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Differentially Functionalyzed Acyclic Cucurbiturils: Synthesis, Self-assembly and CB[6]-Induced Allosteric Guest Binding

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Received (in XXX, XXX) Xth XXXXXXXXXX 20XX, Accepted Xth XXXXXXXXXX 20XX
DOI: 10.1039/c0xx00000x

We report the synthesis of mono- and difunctionalized acyclic cucurbit[6]uril-type containers (1HA, 1HDA, 2HDA) which bear hexylammonium and hexanediammonium arms. The intra- and intermolecular assembly processes of 1HA, 1HDA, 2HDA as well as the ability of CB[n] to trigger allosteric host-guest binding toward guests 9-11 are presented.

The preparation of new molecular container compounds (e.g. crown ethers, cyclodextrins, calixarenes, pillarenes), studies of their molecular recognition behaviour, and their use in advanced technological and biological applications constitute a major facet of contemporary supramolecular chemistry. Since the discovery of cucurbit[6]uril (CB[6]), n = 5, 6, 7, 8, 10, 14; Fig. 1) homologues at the turn of the millennium, CB[n] compounds have played a central role in the field. CB[n] compounds feature two symmetry equivalent ureidyl C=O portals of highly negative electrostatic potential that guard entry to a hydrophobic cavity. CB[n] compounds display extraordinarily high affinity (K_a routinely exceeds 10^8 M^-1) and selectivity toward hydrophobic (diammonium ions and even neutral guests in water. In addition, CB[n] compounds are highly stimuli responsive (e.g. pH, electrochemical, photochemical, chemical) and have been employed in a variety of applications including chemical sensing, drug solubilisation and delivery, molecular machines, supramolecular catalysis, and supramolecular polymers. Recently, the first examples of monofunctionalized CB[6] and CB[7] derivatives have appeared and have been used in several applications including supramolecular Velcro®, chemical sensors, and for targeted drug delivery. Over the years, a number of related structures (CB[n]-type containers) have been prepared including CB[n] derivatives, CB[n] analogues, nor-seco-CB[n], hemicucurbit[n]urils, bambus[n]urils, and biotin[n]urils. Recently, the Isaacs and Sindelar groups have been investigating acyclic CB[n]-type containers (e.g. M2, Fig. 1) which feature a central glycuril oligomer capped with two aromatic walls and sulfonate solubilizing groups and demonstrated their use as solubilizing excipients for insoluble drugs, as components of sensing ensembles, and as an in vivo reversal agent for neuromuscular block. In order to extend the range of applications open to acyclic CB[n]-type receptors we sought to develop synthetic schemes that allow access to differentially functionalyzed acyclic CB[n]-type containers. In this paper we report the preparation of mono- and difunctionalized acyclic CB[n]-type containers 1 and 2 and study their self-association, aggregation and CB[6] induced allosteric guest binding.

For the synthesis of differentially functionalyzed acyclic CB[6]-type containers we decided to allow methylene bridged glycururil tetramer 3 to react with a mixture of two different aromatic sidewalls (e.g. 4 and 6 or 5 and 6) under our standard conditions (CF_3CO_2H / ACO_2, 70 °C) as shown in Scheme 1. The sulfonated naphthalene sidewall 6 was chosen to enhance aqueous solubility whereas the benzene derived sidewalls 4 or 5 were selected to bear one or two reactive primary alkylbromide arms. We find that the benzene derived sidewalls (4 and 5) are more reactive than the naphthalene derived sidewall 6 which results in a reaction mixture that does not contain significant amounts of the tetrasulfonate M2 and thereby simplifies purification. The crude reaction mixtures are initially subjected to Dowex™ ion exchange chromatography (50WX2 resin; SO_4H functional groups on resin) using H_2O as eluent. The desired disulfonates (+)-7 and 8 elute from through the column rapidly due to repulsive sulfonate-sulfonate interactions whereas the major byproduct containing two benzene derived walls (and no sulfonate group) sticks strongly to the resin. Compounds (+)-7 and 8 were obtained by recrystallization from water/acetone mixtures in modest yields of 12% and 20%, respectively. Subsequently, we reacted (+)-7 with hexylamine in water at 100 °C followed by washing with basic MeOH and obtained (+)-1A which has one hexylamine arm in 83% yield. Treatment of (+)-7...
with N-Boc-1,6-hexanediame in water at 100 °C followed by deprotection with TFA and washing with basic MeOH gave (+)-1HA, which contains one hexanediame arm in 73% yield. In an analogous way we reacted 8 with N-Boc-1,6-hexanediame to give C2-symmetric 2HA in 75% yield which features two hexanediame arms. The new compounds were fully characterized by 1H and 13C NMR, IR, and mass spectrometry which were fully consistent with the depicted structures of (+)-1HA, (+)-1HDA, and 2HDA.

After having established a versatile method to prepare monofunctionalized and difunctionalized acyclic CB[n]-type containers (+)-1HA, (+)-1HDA, and 2HDA we turned to an investigation of their supramolecular chemistry. Of course, compounds (+)-1HA, (+)-1HDA, and 2HDA feature alkyl ammonium groups covalently connected to CB[n]-type receptors and therefore have the potential to undergo self-complexation, daisy-chain formation, or supramolecular polymerization.4b-d Figs. 2a–c show the 1H NMR spectra recorded for (+)-1HA, (+)-1HDA, and 2HDA in D2O. Several features of the spectra are noteworthy.

First, four resonances are observed at ≈ 1.8 ppm for the different CH3-groups of the glycoluril tetramer unit which reflect the end-to-end symmetry of this class of compounds. Second, the upfield shifts observed for H2 and H4 relative to model compounds reflect the fact that the o-xylene sidewalls of (+)-1HA, (+)-1HDA, and 2HDA adopts an edge-to-face arrangement with the opposing naphthalene sidewall which places these protons in the anisotropic shielding region of the naphthalene ring. Third, and most importantly, the resonances for the alkane (d)ammonium arms (H8 – H13) are upfield shifted which shows that these arms are bound within the acyclic CB[n]-type cavity (Fig. 2). The diffusion coefficient measured by DOSY spectroscopy for (+)-1HA (2.44 × 10−10 m²/s) and (+)-1HDA (2.56 × 10−10 m²/s) were comparable to those measured for the monomeric control compound 8 (2.52 × 10−10 m²/s) which indicates that these compounds self-include the alkane (d)ammonium arms in their own cavity. The 1H NMR spectrum recorded for 2HDA (Fig. 2c) shows upfield shifts for its hexanediameonum arm (H8 – H13) which indicates it folds into the cavity of 2HDA (Scheme 2); the diffusion coefficient measured for 2HDA (2.69 × 10−10 m²/s) indicates that the broadness of the 1H NMR spectrum (Fig. 2c) is due to dynamic processes rather than aggregation.

After having demonstrated the self-folding of the hexyl ammonium and hexanediameonum arms of (+)-1HA, (+)-1HDA, and 2HDA, we decided to study the use of CB[n] containers as an allosteric switch to promote unfolding and guest binding inside the acyclic CB[n] cavity. Initially, we added CB[7] (1 equiv.) to a solution of 1HDA and observed the unfolded CB[7] (+)-1HDA complex; attempts to add 9, 10, or 11 as guests to bind into the acyclic CB cavity of CB[7] (+)-1HDA were unsuccessful and instead we observed the regeneration of self-folded (+)-1HDA and CB[7] guest complexes (Supporting Information, Fig. S12).

Analogous observations were made with hexanamine ion armed (+)-1HA (Supporting Information, Fig. S11). We surmise

**Scheme 1.** Structures of: a) building blocks and b) guests used in this study. Synthesis of containers (+)-1HA, (+)-1HDA, and 2HDA. Conditions: a) trifluoroacetic acid/ acetic anhydride (1:1), 70 °C, 3h; 12%; b) hexanamide, H2O, 100 °C then 0.1 M NaOH in methanol; 83%; c) N-Boc-1,6-hexanediame, H2O, 100 °C, then trifluoroacetic acid, 2 h, then 0.1 M NaOH in methanol; 73–75%.
that the superior binding affinity of CB[7] toward guests 9 – 11 relative to CB[7]*(+)-1*HDA is responsible for the inability of CB[7] to allosterically activate guest binding to (+)-1*HDA and (+)-1*HA. We also monitored the changes in the self-assembled structures of (+)-1*HDA in the solid state by scanning electron microscopy (SEM). The samples were prepared by dropping their aqueous solution onto a silica wafer followed by freeze drying. Compound (+)-1*HDA forms network structures (Figs. 3a, S19) presumably due to intermolecular interactions between external surfaces of the zwitterionic self-folded form. Fig. 3b shows that upon addition of CB[7] to give CB[7]*1*HDA, the solid state structure transforms into well dispersed aggregates (Supporting Information, Fig. S20). No obvious changes were observed when additional CB[7] was added (Figs. 3c and S21).

Finally, addition of 10 results in the formation of CB[7]*10 and self-folded (+)-1*HDA which again forms networked solid state structures (Figs. 3d, S22).

Although CB[7] is able to induce the unfolding of the ammonium arms of (+)-1*HA and (+)-1*HDA, it is incapable of triggering allosteric binding inside the acyclic CB[n] cavity. To circumvent this issue we decided to investigate CB[6] as the allosteric activator for two reasons: 1) CB[6] binds 5-fold tighter to hexanediammonium units than CB[7] does, 2) the smaller cavity of CB[6] does not form inclusion complexes with 10 or 11 and forms a weak inclusion complex with 9 ($K_d = 550$ M$^{-1}$). 4b) We decided to use 1*HDA for the demonstration of the CB[6] triggered allosteric binding process because it possesses idealized C$_2$ symmetry which makes its $^1$H NMR spectra easier to interpret than asymmetric (+)-1*HA and (+)-1*HDA. Fig. 4a shows the $^1$H NMR spectrum recorded for 1*HDA whereas Fig. 4c shows the $^1$H NMR spectrum recorded for uncomplexed 10. The $^1$H NMR spectrum of the equimolar (0.5 mM) mixture of 1*HDA and 10 (Figure 4b) is simply the sum of its parts which indicates that compound 10 is incapable of competing with the hexanediammonium ion arm of 1*HDA for binding into the cavity of the acyclic CB[n] type container. Fig. 4d shows the $^1$H NMR spectrum recorded for a mixture of 2*HDA and 2 equivalents of CB[6]. Quite interestingly, the presence of two doublets for the o-xylylene protons (H$_1$ and H$_2$) and two resonances for naphthalene protons (H$_3$ and H$_4$; H$_5$ and H$_6$) and the presence of two sets of resonances for H$_7$-H$_{13}$ (one inside a CB[6] cavity and one inside the cavity of 2*HDA) establishes the formation of the 1:1 CB[6]*2*HDA complex rather than the 1:2 CB[6]*2*HDA complex. This indicates that the intramolecular self-folding equilibrium is able to outcompete the formation of the CB[6]*2*HDA complex (Scheme 2). 4e) Fig. 4e shows the $^1$H NMR spectrum that results upon addition of 1 equiv. of 10 to the mixture of CB[6] and 2*HDA. Several changes in the $^1$H NMR spectrum are diagnostic of the formation of the CB[6]*2*HDA*10 tetramolecular complex. For example, the six resonances seen for H$_1$, H$_2$, and H$_5$ of CB[6] + CB[6]*2*HDA simplify to only three resonances for CB[6]*2*HDA*10 complex. In addition, the resonances for uncomplexed 10 (Fig. 4b at 2.16, 1.86, 1.75, and 1.67 ppm) disappear and are shifted upfield into the 1.0 – 0.5 ppm region which indicate binding inside the acyclic CB[n] cavity. We surmise that the sum of the binding free energy associated with the complexation of the second hexanediammonium ion arm inside CB[6] and 10 binding inside the acyclic CB[n] cavity of CB[6]*2*HDA is sufficient to overcome the loss of free energy associated with the release of the self-folded arm from the CB[6]*2*HDA complex. In this manner, CB[6] acts as an allosteric activator that switches on the binding of 10. Fig. 4f shows the $^1$H NMR spectrum recorded upon addition of 1 equiv. CB[7] which partially reverses the allosteric binding process by the formation of the tight binding CB[7]*10 complex. Related observations were made using CB[6] as the allosteric activator to promote the binding of p-xylylenediammonium ion 9 inside CB[6]*2*HDA (Supporting Information, Figs. S13 and S14) which show the clean appearance of resonances for the CB[6]*2*HDA*9 complex (e.g. 9 at 5.99 ppm (H$_2$), the naphthalene sidewall at 8.25 Hz (H$_2$), 7.7 (H$_1$), and the hexanediammonium ion arm complexed inside CB[6] at 0.7 and 0.55 ppm.). Analogous allosteric activation experiments using 1*HDA were unsuccessful due to the formation of precipitates.
In summary, we have reported the preparation of differentially functionalized acyclic CB[n]-type compounds (-)-1_{1\text{H}}, (+)-1_{2\text{HDA}}, and 2_{2\text{HDA}} that contain alkylammonium and alkanediammonium arms. The results of 1H NMR and DOSY spectroscopy indicate that the compounds undergo self-inclusion of their ammonium arms within their own cavity. We find that these self-included ammonium arms can be released by complexation with CB[n] (n = 6, 7) but that only CB[6] is capable of acting as an allostERIC activator that promotes the binding of guests (e.g. 9 or 10) within the acyclic CB[n] cavity of CB[6]•2_{2\text{HDA}}. The work further establishes CB[n]-type containers as outstanding components of stimulus responsive supramolecular systems and suggests application in controlled sequestration and release applications as well as molecular machines.

We thank the US National Science Foundation (CHE-1404911 to L.I.) for financial support. We also acknowledge the use of the facilities of the Maryland NanoCenter and its NispLab.

Notes and references


9) The binding constant for the CB[6]•H2N(CH3)2NH2 complex was previously determined as K0 = 4.5 × 10^8 M^-1. See reference 4b.

10) DOI: 10.1039/cdbc00000x/

† Electronic Supplementary Information (ESI) available: Experimental details of Synthetic procedures, NMR spectroscopy and SEM images.

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