_3\) (0.29 mg, 2.65 µmol, 10 mg/mL), TFA in CHCl\(_3\) (2.3 µL, 1% v/v) and cavitand 85 in CHCl\(_3\) (2.31 mg, 2.65 µmol, 10 mg/mL), 100g formed quantitatively after reacting overnight.
$^1$H NMR spectrum (400 MHz, CDCl$_3$, 25 °C) $\delta_H = 8.55$ (s, 4H, -CHN-), 7.41 (t, 2H, $J = 7.9$ Hz, H$_9$), 7.22 (s, 4H, H$_{a2}$), 7.09 (s, 4H, H$_{a1}$), 6.87 (dd, 4H, $^3J = 7.9$ Hz, $^4J = 2.1$ Hz, H$_8$), 6.79 (t, 2H, $J = 2.1$ Hz, H$_7$), 6.47 (s, 4H, H$_1$), 5.67 (d, 8H, $J = 7.5$ Hz, H$_o$), 4.81 (t, 8H, $J = 8.1$ Hz, H$_m$), 4.56 (d, 8H, $J = 7.5$ Hz, H$_i$), 2.24 (m, 16H, H$_2$), 1.48-1.31 (m, 48H, H$_{3-5}$), 0.92 (t, 24H, $J = 6.9$ Hz, H$_6$). $^{13}$C NMR spectrum (100 MHz, CDCl$_3$, 25 °C): $\delta_C = 157.13, 155.08, 153.68, 153.28, 139.14, 138.63, 130.87, 124.75, 122.28, 120.93, 119.35, 117.50, 115.87, 100.36, 36.71, 32.33, 30.03, 27.87, 23.00, 14.40. MS (MALDI-TOF): m/z (M + H$^+$, 100%), Calcd for C$_{120}$H$_{137}$O$_{16}$N$_4$ + H$: 1891.0061; found: 1890.9877.

4.6.14 Synthesis of 101b and 102b

Prepared by procedure 3 from solutions of propylene-1,3-diamine 17b in CHCl$_3$ (1.70 mg, 22.9 µmol, 10 mg/mL), TFA in CHCl$_3$ (20 µL, 1% v/v) and cavitand 79 in CHCl$_3$ (20 mg, 22.9 µmol, 10 mg/mL), a mixture of 101b and 102b (ratio 1:3) was obtained. Pure 101b was crystallized from CH$_2$Cl$_2$-MeOH. The assignment of these crystals to 101b and the major component in the mixture to 102b is based on their NMR spectroscopic properties and diffusion constants. 101b has $C_{2h}$ symmetry,
whereas 102b has \( C_{2v} \) symmetry. Thus, the two protons on C8 of 101b are nonequivalent and those of 102b are equivalent. The HMQC NMR of 101b obtained by crystallization confirmed that there are two different protons on C8, which is consistent with the crystals being 101b and not 102b. In addition, the diffusivity of 101b, which was measured by DOSY, is smaller than that of 102b and again consistent with the assignment. 101b, which has a more compact structure, is expected to diffuse faster than 102b. Furthermore, proton H^1 of 101b is more shielded, which is in accordance with above assignment, too.

\[ 1^1H \text{ NMR spectrum for 101b (400 MHz, CDCl}_3, 25 \degree C): \delta_H = 8.35 (s, 4H, -CHN-), 7.14 (s, 4H, H_{a1}), 7.05 (s, 4H, H_{a2}), 6.48 (s, 4H, H_1), 5.70 (d, 2H, J = 7.5 Hz, H_o), 5.66 (d, 4H, J = 7.5 Hz, H_o), 5.61 (d, 2H, J = 7.5 Hz, H_o), 4.86 (t, 2H, J = 8.3 Hz, H_m), 4.77 (t, 4H, J = 8.3 Hz, H_m), 4.69 (t, 2H, J = 8.3 Hz, H_m), 4.52 (d, 2H, J = 7.5 Hz, H_i), 4.46-4.38 (m, 6H, H_i), 3.67-3.54 (m, 4H, H_7), 3.54-3.44 (m, 4H, H_7), 2.32-2.15 (m, 16H, H_2), 1.89-1.68 (m, 2H, H_3), 1.48-1.31 (m, 52H, H_3-H_5 and H_8), 0.96-0.88 (m, 24H, H_o). \]

\[ ^{13}C \text{ NMR spectrum for 101b (100 MHz, CDCl}_3, 25 \degree C): \delta_C = 156.70, 155.18, 155.02, 153.35, 153.14, 138.93, 138.84, 138.50, 138.35, 124.56, 121.44, 120.65, 117.10, 100.87, 100.11, 99.50, 61.41, 36.70, 36.60, 33.03, 32.27, 32.23, 30.18, 29.99, 29.94, 27.81, 22.94, 14.35. (MALDI-TOF): m/z (M + H^+, 100\%), \text{Caled for} \]
C_{114}H_{141}O_{16}N_{4} + H^+ : 1823.0371; found: 1823.0248.

4.6.15 Synthesis of 101d and 102d

Prepared by procedure 3 from solutions of pentylene-1,5-diamine 17d in CHCl₃ (0.29 mg, 2.82 µmol, 10 mg/mL), TFA in CHCl₃ (2.5 µL, 1% v/v) and cavitand 79 in CHCl₃ (2.46 mg, 2.82 µmol, 10 mg/mL), a mixture of 101d and 102d (ratio 1:1) was obtained after reacting overnight. Pure 102d was crystallized from CH₂Cl₂-MeOH. According to ¹H and DOSY NMR spectra of these crystals, H¹ is less shielded than that of the other isomer and it diffuses faster than the other isomer, so it is assigned to 102d.

¹H NMR spectrum for 102d (500 MHz, CDCl₃, 25 °C): δ_H = 8.31 (s, 4H, -CHN-), 7.13 (s, 4H, H_{a1}), 7.05 (s, 4H, H_{a2}), 6.51 (s, 4H, H₁), 5.72 (d, 2H, J = 7.4 Hz, H₀), 5.66 (d, 4H, J = 7.4 Hz, H₀), 5.52 (d, 2H, J = 7.4 Hz, H₀), 4.85 (t, 2H, J = 8.3 Hz, Hₘ), 4.76 (t, 4H, J = 8.3 Hz, Hₘ), 4.69 (t, 2H, J = 8.3 Hz, Hₘ), 4.55 (d, 2H, J = 7.4 Hz, H₁), 4.41 (d, 4H, J = 7.4 Hz, H₁), 4.35 (d, 2H, J = 7.4 Hz, H₁), 3.54-3.47 (m, 4H, H₇), 3.46-3.37 (m, 4H, H₇), 2.27-2.16 (m, 16H, H₂), 1.48-1.31 (m, 66H, H₃-H₅, H₈ and H₀), 0.96-0.87 (m, 24H, H₆). ¹³C NMR spectrum for 102d (100 MHz, CDCl₃, 25 °C): δ_C = 156.53, 155.27, 155.10, 153.45, 153.14, 138.94, 138.87, 138.60, 138.39, 124.71, 124.41,
120.71, 117.19, 100.90, 100.05, 99.59, 62.78, 36.64, 32.28, 32.32, 32.29, 32.23, 31.33, 30.22, 30.07, 29.96, 27.86, 25.48, 23.01, 14.41. MS (MALDI-TOF): m/z (M + H⁺, 100%), Calcd for C₁₁₈H₁₄₉O₁₆N₄ + H⁺: 1879.1002; found: 1879.0848.

4.6.16 Synthesis of 101g and 102g and their reduction products 105 and 106

Prepared by procedure 3 from solutions of m-phenylenediamine in CHCl₃ (10.32 mg, 95.56 μmol, 10 mg/mL), TFA in CHCl₃ (80 μL, 1% v/v) and cavitand 79 in CHCl₃ (83.33 mg, 95.56 μmol, 10 mg/mL), a mixture of 101g and 102g (ratio, 2:1) was obtained after overnight. According to the chemical shift of ¹H and the DOSY of both isomers (see above), both isomers were assigned. Since isolation of the isomers by crystallization was not possible, they were reduced to the corresponding amine macrocycles 105 and 106, which could be separated. The mixture was dissolved in THF (20 mL) and Ni(OAc)₂ (52 mg, 196.7 μmol) was added. Under stirring, NaBH₃CN in THF (1.1 mL, 1 M) was added dropwise over 10 minutes. After 20 hours, the solvent was evaporated and a dark solid was obtained. H₂O (20 mL) and NH₃.H₂O (5 mL) were added forming a white precipitate. The mixture was extracted with CH₂Cl₂ (40 mL). The organic layer was washed with sat. NaHCO₃ solution (20 mL). The organic solution was collected, dried over MgSO₄ and concentrated in vacuum to yield a pale yellow solid. 105 (50%) and 106 (21%) were separated and isolated by silica gel column chromatography (EtOAc-CH₂Cl₂ = 1:9).
1H NMR spectrum for 105 (300 MHz, CDCl$_3$, 25 °C): $\delta_H = 7.14$ (s, 4H, H$_{a1}$), 7.12 (s, 4H, H$_{a2}$), 7.02 (t, 2H, $J = 8.2$ Hz, H$_9$), 6.49 (s, 4H, H$_1$), 6.18 (dd, 4H, $^{3}J = 6.2$ Hz, $^{4}J = 1.8$ Hz, H$_8$), 5.91 (d, 2H, $J = 7.2$ Hz, H$_{o7}$), 5.85 (t, 2H, $J = 1.8$ Hz, H$_7$), 5.80 (d, 2H, $J = 7.2$ Hz, H$_{o7}$), 5.75 (d, 4H, $J = 7.2$ Hz, H$_{o8}$), 4.83-4.70 (m, 8H, H$_m$), 4.44 (d, 2H, $J = 7.2$ Hz, H$_{i7}$), 4.42 (d, 2H, $J = 7.2$ Hz, H$_i$), 4.35 (d, 4H, $J = 7.2$ Hz, H$_4$), 4.08-3.97 (m, 4H, H$_{10o}$), 3.97-3.85 (m, 4H, H$_{10i}$), 2.32-2.13 (m, 16H, H$_2$), 1.48-1.30 (m, 48H, H$_3$-H$_5$), 0.96-0.87 (m, 24H, H$_6$).

13C NMR spectrum for 105 (125 MHz, CDCl$_3$, 25 °C): $\delta_C = 155.14$, 155.07, 154.04, 153.98, 149.93, 139.07, 138.86, 138.68, 130.43, 125.09, 121.07, 120.25, 116.56, 104.76, 100.13, 99.93, 99.79, 97.84, 38.62, 37.35, 37.03, 36.67, 32.32, 30.35, 30.29, 30.00, 27.94, 27.87, 22.98, 14.39. MS (MALDI-TOF): m/z (M + Na$^+$, 100%), Calcd for C$_{120}$H$_{144}$O$_{16}$N$_4$ + Na$: 1921.0486$; found: 1921.0560

1H NMR spectrum for 106 (300 MHz, CDCl$_3$, 25 °C): $\delta_H = 7.13$ (s, 4H, H$_{a1}$), 7.06 (s, 4H, H$_{a2}$), 6.93 (t, 2H, $J = 8.2$ Hz, H$_9$), 6.48 (s, 4H, H$_1$), 6.17 (t, 2H, $J = 1.8$ Hz, H$_7$), 6.08 (dd, 4H, $^{3}J = 6.2$ Hz, $^{4}J = 1.8$ Hz, H$_8$), 5.93 (d, 2H, $J = 7.2$ Hz, H$_{o7}$), 5.83 (d, 2H, $J = 7.2$ Hz, H$_7$), 5.76 (d, 4H, $J = 7.2$ Hz, H$_{o7}$), 4.83-4.70 (m, 8H, H$_m$), 4.49-4.26 (m, 8H, H$_4$), 3.97-3.79 (m, 8H, H$_{10}$), 2.32-2.13 (m, 20H, -NH$_2$- and H$_2$), 1.48-1.30 (m, 48H,
H$_3$-H$_5$), 0.96-0.87 (m, 24H, H$_6$). $^{13}$C NMR spectrum for 106 (100 MHz, CDCl$_3$, 25 °C): $\delta$C = 155.20, 155.17, 154.30, 153.48, 149.53, 138.93, 138.38, 138.34, 130.12, 125.63, 121.08, 120.08, 116.61, 105.47, 99.98, 99.66, 99.55, 38.65, 36.96, 36.77, 32.30, 31.97, 30.28, 30.09, 29.92, 27.87, 27.56, 22.97, 14.39. MS (MALDI-TOF): m/z (M + Na$^+$, 100%), Calcd for C$_{120}$H$_{144}$O$_{16}$N$_4$ + Na$: 1921.0486; found: 1921.0358.

4.6.17 Synthesis of 23

Cavitand 19 (29.70 mg, 32.00 µmol) and ethylene-1,2-diamine 17a (3.84 mg, 64 µmol) were dissolved in 1.2 mL CHCl$_3$-p-xylene (4:1). Under stirring, TFA in CHCl$_3$ (10 µL, 1% v/v) was added. After overnight, the solvent was removed and a yellow solid was obtained. It was dissolved in CHCl$_3$ and MeOH was layered on top of the solution. After a week, yellowish needle-shaped crystals of 23 were collected and dried in vacuum (60%).

$^{1}$H NMR spectrum (400 MHz, CDCl$_3$, 25 °C): $\delta$H = 8.28 (s, 24H, -CH/N-), 7.06 (s, 24H, H$_a$), 5.62 (d, 24H, $J$ = 7.37 Hz, H$_o$), 4.78 (t, 24H, $J$ = 7.90, H$_m$), 4.39 (d, 24H, $J$ = 7.55 Hz, H$_i$), 3.69 (s, 48H, H$_1$), 2.13 (m, 48H, H$_2$), 1.30 (m, 144H, H$_3$-H$_5$), 0.85 (t, 72H, $J$ = 7.07 Hz, H$_6$). $^{13}$C NMR spectrum (125 MHz, CDCl$_3$, 25 °C): $\delta$C = 157.58, 153.56, 138.76, 124.42, 121.66, 100.44, 63.12, 36.58, 32.21, 30.07, 27.81, 22.92,
14.30. MS was reported\textsuperscript{13}.

4.7 References


Chapter 5

Synthesis of Hemicarcerands and Kinetic Guest Encapsulation through Mechanochemistry

5.1 Introduction

To date, the majority of chemical reactions are performed in solvents. However, the disadvantages associated with traditional solvent-based methods, that include environmental hazards, high cost and high-energy consumption, combined with rapidly diminishing energy resources, have forced scientists to pursue alternative more efficient ways to synthesize materials. From this perspective, mechanosynthesis in form of grinding or ball-milling is a promising alternative method, since it requires no (neat grinding, NG) or only a small amount of solvent (liquid-assisted grinding, LAG) to form the product within a very short time ranging from several minutes to hours. More importantly, products, in most cases, can be produced quantitatively by simply grinding without further purification. By contrast, reactions in solvents often need longer time or higher temperature and subsequent purification steps are typically needed.

In solvent-based methods, collisions of reactant molecules lead to products, whereas reactions in mechanosynthesis are induced by mechanical energy, which is produced either by manual mortar-and-pestle grinding or by ball-milling. Although the detailed mechanism of mechanical energy transfer is still unclear, several simple models have been proposed attempting to explain the experimental results. According to the ‘hot spot’ or ‘magma-plasma’ model, the local temperature caused by surface friction or impact between the ball and inner surface of the container can reach hundreds to
thousands of degrees. The high temperature helps to overcome the reaction barrier and drives the reaction forward. However, they cannot explain why no decomposition was observed at such high temperature\textsuperscript{3d}. Since molecules are confined in lattice in the solid state, which prevents the mass transport of reactants, another theory proposes that upon grinding, an intermediate bulk phase is formed, which can be vapor, liquid (LAG or eutectic mixture), or an amorphous solid. In the intermediate phase, molecules can move relatively freely, which facilitates their reactivity\textsuperscript{3d, 4a-b}.

Mechanochemistry can be dated back to as early as the 4\textsuperscript{th} century BC and started to attract attention in the 1980’s due to its application in making cocrystals\textsuperscript{3d}. Since then, it has been intensively studied. In the past decade, mechanochemical syntheses have been used in organic synthesis, material sciences and supramolecular chemistry. For example, in the area of organic synthesis, several groups showed that under ball-milling conditions, C-C and C-X bonds form very efficiently\textsuperscript{5}.

In the field of material sciences, zeolite and metal organic frameworks (MOF) are among the most important materials. They can be used for gas storage\textsuperscript{6}, hydrocarbon adsorption\textsuperscript{7} and separation\textsuperscript{8} and sensor applications\textsuperscript{9}. Traditionally, they are prepared by using solvothermal techniques, which involve using a large amount of high boiling solvents under high temperature and pressure, producing a lot of waste and consuming very much energy. However, some MOFs can be produced at room temperature simply by grinding without or with a little added solvent. Some research groups showed that the addition of a small amount of liquid, namely liquid-assisted grinding (LAG), accelerated the reaction or lead to different morphologies\textsuperscript{3a, 10}, which cannot be accessed through solvent-based methods. Presumably, the solvent either
improves the mobility of reactants and/or templates products, which can increase the reaction rate or lead to different products. Similarly, the Friščič group reported that by adding small amounts of templating salts to reactants, so called ion-liquid-assisted grinding (ILAG), MOFs with different topologies could be produced\textsuperscript{11}. Again, in solvents, only one type of MOF forms. It is likely that in solvent-based methods, solvent molecules are present in majority and preferentially enter the cavity of the MOF. Even if external templates are added, they cannot compete with solvent due to their low concentration. On the contrary, by using mechanochemical synthesis, only very small amounts of solvent are added, if any, and the amount of added salts is comparable to that of solvent. As a result, a much stronger template effect is observed.

Recently, mechanosynthesis has also been used to explore supramolecular chemistry. An extraordinary example is the synthesis of hydrogen-bonded octahedral nanocapsule 109, which was reported by Atwood’s group\textsuperscript{12}. A mixture of isovaleraldehyde 37c, pyrogallol 107 and catalytic amount of $p$-toluenesulfonic acid was grinded using a mortar-and-pestle for 5 minutes, yielding quantitatively nanocapsule 109. This is remarkable, because this reaction involved the initial formation of pyrogallol[4]arenes 108, which self-assembled into 109 requiring formation of 48 hydrogen bonds, all in one pot in such a short time, which is significantly faster than in solution (Scheme 5-1).
Scheme 5-1: One-pot synthesis of hydrogen-bonded octahedral nanocapsules 109 through grinding.

The assembly of covalent capsules through grinding is rare and was pioneered by the Severin group. They synthesized two cage molecules 113 and 114 by neat grinding a mixture of pentaerythritol 111, 1,3,5-trisaminomethyl-2,4,6-triethylbenzene 17r and 4-formylphenylboronic acid 110 or 4-(4-formylphenyl)-phenylboronic acid 112, respectively (Scheme 5-2). Surprisingly, the yield obtained by grinding is much higher than that in solution, considering possible competing polymerization at such high concentration. Although there was no direct evidence for polymerization, the author explained the higher yield with capsule templation by reactants during grinding.
Later, the same group successfully made two borasiloxane-based macrocycles through ball-milling\(^{14}\). Again, compared with solvent-based methods, a higher yield was obtained. Inspired by this work, we investigated several imine-based hemicarcerand syntheses through neat and LAG.

![Scheme 5-2: Synthesis of cage molecules 113 and 114 by ball-milling (reprinted with permission from the reference\(^{13}\)).](image)

Guest encapsulation inside hemicarcerands through mechanochemistry was also studied in this chapter. Traditionally, guest encapsulation is performed in solution at high temperature. In solution phase encapsulation, the choice of solvent is very important. In order to avoid solvent competition, the solvent should be bulky, so that it cannot pass through a host portal and only the guest enters the cavity. A second aspect is the solubility of the host and the guest. Both should be good soluble in the
solvent either at ambient temperature or at higher temperature. The third factor that should be considered is the thermo-stability of the host and the guest, since high temperature, typically 100 °C, is required to overcome the activation barrier during encapsulation. In previous studies, bulky phosphorus containing solvents were employed, of which most are highly toxic and environmentally unfriendly. Considering all of this, a solid-state approach for guest encapsulation is highly desirable as an alternative, milder and environmental friendly method.

5.2 Mechanosynthesis of hemicarcerands

5.2.1 Instrument setup

Mechanosynthesis is often performed either manually in the form of mortar-and-pestle or automatically by using a ball mill. The disadvantage of the former method is the low efficiency and sometimes, uneven grinding. The latter suffers from a relative high price. Therefore, in this study, we used a vortex grinding approach, which was recently introduced to the solid state synthesis by the MacGillivray group. As shown in Fig. 5-1, the sample or reactants and two stainless steel balls (diameter ¼ inch) are loaded in a culture glass tube, which is closed with a septum. The tube is mounted onto the vortex and secured with a clamp and vortexed at 2500 rpm. During vortexing, two balls rotate around the walls and the solid in the tube is evenly smeared around the glass walls, which could possible accelerate the mass transport. This method simulates more the effect of mortar-and-pestle grinding, rather than ball-milling, in which the balls collide with the reaction vessel.
5.2.2 Optimizing conditions

Before synthesis of hemicarcerands, the synthesis of capsule 116 was tested as a model reaction. In solution, two equivalents of 17t and three equivalents of 29 react to yield 116 and six newly formed imine bonds. To start the reaction, solid 17t and 29 were loaded in a glass tube together with two stainless steel balls. The mixture was vortexed at 2500 rpm (Scheme 5-3). After several minutes, the mixture became yellowish and gradually reddish, which indicates formation of imines, since cage 116 is a red solid. Within 1 hour, the cage quantitatively formed as determined by $^1$H NMR spectroscopy and MALDI-TOF MS.
Knowing that this method can be used to synthesize imine-based molecular cages, the solid-state synthesis of more complex hemicarcerand 20g was explored. After vortexing a mixture of cavitand 19 and m-phenylenediamine 17g, a yellowish solid was obtained (Scheme 5-4). The product was not fully soluble in CDCl₃ and ¹H NMR spectrum of the supernatant showed a broad imine peak at 8.71-8.35 ppm indicating the formation of fragments and/or polymeric products (Fig. 5-2A). Extending the grinding time only resulted in more precipitate.

**Scheme 5-4:** Synthesis of hemicarcerand 20g through NG or LAG.

In the absence of an acid catalyst, the transamination is very slow in solution and presumably, even slower in the solid state due to inefficient mass transfer. To improve
reaction rate, the catalyst TFA or p-toluenesulfonic acid was grinded together with the mixture, respectively. Indeed, small amounts of hemicarcerand 20g formed, but the precipitate was still the dominant product. In fact, the formation of polymeric product is not surprising considering the high concentration.

According to the effective molarity (EM) studies described in chapter 4, a template can significantly increase the EM of hemicarcerands, which can then effectively compete with polymerization. A suitable template is ferrocene 117, which strongly binds inside hemicarcerand 20g17. Therefore, fifty equivalents of 117 were grinded together with the above mixture for 1 hour. Unfortunately, no obvious improvement was observed.

By carefully analyzing the model reaction system leading quantitatively to 116, we noted that the melting points of both reactants 17t and 115 are relatively low and, during grinding, an eutectic mixture could form, in which the mobility of both reactants is enhanced, thus helping molecules to reorganize themselves and eventually form the cage molecule. However, in the hemicarcerand reaction mixture, formation of an eutectic phase is highly unlikely due to the high melting point of cavitand 19. Therefore, LAG was tested18. Since both reactants are soluble in CH₂Cl₂, and CH₂Cl₂ is also a good template, a small amount of CH₂Cl₂ and TFA was added to the mixture. After 1 hour grinding, the ¹H NMR spectrum of the reaction products showed that 20g quantitatively formed. The added solvent, in a way, could work as “lubricant”, which facilitated the mobility of reactants and quantitatively yielded the product. In fact, it has been suggested that at least one of the reactants has to be soluble in the liquid component for LAG to be effective19.
5.2.3 Optimizing $\eta$ value

The amount of the solvent required in the LAG is characterized by the empirical parameter $\eta$, which is defined as the ratio of volume of solvent in $\mu$L over the mass of reactants in mg$^{19}$. In this chapter, we report $\eta$ as defined. However, $\eta$ may be much smaller in the solid sample and may decrease over time, since the reaction tube cannot be tightly sealed leading to partial evaporation of solvent during the grinding. In fact, based on the $^1$H NMR spectrum of products, only trace amounts of solvent were left after grinding. Therefore, the $\eta$ value reported in this thesis is only the initial ratio. Typically, the $\eta$ value for LAG is between 0.1-2. Therefore, the amount of liquid was optimized within this range.

![Figure 5-2: Partial $^1$H NMR spectra (400 MHz, CDCl$_3$, 25 °C) of grinding mixtures with $\eta$ of 0 (A), 0.2 (B), 0.4 (C) and 0.8 (D).](image)

$^1$H NMR spectra of mixtures with different $\eta$ values are shown in Fig. 5-2. Without added solvent, signals were very broad and no hemicarcerand formed. At $\eta = 0.2$, significant broadening was also observed and small amounts of hemicarcerand started to form. As $\eta$ was increased to 0.4, the amount of hemicarcerand increased
accordingly. At $\eta = 0.8$, the hemicarcerand formed quantitatively. Therefore, $\eta = 0.8$ was used in all of the following studies.

5.2.4 Solvent and template effects in the assembly of hemicarcerands

The nature of the liquid component in LAG was also investigated (Table 5-1, entry 1-8). From previous EM studies, hemicarcerands have highest stability (EM) in CH$_2$Cl$_2$ and CHCl$_3$, and much lower stability (EM) in CHCl$_2$CHCl$_2$, which agrees with the results of LAG. The lower stability (EM) in CHCl$_2$CHCl$_2$ suggests that CHCl$_2$CHCl$_2$ is a poor template, which explains the lower yield in LAG. With added CHCl$_2$CHCl$_2$, polymerization can better compete with hemicarcerand formation as compared to CHCl$_3$ and CH$_2$Cl$_2$. Although EMs in CH$_2$CICH$_2$Cl and THF were not measured, we believe they should be comparable with those in CHCl$_3$ and CH$_2$Cl$_2$, given the quantitative yield with these solvents. $p$-Xylene is likely a poor template for 20g. Based on the CPK models, one $p$-xylene doesn’t fit into the cavity properly, whereas two $p$-xylene molecules are too large to fit into the cavity. On the contrary, two molecules of the smaller toluene and benzene fit very well into the cavity and lead to a quantitative yield. In summary, this study shows that a high mobility of reactants and proper templates are essential for the formation of the hemicarcerand under LAG conditions.
Table 5-1: Solvent effects on the formation of hemicarcerand 20g by LAG.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent or Template</th>
<th>Hemicarcerand</th>
<th>%Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CH₂Cl₂</td>
<td>Y</td>
<td>Quant.</td>
</tr>
<tr>
<td>2</td>
<td>CHCl₃</td>
<td>Y</td>
<td>Quant.</td>
</tr>
<tr>
<td>3</td>
<td>CH₂ClCH₂Cl</td>
<td>Y</td>
<td>Quant.</td>
</tr>
<tr>
<td>4</td>
<td>CHCl₂CHCl₂</td>
<td>Y</td>
<td>45%</td>
</tr>
<tr>
<td>5</td>
<td>THF</td>
<td>Y</td>
<td>Quant.</td>
</tr>
<tr>
<td>6</td>
<td>p-Xylene</td>
<td>Y</td>
<td>55%</td>
</tr>
<tr>
<td>7</td>
<td>Toluene</td>
<td>Y</td>
<td>Quant.</td>
</tr>
<tr>
<td>8</td>
<td>Benzene</td>
<td>Y</td>
<td>Quant.</td>
</tr>
<tr>
<td>9</td>
<td>Menthol</td>
<td>Y</td>
<td>&lt; 10%</td>
</tr>
<tr>
<td>10</td>
<td>Ferrocene</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

5.2.5 Synthesis of hemicarcerands with different linkers

Using above optimized conditions, the condensation of cavitand 19 with linkers 17b-d, g, h, j-o were tested and the results are summarized in Table 5-2. This study shows that linker 17g, j, l, m, n give the corresponding hemicarcerands quantitatively either by LAG or in solution, whereas no hemicarcerand formed with linkers 17k and 17o using above two methods. When linkers 17b-d, h were used (entries 8-11), LAG gave much lower yields than those in solutions. Linkers 17g, j, l, m, n have in common that they are conformationally very rigid, whereas linkers 17b-d, h are conformationally more flexible. Flexibility increases in the order 17h < 17b < 17c < 17d, which roughly correlates inversely with the yields in the LAG.
From this study, it can be concluded that rigid linkers (entries 1-5) give higher yields than flexible linkers (entries 8-11). The aromatic diamines $17_{g,h,j,m,n}$, which are very rigid, led to the quantitative formation of hemicarcerands. For the aliphatic and benzylic diamines $17_{b-d,h}$, the flexibility increases as the length increases and the yield dropped dramatically. For example, compared with propylenediamine $17_{b}$, which yielded 40% hemicarcerand $20_{b}$, butylenediamine $17_{c}$ and pentylenediamine $17_{d}$ only gave trace amounts of hemicarcerands. The reason for the failure of 1,8-diaminonaphthalene $17_{o}$ to yield $20_{o}$ may be the fact that formation of hemicarcerands requires linkers with roughly a 120° angle between the two C-N bonds, which is not possible for $17_{o}$. Therefore, neither method gave the hemicarcerand.
**Table 5-2:** Hemicarcerand synthesis by LAG and in the solution.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Linker</th>
<th>Hemicarcerand</th>
<th>Yield% LAG (assisted solvent)</th>
<th>Yield% Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17b</td>
<td>20b</td>
<td>40% (CH(_2)Cl(_2))</td>
<td>Quant. (CHCl(_3))</td>
</tr>
<tr>
<td>2</td>
<td>17c</td>
<td>20c</td>
<td>&lt; 10% (CH(_2)Cl(_2))</td>
<td>Quant. (CHCl(_3))</td>
</tr>
<tr>
<td>3</td>
<td>17d</td>
<td>20d</td>
<td>&lt; 10% (CH(_2)Cl(_2))</td>
<td>Quant. (CHCl(_3))</td>
</tr>
<tr>
<td>4</td>
<td>17g</td>
<td>20g</td>
<td>Quant. (CH(_2)Cl(_2))</td>
<td>Quant. (CHCl(_3))</td>
</tr>
<tr>
<td>5</td>
<td>17h</td>
<td>20h</td>
<td>&lt; 10% (CH(_2)Cl(_2))</td>
<td>Quant. (CHCl(_3))</td>
</tr>
<tr>
<td>6</td>
<td>17j</td>
<td>20j</td>
<td>Quant. (CH(_2)Cl(_2))</td>
<td>Quant. (CHCl(_3))</td>
</tr>
<tr>
<td>7</td>
<td>17k</td>
<td>20k</td>
<td>NA (CH(_2)Cl(_2))</td>
<td>132 (CHCl(_3))</td>
</tr>
<tr>
<td>8</td>
<td>17l</td>
<td>20l</td>
<td>Quant. (DMSO)</td>
<td>Quant.</td>
</tr>
<tr>
<td>9</td>
<td>17m</td>
<td>20m</td>
<td>Quant. (CH(_2)Cl(_2))</td>
<td>Quant. (CHCl(_3))</td>
</tr>
<tr>
<td>10</td>
<td>17n</td>
<td>20n</td>
<td>Quant. (CH(_2)Cl(_2))</td>
<td>Quant. (CHCl(_3))</td>
</tr>
<tr>
<td>11</td>
<td>17o</td>
<td>20o</td>
<td>NA (CH(_2)Cl(_2))</td>
<td>NA (CHCl(_3))</td>
</tr>
</tbody>
</table>

*Conditions: Reactants, CH\(_2\)Cl\(_2\) (\(\eta = 0.8\)) and catalytic amount of TFA was added and then it was vortexed for 1h; \(^b\)Conditions: cavitand and linker were dissolved in CHCl\(_3\), followed by the addition of catalytic amounts of TFA. The reaction is completed within 1 hour.*

In the case of 1,1'-biphenyl-3,3'-diamine 17k, the \(^1\)H NMR spectrum and MALDI-TOF MS indicated the formation of a tricavitand macrocycle 132 in solution. By grinding, only polymer was produced. Possibly, rotation around the Ph-Ph bond and the twisted conformation of the linker may not be suitable for the formation of 20k.

When studying the effect of solubility of reactants in the liquid component on the formation of cocrystals by LAG, T. Friščič proposed that the solubility of reactants is likely to affect the rate of cocrystalization\(^19\). This conclusion is consistent with our observation in the formation of hemicarcerand 20l. For example, 20l didn’t form in LAG with CH\(_2\)Cl\(_2\). But with DMSO, quantitative hemicarcerand formation was obtained. Since 17l has a very low solubility in CH\(_2\)Cl\(_2\), transamination is very slow and thus didn’t produce 20l. In contrast to this, both reactants have reasonable solubility in DMSO, which enhances the transamination and yielded 20l.
5.3 Guest encapsulation through vortex grinding

5.3.1 Hemicarcerand 20g

5.3.1.1 Guest screen

Earlier, D. J. Cram and coworkers extensively studied the encapsulation properties of hemicarcerand 18g in trippiperidinophosphine oxide, showing that it encapsulates a wide range of guests\textsuperscript{17a}. Among these guests, ferrocene 117 forms the thermodynamically most stable hemicarceplex. Therefore, mechanochemical encapsulation of 117 was first investigated.

Prior to grinding, hemicarcerand 20g was heated overnight at 120 °C in vacuum, which removes all solvent molecules trapped in the cavity. Empty 20g and 117 (1:50}
in moles) were vortexed at 2500 rpm and the encapsulation process was followed by
$^1$H NMR spectroscopy (Fig. 5-3). After 0.5 h vortexing, a new set of signals, which
belongs to hemicarceplex 20g⊙117, appeared in addition to the signals of empty 20g.
Integration indicates a 1:1 mixture of 20g⊙117 and 20g. The singlet at 3.66 ppm is
assigned to the encapsulated guest 117 and is upfield shifted 0.54 ppm compared to
that of free 117 at 4.2 ppm. The complexation induced shift results from the shielding
effect of the aromatic units of 20g and was observed for all guests. Longer vortexing
resulted in increased complex formation until only complex and free 117 were
observed after 3 hours. The mixture was thoroughly washed with hexane, which
removed excess 117 and the yellowish solid of 20g⊙117 was obtained.

![Figure 5-3: $^1$H NMR spectrum (300 MHz, CDCl$_3$, 25 °C) showing the encapsulation progress of 117 after 30 minutes, 1 hour, 2 hours, and 3 hours (from top to bottom). Signals assigned to the complex and the encapsulated guest are marked with red arrows and a triangle, respectively.](image)

Figure 5-3: $^1$H NMR spectrum (300 MHz, CDCl$_3$, 25 °C) showing the encapsulation progress of 117 after 30 minutes, 1 hour, 2 hours, and 3 hours (from top to bottom). Signals assigned to the complex and the encapsulated guest are marked with red arrows and a triangle, respectively.
Chart 5-1: Molecular recognition properties of hemicarcerand 20g.

<table>
<thead>
<tr>
<th>Encapsulated Guests</th>
<th>Not Complexed Guests</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="chart5-1_encapsulated_guests.png" alt="Image" /></td>
<td><img src="chart5-1_not_complexed_guests.png" alt="Image" /></td>
</tr>
</tbody>
</table>

a) Time required for the complete formation of hemicarceplexes is listed in parentheses.

Since 117 can be successfully encapsulated by vortexing, other guests were tested under the same conditions. Chart 5-1 lists the encapsulated guests and those that failed to be encapsulated. Among the encapsulated guests, time required to completely form hemicarceplexes is also shown. For camphor 122, the conditions are slightly modified, since simply vortexing the mixture of 20g and 122 wouldn’t give the complex 20g⊙122. However, the addition of a small amount of TFA to the mixture yielded the complex. Presumably, TFA helps to break imine bonds, which enlarges the portals of 20g, followed by camphor slipping into the cavity. To optimize the TFA amount, two samples were prepared. One sample was the acid-free hemicarcerand (H₀) and the other the mono-protonated hemicarcerand (Hₐ), which was synthesized by adding one equivalent of TFA to the CH₂Cl₂ solution of 20g, followed by evaporation of the solvent. The above two samples were mixed in different ratios and vortexed with 122.
for 1 hour. The resulting mixture was subjected to $^1$H NMR spectroscopy. It demonstrated that the 1:1 mixture of Hf and Ha gave the highest yield. In other word, 6.3% TFA per imine is the optimum. Although 20g⊙117 can be obtained in this way, extended vortexing slowly cleaved the acetal moiety of 20g. Therefore, in the presence of TFA, the reaction time is limited to 3 hours and the product must be further purified by column chromatography.

Encapsulation of 117 and 118 required longer time than that of other guests (Chart 5-1). The possible reason could lie in the different shapes of guest molecules. CPK models revealed that portals of 20g have a rectangular cross section with length of 4-7 Å and width of 2-4 Å, respectively\textsuperscript{17a,20}. Thus, guests with long and thin shapes can enter the cavity easily and those with round shapes need longer time. Therefore, round shaped 117 and 118 are expected to enter the cavity slower. Another example showing that 20g is sensitive to the molecular shape is the pair 4-acetoxbenzoic acid 124 and aspirin 128. They have the same functional groups but different substitution patterns. 124 can form the complex completely within a short time but 128 cannot give the corresponding complex, which is consistent with binding studies and template studies of other hemicarcerands\textsuperscript{21}.

5.3.1.2 Guest selectivity

In order to explore differences in the guest selectivity in the solid state and liquid phase, competitive complexation studies were carried out in a solvent that is too bulky to enter the cavity of 20g and in the solid state. In vortex grinding studies, 20g and an excess of two guests were vortexed for 3 hours and the resulting product mixture was analyzed by $^1$H NMR spectroscopy. In the solution studies, 1,3,5-triisopropylbenzene
was used. Accordingly, the reactants were suspended in the solvent, heated to 100 °C, which gave a transparent solution, and were stirred at that temperature overnight. After cooling to room temperature, the solution was treated with methanol and the hemicarceplex mixture was filtered and analyzed by $^1$H NMR spectroscopy (Table 5-3).

**Table 5-3:** Guest selectivity in the solvent and in the solid state.

<table>
<thead>
<tr>
<th>Entry</th>
<th>G1 (equivalent)</th>
<th>G2 (equivalent)</th>
<th>20g⊙G1: 20g⊙G2 vortex grinding</th>
<th>20g⊙G1: 20g⊙G2 1,3,5-triisopropylbenzene</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>117 (50)</td>
<td>118 (50)</td>
<td>1:6</td>
<td>1:4.5</td>
</tr>
<tr>
<td>2</td>
<td>117 (50)</td>
<td>121 (50)</td>
<td>1:14</td>
<td>3.5:1</td>
</tr>
<tr>
<td>3</td>
<td>117 (50)</td>
<td>119 (50)</td>
<td>0:1</td>
<td>1:3.7</td>
</tr>
<tr>
<td>4</td>
<td>121 (50)</td>
<td>119 (50)</td>
<td>0:1</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>121 (50)</td>
<td>119 (5)</td>
<td>1:9</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>122 (50)</td>
<td>119 (50)</td>
<td>0:1</td>
<td>0:1</td>
</tr>
</tbody>
</table>

For the guest pair of 117 and 118, the ruthenocene hemicarceplex 20g⊙118 is the major product in both methods but higher selectivity was observed in the vortex grinding method. We explain the higher selectivity for 20g⊙118 with the different sandwich structures of 117 and 118. The two cyclopentadienyl rings in 117 are staggered but are eclipsed in 118 due to the larger radius of the ruthenium atom. Therefore, 117 is slightly wider than 118 and more difficult to pass through a host’s portal. In solution, the portal size of 20g can be altered through gating mechanisms and only a small selectivity is observed. However, in the solid state, 20g is likely very rigid, which may increase the barrier for 117 even more compared to that of 118. This could be the reason for the higher selectivity.
For the pair of 117 and 121, the selectivity is reversed by the two methods. In the vortex grinding method, the formation of 20g⊙121 was much more favored. However, in solution, 20g⊙117 was the major product. This difference may originate from the fact that encapsulation by vortex grinding is under kinetic control, whereas it is under thermodynamic control at 100 °C in solution. 121 is a molecule with a small cross-section and preferentially enters the cavity during vortex grinding as compared to 117, which has a larger cross-section. On the other hand, as will be discussed in the next section, 20g⊙117 is thermodynamically more stable so that in solution, 20g⊙117 becomes the major product after equilibration.

Remarkably, only the menthol hemicarceplex 20g⊙119 was obtained by vortex grinding equal amounts of 119 and 121 with 20g. Selectivity could only be measured when a 10-fold excess of 121 was used. Under the same conditions, much lower selectivity was observed in solution. Again, the different results may arise from the different control processes.

Finally, as noted in section 5.3.1.1, without addition of TFA, camphor hemicarceplex 20g⊙122 cannot be formed by vortex grinding, which explains that 20g⊙119 is the only product in entry 6.

5.3.1.3 Kinetic versus thermodynamic control in solid-state encapsulation via vortex grinding

In order to confirm that the encapsulation by vortex grinding is under kinetic control, pre-made 20g⊙117 was vortex grinded with fifty equivalents of 119. Since 20g⊙119 is favored in the solid state, as discussed above, 20g⊙119 is expected to form, if
guests can exchange in the solid-state and encapsulation is under thermodynamic control. However, after 1 hour grinding, no $20g \bigcirc 119$ was detected by $^1$H NMR spectroscopy. Longer grinding gave the same result. To further confirm kinetic control, a dichloromethane hemicarceplex $20g \bigcirc CH_2Cl_2$ was prepared and grinded with fifty equivalents of $117$ for 1 hour. No $20g \bigcirc 117$ was detected by $^1$H NMR spectroscopy. Since CH$_2$Cl$_2$ has a very low binding affinity for $20g$, and is small enough to be exchanged without high activation barrier, the absence of CH$_2$Cl$_2$-$117$ exchange suggests that guest encapsulation in the absence of TFA must be under kinetic control.

On the other hand, since $122$ can be encapsulated in the presence of TFA, but not in its absence, we propose that in the presence of TFA, guest exchange by vortex grinding is under thermodynamic control. For example, in the presence of TFA, a mixture of $20g \bigcirc CH_2Cl_2$ and excess $117$ gave a small amount of $20g \bigcirc 117$ after grinding for 1 hour. Longer grinding (e.g. 2 hours) resulted in the formation of 20% $20g \bigcirc 117$ but also resulted in an increased amount of acetal cleavage. Therefore, the guest selectivity in the solid state can be controlled by addition of TFA.
5.3.1.4 Kinetics of ferrocene and anthracene complexation in 1,3,5-triisopropylbenzene

Figure 5-4: Partial $^1$H NMR spectrum (500 MHz, CDCl$_3$, 25 °C) showing the guest exchange between ferrocene and anthracene after 10 (A), 20 (B), 40 (C), 60 (D), 180 (E), 240 (F), 300 (G), 360 minutes (H). Signals of ferrocene hemicarceplex are marked with arrows in (A).
In order to determine kinetic and thermodynamic selectivity in the complexation of 117 and 121 in 1,3,5-triisopropylbenzene at 100 °C, complexation was followed by \textsuperscript{1}H NMR spectroscopy. Empty hemicarcerand 20g, 117 and 121 were dissolved in 1 mL 1,3,5-triisopropylbenzene at 100 °C. Samples were taken after 10, 20, 40, 60, 180, 240, 300 and 360 minutes and transferred to 500 µL CDCl\textsubscript{3} (Fig 5-4). The ratio of the two hemicarceplexes was determined by \textsuperscript{1}H NMR spectroscopy. A plot of %hemicarceplex formed versus time shows that initially 20g⊙121 mainly forms, which is the kinetic product (Fig. 5-5). But, over time, it slowly exchanged and 20g⊙117 dominated after ~ 50 minutes. The kinetic selectivity is about \[\frac{[20g⊙121]}{[20g⊙117]} = \sim 9:1\], which is similar to the selectivity in the vortex grinding experiment. This further supports our conclusion that guest exchange by vortex grinding is under kinetic control in the absence of TFA.

\textbf{Figure 5-5}: Percentage of hemicarceplex at different time upon heating 20g with 1:1 mixture of 121 and 117 in 1,3,5-triisopropylbenzene at 100 °C.
5.3.2 Encapsulation properties of hemicarcerands 20b and 20c

Besides hemicarcerand 20g, the encapsulation properties of the more flexible hemicarcerands 20b and 20c were also studied. For 20b, only 119, 120 and 123 can be encapsulated in the solid state. 117 and 121 could not be encapsulated probably due to the smaller cavity size of 20b as compared to that of 20g (Chart 5-2). Additionally, 20b may easily collapse due to the flexible linkers, which makes the portals smaller in the solid state than those of the rigid hemicarcerand 20g, which is supported by the longer time required to form hemicarceplexes with the same guests in the solid-state. For example, hemicarerand 20g can completely encapsulate 123 within 1 hour, whereas 3 hours are needed for hemicarcerand 20b.

Chart 5-2: Molecular recognition properties of hemicarcerands 20b and 20c in the solid-state.
Hemicarcerand 20c assembled from the longer diamine 17c and has larger portals and a larger cavity than those of 20b and 20g. As a consequence, it encapsulates adamantane 125 and amantadine 126, which cannot be encapsulated by the other two hemicarcerands in the solid-state (Chart 5-2). Although hemicarceplexes 20c⊙119 and 20c⊙122 were not observed by ¹H NMR spectroscopy, it doesn’t mean that 119 and 122 cannot enter 20c through vortex grinding, but simply exchange with solvent molecules before an ¹H NMR spectrum of the products can be recorded.

5.3.3 Decomplexation

As discussed in chapter 1, guests are not permanently trapped in hemicarceplexes. In appropriate solvents, guests can be displaced with solvent molecules either at ambient temperature or at higher temperatures (Eq. 5-1). In principle, the reaction is reversible, but the solvent is present in large excess compared to the guest. Therefore, the reverse reaction is negligible and the decomplexation process can be treated as a pseudo first order reaction (Eq. 5-2) with a half-life (t₁/₂) that is given by Eq. 5-3.

\[
H⊙G + S \rightleftharpoons \underset{k^-}{k^+} \rightarrow H⊙S + G
\]  \hspace{1cm} (5-1)

\[ r = k_1 \times [H⊙G] \]  \hspace{1cm} (5-2)

\[ t_{1/2} = \frac{\ln 2}{k_1} \]  \hspace{1cm} (5-3)
**Figure 5-6**: Partial $^1$H NMR spectrum (400 MHz, CDCl$_3$, 25°C) showing the decomplexation process of $20g$⊙123 after 0, 30, 60, 180, 360 and 480 minutes (top to bottom). Signals of $20g$ and 123 are labeled with red and black arrows, respectively.

For example, Fig. 5-6 shows the decomplexation profile of $20g$⊙123. Over time, the signals of empty $20g$ gradually grow. The amount of empty $20g$, [H], can be calculated from the integration of host signals. A plot of ln[H] against time t gave a straight line, whose slope is rate constant $k_1$. The half-life ($t_{1/2}$) was calculated according to Eq. 5-3.

Half-life ($t_{1/2}$) of selected hemicarceplexes are summarized in Table 5-4. Interestingly, guests 120 and 123, show different stability with hemicarcerands 20b and 20g in CDCl$_3$. $20g$⊙120 is more stable than $20b$⊙120, but $20g$⊙123 is less stable than $20b$⊙123. Presumably, since the methyl groups of 123 can form CH-π interactions
with the cavitands of hemicarcerands and the flexible hemicarcerand 20b can better rearrange itself, a tighter hemicarceplex 20b⊙123 forms, which increases its stability. This is supported by the $^1$H NMR spectra of both hemicarceplexes. For example, the spectrum of 20g⊙123 shows only one multiplet of host signals although the guest is asymmetric. It indicates that the tumbling rate of the guest is fast on the NMR time scale. In contrast, 20b⊙123 gives two sets of host signals indicating a slow tumbling rate.

On the other hand, 120 may not form very strong interactions with hemicarcerands. Thus, the stability of these hemicarceplexes is mainly governed by constrictive binding. Constrictive binding is expected to be lower for the flexible hemicarcerand 20b, leading to a faster decomplexation.

**Table 5-4:** Half-life ($t_{1/2}$) of selected hemicarceplexes in solution at room temperature.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Complex</th>
<th>Solvent</th>
<th>$t_{1/2}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20g⊙120</td>
<td>CDCl$_3$</td>
<td>7.7</td>
</tr>
<tr>
<td>2</td>
<td>20g⊙123</td>
<td>CDCl$_3$</td>
<td>10.5</td>
</tr>
<tr>
<td>3</td>
<td>20g⊙124</td>
<td>CDCl$_3$</td>
<td>Decomposition</td>
</tr>
<tr>
<td>4</td>
<td>20b⊙120</td>
<td>CDCl$_3$</td>
<td>1.6</td>
</tr>
<tr>
<td>5</td>
<td>20b⊙123</td>
<td>CDCl$_3$</td>
<td>23</td>
</tr>
<tr>
<td>6</td>
<td>20d⊙125</td>
<td>CDCl$_2$CDCl$_2$</td>
<td>22</td>
</tr>
<tr>
<td>7</td>
<td>20d⊙126</td>
<td>CDCl$_2$CDCl$_2$</td>
<td>9</td>
</tr>
</tbody>
</table>

Amantadine 126 is larger than adamantane 125. Thus, 126 is expected to form a more stable hemicarceplex than 125 due to the high constrictive barrier of 126. However, contrary to expectation, 20c⊙125 is more stable. Presumably, the amine group of 126 temporarily breaks imine bonds and therefore self-catalyzes its escape from the cavity.
5.4 Conclusion and outlook

In this chapter, the synthesis of hemicarcerands through LAG was explored. It was found that the addition of small amounts of solvent is very important for the formation of hemicarcerands under vortex grinding conditions. Without solvents, only polymers were produced. In addition, high rigidity and correct conformation of linkers are required for the quantitative formation of hemicarcerands. Encapsulation properties of 20b, 20c and 20g through vortex grinding were also investigated. Although fewer guests could be encapsulated by 20g through the vortex grinding method than thermally through the solution phase method, a higher selectivity, which is kinetically controlled, was achieved. The vortex grinding method can be applied to less stable hemicarcerands 20b and 20c, suggesting more applications and it provides an alternative way to encapsulate guests.

Currently, the synthesis and encapsulation properties of polyimine nanocapsules by vortex grinding are under investigation. Potentially, larger guests like fullerenes and biomolecules can be encapsulated in nanocapsules by this method.

5.5 Experimental section

5.5.1 General procedure

Reagents and chromatography solvents were purchased and used without further purification. $^1$H NMR spectra recorded in CDCl$_3$ were referenced to residual CHCl$_3$ at $\delta_H = 7.26$. $^{13}$C NMR spectra recorded in CDCl$_3$ were referenced to $^{13}$CDCl$_3$ at $\delta_C = 77.5$ ppm. Mass spectra were recorded on an Applied Biosystems Voyager DE-Pro mass spectrometer (MALDI-TOF). External standards were used for calibration and 2,4,6-trihydroxylacetophenone (THAP) as matrix. Two ¼ inch stainless steel balls
and a VWR® advanced heavy-duty vortex mixer (2500 rpm) were used for vortex grinding.

5.5.2 Synthesis of 116

1,3,5-tris(aminomethyl)-2,4,6-trimethylbenzene 17t (20 mg, 97 µmol) and 5'-butyl-2-hydroxyisophthalaldehyde 29 (29.9 mg, 145 µmol) were vortex grinded for 1 hour. The solid was collected and dried.

![Chemical Structure](image)

$^1$H NMR spectrum (500 MHz, CDCl$_3$, 25 °C) $\delta$: 12.66 (br, 3H, -OH), 8.42 (s, 6H, -CHN), 7.70 (s, 6H, H$_3$), 4.93 (s, 12H, H$_2$), 2.20 (s, 18H, H$_1$), 1.34 (s, 27H, H$_4$). $^{13}$C NMR spectrum (100 MHz, CDCl$_3$, 25 °C) $\delta$: 160.84, 158.85, 141.02, 137.14, 133.21, 128.50, 121.53, 57.02, 34.38, 31.69, 16.54. MS (MALDI-TOF) $m/z$: 925.5866 (M + H$^+$, 100%); Calcd for C$_{60}$H$_{72}$O$_3$N$_6$ + H$^+$: 925.5739.

5.5.3 Synthesis of hemicarcerands (Procedure I)

Tetraformylcavitand 19 and diamines (mole ratio 1:2) were mixed well in a glass tube. Subsequently, a small amount of CH$_2$Cl$_2$ or another solvent ($\eta = 0.8$) containing catalytic amounts of TFA and two stainless steel balls were added to the mixture. The tube was closed with a septum and secured on the vortex mixer. After 1 hour vortexing at 2500 rpm, 0.5 mL TEA was added to neutralize TFA and to terminate the dynamics of imine bonds. The yellow to orange suspension was dissolved in 1 mL
CH₂Cl₂ and then precipitated by adding methanol. The precipitate was filtered and dried in vacuum.

**Synthesis of 20l**: Prepared by procedure 1 from tetraformylcavitand 19 (31 mg, 33.4 µmol), 3,5-diaminobenzoic acid 17l (10.2 mg, 66.8 µmol) and 30 µL DMSO-CH₂Cl₂ (1:1) or pure DMSO containing 0.3 µL TFA, hemicarcerand 20l formed quantitatively within 1 hour.

\[
\text{1H NMR spectrum (400 MHz, DMSO-d6-CDC}_3 = 1:9 25 ^\circ \text{C}):} \quad \delta_H = 12.57 \text{ (s, 4H, -COOH), 8.37 (s, 4H, H}_{\text{im}} \text{)), 7.63 (s, 8H, H}_{\text{a1}} \text{)), 7.27 (d, 8H, J = 1.9 Hz, H}_{\text{a3}} \text{)), 6.61 (s, 4H, H}_{\text{a2}} \text{), 5.61 (d, 8H, J = 7.8 Hz, H}_0 \text{), 4.79 (t, 8H, J = 7.7 Hz, H}_{\text{m}} \text{), 4.51 (d, 4H, J = 7.8 Hz, H}_3 \text{), 2.39 (m, 16H, H}_1 \text{), 1.55-1.23 (m, 48H, H}_{2-4} \text{), 0.87 (t, 24H, J = 7.2 Hz, H}_5 \text{).} \]

\[
\text{13C NMR spectrum (125 MHz, DMSO-d6, 100 ^\circ \text{C'):} \quad \delta_C = 167.17, 157.72, 154.22, 153.73, 139.81, 134.48, 125.10, 124.13, 116.42, 100.84, 37.18, 31.87, 29.64, 27.89, 22.77. MS (MALDI-TOF) m/z: 2323.0406 (M + H⁺, 100%); Calcd for C_{140}H_{144}O_{24}N_8 + H⁺: 2320.0381.}
\]

**Synthesis of 20m**: Prepared by procedure 1 from tetraformylvccavitand 19 (30.5 mg, 32.9 µmol), 4,4'-oxydianiline 17m (13.2 mg, 65.8 µmol) and 30 µL CH₂Cl₂ (1:1)
containing 0.3 µL TFA, hemicarcerand 30m formed quantitatively within 1 hour.

\[
\begin{align*}
\text{H NMR spectrum (400 MHz, CDCl}_3, 25 \degree C) : \delta_H = 8.60 \text{ (s, 8H, } H_{im},) \text{, 7.26 (s, 8H, } H_{a1},) \\
\text{overlap with } CHCl_3), 7.09-6.94 \text{ (m, 32H, } H_{a2}, H_{a3}), 5.80 \text{ (d, 8H, } J = 8.4 \text{ Hz, } H_o), 4.99 \text{ (t, 8H, } J = 8.4 \text{ Hz, } H_m,) \text{, 4.63 (d, 8H, } J = 8.4 \text{ Hz, } H_i), 2.30 \text{ (m, 16H, } H_1), 1.57-1.33 \text{ (m, 48H, } H_2-4\text{), 0.95 (t, 24H, } J = 7.1 \text{ Hz, } H_5). \text{ } ^{13}C \text{ NMR spectrum (125 MHz, CDCl}_3, 25 \degree C): } \delta_C = 155.68, 155.08, 153.97, 147.87, 139.11, 124.18, 122.26, 119.62, 119.38, 100.73, 36.69, 32.18, 29.93, 27.81, 22.95, 14.34. \text{ MS (MALDI-TOF) } m/z: 2516.1596 (M + H\textsuperscript{+}, 100\%); \text{ Calcd for } C_{140}H_{144}O_{24}N_8 + H\textsuperscript{+}: 2596.1908. \\
\end{align*}
\]

**Synthesis of 20n:** Prepared by procedure 1 from tetraformylcavitand 19 (31.34 mg, 33.8 µmol), naphthalene-2,7-diamine 17n (10.67 mg, 67.6µmol) and 30 µL CH\textsubscript{2}Cl\textsubscript{2} (1:1) containing 0.3 µL TFA, hemicarcerand 30n formed quantitatively within 1 hour.
H NMR spectrum (300 MHz, CDCl₃, 25 °C): δ_H = 8.52 (s, 8H, H_m), 7.81 (d, 8H, J = 8.7 Hz, H_a4), 7.41 (s, 8H, H_a2), 7.32 (s, 8H, H_a1), 7.14 (d, 8H, J = 7.8 Hz, H_o), 5.62 (d, 8H, J = 7.8 Hz, H_m), 4.99 (t, 8H, J = 8.0 Hz, H_m), 4.67 (d, 8H, J = 7.8 Hz, H_i), 2.32 (m, 16H, H_1), 1.59-1.29 (m, 48H, H_2-H_4), 0.96 (t, 24H, J = 7.1 Hz, H_5). ¹³C NMR spectrum (125 MHz, CDCl₃, 25 °C): δ_C = 156.97, 153.97, 150.64, 139.07, 133.91, 130.62, 129.55, 124.30, 122.44, 120.93, 118.45, 100.79, 36.66, 32.17, 30.02, 79.81, 22.95, 14.34. MS (MALDI-TOF) m/z: 2347.1373 (M + H⁺, 100%); Calcd for C₁₅₂H₁₅₂O₁₆N₈ + H⁺: 2347.1428.

5.5.4 Synthesis of hemicarceplexes (Procedure 2)

Hemicarcerand and excess guest (mole ratio 1:50-100) were mixed well in a glass tube. Two stainless steel balls were added. The tube was sealed and vortexed. The encapsulation process was monitored by ¹H NMR spectroscopy. Upon completion, the excess guest was removed either by washing with solvents or by silica gel column chromatography.

Synthesis of 20b⊙119: Prepared by procedure 2 from hemicarcerand 20b (11.23 mg, 5.59 µmol) and menthol 119 (87 mg, 0.56 mmol), about 61% complex formed after 5
hours vortexing. Longer time did not increase the yield. The resulting mixture was dissolved in a small amount of CH$_2$Cl$_2$ and was precipitated by adding methanol. The white precipitate was filtered off and dried in vacuum. Since the host and complex are not stable towards column chromatography, no pure complex was obtained.

\[\text{H NMR spectrum (400 MHz, CDCl}_3, 25 ^\circ \text{C): } \delta_H = 8.38 \text{ (s, 4H, H}_\text{im}), 8.37 \text{ (s, 4H, H}_\text{im}), 7.21 \text{ (s, 4H, H}_\text{a}), 7.17 \text{ (s, 4H, H}_\text{a1}), 5.70 \text{ (d, 4H, } J = 7.7 \text{ Hz, H}_\text{o}), 5.65 \text{ (d, 4H, } J = 7.7 \text{ Hz, H}_\text{o}), 4.94-4.80 \text{ (m, 8H, H}_m), 4.47 \text{ (d, 4H, } J = 7.7 \text{ Hz, H}_i), 4.38 \text{ (d, 4H, } J = 7.7 \text{ Hz, H}_i), 3.68-3.46 \text{ (m, 16H, H}_1), 3.16-3.04 \text{ (m, 1H, H}_17), 2.42-2.11 \text{ (m, 16H, H}_6), 1.91-1.73 \text{ (m, 8H, H}_7), 1.49-1.29 \text{ (m, 48H, H}_2-H_4), 0.92 \text{ (t, 24H, } J = 6.9 \text{ Hz, H}_5), 0.71-0.55 \text{ (m, 10H, H}_9-H_13, H_16 \text{ and -OH), -1.03 \text{ (d, 3H, } J = 8.1 \text{ Hz, H}_14), -1.11 \text{ (d, 3H, } J = 8.1 \text{ Hz, H}_13), -2.89 \text{ (br, 3H, H}_8). MS (MALDI-TOF) m/z: 2181.2888 (M + H$^+$, 100%); Calcd for C$_{134}$H$_{172}$O$_{17}$N$_9$ + H$^+$: 2181.3001.

**Synthesis of 20b⊙120**: Prepared by procedure 2 from hemicarcerand 20b (10 mg, 4.66 µmol) and 9-fluorenone 120 (86 mg, 0.47 mmol), about 80% complex formed after 2 hours vortexing. Longer time did not increase the yield. The resulting mixture was dissolved in a small amount of CH$_2$Cl$_2$ and was precipitated by adding methanol. The slightly yellowish precipitate was filtered off and dried in vacuum. Since the host and complex are not stable towards column chromatography, no pure complex was
obtained.

\[ \text{\H NMR spectrum (500 MHz, CDCl}_3, 25 \degree \text{C): } \delta_H = 8.28 \text{ (s, 8H, } H_{im} \text{), 7.33 \text{ (s, 8H, } H_a \text{), 6.81 \text{ (d, 2H, } J = 6.7 \text{ Hz, } H_8 \text{), 5.70 \text{ (d, 2H, } J = 6.7 \text{ Hz, } H_{11} \text{), 5.52 \text{ (d, 8H, } J = 7.7 \text{ Hz, } H_o \text{), 4.93 \text{ (t, 8H, } J = 7.2 \text{ Hz, } H_m \text{), 4.79 \text{ (t, 2H, } J = 6.7 \text{ Hz, } H_o \text{), 4.25 \text{ (d, 8H, } J = 7.7 \text{ Hz, } H_i \text{), 3.44 \text{ (br, 16H, } H_1 \text{), 3.32 \text{ (t, 2H, } J = 6.7 \text{ Hz, } H_{10} \text{), 2.29 \text{ (m, 16H, } H_6 \text{), 1.74 \text{ (m, 8H, } H_7 \text{), 1.49-1.29 \text{ (m, 48H, } H_2 - H_4 \text{), 0.92 \text{ (t, 24H, } J = 6.9 \text{ Hz, } H_5 \text{). Only empty host was observed by MS.)}}

Synthesis of 20b⊙123: Prepared by procedure 2 from hemicarcerand 20b (11.39 mg, 5.67 \mu mol) and 2, 3-dimethoxyl-5-methyl-p-benzoquinone 123 (52 mg, 0.28 mmol), the complex completely formed after 3 hours vortexing. The resulting mixture was dissolved in a small amount of CH$_2$Cl$_2$. The addition of methanol precipitated the product. The red precipitate was filtered and dried in vacuum.
$^1$H NMR spectrum (300 MHz, CDCl₃, 25 °C): $\delta_H = 8.37$ (s, 8H, $H_{im}$), 7.19 (s, 8H, $H_a$), 5.79 (s, 1H, $H_8$), 5.71 (d, 4H, $J = 7.6$ Hz, $H_o$), 5.61 (d, 4H, $J = 7.6$ Hz, $H_o$), 4.97-4.81 (m, 8H, $H_m$), 4.41 (d, 4H, $J = 7.6$ Hz, $H_i$), 4.18 (d, 4H, $J = 7.6$ Hz, $H_i$), 3.48 (m, 16H, $H_6$), 1.77 (m, 8H, $H_7$), 1.48-1.28 (m, 48H, $H_2-H_4$), 0.92 (br, 24H, $H_5$), 0.37 (s, 3H, $H_9$), -0.98 (s, 3H, $H_{11}$). MS (MALDI-TOF) $m/z$: 2193.2317 (M + H⁺, 100%); Calcld for C₁₃₃H₁₆₂O₂₀N₈ + H⁺: 2193.2073.

**Synthesis of 20c⊙125**: Prepared by procedure 2 from hemicarcerand 20c (9.5 mg, 4.6 µmol) and adamantane 125 (63 mg, 0.46 mmol), the complex completely formed after 9 hours vortexing. The resulting mixture was suspended in methanol and sonicated for 30 minutes. The white precipitate was filtered off and dried in vacuum. During sonication, the complex partially dissociated.
$^1$H NMR spectrum (300 MHz, CDCl$_3$, 25 °C): $\delta_H = 8.39$ (s, 8H, H$_m$), 7.20 (s, 8H, H$_a$), 5.68 (d, 8H, $J = 7.5$ Hz, H$_o$), 4.89 (br, 8H, H$_m$), 4.07 (d, 8H, $J = 7.5$ Hz, H$_i$), 3.67 (br, 16H, H$_1$), 2.23 (m, 16H, H$_6$), 1.83 (br, 16H, H$_7$), 1.48-1.29 (m, 48H, H$_2$-H$_4$), 0.92 (m, 24H, H$_5$), 0.68 (s, 4H, H$_8$), 0.41 (s, 12H, H$_9$). MS (MALDI-TOF) $m/z$: 2203.3270 (M + H$^+$, 100%); Calcd for C$_{136}$H$_{176}$O$_{16}$N$_8$ + H$^+$: 2202.9343.

*Synthesis of 20c*\textsuperscript{126}: Prepared by procedure 2 from hemicarcerand 20c (10.07 mg, 4.88 µmol) and 1-adamantylamine 126 (73.7 mg, 0.49 mmol), the complex completely formed after 9 hours vortexing. The resulting mixture was suspended in methanol and sonicated for 30 minutes. The white precipitate was filtered off and dried in vacuum. During sonication, the complex partially dissociated.

$^1$H NMR spectrum (500 MHz, CDCl$_3$, 25 °C): $\delta_H = 8.39$ (s, 8H, H$_m$), 7.21 (s, 8H, H$_a$), 5.66 (d, 8H, $J = 7.2$ Hz, H$_o$), 4.88 (br, 8H, H$_m$), 4.26 (d, 8H, $J = 7.5$ Hz, H$_i$), 3.66 (br, 16H, H$_1$), 2.23 (m, 16H, H$_6$), 1.83 (br, 16H, H$_7$), 1.48-1.29 (m, 48H, H$_2$-H$_4$), 1.05 (s, 3H, H$_{10}$), 0.92 (m, 24H, H$_5$), 0.54 (d, 3H, $J = 0.53$ Hz, H$_9$), 0.32 (s, 6H, H$_{11}$), -0.15 (d, 3H, $J = 0.53$ Hz, H$_8$), -1.45 (br., 2H, -NH$_2$). MS (MALDI-TOF) $m/z$: 2218.3441 (M + H$^+$, 100%); Calcd for C$_{138}$H$_{177}$O$_{16}$N$_9$ + H$^+$: 2218.3416.
Synthesis of 20g⊙117: Prepared by procedure 2 from hemicarcerand 20g (9.78 mg, 4.56 µmol) and ferrocene 117 (85 mg, 0.46 mmol), the complex completely formed within 3 hours. The resulting mixture was dissolved in a small amount of CH₂Cl₂ and was precipitated by adding methanol. The orange precipitate was filtered off and washed with hexane to remove excess guest.

\[ \text{H NMR spectrum (300 MHz, CDCl₃, 25 °C):} \delta_H = 8.51 \text{ (s, 8H, H}_{\text{im}}), 7.44 \text{ (t, 4H, } J = 8.2 \text{ Hz, H}_{a1}), 7.19 \text{ (s, 8H, H}_{a1}), 7.13 \text{ (t, 4H, } J = 1.9 \text{ Hz, H}_{a2}), 6.85 \text{ (dd, 8H, } ^3J = 8.2 \text{ Hz, } J = 1.9 \text{ Hz, H}_{a3}), 5.79 \text{ (d, 8H, } J = 7.9 \text{ Hz, H}_o), 4.88 \text{ (t, 8H, } J = 7.9 \text{ Hz, H}_m), 4.48 \text{ (d, 8H, } J = 7.9 \text{ Hz, H}_i), 3.66 \text{ (s, 10H, H}_6), 2.23 \text{ (m, 16H, H}_1), 1.50\text{-}1.28 \text{ (m, 48H, H}_2\text{-}H_4), 0.93 \text{ (t, 24H, } J = 6.8 \text{ Hz, H}_5). \text{ MS (MALDI-TOF) } m/z: 2333.1132 \text{ (M} + \text{H}^+, 100\%); \text{ Calcd for } C_{146}H_{154}O_{16}N_8Fe + H^+: 2333.0940. \]

Synthesis of 20g⊙118: Prepared by procedure 2 from hemicarcerand 20g (10 mg, 4.67 µmol) and ruthenocene 118 (54 mg, 0.23 mmol), the complex completely formed within 2.5 hours. The resulting mixture was dissolved in a small amount of CH₂Cl₂ and was precipitated by adding methanol. The white precipitate was filtered off and washed with hexane to remove excess guest.
\( ^1 \)H NMR spectrum (300 MHz, CDCl₃, 25 °C): \( \delta_H = 8.49 \) (s, 8H, H_m), 7.40 (t, 4H, \( J = 8.3 \) Hz, H_a4), 7.25 (s, 8H, H_a), 6.87 (t, 4H, \( J = 7.6 \) Hz, H_a2), 6.81 (dd, 8H, \( J_1 = 8.3 \) Hz, \( J_2 = 1.7 \) Hz, H_a3), 5.76 (d, 8H, \( J = 7.6 \) Hz, H_o), 4.91 (t, 8H, \( J = 7.5 \) Hz, H_m), 4.62 (d, 8H, \( J = 7.6 \) Hz, H_i), 4.06 (s, 10H, H_9), 2.27 (m, 16H, H_1), 1.50-1.28 (m, 48H, H_2-H_4), 0.93 (t, 24H, \( J = 6.8 \) Hz, H_5). MS (MALDI-TOF) m/z: 2379.0913 (M + H\(^+\), 100%); Calcd for C_{146}H_{154}O_{16}N_8Ru + H\(^+\): 2379.0663.

**Synthesis of 20g⊙119**: Prepared by procedure 2 from hemicarcerand 20g (9.66 mg, 4.51 µmol) and menthol 119 (70 mg, 0.45 mmol), the complex completely formed within 1.5 hours. The resulting mixture was dissolved in a small amount of CH₂Cl₂ and was precipitated by adding methanol. The white precipitate was filtered off and dried in vacuum.
$^1$H NMR spectrum (300 MHz, CDCl$_3$, 25 °C): $\delta_H = 8.46$ (s, 4H, H$_{im}$), 8.42 (s, 4H, H$_{im}$) 7.45-7.28 (m, 12H, H$_{a3}$ and H$_{a4}$), 6.90-6.71 (m, 12H, H$_{a1}$ and H$_{a2}$), 5.75 (d, 4H, $J = 8.4$ Hz, H$_o$), 5.70 (d, 4H, $J = 8.4$ Hz, H$_o$), 5.05-4.85 (m, 8H, H$_m$), 4.66-4.50 (m, 8H, H$_i$), 2.81 (m, 1H, H$_{15}$), 2.30 (m, 16H, H$_1$), 1.52-1.33 (m, 48H, H$_2$-H$_4$), 1.23-1.05 (m, 3H, H$_{10}$ and H$_{14}$), 0.94 (t, 24H, $J = 6.8$ Hz, H$_3$), 0.83- 0.11 (m, 7H, H$_7$-H$_9$, H$_{11}$ and OH), -0.58 (d, 3H, $J = 7.5$ Hz, H$_{12}$), -0.69 (d, 3H, $J = 7.5$ Hz, H$_{13}$), -2.61 (s, 3H, H$_6$). MS (MALDI-TOF) $m/z$: 2303.2451 (M + H$^+$, 100%); Calcd for C$_{146}$H$_{164}$O$_7$N$_8$ + H$^+$: 2303.2300.

**Synthesis of 20g**: Prepared by procedure 2 from hemicarcerand 20g (9.9 mg, 4.62 µmol) and 9-fluorenone 120 (75 mg, 0.46 mmol), the complex completely formed within 1 hour. The resulting mixture was suspended in methanol and sonicated for 30 minutes. The yellowish precipitate was filtered off and dried in vacuum. During sonication, the complex partially dissociated.
1H NMR spectrum (300 MHz, CDCl₃, 25 °C): δ_H = 8.37 (s, 8H, H_m), 7.45 (s, 8H, H_a1), 7.29 (t, 4H, J = 8.0 Hz, H_a4), 7.25-2.18 (m, 2H, overlap with CHCl₃), 6.71 (d, 8H, J = 6.7 Hz, H_a3), 6.31 (s, 4H, H_a2), 6.11 (d, 2H, J = 7.7 Hz, H_o), 5.58 (d, 8H, J = 7.6 Hz, H_o), 5.23 (t, 2H, J = 7.7 Hz, H_7), 5.01 (t, 8H, J = 7.6 Hz, H_m), 4.40 (d, 8H, J = 7.6 Hz, H_i), 3.74 (t, 2H, J = 7.3 Hz, H_8), 2.36 (m, 16H, H_1), 1.50-1.30 (m, 48H, H_2-H_4), 0.96 (t, 24H, J = 6.8 Hz, H_5). Only empty host was observed by MS.

Synthesis of 20g⊙121: Prepared by procedure 2 from hemicarcerand 20g (10 mg, 4.66 µmol) and anthracene 121 (83 mg, 0.46 mmol), the complex completely formed within 2 hours. The resulting mixture was purified on an alumina column (hexane-CH₂Cl₂ = 1:1). The product was obtained as a white solid.
\( ^1 \text{H NMR spectrum (300 MHz, CDCl}_3, 25 \, ^\circ \text{C)}: \delta_H = 8.36 \, (s, 8H, H_{\text{im}}), 7.97 \, (s, 2H, H_8), 7.53 \, (s, 8H, H_{a1}), 7.32 \, (t, 4H, J = 8.2 \, \text{Hz}, H_{a4}), 7.12 \, (m, 4H, H_7), 6.74 \, (dd, 8H, J = 7.8 \, \text{Hz} \text{ and } J = 2.0 \, \text{Hz}, H_{a3}), 6.34 \, (t, 4H, J = 2.0 \, \text{Hz}, H_{a2}), 5.51 \, (d, 8H, J = 7.9 \, \text{Hz}, H_o), 5.03 \, (t, 8H, J = 8.2, H_m), 4.44 \, (d, 8H, J = 7.9 \, \text{Hz}, H_i), 3.35 \, (m, 4H, H_6), 2.41 \, (m, 16H, H_1), 1.52-1.33 \, (m, 48H, H_2-H_4), 0.97 \, (t, 24H, J = 0.97 \, \text{Hz}, H_5). \) Only empty host was observed by MS.

**Synthesis of 20g⊙122**: Prepared by procedure 2 from hemicarcerand 20g (5 mg, 2.33 \( \mu \text{mol}), \) mono-protonated hemicarcerand 20g·TFA (5 mg, 2.21 \( \mu \text{mol}) and camphor 122 (72 mg, 0.23 mmol), the complex formed after 4.5 hours grinding. The resulting mixture was neutralized with triethylamine and purified on an alumina column (1/4 hexane/CH\( _2 \text{Cl}_2 \)).

\( ^1 \text{H NMR spectrum (400 MHz, CDCl}_3, 25 \, ^\circ \text{C)}: \delta_H = 8.41 \, (s, 8H, H_m), 7.42-7.32 \, (m, 12H, H_{a1} \text{ and } H_{a4}), 6.82 \, (dd, 8H, J = 6.8 \, \text{Hz} \text{ and } J = 2.0 \, \text{Hz}, H_{a3}), 6.58 \, (t, 4H, J = 2.0 \, \text{Hz}, H_{a2}), 5.82 \, (d, 8H, J = 7.6 \, \text{Hz}, H_o), 4.97 \, (t, 8H, J = 8.1 \, \text{Hz}, H_m), 4.31 \, (d, 8H, J = 7.6 \, \text{Hz}, H_i), 2.30 \, (m, 16H, H_1), 1.50-1.30 \, (m, 48H, H_2-H_4), 1.00-0.70 \, (m, 29H, H_5, H_8, H_9 \text{ and } H_{10}), 0.23 \, (s, 3H, H_6), 0.19 \, (s, 3H, H_{12}), 0.51 \, (s, 3H, H_7). \) MS (MALDI-TOF) \( m/z \): 2299.1820 (M + H\(^+\), 100%); Calcd for C\( _{146} \text{H}_{160} \text{O}_{17} \text{N}_8 + \text{H}^+\): 2299.2022.
**Synthesis of 20g ⊙ 123**: Prepared by procedure 2 from hemicarcerand 20g (10.08 mg, 4.70 µmol) and 2, 3-dimethoxy-5-methyl-p-benzoquinone 123 (85 mg, 0.47 mmol), the complex completely formed within 1 hour. The resulting mixture was suspended in methanol and sonicated for 0.5 hour. The red precipitate was filtered off, collected and dried in vacuum. During sonication, the complex partially dissociated.

\[
\begin{align*}
1^1H \text{ NMR spectrum (300 MHz, CDCl}_3, 25^\circ C):} & \quad \delta_{H} = 8.39 \text{ (s, 8H, } H_{im}) , 7.37-7.28 \text{ (m, 12H, } H_{a1} \text{ and } H_{a4}) , 6.74 \text{ (d, 8H, } J = 7.8 \text{ Hz, } H_{a3}) , 6.32 \text{ (s, 4H, } H_{a2}) , 5.91 \text{ (s, 1H, } H_7) , 5.65 \text{ (d, 8H, } J = 7.8 \text{ Hz, } H_6) , 4.97 \text{ (t, 8H, } J = 8.5 \text{ Hz, } H_m) , 8.48 \text{ (br, 8H, } H_i) , 2.88 \text{ (s, 3H, } H_8) , 2.30 \text{ (m, 16H, } H_1) , 1.50-1.30 \text{ (m, 48H, } H_2-H_4) , 1.04-0.79 \text{ (m, 27H, } H_5 \text{ and } H_6) , -0.27 \text{ (s, 3H, } H_9) . \text{ MS (MALDI-TOF) } m/z: 2329.1609 (M + H^+, 100\%); \text{ Calcd for } C_{145}H_{154}O_{20}N_8 + H^+: 2329.1381. \\
\end{align*}
\]

**5.5.5 Synthesis of 132**

Tetraformycavitand 19 (4.08 mg, 4.40 µmol) and 3,3’-diaminobiphenyl 17k (1.62 mg, 8.8 µmol) were dissolved in 500 µL CDCl3 containing catalytic amounts of TFA. After 1 hour, the \(^1H\) NMR spectrum showed that 132 formed quantitatively.
\[ \text{H NMR spectrum (300 MHz, CDCl}_3, 25 ^\circ \text{C): } \delta_H = 8.59 \text{ (s, 12H, H}_\text{im}), \ 7.42 \text{ (m, 12H, H}_\text{a4 and H}_\text{a5}), \ 7.39 \text{ (d, 12H, } J = 8.0 \text{ Hz, H}_\text{a6}), \ 7.27 \text{ (s, 12H, H}_\text{a1}), \ 7.18 \text{ (s, 12H, H}_\text{a2}), \ 7.01 \text{ (d, 12H, } J = 7.7 \text{ Hz, H}_\text{a3}), \ 5.84 \text{ (d, 6H, } J = 7.7 \text{ Hz, H}_\text{o}), \ 5.57 \text{ (d, 6H, } J = 7.7 \text{ Hz, H}_\text{o}), \ 5.07 \text{ (t, 6H, } J = 8.0 \text{ Hz, H}_\text{m}), \ 4.96 \text{ (d, 6H, } J = 7.7 \text{ Hz, H}_\text{i}), \ 4.90 \text{ (t, 6H, } J = 8.0 \text{ Hz, H}_\text{m}), \ 4.45 \text{ (d, 6H, } J = 7.7 \text{ Hz, H}_\text{o}), \ 2.32 \text{ (m, 24H, H}_1), \ 1.56-1.35 \text{ (m, 72H, H}_2-\text{H}_4), \ 1.02-0.87 \text{ (m, 36H, H}_5) \].

\[ \text{MS (MALDI-TOF) } m/z: \ 3676.7667 (M + H}^+, \ 100\%); \text{ Calcd for C}_{240}H_{240}O_{24}N_{12} + H}^+: \ 3676.8047. \]

5.5.6 Synthesis of 133

Hemicarceplex 20g⊙117 (37 mg, 15.8 µmol) and Ni(OAc)$_2$ (25 mg, 0.13 mmol) were suspended in 10 mL THF. Under argon, NaBH$_3$CN (80 mg, 1.3 mmol) was added to the solution. The mixture was heated to 80 °C and allowed to react for 1 hour. Then, it was cooled to room temperature and diluted with 10 mL water. 3 × 10 mL CH$_2$Cl$_2$ were used to extract the product and the combined organic solutions were dried over MgSO$_4$, filtered and concentrated in vacuum. The crude product was purified by silica gel column chromatography (EtOAc-CH$_2$Cl$_2$ = 1:99). 11 mg of 133 (30%) was obtained as a white solid.
1H NMR spectrum (500 MHz, CD₂Cl₂, 25 °C): δ_H = 7.23 (s, 8H, H_a1), 7.06 (t, 4H, J = 8.2 Hz, H_a4), 7.62 (dd, 8H, J = 8.2 Hz, J = 2.1 Hz, H_a3), 5.94 (d, 8H, J = 7.1 Hz, H_o), 5.82 (t, 4H, J = 2.1 Hz, H_a2), 4.77 (t, 8H, J = 8.2 Hz, H_m), 4.07 (d, 8H, J = 8.2 Hz, H_i), 4.02 (d, 16H, J = 5.4 Hz, H_6), 3.51 (t, 8H, J = 5.4 Hz, -NH-), 3.17 (s, 10H, H_7), 2.27 (s, 16H, H_1), 1.52-1.32 (m, 48H, H_2-H_4), 0.95 (t, 24H, J = 7.3, H_5).

13C NMR spectrum (125 MHz, CD₂Cl₂, 25 °C): δ_C = 154.29, 149.61, 138.38, 130.43, 123.96, 120.90, 103.72, 100.75, 97.54, 67.61, 39.13, 37.35, 32.22, 30.47, 27.84, 22.91, 14.08.

MS (MALDI-TOF) m/z: 2349.2308 (M + H^+, 100%); Calcd for C_{146}H_{170}O_{16}N_{8}Fe + H^+: 2349.2174.

5.6 References


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Chapter 6

Synthesis of Water-Soluble Acylhydrazone Nanocapsules and Attempt to Encapsulate Proteins

6.1 Introduction

The majority of artificial nanocapsules have been studied in organic solvents\(^1\). However, the demand for greener chemistry and potential applications of these capsules in biochemistry is shifting the research focus from organic media to aqueous solutions\(^2\). In biomedical research, for example, nanocapsules can be potentially used for drug delivery and to tune the functionality of biomolecules. Compared to other systems, nanocapsules have uniform cavities and portal sizes and their sizes can be easily engineered through modular methods.

Compared with common organic solvents, water exhibits many unique properties, which may impose a great challenge for the development of water-soluble nanocapsules. For example, water molecules are both H-bonding donors and acceptors. Therefore, hydrogen bonded nanocapsules, which are well developed in organic solvents, cannot form in water due to disruption by water\(^1c,3\). Additionally, most organic molecules are not soluble in water and require further functionalization to increase their solubility.

By far, coordination nanocapsules are the most explored water-soluble nanocapsules since many coordination bonds are pretty stable in water and highly charged coordination capsules have often good water solubility\(^4\). As mentioned in previous chapters, the coordination nanocapsules developed in the Fujita group and in the
Raymond group bind a wide spectrum of guests and show excellent catalytic properties in water\(^5\). For example, it was found that cage 8 could selectively recognize a ‘-Trp-Trp-Ala-’ (-W1-W2-A3-) peptide sequence\(^6\). The crystal structure of 8⊕NH\(_2\)-Trp-Trp-Ala-Ac shows that the tripeptide has multiple interactions (W1, \(\pi\)-\(\pi\); W2, \(\pi\)-\(\pi\); A3, CH-\(\pi\)) with 8, which explains the strong binding affinity. Interestingly, the sequences ‘-Trp-Ala-Trp-’ and ‘-Ala-Trp-Ala-’, which contain the same amino acids but in a different order, show relative low affinity. Recently, the same group reported the successful encapsulation of ubiquitin inside a discrete M\(_{12}\)L\(_{24}\) nanocapsule, in which one of the ligands is tethered to ubiquitin\(^7\). Another type of discrete water-soluble nanocapsules is assembled through the hydrophobic effect (Fig. 1-8 and 1-9), which has been discussed in chapter 1\(^2d\).

**Figure 6-1**: Crystal structure (a) and binding sites W1, W2 and A3 (b) of 8⊕Ac-Trp-Trp-Ala-NH\(_2\) (reprinted with permission from the reference\(^6\)).

Although water-soluble covalent hosts, such as calixarenes, cyclodextrins, cucurbiturils and water-soluble cavitands\(^2a\), were studied very early, they can only partially shield guests and thus won’t affect properties of guests significantly. By contrast, nanocapsules are close-shell molecules and can cover most parts of the
guests. They provide a microenvironment that is totally different from the bulk solution. Therefore, it is necessary to develop water-soluble covalent nanocapsules. In 1997, D. J. Cram and coworkers reported the first water soluble hemicarcerand 133. As compared to the parent host, which lacked the COOH groups and is only soluble in organic solvents, 133 can encapsulate guests much faster due to the hydrophobic effect\(^8\). Recently, it was also found that 133 has a very high binding affinity towards cytochrome \(c^9\). Later, in 2009, our group reported the synthesis and property investigation of giant water-soluble, positively charged octahedral nanocapsules. It was shown that they could encapsulate small guests that are negatively charged and also possess hydrophobic groups\(^10\).

In order to avoid laborious post-assembly modifications of nanocapsules, it is highly desirable that water-soluble covalent nanocapsules can be formed through self-assembly processes. One option is to use dynamic imine chemistry as we did previously in organic solvents. However, the formal equilibrium constant of imine formation in water is much smaller than that in non-aqueous medium due to the high molarity of water (\(\sim 55 \text{ M}\)) and polyimine capsule assembly in water is likely unfavorable. However, our study of EM indicates that template effects can enhance
the stability of octaamine hemicarcerands dramatically, which may promote the formation of capsules in water. In fact, in basic water, a mixture of two equivalents of triformyltribenzylene (CTB) 134 and three equivalents of ethylenediamine 17a yielded short oligomers (Fig. 6-2A), but quantitative cryptophane complex 136⊙G after the addition of a small amount of guest molecules\(^{11}\). Later, the same idea was applied to construct hemicarcerands\(^{12}\). Fig. 6-2B shows the formation of water-soluble hemicarceplexes 137⊙G. 137 could not be assembled in organic solvents other than HMPA containing \(\rho\)-dimethoxybenzene as template in low yield (see Chapter 5)\(^{13}\). However, it formed quantitatively in water upon addition of an appropriate guest. Since 137 is highly strained, the formation is most likely driven by entropy, considering that partial dehydration of guest, linkers and cavitands is involved during the formation 137⊙G.

Figure 6-2: Templated self-assembly of cryptophane 136 (A) and hemicarcerand 137 (B).
So far, only small hemicarcerands have been synthesized through the above methods, even though EM predicts that larger imine nanocapsules may be stable in water even in the absence of guest molecules. We are currently underway in the Warmuth group to support this hypothesis.

Acylhydrazones are thermodynamically more stable in water than imines\textsuperscript{14}. Also, acylhydrazone hosts can be synthesized in organic solvents in very high yields\textsuperscript{15}. Therefore, the acylhydrazone bond can be potentially used for assembling water-soluble Schiff-base nanocapsules that are large enough to encapsulate a protein molecule or other biomacromolecules.

By encapsulating proteins in a closed-shell nanocapsule, we can potentially modify their properties. For example, the stability of proteins towards denaturants could be enhanced due to the protection by the host, the reactivity of enzymes may be altered and the conformation of biomolecules in the confined space inside the capsule could be changed. However, the development of proper nanocapsules with large enough cavity sizes and high binding affinity towards proteins is still challenging. Therefore, this field is largely unexplored. Although several different materials have shown to be able to immobilize proteins, such as micelles\textsuperscript{16}, nanogels\textsuperscript{17}, MOFs\textsuperscript{18}, and capsids\textsuperscript{19}, they suffer from disadvantages in several aspects. For example, materials like micelles and nanogels don’t have uniform sizes and different number of proteins can be encapsulated. As for capsids, they have defined cavity sizes, but cavity sizes are difficult to modify. MOFs don’t have the above problems, but only solid-state analytical techniques can be used, which limits their applications. Therefore, it is necessary to develop nanocapsules that have uniform and easily tuned sizes and can
be analyzed easily. So far, only two examples of encapsulated proteins have been reported\textsuperscript{20,7}. However, in these studies, the proteins were tethered to one of the capsule building blocks, which limits the range of proteins that can be encapsulated by this approach. In our study, we are more interested in thermodynamically driven encapsulation, namely, encapsulation occurring purely through non-covalent interactions. We propose that encapsulation can be driven by hydrophobic interactions between the hydrophobic surface of proteins and the inner surface of Schiff-base nanocapsules and electrostatic interactions between positive charges on the protein surface and negatively charged groups of the nanocapsules.

In this chapter, we successfully prepared acylhydrazone hemicarcerands and rhombicuboctahedral nanocapsules in water and the encapsulation of proteins was also investigated.

6.2 Synthesis of water-soluble hemicarcerands and rhombicuboctahedral nanocapsules

6.2.1 Synthesis of cavitand 146

The synthesis of deep cavitands that were chosen as building blocks for nanocapsules is outlined in Scheme 6-1. Tetrabromocavitand 138 was treated with BuLi at -78 °C for 1 hour, which was followed by addition of borate MeO-Bpin 139, yielding a tetraboronic ester cavitand 140. With 140 in hand, two synthetic routes were tested (Scheme 6-3). Firstly, the coupling reaction\textsuperscript{21} of 140 and 4-bromo-2-hydroxybenzaldehyde 141 yielded deep cavitand 142. Alkylation of the phenol groups gave deep cavitand 144. Unfortunately, the yield of 142 was not reproducible, especially at large scale. Presumably, the active phenol groups
competitively bind to the palladium catalyst, which might give unreliable results. Therefore, another synthetic route was developed, in which the phenol groups were alkylated before the coupling reaction. This route gave 144 in much higher yield.

Scheme 6-1: Synthesis of deep cavitand 145.
Treating deep cavitand 144 with neat TFA deprotected 'BuO- and TBDPS- groups at the same time. Under these conditions, the released alcohol groups further reacted with TFA to produce esters. Thus, an additional basic hydrolysis step was required to prepare 145. Finally, O-sulfonation of alcohol groups with PySO₃ produced the water-soluble cavitand 146 (Scheme 6-2). Conditions were optimized by ¹H NMR spectroscopy (Fig. 6-3). Initially, three equivalents of PySO₃ per -OH were used. After 1 hour, two new signals at 3.8 ppm and 1.7 ppm appeared in the ¹H NMR spectrum, which are methylene protons of -CH₂CH₂OSO₃H. Methylene protons of –CH₃CH₂OH are at 3.4 ppm and 1.6 ppm, indicating only partial conversion. Extending the reaction time didn’t increase the conversion significantly. However, six equivalents of PySO₃ per -OH resulted in complete conversion within 1 hour.

![Scheme 6-2: Synthesis of water-soluble cavitand 146.](image)

The ¹H NMR spectrum of the pure water-soluble cavitand 146 with assignment is shown in Fig. 6-4. Signals are fairly sharp indicating that the phenyl rings can freely rotate regardless of their large substituents. The presence of roughly one DMSO per cavitand could not be removed even after prolonged heating in vacuum, suggesting that one DMSO is trapped in the cavitand.
Figure 6-3: $^1$H NMR spectra (DMSO-d$_6$, 400 MHz) of O-sulfonation products of reaction of 145 with three equivalents of PySO$_3$ per -OH for 1 hour (A) and 24 hours (B) and six equivalents of PySO$_3$ per -OH for 1 hour at room temperature (C). Methylene protons of -CH$_2$CH$_2$OH and -CH$_2$CH$_2$OSO$_3$H groups are labeled with black dots and black arrows, respectively.

Figure 6-4: $^1$H NMR spectrum (400 MHz, D$_2$O, 25 °C) of water-soluble cavitand 146.
6.2.2 Synthesis of hemicarcerands 148a, b

Before attempting to assemble nanocapsules in water, hemicarcerand 148a was first assembled by mixing two equivalents of 146 and four equivalents of isophthalic dihydrazide 147a in buffer (pD = 3.5, acetate buffer, 10 mM) (Scheme 6-3). At room temperature, the $^1$H NMR spectrum of the products shows very strong signal broadening. However, higher temperatures resulted in sharper signals and at or above 70 °C, signals are well resolved and consistent with formation of hemicarcerand 148a (Figure 6-5).

![Scheme 6-3: Synthesis of water-soluble hemicarcerands 148a, b.](image)

An assignment was possible by GCOSY (Fig. 6-6A). The singlet at 8.68 ppm is assigned to CH=N and is in the region of chemical shifts of hydrazone protons. In the aromatic region, singlets at 8.35 and 6.54 ppm are assigned to H$_5$ and H$_4$. The integration of H$_5$ is smaller probably due to its longer relaxation time (d1) and/or broad features. Doublets at 7.95, 7.86 and 6.57 ppm, ratio 1:1:1, are assigned to H$_6$, H$_2$ and H$_3$, respectively. Resonance of H$_1$ and H$_7$ overlapped to yield a broad signal at 7.53 ppm.
Figure 6-5: VT-NMR spectra (500 MHz, D$_2$O) of products of the reaction between two equivalents of cavitand 146 and four equivalents of linker 147a at 25 (A), 50 (B), 70 (C) and 90 °C (D). These spectra are referenced to DMSO.

Figure 6-6: $^1$H NMR spectra (500 MHz, D$_2$O) of water-soluble hemicarcerands 148a at 70 °C (A) and 148b at room temperature (B). Spectra are referenced to DMSO. An impurity from 147b is labeled with a black arrow.
To our best knowledge, this is the first covalent water-soluble hemicarcerand, which assembles in water without being assisted by templates. More importantly, it is stable over a wide pH range due to the high stability of acylhydrazone bonds. The strong broadening in the $^1$H NMR spectrum at room temperature can be explained by partial aggregation of hemicarcerand 148a due to the hydrophobic linkers and/or slowly exchanging hydrazone bond conformations, which have similar energy$^{15}$. At higher temperature, aggregation is weakened and conformation exchange becomes faster, which results in much sharper signals. To suppress aggregation, the more hydrophilic linker 147b was tested and gave hemicarcerand 148b under the same conditions as above. The $^1$H NMR signals of 148b are fairly sharp even at room temperature (Fig. 6-6B), indicating that the aggregation is the major reason for signal broadening of 148a.

![Chart 6-1: Guest molecules 149-153 for encapsulation study.](image)

The recognition properties of both hemicarcerands were studied, but no encapsulation was observed for selected guests (Chart 6-1). Possibly, guest exchange is fast on the NMR time scale due to their large portal sizes.
6.2.3 Synthesis of rhombicuboctahedral nanocapsules 155a-c

Encouraged by the formation of hemicarcerands 148a, b, the assembly of rhombicuboctahedral nanocapsules was investigated (Scheme 6-4). Six equivalents of cavitand 146 and eight equivalents of linker 154a were mixed in buffer containing 25% DMSO-d6. DMSO was added to increase the solubility of 154a. However, the $^1$H NMR spectrum shows extremely broad signals even at 70 °C. Therefore, to reduce signal broadening caused by aggregation, the hydrophilicity of 154a was increased by aromatic sulfonation.

![Scheme 6-4: Synthesis of nanocapsule 155a.](image)

6.2.3.1 Synthesis of water-soluble linkers

1,3,5-Tris(4-carboxyl-2-sulfophenyl)benzene 157 is expected to form via aromatic sulfonation of 1,3,5-tris(4-carboxyphenyl)benzene 156. However, the unexpected sulfone product 158 was obtained upon treating 156 with fuming sulfuric acid (Scheme 6-5). Likely, the ‘meta’ position to –COOH group or the central ring was sulfonated first, followed by intramolecular sulfonation under the harsh conditions. The milder sulfonating reagent, ClSO$_3$H, was also tested. However, in this case, the obtained product could not dissolve in any solvents and its composition could not be identified. These results indicate that sulfonation at high temperatures (60-120 °C)
seem not to give the desired product. Therefore activating OMe groups were incorporated to allow aromatic sulfonation of the peripheral phenyl groups of 158 at room temperature.

Scheme 6-5: Sulfonation of 1,3,5-tris(4-carboxylphenyl)benzene 156.

Scheme 6-6: Synthesis and sulfonation of 1,3,5-tris(4-carboxyl-3-methoxylphenyl)benzene 164.

The synthesis of 1,3,5-tris(4-carboxyl-3-methoxylphenyl)benzene 164 is outlined in Scheme 6-6. Miyaura borylation of methyl 4-iodo-2-methoxybenzoate 160 lead to methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate 162. Three-fold Suzuki coupling with 1,3,5-tribromobenzene 163 gave 164. Unfortunately, at room
temperature, aromatic sulfonation of 164 again gave only sulfone products.

However, sulfonation of 1,3,5-tris(4-hydrazinecarbonyl-3-methoxylphenyl)benzene 165 with ClSO$_3$H at room temperature gave the desired product, 1,3,5-tris(4-carboxyl-5-methoxy-2-sulfophenyl)benzene 166. The amide groups might be hydrolyzed during the sulfonation (Scheme 6-7). After converting 166 to the methyl ester, reflux with hydrazine hydrate solution in abs. EtOH yielded 1,3,5-tris(4-hydrazinecarbonyl-5-methoxy-2-sulfophenyl)benzene 154b. Since the biaryl torsional angle of the triphenyl unit has a profound influence on the formation of nanocapsules, linker 154c with the -SO$_3$H groups ‘ortho’ to the hydrazine groups was also synthesized.

Scheme 6-7: Synthesis of water-soluble linker 154b.

The synthesis of 1,3,5-tris(4-hydrazinecarboxyl-3-sulfophenyl)benzene 154c is illustrated in Scheme 6-8. Sulfonation of 1,3,5-tris(4-methylphenyl)benzene 167 in hot fuming sulfuric acid yields 1,3,5-tris(4-methyl-3-sulfophenyl)benzene 168. Oxidation with KMnO$_4$ in aqueous NaOH solution gave sodium 1,3,5-tris(4-carboxylato-3-sulfonatophenyl)benzene 169 and sodium 2-sulfonatoterephthalate 170. Finally, 1,3,5-tris(4-hydrazinecarbonyl-3-sulfophenyl)benzene 154c was obtained by converting 169 to the methyl ester, followed by reflux with hydrazine hydrate in abs. EtOH.
6.2.3.2 Synthesis of water-soluble rhombicuboctahedral nanocapsules

The assembly of rhombicuboctahedral nanocapsule was attempted with linkers 154b and 154c in buffer (pD = 3.5, acetate buffer, 10 mM). The reaction was followed by $^1$H NMR spectroscopy. The $^1$H NMR spectrum of the reaction of six equivalents of cavitand 146 and eight equivalents of 154b shows much sharper signals than observed for nanocapsule 155a. Integration and chemical shifts of signals are consistent with formation of 155b. Upon formation of 155b, all linker signals are slightly downfield shifted as compared to those of 154b. Those of the cavitand are not much affected except that they are broadened (Fig. 6-7). Although MS data could not be obtained yet, the structure of 155b is supported by DOSY NMR spectroscopy, based on which all signals have the same diffusion constant, indicating that they all belong to a single species (Fig. 6-8). According to the ‘Stokes-Einstein’ equation, a diameter of 5.92 nm was calculated for 155b from its diffusion constant, which is consistent with estimates from force field calculations and CPK models.
Figure 6-7: $^1$H NMR spectra (500MHz, D$_2$O, 25 °C) of cavitand 146 (A), rhombicuboctahedral nanocapsule 155b (B) and linker 154b (C).

Figure 6-8: DOSY NMR spectrum (500 MHz, D$_2$O, pD = 9, 25 °C) of nanocapsule 155b.
Although the product formed from six cavitands 146 and eight linkers 154c shows very broad signals in the $^1$H NMR spectrum, DOSY NMR again supports formation of rhombicuboctahedral nanocapsule 155c, with a diameter of 5.90 nm (Fig. 6-9). It is unlikely that the broad signals in the NMR spectrum result from aggregation, since the linkers are quite hydrophilic. Possibly, the bulky ortho substituents may affect the conformational exchange of the hydrazone bonds, leading to broad signals. Unfortunately, 155c could not be characterized by ESI MS or MALDI-TOF MS, either.

Figure 6-9: $^1$H and DOSY NMR spectra (500 MHz, D$_2$O, 25 °C) of rhombicuboctahedral nanocapsule 155c.
6.3 Preliminary results for protein encapsulation

Cytochrome c was chosen as protein for the first protein encapsulation attempts inside 155b. Cytochrome c is a globular protein, which can fit into the cavity of 155b according to CPK models. It is also very stable towards denaturation under the experimental conditions.

![Figure 6-10](image)

**Figure 6-10:** $^1$H NMR spectra (500 MHz, D$_2$O, pD = 3.5, 10 mM acetate buffer, 25 °C) of cavitand 146 (A), a 1:1 mixture of 146 and cytochrome c (B), nanocapsule 155b (C), a 1:1 mixture of nanocapsule 155b and cytochrome c immediately after mixing (D) and after 3 days (E).

Since complexation-induced shifts are normally observed for host-guest complexation, $^1$H NMR spectroscopy was initially used as analytical tool to follow the encapsulation
process. Fig. 6-10 shows the $^1$H-NMR spectra of cavitand 146, a 1:1 mixture of 146 and cytochrome c, and a 1:1 mixture of cytochrome c and nanocapsule 155b. Due to the low intensity and possibly broadening of cytochrome c, its signals did not come out of the baseline. As compared to the $^1$H NMR spectrum of free 146 (A), H$_1$ and H$_3$ of 146 in the mixture are slightly shifted, but protons of alkyl feet do not obviously change. This indicates that cytochrome c is likely binding to the upper rim of 146, possibly interacting with the carboxylic acid groups. The –SO$_3^-$ groups in the feet seem to have lower binding affinity towards cytochrome c. Addition of cytochrome c to nanocapsule 155b did not induce obvious chemical shift changes. However, considering the strong broadening of the nanocapsule spectrum, a slight change may be difficult to observed. Therefore, the encapsulation was also investigated by reverse phase HPLC (RP-HPLC).

We expect, that the retention time of 155b and cytochrome c will be altered upon complexation/encapsulation. Fig. 6-11A shows the HPLC trace of empty nanocapsule 155b, which has a retention time of 10.3 minutes. After 155b was mixed with cytochrome c and allowed to equilibrate for two weeks at pD = 3.5, the retention time changed to 17.3 minutes. The change of retention time indicates the presence of interactions between nanocapsule 155b and cytochrome c. However, cytochrome c could bind outside or inside of 155b. Fig. 6-11C shows the chromatogram upon immediate injection of a freshly prepared mixture of nanocapsule 155b and cytochrome c. The retention time is identical to that of the above sample. Since the encapsulation of such a large molecule is likely to be slow, the sample shown in Fig. 6-11C must be a ‘outside-binding’ complex. Therefore, cytochrome c simply binds outside. Possibly, the electrostatic interactions between the negatively charged
nanocapsule and positively charged cytochrome c are too strong to allow cytochrome c to move inside the nanocapsule. In addition, the encapsulation of cytochrome c may require temporary opening of one or more acylhydrazone bonds, but the slow dynamic of hydrazone bonds may prevent the opening of bonds within the time frame of the experiment and thus, prevent the encapsulation.

Figure 6-11: HPLC trace of empty nanocapsule (A), mixture of nanocapsule 155b and cytochrome c for 2 weeks (B) and mixture after immediate mixing (C). (Conditions: column, Vydac RP-18, 5 mm, 300 Å*, 4.6 × 250 mm; mobile phase, CH₃OH/10 mM, pH = 7, Na₂HPO₄-NaH₂PO₄ buffer, pH = 7; gradient 10:90 to 90:10 over 20 minutes, then isocratic; flow, 1 ml/minute; detection λ = 280 nm)

The other too smaller and less charged proteins, insulin and ubiquitin, and nanocapsule 155c were also tested, which, unfortunately, gave the same results.
6.4 Conclusion and outlook

In this chapter, water-soluble hemicarcerands and rhombicuboctahedral nanocapsules were synthesized for the first time by using dynamic acylhydrazone bonds. They are stable at or above pD = 3.5, which is suitable for use in biological systems. Since acylhydrazone bonds are dynamic under acidic conditions, but become permanent under basic conditions, these capsules can be potentially used for drug delivery. Although preliminary protein encapsulation experiments were not successful and led to the electrostatic binding of cytochrome c to the outside of the capsule, the modification of water-soluble nanocapsules to avoid outside binding is currently under investigation. One way is to solubilizing acylhydrazone nanocapsules in water by attaching polyethylene glycol (PEG) chains, which both minimize the electrostatic interactions between nanocapsules and proteins and increase the biocompatibility of nanocapsules. Advantageous may also be the use of imine bonds and the assembly of imine nanocapsules in water, which should be possible, based on the EMs of rhombicuboctahedral nanocapsule. Imine nanocapsules are likely to give sharper signals, which improves analysis by NMR spectroscopy. Additionally, imine bonds are more dynamic than acylhydrazones, which can accelerate the guest exchange. Especially for large biomolecules, which require temporarily opening of one or more imine bonds, fast imine bond hydrolysis is critical for the encapsulation within a reasonable time.

6.5 Experimental section

6.5.1 General procedures

Reagents and chromatography solvents were purchased and used without further purification except for chloroform, which was passed through K$_2$CO$_3$ prior to use.
THF was dried over Na/benzophenone and distilled under argon. $^1$H NMR spectra recorded in CDCl$_3$ were referenced to residual CHCl$_3$ at $\delta_H = 7.26$. $^{13}$C NMR spectra recorded in CDCl$_3$ were referenced to $^{13}$CDCl$_3$ at $\delta_C = 77.25$ ppm. NMR spectra were recorded on VARIAN 500, 400 and 300MHz NMR instruments. Mass spectra were recorded with an Applied Biosystems Voyager DE-Pro mass spectrometer (MALDI-TOF) with 2,4,6-trihydroxylacetophenone (THAP) as matrix.

6.5.2 Synthesis of 140

Tetrabromocavitand 138 (1.1 g, 0.53 mmol) was dried in vacuum overnight at 100 °C in a flask and then dissolved in THF (50 mL). BuLi/hexane (2.5 M, 2.1 mL, 5.25 mol) was slowly added to the solution at -78 °C under argon. After 2 hours, MeO-Bpin 139 (1.74 mL, 8.4 mmol) was added to the above solution at the same temperature. After 1 hour, the solution was slowly heated up to room temperature. Subsequently, the reaction was quenched by adding saturated NH$_4$Cl solution (60 mL). After stirring for another 2 hours, the reaction mixture was extracted with EtOAc (3 × 50 mL). The combined organic solutions were washed with saturated NaCl solution, dried over anhydrous MgSO$_4$ and concentrated in vacuum. The crude product was purified by silica gel column chromatography (CH$_2$Cl$_2$) and 138 (82%) was obtained as a white solid.
\[ \text{H NMR spectrum (300 MHz, CDCl}_3, 25 ^\circ \text{C):} \quad \delta_H = 7.61 \ (m, \ 16H, H_{a2}), \ 7.41-7.27 \ (m, \ 24H, H_{a3} \text{ and } H_{a4}), \ 7.02 \ (s, 4H, H_{a1}), \ 5.59 \ (d, 4H, J = 7.54 Hz, H_o), \ 4.73 \ (t, 4H, J = 7.98 Hz, H_m), \ 4.53 \ (d, 4H, J = 7.54 Hz, H_i), \ 3.57 \ (t, 8H, J = 6.21 Hz, H_4), \ 2.10 \ (m, 8H, H_1), \ 1.59 \ (m, 8H, H_3), \ 1.39-1.21 \ (m, 56H, H_3 \text{ and } H_6), \ 1.00 \ (s, 36H, H_5). \]

\[ \text{13}^C \text{ NMR spectrum (125 MHz, CDCl}_3, 25 ^\circ \text{C):} \quad \delta_C = 158.05, \ 137.86, \ 135.76, \ 134.25, \ 129.69, \ 127.79, \ 122.51, \ 99.66, \ 83.76, \ 63.98, \ 36.01, \ 32.63, \ 29.78, \ 27.12, \ 24.96, \ 24.05, \ 19.39. \]

\[ \text{MS (MALDI-TOF) m/z:} \quad 2305.1971 \ (M + \text{Na}^+, 100\%); \quad \text{Calcd for C}_{136}H_{172}O_{20}Si_4B_4 + \text{Na}^+: \quad 2305.1852. \]

6.5.3 Synthesis of 142 (small scale reaction)

Cavitand 140 (32.92 mg, 57.7 µmol), 4-bromo-2-hydroxybenzaldehyde 141 (17.4 mg, 86.6 µmol), K$_3$PO$_4$ (24.5 mg, 115.4 µmol), Pd(OAc)$_2$ (0.65 mg, 2.9 µmol) and S-PHOS (2.4 mg, 5.8 µmol) were weighed in a Schlenk flask. Under argon, degassed THF (10 mL) and water (1 mL) were added to the flask. After sonicking for 10 minutes, the sealed flask was heated to 100 °C for 24 hours. The obtained solution was acidified with 0.1 M HCl solution and extracted with EtOAc (3×20 mL). The combined organic solutions were washed with saturated NaCl solution, dried over anhydrous MgSO$_4$ and concentrated in vacuum. The crude product was purified by
silica gel column chromatography (EtOAc-CH₂Cl₂ = 2:98). The product was obtained in 70% as a white solid.

![Chemical structure]

$^1$H NMR spectrum (300 MHz, CDCl₃, 25 °C): $\delta_H = 11.00$ (s, 4H, -OH), 9.87 (s, 4H, -CHO), 7.70-7.59 (m, 16H, $H_{a2}$), 7.51 (d, 4H, $J = 7.50$ Hz, $H_{a5}$), 7.38-7.27 (m, 28H, $H_{a3}$, $H_{a4}$ and $H_{a7}$), 6.71 (d, 4H, $J = 7.50$ Hz, $H_{a6}$), 6.61 (s, 4H, $H_{a1}$), 5.27 (d, 4H, $J = 7.19$ Hz, $H_{o}$), 4.82 (t, 4H, $J = 8.15$ Hz, $H_{m}$), 4.18 (d, 4H, $J = 7.19$ Hz, $H_{i}$), 3.65 (t, 8H, $J = 6.23$ Hz, $H_{l}$), 2.28 (m, 8H, $H_{l}$), 1.68 (m, 8H, $H_{h}$), 1.45 (m, 8H, $H_{h}$), 1.01 (s, 36H, $H_{h}$). $^{13}$C NMR spectrum (100 MHz, CDCl₃, 25 °C): $\delta_C = 196.30$, 161.33, 152.30, 143.62, 138.53, 135.75, 134.15, 133.34, 129.79, 128.58, 127.83, 122.10, 120.77, 119.81, 118.91, 100.76, 64.00, 36.84, 32.62, 30.03, 27.13, 24.26, 19.43. MS (MALDI-TOF) m/z: 2280.9250 (M + Na⁺, 100%); Calcd for C₁₄₀H₁₄₄O₂₀Si₄ + Na⁺: 2280.8687.

### 6.5.4 Synthesis of 144

Cs₂CO₃ (0.7 g, 2.13 mmol) was added to a solution of cavitand 144 (0.6 g, 0.27 mmol) in DMF (20 mL). Under argon, tert-butyl bromoacetate (470 µL, 3.2 mmol) was added to the above suspension. The mixture was warmed up to 60 °C and kept stirring
overnight. The solvent was evaporated in vacuum and the residue was taken up in CH$_2$Cl$_2$, washed with water, dried over MgSO$_4$ and concentrated in vacuum. The crude product was purified by silica gel column chromatography (EtOAc-CH$_2$Cl$_2$ = 1:9). The product was obtained in 53% yield as a white solid.

\[ \text{1H NMR spectrum (400 MHz, CDCl$_3$, 25 °C): } \delta_H = 10.51 (s, 4H, -CHO), 7.80 (d, 4H, } J = 7.79 \text{ Hz, H}_a5), 7.62 (m, 16H, H$_{a2}$), 7.37-7.27 (m, 28H, H$_{a3}$, H$_{a4}$ and H$_{a7}$), 6.64 (s, 4H, H$_{a1}$), 6.59 (d, 4H, } J = 7.79 \text{ Hz, H}_{a6}), 5.24 (d, 4H, } J = 7.19 \text{ Hz, H}_o), 4.82 (t, 4H, } J = 7.79 \text{ Hz, H}_m), 4.49 (s, 8H, H$_7$), 4.17 (d, 4H, } J = 7.79 \text{ Hz, H}_i), 3.66 (t, 8H, } J = 6.29 \text{ Hz, H}_4), 2.30 (m, 8H, H$_1$), 1.69 (m, 8H, H$_3$), 1.48 (m, 8H, H$_2$), 1.39 (s, 36H, H$_8$), 1.02 (s, 36H, H$_5$). \]

\[ \text{13C NMR spectrum (125 MHz, CDCl$_3$, 25 °C): } \delta_C = 189.30, 167.13, 159.90, 152.59, 141.74, 138.52, 135.73, 134.12, 129.81, 128.66, 128.26, 127.85, 124.62, 122.82, 120.69, 115.85, 100.74, 83.06, 66.42, 64.01, 36.81, 32.60, 30.12, 29.91, 28.20, 27.13, 24.20, 19.43. \]

**6.5.5 Synthesis of 145**

At 0 °C, cavitand 144 (212.7 mg, 94.6 μmol) was dissolved in TFA (4 mL). After 10 minutes, it was slowly warmed up to room temperature and kept stirring for 2 hours.
Then, the solution was washed with hexane (5 × 1 mL) to remove TBDPS-H and concentrated in vacuum. Without purification, the obtained residue and Na₂CO₃ (220 mg, 1.59 mmol) were dissolved in 6 mL MeOH-H₂O (1:1). After 1 hour, the solvent was removed in vacuum. The residue was dissolved in water and acidified with 1 M HCl solution. The precipitate was filtered off and dried in vacuum. The product was obtained in 95% as an off-white solid.

![Chemical structure](image)

**1H NMR spectrum (400 MHz, DMSO-d6, 25 °C):** δH = 10.40 (s, 4H, -CHO), 7.87 (s, 4H, H₄), 7.69 (d, 4H, J = 8.22 Hz, H₆), 6.97 (s, 4H, H₁), 6.72 (d, 4H, J = 8.22 Hz, H₃), 5.22 (d, 4H, J = 7.64 Hz, H₅), 4.84 (s, 8H, H₃), 4.69 (t, 4H, J = 8.22 Hz, H₆), 4.30 (d, 4H, J = 7.64 Hz, H₂), 3.46 (t, 8H, J = 5.87 Hz, H₄), 2.50 (m, 8H, H₁, overlap with DMSO-d5), 1.62 (m, 8H, H₃), 1.38 (m, 8H, H₂). **13C NMR spectrum (100 MHz, DMSO-d6, 25 °C):** δC = 188.96, 169.87, 159.61, 151.57, 141.16, 138.40, 127.99, 127.31, 123.34, 123.07, 122.52, 115.74, 99.79, 65.18, 60.75, 37.24, 32.59, 29.43, 24.31. MS (MALDI-TOF) m/z 1559.4847 (M + Na⁺, 100%); Caled for C₈₄H₆₀O₂₈ + Na⁺: 1559.4728.

**6.5.6 Synthesis of 146·DMSO**

Cavitand 145 (46.3 mg, 43.2 µmol) and pyridine·SO₃ (165 mg, 1.04 mmol) were dissolved in anhydrous DMSO (1 mL) and the solution was stirred at 80 °C for 1 hour.
After the solution was cooled down to room temperature, saturated Na₂CO₃ solution was added to the solution until pH = 11. Then, the solvent was removed in vacuum and the residue was sonicated in DMSO (3 mL) for 10 minutes. The insoluble salt was filtered off and the filtrate was concentrated in vacuum. CH₃CN (10 mL) was added to the concentrated DMSO solution. The precipitate was filtered off, washed with CH₃CN several times and dried in vacuum. The product with one encapsulated DMSO molecule was obtained in 53% yield as a yellowish solid.

¹H NMR spectrum (400 MHz, D₂O, 25 °C): δ_H = 10.37 (s, 4H, -CHO), 7.86 (d, 4H, J = 8.14 Hz, H_a2), 7.81 (s, 4H, H_a4), 6.90 (d, 4H, J = 8.14 Hz, H_a3), 6.82 (s, 4H, H_a1), 5.22 (d, 4H, J = 7.32 Hz, H_o), 4.78 (m, 4H, H_m, overlap with H₂O), 4.60 (s, 8H, H_3), 4.38 (d, 4H, J=7.32 Hz, H_i), 4.20 (t, 8H, J = 6.10 Hz, H_4), 2.64 (m, 8H, H_1), 1.95 (m, 8H, H_3), 1.59 (m, 8H, H_2). ¹³C NMR spectrum (100 MHz, D₂O, 25 °C): δ_C = 194.15, 176.52, 161.06, 152.35, 143.59, 139.31, 130.31, 130.04, 124.22, 123.24, 122.74, 115.44, 101.26, 70.35, 87.93, 37.84, 29.41, 29.08, 24.48. MS (ESI) m/z 993.3 (M²⁻, 100%), 654.6 (M³⁻, 100%); Calcd for M²⁻, 993.1; M³⁻, 654.4.

6.5.7 Synthesis of 148a (Procedure 1)

Cavitand 146·DMSO (3.47 mg, 1.64 µmol) was dissolved in D₂O (300 µL) and a solution of isophthalohydrazide 147a in D₂O (64 µL, 10 mg/mL, 3.29 µmol) was
added. Subsequently, acetate buffer (36.4 µL, 100 mM, pD = 3.5) was added to the mixture. **148a** quantitatively formed overnight as determined by $^1$H NMR spectroscopy.

$^1$H NMR spectrum (500 MHz, D$_2$O, 70 °C): $\delta_H = 9.39$ (s, 8H, H$_{im}$), 9.05 (s, 4H, H$_{a5}$), 8.65 (d, 8H, $J = 8.81$ Hz, H$_{a6}$), 8.56 (d, 8H, $J = 7.71$ Hz, H$_{a2}$), 8.27-8.21 (m, 12H, H$_{a4}$ and H$_{a7}$), 7.29 (d, 8H, $J = 7.71$ Hz, H$_{a3}$), 7.25 (s, 8H, H$_{a1}$), 5.74 (d, 8H, $J = 6.61$ Hz, H$_o$), 5.33 (t, 8H, $J = 7.71$ Hz, H$_m$), 5.09 (s, 16H, H$_5$), 4.80 (d, 8H, $J = 6.61$ Hz, H$_i$, overlap with HOD), 4.67 (t, 16H, $J = 6.61$ Hz, H$_4$), 3.10 (m, 16H, H$_1$), 2.43 (m, 16H, H$_3$), 2.09 (m, 8H, H$_2$).

**6.5.8 Synthesis of 148b**

Prepared by procedure 1 from cavitand **146** (3.06 mg, 1.51 µmol), sodium 3,5-di(hydrazinecarbonyl)benzenesulfonate **147b** (0.89 mg, 3.02), **148b** formed quantitatively overnight.
$^{1}$H NMR spectrum (400 MHz, D$_2$O, 25 °C): δ$_H$ = 8.80 (s, 8H, H$_{im}$), 8.63 (s, 4H, H$_{a5}$),
8.44 (s, 8H, H$_{a6}$), 7.98 (d, 8H, $J = 8.0$ Hz, H$_{a2}$), 7.72 (s, 8H, H$_{a4}$), 6.80-6.46 (m, 16H, $J$
= 7.7 Hz, H$_{a1}$ and H$_{a3}$), 5.17 (br, 8H, H$_o$), 4.48 (s, 16H, H$_5$), 4.18 (br, 8H, H$_i$), 4.11 (t,
16H, $J = 6.3$ Hz, H$_4$), 2.55 (br, 16H, H$_1$), 1.85 (m, 16H, H$_3$), 1.50 (m, 8H, H$_2$).

6.5.9 Synthesis of 155b

Prepared by procedure 1 from cavitand 146 (6.16 mg, 3.03 µmol),
1,3,5-tris(4-hydrazinecarbonyl-5-methoxyl-2-sulfophenyl)benzene 154b (1.64 mg,
2.02 µmol), 155b formed quantitatively overnight.
1H NMR spectrum (400 MHz, D2O, 25 °C): δH = 8.71 (s, 24H, Him), 8.43 (s, 24H, H5), 7.97 (s, 8H, H7), 7.87-7.38 (m, 48H, J = 8.0 Hz, H2 and H4), 7.00-6.43 (m, 48H, H1 and H3), 5.18 (br, 24H, H0), 4.32-3.64 (m, 144H, H1 and -OCH3), 2.52 (br, 48H, H1), 1.81 (m, 48H, H3), 1.47 (m, 48H, H2).

6.5.10 Synthesis of 164

Methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate 162 (1.57 g, 5.38 mmol), 1,3,5-tribromobenzene 163 (585.5 mg, 1.49 mmol), K2CO3 (1.85g, 13.4 mmol), Pd(OAc)2 (109.3 mg, 0.448 mmol) and PPh3 (234.8 mg, 0.896 mmol) were weighed in a Schlenk flask. Under argon, degassed THF (10 mL) and H2O (1 mL) were added to the reactants. Then, the sealed tube was heated at 100 °C overnight. The resulting solution was diluted with water and extracted with CH2Cl2 (3 × 50 mL). The combined organic solutions were dried over MgSO4 and concentrated in vacuum. The crude product was purified by silica gel column chromatography (EtOAc-CH2Cl2 = 5:95). The product was obtained in 32% yield as a white solid.
1H NMR spectrum (500 MHz, CDCl₃, 25 °C): δ_H = 7.93 (d, 3H, J = 8.13 Hz, Hₐ1), 7.77 (s, 3H, Hₐ4), 7.28 (dd, 3H, 3_J = 8.13 Hz, 4_J = 1.48 Hz, Hₐ2), 7.21 (d, 3H, J = 1.48 Hz, Hₐ3), 3.99 (s, 9H, -COOMe), 3.92 (s, 9H, -OMe). 13C NMR spectrum (125 MHz, CDCl₃, 25 °C): δ_C = 166.58, 159.88, 146.28, 142.14, 132.67, 126.40, 119.52, 111.36, 56.47, 52.33.

6.5.11 Synthesis of 165

Compound 164 (51 mg, 89.5 mmol) was dissolved in abs. EtOH (3 mL). Hydrazine hydrate (78 µL, 1.61 mmol) was added to the solution. The mixture was refluxed overnight. Then, the solvent and excess hydrazine hydrate were removed in vacuum. The product was obtained in 95% yield as a white solid.

1H NMR spectrum (500 MHz, DMSO, 25 °C): δ_H = 9.26 (s, 3H, -CONH₂), 8.02 (s, 3H, Hₐ4), 7.83 (d, 3H, J = 8.24 Hz, Hₐ1), 7.53 (dd, 3H, 3_J = 8.24 Hz, 4_J = 1.54 Hz, Hₐ2), 7.51 (d, 3H, J = 1.54 Hz, Hₐ3), 4.59 (broad, 6H, -NH₂), 4.00 (s, 9H, -OMe). 13C NMR spectrum (125 MHz, DMSO, 25 °C): δ_C = 165.07, 157.99, 144.50, 141.75, 131.58, 126.31, 121.68, 120.27, 111.50, 56.82.
6.5.12 Synthesis of 166

At 0 °C, 1,3,5-tris(4-hydrazinecarbonyl-3-methoxyphenyl)benzene 165 (125 mg, 0.22 mmol) was added to 3 mL HSO$_3$Cl and stirred under argon at room temperature for 3 days. The resulting solution was carefully poured onto crushed ice. The precipitate was filtered off and washed with water. The residue was suspended in water and neutralized with Na$_2$CO$_3$. The solid was filtered off and the filtrate was concentrated in vacuum to give 166 as a yellowish solid, which was used for the next step without further purification.

$^1$H NMR spectrum (400 MHz, D$_2$O, 25 °C): $\delta_H = 8.53$ (s, 3H, $H_{a1}$), 7.85 (s, 3H, $H_{a3}$), 7.37 (s, 3H, $H_{a2}$), 4.05 (s, 9H, -OMe). $^{13}$C NMR spectrum (100 M, D$_2$O, 25 °C): $\delta_C =$ 168.89, 160.17, 146.51, 138.58, 1330.50, 132.24, 129.70, 117.34, 116.86, 56.83. MS (ESI) m/z 788.99 ([M + Na$^-$], 100%), 393.99 ([M + Na]$^{2-}$, 100%), 810.97 ([M + 2Na$^-$], 100%); Calcd for [M + Na$^-$], 789.1, [M + Na]$^{2-}$, 394.2, [M + 2Na$^-$], 811.0.

6.5.13 Synthesis of 154b

Crude 166 was suspended in 20 mL methanol. HCl gas was bubbled through the solution for 10 minutes at 0 °C. The suspension was warmed up to room temperature and kept stirring for 2 days. Upon completion of the esterification, the precipitate was filtered off and the filtrate was concentrated in vacuum. The obtained orange solid was suspended in 3 mL abs. ethanol and refluxed with 1 mL hydrazine hydrate. After 20 hours, the solvent was removed and the yellowish solid was dissolved in water,
which was acidified with 2 M HCl. The precipitate was filtered and dried in vacuum. 24 mg yellowish solid was obtained. The overall yield starting from 165 is 14%.

![Chemical structure of compound](image)

$^{1}$H NMR (500 MHz, D$_2$O, 25 °C): $\delta_H = 8.35$ (s, 3H, H$_{a1}$), 7.84 (s, 3H, H$_{a3}$), 7.34 (s, 3H, H$_{a2}$), 4.04 (s, 9H, -OMe). MS (ESI) m/z 855.1 ([MD$_2$Na$_2$]$^-$, 100%), 416.4 ([MD$_2$Na]$^-$, 100%); Calcd for [MD$_2$Na$_2$], 855.06, [MD$_2$Na]$^-$, 416.04.

6.5.14 Synthesis of 168

A flask containing 35 mL conc. H$_2$SO$_4$ was cooled in an ice bath. 1,3,5-Tri(4-methylphenyl)benzene 167 (3 g, 8.62 mmol) was slowly added to the acid under vigorously stirring. Then, it was warmed up to room temperature. After 1 hour, it was heated to 90 °C for 12 hours. Upon completion, it was cooled down to room temperature and carefully poured into 30 mL ice water and a brown precipitate was formed. The precipitate was filtered off, dissolved in water and then salted out by adding NaCl. It was filtered again and the residue was dissolved in water. Subsequently, it was neutralized with NaOH. 3.26 g (58%) product was obtained as a white solid after recrystallization.

![Chemical structure of compound](image)

$^{1}$H NMR spectrum (300 MHz, D$_2$O, 25 °C) $\delta_H = 8.09$ (s, 3H, H$_{a3}$), 7.61 (s, 3H, H$_{a4}$), 7.47 (d, 3H, $J = 8.23$ Hz. H$_{a2}$), 7.19 (d, 3H, $J = 8.23$ Hz. H$_{a1}$), 2.60 (s, 9H, -Me).
6.5.15 Synthesis of 169

1,3,5-Tris(4-methyl-3-sulfophenyl)benzene 168 (2.3 g, 3.5 mmol) and 70 mg NaOH were dissolved in 10 mL water. KMnO₄ (3 × 0.83 g, 10.6 mmol) was added to the solution in portions. The reaction was monitored by ¹H NMR spectroscopy until all methyl groups were consumed. Upon completion, MnO₂ was filtered off and the filtrate was acidified with 2 M HCl to pH 3. A mixture of 169 and 170 crystalized as a white solid. Subsequently, 500 mg (20%) of pure 169 was obtained by RP-HPLC.

\[
\begin{align*}
\text{COOH} & \\
n_1 & \\
n_2 & \\
n_3 & \\
n_4 & \\
\text{SO₃H} & \\
\end{align*}
\]

¹H NMR spectrum (400 MHz, D₂O, 25 °C) δ_H = 8.15 (d, 3H, J = 8.23, Hₐ₃), 8.01 (s, 3H, Hₐ₄), 7.88 (dd, 3H, J₃ = 8.3 Hz, J₄ = 1.9 Hz, Hₐ₂), 7.19 (d, 3H, J = 7.4 Hz, Hₐ₁).

6.5.16 Synthesis of 154c

1,3,5-Tris(4-carboxylato-3-sulfonatophenyl)benzene 169 was suspended in MeOH and purged with HCl gas for 10 minutes. After two days, the precipitate was filtered off and the filtrate was concentrated in vacuum. Without purification, the residue was dissolved in 5 mL hydrazine hydrate and refluxed for 24 hours. The excess hydrazine was removed and the residue was dissolved in water. The solution was acidified with 1 M HCl and the formed precipitate was filtered off and recrystallized from hot DMSO. 155 mg (33%) of product was obtained as off-white solid.
\[ \text{CONNHNH}_2 \]
\[ \text{SO}_3\text{H} \]

$^1$H NMR spectrum (400 MHz, D$_2$O, 25 °C) $\delta_H = 7.22$ (s, 3H, H$_{a3}$), 6.94 (s, 3H, H$_{a4}$), 6.88 (d, 3H, $J = 7.9$ Hz, H$_{a2}$), 6.60 (d, 3H, $J = 8.4$ Hz, H$_{a1}$).

6.6 References


### Appendix A. Compound numbers and structures

<table>
<thead>
<tr>
<th>Compd #</th>
<th>Structure</th>
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<td>11</td>
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</tr>
<tr>
<td>12 a-c</td>
<td><img src="" alt="Image" /></td>
</tr>
<tr>
<td>a. R = (CH₂)₄CH₃</td>
<td>b. R = (CH₂)₂Ph</td>
</tr>
<tr>
<td>c. R = CH₃</td>
<td></td>
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<tr>
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<td><img src="" alt="Image" /></td>
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</tbody>
</table>
| 16 | \[
\text{R}=(\text{CH}_2)_2\text{Ph}
\] |
|---|---|
| 17a-t | \[
\text{H}_2\text{N}^-\text{A}^+\text{NH}_2
\] |
| a: A = -(CH\(_2\))\(_2\) | j: A = \[
\text{O}_2\text{C}_2\text{H}_3
\] |
| b: A = -(CH\(_2\))\(_3\) | n: A = \[
\text{O}_2\text{C}_2\text{H}_3
\] |
| c: A = -(CH\(_2\))\(_4\) | k: A = \[
\text{O}_2\text{C}_2\text{H}_3
\] |
| d: A = -(CH\(_2\))\(_5\) | o: A = \[
\text{O}_2\text{C}_2\text{H}_3
\] |
| e: A = -(CH\(_2\))\(_6\) | f: A = -(CH\(_2\))\(_7\) |
| g: A = \[
\text{O}_2\text{C}_2\text{H}_3
\] |
| h: A = \[
\text{O}_2\text{C}_2\text{H}_3
\] |
| i: A = \[
\text{O}_2\text{C}_2\text{H}_3
\] |
| j: A = \[
\text{O}_2\text{C}_2\text{H}_3
\] |
| k: A = \[
\text{O}_2\text{C}_2\text{H}_3
\] |
| I: A = \[
\text{O}_2\text{C}_2\text{H}_3
\] |
| p: A = \[
\text{O}_2\text{C}_2\text{H}_3
\] |
| m: A = \[
\text{O}_2\text{C}_2\text{H}_3
\] |
| q: A = \[
\text{O}_2\text{C}_2\text{H}_3
\] |
| r: A = \[
\text{O}_2\text{C}_2\text{H}_3
\] |
| s: A = \[
\text{O}_2\text{C}_2\text{H}_3
\] |
| t: A = \[
\text{O}_2\text{C}_2\text{H}_3
\] |
| 17r | H\(_2\)N | 17s | H\(_2\)N | 17t | H\(_2\)N |
| 18a | \[
\text{R}=(\text{CH}_2)_2\text{Ph}
\] |
| 19 | \[
\text{R}=(\text{CH}_2)_4\text{CH}_3
\] |
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### Equations

\[
R = (\text{CH}_2)_4 \text{CH}_3
\]

\[
-\text{CH} = \text{N(CH}_2)_2 \text{N=CH-}
\]

\[
R = (\text{CH}_2)_4 \text{CH}_3
\]

\[
-\text{CH} = \text{N(CH}_2)_2 \text{N=CH-}
\]

\[
R = (\text{CH}_2)_4 \text{CH}_3
\]

\[
-\text{CH} = \text{N(CH}_2)_2 \text{N=CH-}
\]

\[
R = \text{C}_{10}^6 \text{H}_{13}
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<td>35</td>
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</tr>
<tr>
<td>36</td>
<td><img src="https://example.com/image5.png" alt="Image" /></td>
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</tbody>
</table>
| 37a-c | a. R = (CH\(_2\))\(_4\)CH\(_3\)  
b. R = (CH\(_2\))\(_2\)Ph  
c. R = CH\(_3\)CH\((\text{CH}_3)\_2\) |
| 38a-c | a. R = (CH\(_2\))\(_4\)CH\(_3\)  
b. R = (CH\(_2\))\(_2\)Ph  
c. R = (CH\(_2\))\(_4\)OH |
| 39a-d | a. R = (CH\(_2\))\(_3\)OH  
b. R = (CH\(_2\))\(_2\)Ph  
c. R = (CH\(_2\))\(_4\)OH  
d. R = (CH\(_2\))\(_4\)OTBDPS |
40a-e

- $R = (\text{CH}_2)_4 \text{CH}_3$
- $R = (\text{CH}_2)_2 \text{Ph}$
- $R = (\text{CH}_2)_4 \text{OH}$
- $R = (\text{CH}_2)_4 \text{OTBDPS}$
- $R = (\text{CH}_2)_4 \text{O}$

41a, b

- $\text{CH}=\text{N}(\text{CH}_2)_2 \text{N}=\text{CH}$
- $R = (\text{CH}_2)_4 \text{CH}_3$
- $R = (\text{CH}_2)_2 \text{Ph}$

42

43

44

45

46

47

48

49

50

51

52
| 53 | ![Chemical Structure 53](image) |
| 54 | ![Chemical Structure 54](image) |
| 55 | ![Chemical Structure 55](image) |
| 56 | ![Chemical Structure 56](image) |
| 57 | ![Chemical Structure 57](image) |
| 58 | ![Chemical Structure 58](image) |
$R = (CH_2)_4CH_3$

67a-c

68a-c

69
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<td>74</td>
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</table>
R = (CH₂)₄CH₃

a: A = -(CH₂)₃-
b: A = -(CH₂)₄-
c: A = -(CH₂)₅-
d: A = -(CH₂)₆-
e: A = -(CH₂)₇-
f: A = -(CH₂)₈-
g: A = -(CH₂)₉-

H₂N-A-NHAc

76a-g, i, s

H₂N-A-NHAc

76a-g, i

R = (CH₂)₄CH₃

77

R = (CH₂)₄CH₃

78

R = (CH₂)₄CH₃

79

R = (CH₂)₄CH₃

80

R = (CH₂)₄CH₃

81

R = (CH₂)₄CH₃
$R = (\text{CH}_2)_4\text{CH}_3$

95a, b

a: $p$-NHAc
b: $m$-NHAc

$R = (\text{CH}_2)_4\text{CH}_3$

96a, b

a: $p$-NHAc
b: $m$-NHAc

$R = (\text{CH}_2)_4\text{CH}_3$

97

$R = (\text{CH}_2)_4\text{CH}_3$
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<th>98b, d, g</th>
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</table>
|   | ![Image](image1.png) | b: A = (CH₂)₃  
   |   |   | d: A = (CH₂)₅  
   |   |   | g: A = ![Image](image2.png)  
   | R = (CH₂)₄CH₃ |   |
|   | 99d, d |   |
|   | ![Image](image3.png) | b: A = (CH₂)₃  
   |   |   | d: A = (CH₂)₅  
   |   |   | g: A = ![Image](image4.png)  
   | R = (CH₂)₄CH₃ |   |
|   | 100b, d, g |   |
|   | ![Image](image5.png) | b: A = (CH₂)₃  
   |   |   | d: A = (CH₂)₅  
   |   |   | g: A = ![Image](image6.png)  
   | R = (CH₂)₄CH₃ |   |
|   | 101b, d, g |   |
|   | ![Image](image7.png) | b: A = (CH₂)₃  
   |   |   | d: A = (CH₂)₅  
   |   |   | g: A = ![Image](image8.png)  
   | R = (CH₂)₄CH₃ |   |
|   | 102b, d, g |   |
|   | ![Image](image9.png) | b: A = (CH₂)₃  
   |   |   | d: A = (CH₂)₅  
   |   |   | g: A = ![Image](image10.png)  
<p>| R = (CH₂)₄CH₃ |   |</p>
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<td>137G</td>
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</table>
147a, b

\[
\begin{align*}
R & \equiv CONHNH_2 \\
ap & \equiv R = H \\
b & \equiv R = SO_3Na
\end{align*}
\]

148a, b

\[
\begin{align*}
Z & \equiv CH_3COONa \\
r'' & \equiv (CH_2)_4OSO_3Na
\end{align*}
\]

149

\[
\begin{align*}
\text{COOMe}
\end{align*}
\]

150

\[
\begin{align*}
\text{COOEt}
\end{align*}
\]

151

\[
\begin{align*}
\text{PrBr}
\end{align*}
\]

152
\[ R'' = (\text{CH}_2)_2\text{SO}_3\text{Na} \]
\[ Z = \text{CH}_2\text{COOH} \]
Appendix B. $^1$H NMR spectra, $^{13}$C NMR spectra and MALDI-TOF MS of compounds
Figure 61: Chemical structure and NMR spectra for compound 61.
98b

![Chemical Structure](image)

**Dimensions:** Hm, H1, H2, H3, H4, H5, H6, H7

**PPM:**
- 8.0
- 7.0
- 6.0
- 5.0
- 4.0
- 3.0
- 2.0
- 1.0
- 0.0

**Labels:**
- Hm
- H1
- H2
- H3
- H4
- H5
- H6
- H7

**Peak Intensities:**
- 2.318
- 0.752
- 1.885
- 3.123
- 8.005
- 26.21
- 12.55

---

98b

![Chemical Structure](image)

**Dimensions:** Hm, H1, H2, H3, H4, H5, H6, H7

**PPM:**
- 140.0
- 120.0
- 100.0
- 80.0
- 60.0
- 40.0
- 20.0
98d
20b ○ 119

- 20b
20b \odot 120

- 20b
20c ◊ 126

- 20c

- NH2
20g\(\circ\) 120

- 20g
329

144

CHO

PPM

10.0
8.0
6.0
4.0
2.0

180.0
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
Curriculum Vita

Junling Sun

Education

2008-Present  Rutgers, The State University of New Jersey, NJ, USA
Ph.D. Candidate in Chemistry

2004-2008  Wuhan University, Hubei, China
B.S. in Chemistry

Publications