HHS Public Access

Author manuscript

Supramol Chem. Author manuscript; available in PMC 2017 April 13.

Published in final edited form as:

Supramol Chem. 2016 ; 28(9-10): 825–834. doi:10.1080/10610278.2016.1167893.

Cationic acyclic cucurbit[n]uril-type containers: synthesis and molecular recognition toward nucleotides

David Sigwalt, **Peter Y. Zavalij**, and **Lyle Isaacs***

Department of Chemistry and Biochemistry, University of Maryland, College Park, MD 20742, USA

Abstract

We report the synthesis of **M2NH3** which is a tetracationic analogue of our prototypical acyclic CB[n]-type molecular container **M2**. Both **M1NH3** and **M2NH3** possess excellent solubility in D_2O and do not undergo intermolecular self-association processes that would impinge on their molecular recognition properties. Compounds **M1NH3** and **M2NH3** do, however, undergo an intramolecular self-complexation process driven by ion-dipole interactions between the ureidyl C=O portals and the $OCH_2CH_2NH_3$ arms along with inclusion of one aromatic wall in its own hydrophobic cavity. The K_a values for **M1NH3** and **M2NH3** toward seven nucleotides were determined by ^1H NMR titration and found to be quite modest (K_a in the $10^2 – 10^3$ M^{-1} range) although **M2NH3** is slightly more potent. The more highly charged guests (e.g. ATP) form stronger complexes with **M1NH3** and **M2NH3** than the less highly charged guest (e.g. ADP, AMP). The work highlights the dominant influence of the ureidyl C=O portals on the molecular recognition behavior of acyclic CB[n]-type receptors and suggests routes (e.g. more highly charged arms) to enhance their recognition behavior toward anions.

Introduction

Since the pioneering work of Pedersen, Lehn, and Cram more than 40 years ago the development of the field of supramolecular chemistry has witnessed the creation ever more complex receptor molecules whose structures are tailored toward specific molecular targets.[1] For example, macrocyclic molecular container compounds (e.g. crown ethers, cyclodextrins, calixarenes, cyclophanes, pillararenes)[2] have served as core structures of more complex receptors prepared by covalent reactions or by self-assembly processes. These exquisitely designed hosts have been used for a variety of applications including chemical sensing,[3] drug delivery,[4] to promote supramolecular polymerization,[5] supramolecular approaches to catalysis,[6] to stabilize otherwise unstable compounds and conformations of molecules,[7] to promote the assembly and/or disassembly of protein-protein interactions,[8] and for various imaging applications.[9]

Over the past 15 years, the synthetic and supramolecular chemistry of the cucurbit[n]uril (CB[n], n = 5, 6, 7, 8, 10, 14) family of molecular containers has been unfolding rapidly.[10,11] CB[n] compounds are prepared by condensation of glycoluril and formaldehyde

*To whom correspondence should be addressed.

under aqueous acidic conditions and feature *n* glycoluril units connected by *2n* CH_2-bridges.[12] CB[n] compounds possess a hydrophobic cavity that is guarded by two symmetry equivalent ureidyl carbonyl lined portals. The molecular recognition properties of CB[n] containers are unrivaled among synthetic molecular containers in that they display remarkable binding affinity (K_a up to 10^{17} M^{-1}) and very high selectivity toward their guests (hydrophobic cations and even neutral molecules) in aqueous solution.[13,14] CB[n] host-guest complexes form the basis for complex multistate supramolecular systems because they undergo changes in constitution and conformation in response to appropriate stimuli (e.g. pH, chemical, electrochemical, photochemical).[15] For example, unfunctionalized CB[n] containers have featured prominently in a variety of applications including drug delivery,[16] chemical sensing ensembles,[17] supramolecular catalysis, peptide and protein recognition,[18] supramolecular materials,[11,19] and affinity capture materials.[20] In order to further augment the recognition properties of the CB[n] systems, the development of methods for the selective (mono)functionalization of CB[n] have been pursued. As early as 2003, Kim's group developed the perhydroxylation of CB[n] to generate $(HO)_{2n}$CB[n] and has used these compounds as starting materials to prepare supramolecular systems capable of affinity capture, targeted drug delivery, and tissue engineering.[14,21] In 2011 and 2012, the Isaacs group utilized their mechanistic knowledge of the CB[n] forming reaction to devise the first routes to CB[6] and CB[7] derivatives containing a single reactive functional group.[22] Subsequently, the perhydroxylation reaction was tamed which allowed the isolation of monohydroxylated CB[n] compounds by the groups of Scherman, Kim, and Bardelang.[23] The Isaacs group along with the Sindelar group has also been pursuing a different approach to the preparation of functionalized CB[n]-type receptors for advanced applications in the form of acyclic CB[n]-type receptors whose structures are typified by that of **M1** (Figure 1).[24,25-27] **M1** features a central C-shaped glycoluril tetramer which endows it with the ability to bind to hydrophobic cations, two aromatic walls which form **π−π** interactions with aromatic target compounds, and four sodium sulfonate solubilizing groups. **M1** and its relatives have been used in a variety of applications including the solubilization of insoluble pharmaceutical agents and carbon nanotubes, the creation of sensing ensembles, and the *in vivo* reversal of neuromuscular block.[24,25,26] We have studied the influence of several structural variables (e.g. aromatic sidewall identity, solubilizing group identity (NH_3^+ versus OH versus SO_3Na), and length of central glycoluril oligomer (monomer – tetramer)) on their function as solubilizing excipients for insoluble drugs.[24,25,26] Interestingly, in our study of the recognition properties of the cationic analogue of **M1** (**M1NH3**, Figure 1) we found that the container is not a good host for cationic compounds due to self-complexation of the NH_3^+ arms at the ureidyl carbonyl portals, but displayed modest affinity toward adamantanecarboxylate (K_a = 678 M^{-1}) in 20 mM sodium phosphate buffer at pH 7.4.[26] Accordingly, it appeared that the cationic arms turned **M1NH3** into an anion receptor. Based on this observation, we recently prepared cationic acyclic cucurbit[n]uril dendrimers and demonstrated their ability to bind to and compact plasmid DNA although, unfortunately, gene transfection ability was quite modest.[28] Previous supramolecular researchers have shown that polycationic receptors are capable of recognizing nucleotide anions in aqueous solution due to electrostatic and other non-covalent interactions.[29] Within the CB[n] field, bambusurils and related compounds are known to be potent receptors for anions in water.[30] Given the generally high affinity of CB[n] compounds toward their guests, we decided to

investigate the binding properties of tetracationic hosts **M1NH3** and **M2NH3** toward nucleotides because of the confluence of aromatic moieties which are known to bind nicely to acyclic CB[n] and the anionic phosphates which should interact with the ammonium ion arms.

Results and Discussion

This results and discussion section is organized as follows. First, we describe the synthesis of **M2NH3** and determination of its solubility and self-association in water. Next, we describe the structural features of this class of host molecules. Then, we measure the binding constants of **M1NH3** and **M2NH3** toward seven nucleotide guests by ^{1}H NMR spectroscopic titration. Finally, we discuss the trends in binding affinity as a function of guest structure and as a function of ionic strength of the aqueous binding media.

Synthesis of M2NH3

Scheme 1 shows the synthesis of tetracationic acyclic CB[n]-type molecular container **M2NH3**. As starting materials, we selected methylene bridged glycoluril tetramer bis (cyclic ether) **1**[31] and 1,4-bis(2-chloroethoxy)naphthalene **2**.[32] Upon heating in a 1:1 mixture of TFA and Ac_2O, **1** and **2** undergo double electrophilic aromatic substitution reactions to install the two naphthalene walls yielding **3** in 36% yield. Tetrachloro acyclic CB[n] derivative **3** could be transformed into the correspond tetraazide functionalized acyclic CB[n]-type molecular container **4** by treatment with sodium azide in hot DMSO (80 °C) by simple S_N2 chemistry. Finally, **4** could be transformed into the target tetracationic acyclic CB[n]-type molecular container **M2NH3** by treatment with triphenylphospine in aqueous DMSO followed by treatment with HCl. Compound **M2NH3** was characterized by all of the usual spectroscopic techniques including ^{1}H NMR, ^{13}C NMR, infrared spectroscopy, and electrospray ionization mass spectrometry (ES-MS). The high resolution ES-MS spectrum showed a molecular ion that was consistent with the depicted molecular formula of the singly protonated form of the free base of **M2NH3** ($C_{58}H_{69}N_{20}O_{12}$) at m/z = 1237.5409. The ^{1}H NMR and ^{13}C NMR spectra recorded for **3** and **4** (in DMSO) and **M2NH3** in D_2O are relatively simple which reflects their overall C_{2v}-symmetry. For example, the ^{13}C NMR spectra (Supporting Information) display only two resonances for the ureidyl C=O groups, five resonances for the C-atoms of the aromatic ring, two CH_3 signals, two resonances for the OCH_2CH_2X arms, along with three CH_2 and four signals for the equatorial glycoluril C-atoms. Similarly, the ^{1}H NMR spectrum of **M2NH3** displays the typical AA'BB' pattern for aromatic protons H_a and H_b, two singlets for the CH_3-groups, two resonances for the equatorial glycoluril CH groups (H_k and H_l) and three pairs of doublets for the diastereotopic CH_2-groups connecting two glycoluril units or a glycoluril unit with an aromatic sidewall. As expected based on theory, the resonances for the $OCH_2CH_2NH_3$ arms display patterns that reflect the fact that the protons on each CH_2-group are diastereotopic. Interestingly, the chemical shift of H_a and H_b of the naphthalene sidewalls of **M2NH3** measured in D_2O as solvent are significantly upfield shifted relative to the related chemical shifts measured for **4** in DMSO-d_6 (Figure 2). This observation suggests that **M2NH3**, similar to **M1NH3**,[26] assumes a self-folded conformation in which the naphthalene rings undergo π−π interactions.

X-ray Crystal Structure of 4

We were unable to obtain single crystals of **M2NH3**, but were lucky enough to obtain crystals and solve the x-ray crystal structure for compound **4** (CCDC-1454834). Figure 3 shows a cross-eyed stereoview of one molecule of **4** in the crystal. As seen previously for **M2**, compound **4** assumes a helical conformation in which its two terminal aromatic rings are splayed out of plane with respect to the glycoluril tetramer backbone. A molecule of formic acid fills the cavity of **4** in the crystal and forms one H(C=O)O-H•••O=C H-bond (O•••O distance: 2.77 Å) to the ureidyl C=O portal of **4**. Reciprocally, two molecules of H_2O form a H-bonded chain from the included formic acid terminated at the opposite ureidyl C=O portal. The individual molecules of **4** exhibit an interesting three dimensional packing in the crystal whereby molecules of one handedness form zig-zag chains along the a-axis which repeat along the b-axis to give sheets of a single handedness in the ab-plane (Supporting Information). These sheets pack along the z-axis with alternating senses of chirality driven in part by interactions between naphthalene sidewalls in adjacent slabs.

Solubility Properties of M1NH3 and M2NH3

The ability to use a given molecular container compound in relevant applications requires sufficient solubility in appropriate solvents. Unfunctionalized CB[n] (n = 6, 8, 10) exhibit low solubility in water (< 100 μM) whereas CB[n] derivatives and acyclic CB[n] can exhibit dramatically enhanced solubility. For this reason we decided to determine the inherent solubility of **M1NH3** and **M2NH3** before proceeding to perform molecular recognition studies. Experimentally, we added **M1NH3** or **M2NH3** to water until a heterogenous mixture was formed and then excess insoluble host was filtered off and the concentration of **M1NH3** and **M2NH3** in solution was measured by ^{1}H NMR spectroscopy relative to trimesic acid as an internal standard of known concentration. In this manner we measured the high inherent solubility of **M1NH3** (250 mM) and **M2NH3** (≥ 207 mM).

Container M2NH3 Does Not Undergo Significant Self-Association

Before proceeding to study any new host molecule – particularly for studies in aqueous solution – it is important to determine whether the host undergoes self-association processes that would impinge upon the expected host-guest recognition processes. Previously, we have investigated the use of **M1NH3** as a solubilizing excipient for insoluble drugs and studied its self-association by ^{1}H NMR dilution experiments in 20 mM sodium phosphate buffered D_2O and did not observe any changes in chemical shift that would be indicative of self-association processes.[26] Accordingly, we performed an analogous dilution experiment for **M2NH3** (20 mM to 0.125 mM) and looked for changes in ^{1}H NMR chemical shift as a function of concentration (Figure 4). As can be readily seen the host protons – most significantly those of the aromatic sidewalls – do not undergo any significant changes in chemical shift which would be indicative of self-association processes over the experimentally accessible concentration range. Accordingly, we conclude that **M2NH3** exists in its monomeric form under the conditions used for K_a determination by ^{1}H NMR titrations decribed below.

Structures of M1NH3 and M2NH3

Previously, we have determined the x-ray crystal structures of **M1** and **M2** whose aromatic sidewalls are roughly orthogonal and help define a hydrophobic box whereas the sulfonate solubilizing groups do not interact with the ureidyl C=O groups of the host. In sharp contrast, the x-ray crystal structure of **M1NH3** [26] (Figure 5) displays a self-folding phenomenon whereby the two o-xylylene walls undergo π−π interactions with each other which reduces the available cavity volume. In addition, one of the $CH_2CH_2NH_3$ sidearms of **M1NH3** folds back to the ureidyl C=O portal to form ion-dipole interactions which helps explain the fact that **M1NH3** is not a good receptor for cations. Unfortunately, we have not been able to obtain x-ray quality crystals of **M2NH3**. However, on the basis of the x-ray crystal structure of **M1NH3**, we infer that **M2NH3** may undergo a related self-folding phenomenon although the presence of the longer naphthalene walls of **M2NH3** may induced some strain within a self-folded geometry. As described above, the observation of upfield shifting of H_a and H_b for **M2NH3** in water relative to **4** as model compound strongly suggests a self-folded geometry for **M2NH3** driven by C=O•••NH_3^+ ion-dipole interactions and resulting in intramolecular π−π interactions between the naphthalene walls of **M2NH3**.

Binding Studies of M1NH3 and M2NH3 Toward the Nucleotide Guests

Next, we decided to measure the binding constants for the interaction of **M1NH3** and **M2NH3** with the various nucleotide guests. For this purpose, we decided to use ^{1}H NMR spectroscopy and monitor the change in chemical shift of the protons on the nucleotide upon titration with the acyclic CB[n]-type molecular containers **M1NH3** and **M2NH3** with the expectation that the direction and magnitude of the induced changes in chemical shift (Δδ) would deliver information about the structure of the host-guest complex. Figure 7 shows the ^{1}H NMR recorded for the titration of a fixed concentration of GTP (1 mM) with **M1NH3** (0 – 8 mM) in 20 mM sodium phosphate buffered D_2O at pD 7.4. The ^{1}H NMR chemical shifts of the aromatic proton of GTP (H_p) shifts slightly upfield upon complex formation with **M1NH3**. In addition, the proton attached to the anomeric center (H_q) and the sugar ring (H_s) also experience small upfield shifts upon complex formation. We performed a simultaneous global fitting of the plot of change in chemical shift data versus [**M1NH3**] (Figure 6f) to extract the binding constant for the **M1NH3**•GTP complex (K_a = 234 M^{-1}). Analogous ^{1}H NMR titrations were performed for the **M1NH3** and **M2NH3** with the other nucleotides and the binding constants are presented in Table 1.

The complexes between **M1NH3** or **M2NH3** with the seven nucleotides are all relatively weak with binding constants in the 10^2 – 10^3 M^{-1} range. However, there are useful trends in the binding affinity data that can be discerned. First, the binding constants of **M2NH3** toward a given nucleotide are generally larger than that of **M1NH3**. Previously, we have observed similar trends in the binding affinity of **M1** and **M2** toward insoluble drugs and attributed this effect to the fact that **M2** is shaped by two naphthalene rings which results in a larger hydrophobic cavity and one that offers a larger amount of π-surface area to its guests.[25] We surmise that the superior binding affinity of **M2NH3** relative to **M1NH3** may be due to similar effects; confirmation of this interpretation awaits an x-ray crystal structure of **M2NH3**. Second, across the series of guests ATP, ADP, AMP, cAMP that differ only in

the number of negative charges we observe a decrease in K_a toward **M1NH3** and **M2NH3** as guest charge decreases. The 5-fold (18-fold) decrease observed between the complexes of **M1NH3•**ATP versus **M1NH3•**cAMP (**M2NH3•**ATP versus **M2NH3•**cAMP) suggest that a significant portion of the binding free energy of these complexes are due to electrostatic interactions between the negatively charged phosphate groups and the positively charged arms of **M1NH3** and **M2NH3**. Third, among the purines, we find that ATP forms tighter complexes than GTP toward both **M1NH3** and **M2NH3**. We believe that this trend is due to the fact that the nucleobase of GTP is more hydrophilic than that of ATP because of the additional oxo-group. Accordingly, binding GTP in the hydrophobic cavity of **M1NH3** or **M2NH3** would require more extensive desolvation of the guest. Consistently, we do not observe a similar trend in the binding constants of UTP and CTP toward **M1NH3** and **M2NH3** because of the more comparable substituents on the bases.

To obtain structural information about the complexes between **M1NH3** or **M2NH3** and the seven nucleotides we obtained the maximal complexation induced changes in chemical shift (Δδ) from the simultaneous global fitting of the titration data which are summarized in Table 2. Table 2 presents Δδ values for the protons on the aromatic rings (H_o and H_p), the anomeric center (H_q), and for GTP proton (H_s) on the sugar residue. Although we were unable to monitor the other protons across the full titration because of spectral complexity or the obscuring effect of the D2O solvent we do observe that most resonances tend to shift slightly upfield upon complex formation which is consistent with the nucleotides residing in the hydrophobic cavity of **M1NH3** and **M2NH3**. Figure 8 presents one MMFF minimized geometry of the **M2NH3•**ATP complex although given the relatively low K_a values we suspect a range of similar geometries are plausible. As can be seen, the aromatic base and the sugar ring are bound within the anisotropic shielding region of the acyclic CB[n]-type cavity whereas the ammonium ion arms extend toward the cavity to complement the anionic triphosphate. We believe that analogous complex geometries are likely for the complexes of **M1NH3** and **M2NH3** with the other nucleotides. ^{31}P NMR measured with ATP in presence of **M1NH3** or **M2NH3** show a slight downshift of phosphate units compare to ATP only (for **M1NH3** Δδ: P_α 0.21; P_β 0.33; P_γ 0.39 ppm; for **M2NH3** Δδ: P_α 0.02; P_β 0.17; P_γ 0.32 ppm; Supporting Information). This observation is consistent with electrostatic / H-bonding interactions between the phosphate chain and the ammonium ion arms. To assess the importance of electrostatic interactions on the strength of the **M2NH3•**ATP complex we decided to perform ^{1}H NMR titrations as a function of the concentration of the sodium phosphate buffer. Figure 9 shows a plot of K_a as a function of sodium phosphate concentration. As expected the observed K_a values decrease as sodium phosphate concentration increases presumably due to the competition / screening effect of the added salt.

Conclusion

In summary, we have reported the synthesis of the tetracationic analogue of **M2** which we refer to as **M2NH3**. We find that both **M1NH3** (250 mM) and **M2NH3** (≥ 207 mM) possess outstanding solubility in D_2O. The results of ^{1}H NMR dilution experiments establish that neither **M1NH3** nor **M2NH3** undergo significant self-association which enables their ability

to act as hosts. By virtue of their electrostatically negative ureidyl C=O portals and their cationic $(CH_2)_2NH_3^+$ arms both **M1NH3** and **M2NH3** form self-complexed structures due to ion-dipole interactions at the portals and π−π interactions of one sidewall folding into its own cavity. The results of ^{1}H NMR titrations establish that both **M1NH3** and **M2NH3** are relatively weak hosts toward nucleotides with K_a in the $10^2 – 10^3$ M^{-1} range although **M2NH3** is somewhat more potent than **M1NH3** toward a given guest. We attribute this relatively modest affinity to the electrostatically negative ureidyl C=O portals which interact with the NH_3^+ arms and thereby decrease electrostatic interactions toward the negatively charged guests. As expected, along the series from ATP, ADP, AMP, to cAMP we observe that the K_a values toward **M1NH3** or **M2NH3** decreases as the charge on the guest decreases. In conclusion, the work highlights the powerful influence of the ureidyl C=O portals on acyclic cucurbituril recognition processes and suggests that future designs of acyclic CB[n] containers for anion recognition requires the use of arms that are more preorganized, more highly charged, and coordinate less well with the C=O portals to enhance their anion recognition abilities.

Experimental Details

Starting materials were purchased from commercial suppliers and were used without further purification. Compounds **1**, **2**, and **M1NH3** were synthesized following the literature procedures.[26,31,32] Melting points were measured on a Meltemp apparatus in open capillary tubes and are uncorrected. IR spectra were recorded on a JASCO FT/IR 4100 spectrometer and are reported in cm^{-1}. ^{1}H and ^{13}C NMR spectra were measured on 400, 500, or 600 MHz instruments. Mass spectrometry was performed using a JEOL AccuTOF electrospray instrument (ESI).

Compound 3

A solution of **1** (1.498 g, 1.95 mmol) in TFA/Ac_2O (1:1, 15 mL) was treated with 1,4-bis(2-chloroethoxy)naphthalene (**2**) (1.944 g, 6.81 mmol) and the mixture was stirred at 70 °C for 3 h under nitrogen. The mixture was poured into MeOH (100 mL) and the precipitate was isolated by filtration. The crude solid was washed with water (100 mL) and filtered. The solid was resuspended in acetone (100 mL) and filtered. Finally, the crude solid was dissolved in formic acid (10 mL), precipitated by addition of water (7 mL) and centrifuged, three times. Compound **3** was dried under high vacuum and obtained as a white solid (932 mg, 36%). M.p. > 300 °C (dec.). IR (ATR, cm^{-1}): 2927w, 1723s, 1462s, 1310s, 1221s, 1183m, 1081m, 796s. ^{1}H NMR (500 MHz, DMSO-d_6): 8.11-8.09 (m, 4H), 7.70-7.68 (m, 4H), 5.55 (d, J = 14.6 Hz, 2H), 5.48 (d, J = 15.0 Hz, 4H), 5.38 (d, J = 9.0 Hz, 2H), 5.37 (d, J = 16.1 Hz, 4H), 5.26 (d, J = 9.0 Hz, 2H), 4.66-4.61 (m, 4H), 4.37 (d, J = 16.1 Hz, 4H), 4.10-3.93 (m, 18H), 1.74 (s, 6H), 1.72 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): 155.3, 154.2, 147.7, 127.8, 127.5, 126.7, 122.6, 77.5, 76.1, 74.0, 70.8, 70.4, 52.9, 48.4, 44.7, 35.9, 16.0, 15.6. MS (ESI): *m/z* 726 ([M+*p*-xylenediamine+2H]$^{2+}$, calculated for $C_{66}H_{74}Cl_4N_{18}O_{12}^{2+}$: 726).

Compound 4

A mixture of tetrachloride **3** (673 mg, 0.51 mmol) and NaN_3 (667 mg, 10.26 mmol) was stirred in DMSO (10 mL) at 80 °C for 12 h. The reaction mixture was cooled to RT and water (40 mL) was added. The mixture was filtered and the solid was washed with water (40 mL) and then filtered again. Subsequently, the solid was suspended in MeOH (40 mL) and filtered, twice. Compound **4** was dried under high vacuum and obtained as a white solid (606 mg, 88%). M.p. > 300 °C (dec.). IR (ATR, cm^{-1}): 2930w, 2102m, 1721s, 1461s, 1307s, 1220s, 1182s, 1081m, 796s. ^{1}H NMR (400 MHz, DMSO-d_6): 8.07-8.05 (m, 4H), 7.74-7.72 (m, 4H), 5.54 (d, J = 14.5 Hz, 2H), 5.49 (d, J = 15.1 Hz, 4H), 5.39 (d, J = 9.0 Hz, 2H), 5.36 (d, J = 16.0 Hz, 4H), 5.26 (d, J = 9.0 Hz, 2H), 4.55-4.51 (m, 4H), 4.39 (d, J = 16.0 Hz, 4H), 4.09 (d, J = 15.1 Hz, 4H), 4.07 (d, J = 14.5 Hz, 2H), 3.98-3.93 (m, 4H), 3.76-3.59 (m, 8H), 1.74 (s, 6H), 1.73 (s, 6H). ^{13}C NMR (125 MHz, DMSO-d_6): 155.3, 154.2, 147.8, 127.7 127.3, 126.7, 122.6, 77.5, 76.1, 73.3 70.8, 70.4, 52.9, 50.9, 48.4, 35.9, 16.1, 15.6. MS (ESI): *m/z* 739 ([M+*p*-xylenediamine+2H]$^{2+}$, calculated for $C_{66}H_{74}N_{30}O_{12}^{2+}$: 739). X-ray crystal structure (CCDC-1454834).

Compound M2NH3

A mixture of tetraazide **4** (212 mg, 0.16 mmol) and PPh_3 (314 mg, 1.20 mmol) were stirred in DMSO/H_2O (5:1, 6 mL) at 80 °C for 4 h. The mixture was cooled to RT and acidified to pH 1 with HCl (6 M). Acetone (40 mL) was added and the precipitate was isolated by centrifugation. The solid was dissolved in water (2 mL) and precipitated with acetone (40 mL), four times. The solid was dried under high vacuum and obtained as a yellow solid (178 mg, 81%). M.p. > 300°C (dec.). IR (ATR, cm^{-1}): 3359w, 2975w, 1722s, 1692s, 1461s, 1311s, 1227s, 1151s, 1064s, 1007m, 977m, 821m, 797s. ^{1}H NMR (400 MHz, D_2O): 7.36-7.34 (m, 4H), 7.24-7.22 (m, 4H), 5.49-5.41 (m, 8H), 5.33 (d, J = 8.6 Hz, 2H), 5.15 (d, J = 16.0 Hz, 4H), 4.56 (d, J = 16.2 Hz, 4H), 4.33 (d, J = 15.8 Hz, 4H), 4.05 (d, J = 15.5 Hz, 2H), 3.95-3.86 (m, 8H), 3.32-3.14 (m, 8H), 1.84 (s, 6H), 1.76 (s, 6H). ^{13}C NMR (125 MHz, D_2O, dioxane as reference): 156.9, 148.9, 128.2, 127.9, 126.5, 122.9, 80.0, 78.8, 72.2, 71.6, 71.2, 53.6, 48.9, 40.4, 37.0, 16.1, 15.4 (only 17 of the 18 expected resonances were observed. HR-MS (ESI): *m/z* 1237.5409 ([M+H-4HCl]$^{1+}$, calculated for $C_{58}H_{69}N_{20}O_{12}^{+}$: 1237.5404), 413.1853 ([M+3H-4HCl]$^{3+}$, calculated for $C_{58}H_{71}N_{20}O_{12}^{3+}$: 413.1854).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

We thank the National Cancer Institute of the National Institutes of Health (CA168365 to L. I.) for financial support.

References

1. Cram DJ. Angew. Chem., Int. Ed. Engl. 1988; 27:1009.Lehn J-M. Angew. Chem., Int. Ed. Engl. 1988; 27:89.Pedersen CJ. Angew. Chem. Int. Ed. Engl. 1988; 27:1021.Gutsche CD. Acc. Chem. Res. 1983; 16:161.Rebek J. Acc. Chem. Res. 2009; 42:1660. [PubMed: 19603810] Pluth MD. Raymond KN. Chem. Soc. Rev. 2007; 36:161. [PubMed: 17264920] Yoshizawa M. Klosterman J.

Fujita M. Angew. Chem., Int. Ed. 2009; 48:3418.Kumari H. Deakyne CA. Atwood JL. Acc. Chem. Res. 2014; 47:3080. [PubMed: 25198830] Kay ER. Leigh DA. Angew. Chem., Int. Ed. 2015; 54:10080.

2. Diederich F. Angew. Chem., Intl. Ed. Engl. 1988; 27:362.Boehmer V. Angew. Chem., Int. Ed. Engl. 1995; 34:713.Rekharsky MV, Inoue Y. Chem. Rev. 1998; 98:1875. [PubMed: 11848952] Ogoshi T, Kanai S, Fujinami S, Yamagishi T-A, Nakamoto Y. J. Am. Chem. Soc. 2008; 130:5022. [PubMed: 18357989] Xue M, Yang Y, Chi X, Zhang Z, Huang F. Acc. Chem. Res. 2012; 45:1294. [PubMed: 22551015]
3. You L, Zha D, Anslyn EV. Chem. Rev. 2015; 115:7840. [PubMed: 25719867]
4. Ma X, Zhao Y. Chem. Rev. 2015; 115:7794. [PubMed: 25415447]
5. Harada A, Takashima Y, Nakahata M. Acc. Chem. Res. 2014; 47:2128. [PubMed: 24911321]
6. Garcia-Simon C, Gramage-Doria R, Raoufmoghaddam S, Parella T, Costas M, Ribas X, Reek Joost NH. J. Am. Chem. Soc. 2015; 137:2680. [PubMed: 25632976] Ramamurthy V. Acc. Chem. Res. 2015; 48:2904. [PubMed: 26488308]
7. Warmuth R. Angew. Chem. Int. Ed. 1997; 36:1347.Mal P, Breiner B, Rissanen K, Nitschke JR. Science. 2009; 324:1697. [PubMed: 19556504] Cram DJ, Tanner ME, Thomas R. Angew. Chem., Int. Ed. 1991; 30:1024.
8. Cummings CG, Hamilton AD. Curr. Opin. Chem. Biol. 2010; 14:341. [PubMed: 20430687]
9. Gassensmith JJ, Baumes JM, Smith BD. Chem. Commun. 2009:6329.
10. Lee JW, Samal S, Selvapalam N, Kim H-J, Kim K. Acc. Chem. Res. 2003; 36:621. [PubMed: 12924959] Lagona J, Mukhopadhyay P, Chakrabarti S, Isaacs L. Angew. Chem., Int. Ed. 2005; 44:4844.Nau WM, Florea M, Assaf KI. Isr. J. Chem. 2011; 51:559.Assaf KI, Nau WM. Chem. Soc. Rev. 2015; 44:394. [PubMed: 25317670] Masson E, Ling X, Joseph R, Kyeremeh-Mensah L, Lu X. RSC Adv. 2012; 2:1213.
11. Barrow SJ, Kasera S, Rowland MJ, del Barrio J, Scherman OA. Chem. Rev. 2015; 115:12320. [PubMed: 26566008]
12. Freeman WA, Mock WL, Shih N-Y. J. Am. Chem. Soc. 1981; 103:7367.Kim J, Jung I-S, Kim S-Y, Lee E, Kang J-K, Sakamoto S, Yamaguchi K, Kim K. J. Am. Chem. Soc. 2000; 122:540.Day AI, Arnold AP, Blanch RJ, Snushall B. J. Org. Chem. 2001; 66:8094. [PubMed: 11722210] Day AI, Blanch RJ, Arnold AP, Lorenzo S, Lewis GR, Dance I. Angew. Chem., Int. Ed. 2002; 41:275.Liu S, Zavalij PY, Isaacs L. J. Am. Chem. Soc. 2005; 127:16798. [PubMed: 16316221] Cheng X-J, Liang L-L, Chen K, Ji N-N, Xiao X, Zhang J-X, Zhang Y-Q, Xue S-F, Zhu Q-J, Ni X-L, Tao Z. Angew. Chem., Int. Ed. 2013; 52:7252.
13. Mock WL, Shih N-Y. J. Org. Chem. 1986; 51:4440.Liu S, Ruspic C, Mukhopadhyay P, Chakrabarti S, Zavalij PY, Isaacs L. J. Am. Chem. Soc. 2005; 127:15959. [PubMed: 16277540] Rekharsky MV, Mori T, Yang C, Ko YH, Selvapalam N, Kim H, Sobransingh D, Kaifer AE, Liu S, Isaacs L, Chen W, Moghaddam S, Gilson MK, Kim K, Inoue Y. Proc. Natl. Acad. Sci. U. S. A. 2007; 104:20737. [PubMed: 18093926] Cao L, Šekutor M, Zavalij PY, Mlinarić-Majerski K, Glaser R, Isaacs L. Angew. Chem., Int. Ed. 2014; 53:988.Biedermann F, Uzunova VD, Scherman OA, Nau WM. De Simone A. J. Am. Chem. Soc. 2012; 134:15318. [PubMed: 22881280] Moghaddam S, Yang C, Rekharsky M, Ko YH, Kim K, Inoue Y, Gilson MK. J. Am. Chem. Soc. 2011; 133:3570. [PubMed: 21341773]
14. Shetty D, Khedkar JK, Park KM, Kim K. Chem. Soc. Rev. 2015; 44:8747. [PubMed: 26434388]
15. Isaacs L. Acc. Chem. Res. 2014; 47:2052. [PubMed: 24785941] Del Barrio J, Horton P, Lairez D, Lloyd G, Toprakcioglu C, Scherman O. J. Am. Chem. Soc. 2013; 135:11760. [PubMed: 23879174] Ko YH, Kim E, Hwang I, Kim K. Chem. Commun. 2007:1305.Loh XJ, del Barrio J, Toh PPC, Lee T-C, Jiao D, Rauwald U, Appel EA, Scherman OA. Biomacromolecules. 2012; 13:84. [PubMed: 22148638]
16. Vazquez J, Remon P, Dsouza RN, Lazar AI, Arteaga JF, Nau WM, Pischel U. Chem. - Eur. J. 2014; 20:9897. [PubMed: 25043661] Walker S, Oun R, McInnes FJ, Wheate NJ. Isr. J. Chem. 2011; 51:616.Dong N, Xue S-F, Zhu Q-J, Tao Z, Zhao Y, Yang L-X. Supramol. Chem. 2008; 20:659.Macartney DH. Isr. J. Chem. 2011; 51:600.

17. Dsouza R, Hennig A, Nau W. Chem. Eur. J. 2012; 18:3444. [PubMed: 22367854] Ghale G, Lanctot AG, Kreissl HT, Jacob MH, Weingart H, Winterhalter M, Nau WM. Angew. Chem., Int. Ed. 2014; 53:2762.Norouzy A, Azizi Z, Nau WM. Angew. Chem., Int. Ed. 2015; 54:792.

18. Dang D, Nguyen H, Merkx M, Brunsveld L. Angew. Chem., Int. Ed. 2013; 52:2915.Lee HH, Choi TS, Lee SJC, Lee JW, Park J, Ko YH, Kim WJ, Kim K, Kim HI. Angew. Chem., Int. Ed. 2014; 53:7461.Chinai JM, Taylor AB, Ryno LM, Hargreaves ND, Morris CA, Hart PJ, Urbach AR. J. Am. Chem. Soc. 2011; 133:8810. [PubMed: 21473587] Urbach AR, Ramalingam V. Isr. J. Chem. 2011; 51:664.Smith LC, Leach DG, Blaylock BE, Ali OA, Urbach AR. J. Am. Chem. Soc. 2015; 137:3663. [PubMed: 25710854] Rekharsky MV, Yamamura H, Ko YH, Selvapalam N, Kim K, Inoue Y. Chem. Commun. 2008:2236.

19. Appel E, del Barrio J, Loh X, Scherman O. Chem. Soc. Rev. 2012; 41:6195. [PubMed: 22890548]

20. Lim S, Kim H, Selvapalam N, Kim K-J, Cho SJ, Seo G. Kim K. Angew. Chem. Int. Ed. 2008; 47:3352.Miyahara Y, Abe K, Inazu T. Angew. Chem., Int. Ed. 2002; 41:3020.Tian J-A, Ma S-Q, Thallapally PK, Fowler D, McGrail BP, Atwood JL. Chem. Commun. 2011; 47:7626.

21. Jon SY, Selvapalam N, Oh DH, Kang J-K, Kim S-Y, Jeon YJ, Lee JW, Kim K. J. Am. Chem. Soc. 2003; 125:10186. [PubMed: 12926937] Kim E, Kim D, Jung H, Lee J, Paul S, Selvapalam N, Yang Y, Lim N, Park CG, Kim K. Angew. Chem., Int. Ed. 2010; 49:4405.Jung H, Park KM, Yang J-A, Oh EJ, Lee D-W, Park K, Ryu SH, Hahn SK, Kim K. Biomaterials. 2011; 32:7687. [PubMed: 21788071] Lee D-W, Park K, Banerjee M, Ha S, Lee T, Suh K, Paul S, Jung H, Kim J, Selvapalam N, Ryu S, Kim K. Nat. Chem. 2011; 3:154. [PubMed: 21258389] Jung H, Park JS, Yeom J, Selvapalam N, Park KM, Oh K, Yang J-A, Park KH, Hahn SK, Kim K. Biomacromolecules. 2014; 15:707. [PubMed: 24605794] Jang M, Kim H, Lee S, Kim HW, Khedkar JK, Rhee YM, Hwang I, Kim K, Oh JH. Adv. Funct. Mater. 2015; 25:4882.Yeom J, Kim SJ, Jung H, Namkoong H, Yang J, Hwang BW, Oh K, Kim K, Sung YC, Hahn SK. Adv. Healthcare Mater. 2015; 4:237.

22. Lucas D, Minami T, Iannuzzi G, Cao L, Wittenberg JB, Anzenbacher P, Isaacs L. J. Am. Chem. Soc. 2011; 133:17966. [PubMed: 21970313] Cao L, Isaacs L. Org. Lett. 2012; 14:3072. [PubMed: 22650758] Vinciguerra B, Cao L, Cannon JR, Zavalij PY, Fenselau C, Isaacs L. J. Am. Chem. Soc. 2012; 134:13133. [PubMed: 22799491] Cao L, Hettiarachchi G, Briken V, Isaacs L. Angew. Chem., Int. Ed. 2013; 52:12033.

23. Zhao N, Lloyd G, Scherman O. Chem. Commun. 2012; 48:3070.Ahn Y, Jang Y, Selvapalam N, Yun G, Kim K. Angew. Chem., Int. Ed. 2013; 52:3140.Ayhan MM, Karoui H, Hardy M, Rockenbauer A, Charles L, Rosas R, Udachin K, Tordo P, Bardelang D, Ouari O. J. Am. Chem. Soc. 2015; 137:10238. [PubMed: 26197228]

24. Ma D, Hettiarachchi G, Nguyen D, Zhang B, Wittenberg JB, Zavalij PY, Briken V, Isaacs L. Nat. Chem. 2012; 4:503. [PubMed: 22614387] Ma D, Zhang B, Hoffmann U, Sundrup MG, Eikermann M, Isaacs L. Angew. Chem., Int. Ed. 2012; 51:11358.Shen C, Ma D, Meany B, Isaacs L, Wang Y. J. Am. Chem. Soc. 2012; 134:7254. [PubMed: 22512431] Gilberg L, Zhang B, Zavalij PY, Sindelar V, Isaacs L. Org. Biomol. Chem. 2015; 13:4041. [PubMed: 25731639]

25. Zhang B, Isaacs L. J. Med. Chem. 2014; 57:9554. [PubMed: 25369565]

26. Zhang B, Zavalij PY, Isaacs L. Org. Biomol. Chem. 2014; 12:2413. [PubMed: 24595500]

27. Stancl M, Necas M, Taraba J, Sindelar V. J. Org. Chem. 2008; 73:4671. [PubMed: 18494528] Stancl M, Hodan M, Sindelar V. Org. Lett. 2009; 11:4184. [PubMed: 19705866] Stancl M, Gargulakova Z, Sindelar V. J. Org. Chem. 2012; 77:10945. [PubMed: 23151064] Stancl M, Gilberg L, Ustrnul L, Necas M, Sindelar V. Supramol. Chem. 2014; 26:168.

28. Sigwalt D, Ahlbrand S, Zhang M, Vinciguerra B, Briken V, Isaacs L. Org. Lett. 2015:5914. [PubMed: 26593638]

29. Kuchelmeister HY. Schmuck C. Chem. Eur. J. 2011; 17:5311. [PubMed: 21462273] Inclan M, Albelda MT, Carbonell E, Blasco S, Bauza A, Frontera A. Garcia-Espana E. Chem. Eur. J. 2014; 20:3730. [PubMed: 24574302] Hosseini MW, Blacker AJ, Lehn J-M. J. Am. Chem. Soc. 1990; 112:3896.Guo Y-H, Ge Q-C, Lin H, Lin H-K, Zhu S-R. Polyhedron. 2002; 21:1005.Delepine A-S, Tripier R, Baccon ML, Handel H. Eur. J. Org. Chem. 2010:5380.Moreno-Corral R, Lara KO. Supramol. Chem. 2008; 20:427.Bazzicalupi C, Bencini A, Biagini S, Faggi E, Meini S, Giorgi C, Spepi A, Valtancoli B. J. Org. Chem. 2009; 74:7349. [PubMed: 19743804] Arranz-Mascaros P, Bazzicalupi C, Bianchi A, Giorgi C, Goldino-Salido ML, Gutierrez-Valero MD, Lopez-Garzon R, Valtancoli B. New J. Chem. 2011; 35:1883.Abe H, Mawatari Y, Teraoka H, Fujimoto K, Inouye

M. J. Org. Chem. 2004; 69:495. [PubMed: 14725465] Bencini A, Biagini S, Giorgi C, Handel H, Baccon ML, Mariani P, Paoletti P, Paoli P, Rossi P, Tripier R, Valtancoli B. Eur. J. Org. Chem. 2009:5610.

30. Svec J, Necas M, Sindelar V. Angew. Chem., Int. Ed. 2010; 49:2378.Svec J, Dusek M, Fejfarova K, Stacko P, Klan P, Kaifer AE, Li W, Hudeckova E, Sindelar V. Chem. - Eur. J. 2011; 17:5605. [PubMed: 21484899] Havel V, Sindelar V, Necas M, Kaifer AE. Chem. Commun. 2014; 50:1372.Yawer MA, Havel V, Sindelar V. Angew. Chem., Int. Ed. 2015; 54:276.Prigorchenko E, Oeren M, Kaabel S, Fomitsenko M, Reile I, Jarving I, Tamm T, Topic F, Rissanen K, Aav R. Chem. Commun. 2015; 51:10921.Lisbjerg M, Jessen BM, Rasmussen B, Nielsen BE, Madsen AO, Pittelkow M. Chem. Sci. 2014; 5:2647.Lisbjerg M, Valkenier H, Jessen BM, Al-Kerdi H, Davis AP, Pittelkow M. J. Am. Chem. Soc. 2015; 137:4948. [PubMed: 25851041] Singh M, Solel E, Keinan E, Reany O. Chem. - Eur. J. 2015; 21:536. [PubMed: 25417852] Parvari G, Annamalai S, Borovoi I, Chechik H, Botoshansky M, Pappo D, Keinan E. Chem. Commun. 2014; 50:2494.
31. Ma D, Zavalij PY, Isaacs L. J. Org. Chem. 2010; 75:4786. [PubMed: 20540586]
32. Holliday BJ, Jeon Y-M, Mirkin CA, Stern CL, Incarvito CD, Zakharov LN, Sommer RD, Rheingold AL. Organomettalics. 2002; 21:5713.

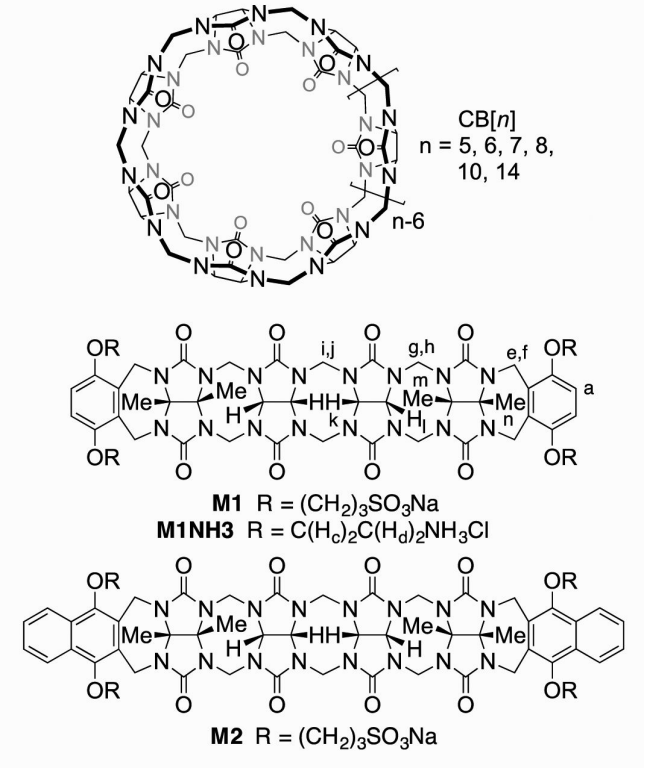


Figure 1.
Chemical structures of CB[n] and prototypical acyclic CB[n]-type molecular containers.

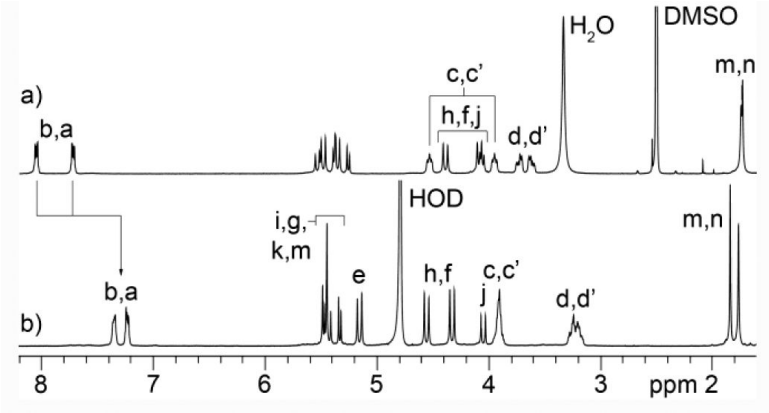


Figure 2.

NMR spectra recorded for: a) **4** (DMSO-d_6, 400 MHz, RT), b) **M2NH3** (D_2O, 400 MHz, RT).

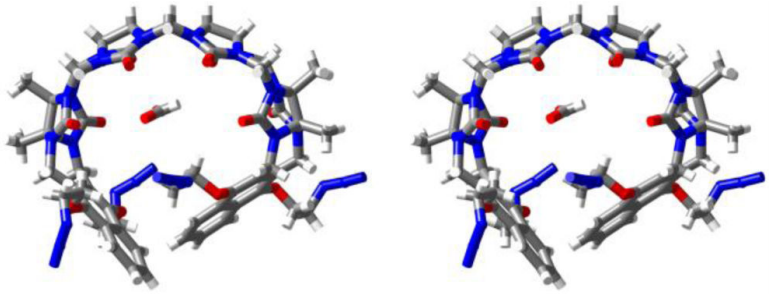


Figure 3.
Cross-eyed stereoview of the x-ray crystal structure of one molecule of **4** in the crystal. Color code: C, grey; H, white; N, blue; O, red; H-bonds, red-yellow stripped.

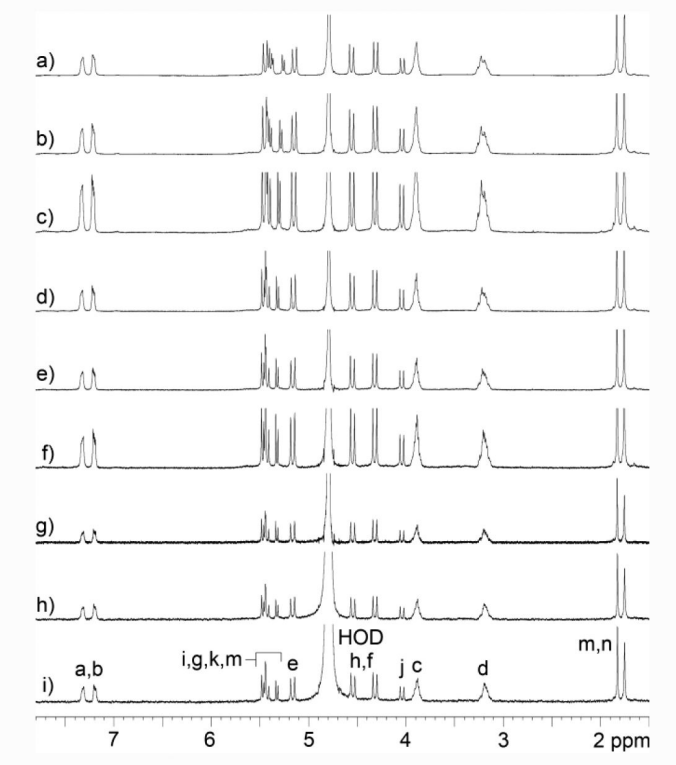


Figure 4.

^{1}H NMR spectra recorded (D_2O, 400 MHz, 20 mM sodium phosphate, pD = 7.4, RT) during the dilution experiment for **M2NH3**: a) 20 mM, b) 15 mM, c) 10 mM, d) 5 mM, e) 2.5 mM, f) 1 mM, g) 0.5 mM, h) 0.25 mM, i) 0.125 mM.

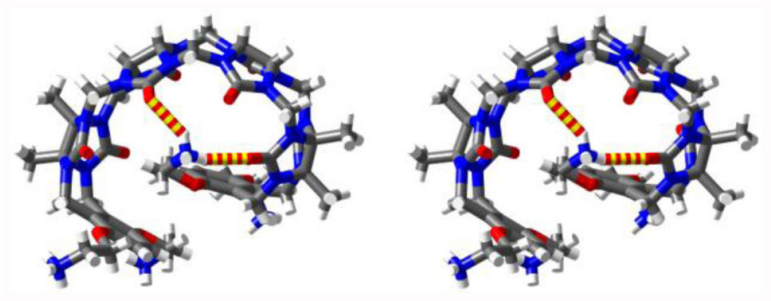


Figure 5.

Cross-eyed stereoview of the X-ray crystal structure of **M1NH3**. Color code: C, grey; H, white; N, blue; O, red; H-bonds, red-yellow striped.

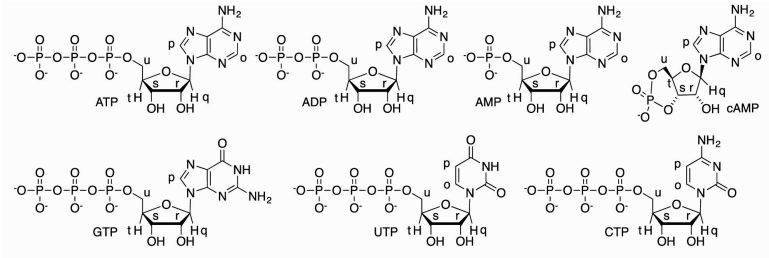


Figure 6.
Structures and selected proton labeling of guests used in this study.

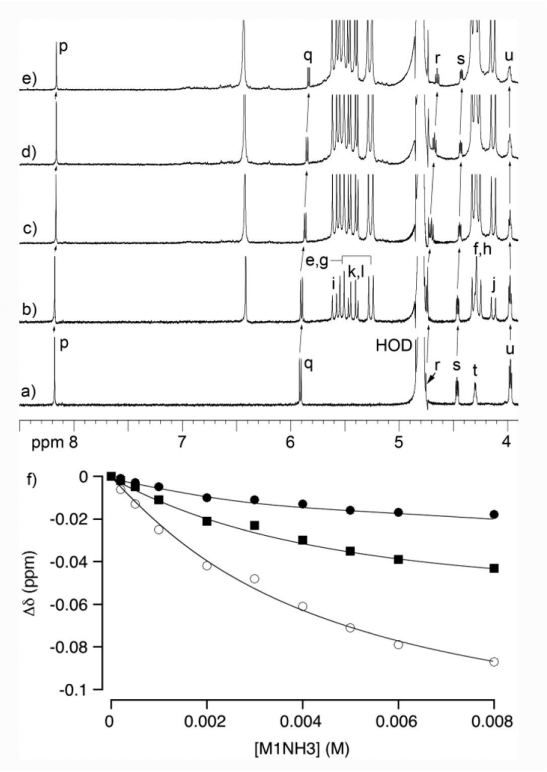


Figure 7.

a) ^{1}H NMR spectra (400 MHz, 20 mM sodium phosphate, pD = 7.4, RT) recorded during the titration of GTP (1 mM) with **M1NH3**: a) 0 mM, b) 0.5 mM, c) 2 mM, d) 4 mM, e) 6 mM. Global fitting of the Δδ versus [**M1NH3**] data to a 1:1 binding model to determine K_a = 234 ± 20 M^{-1}.

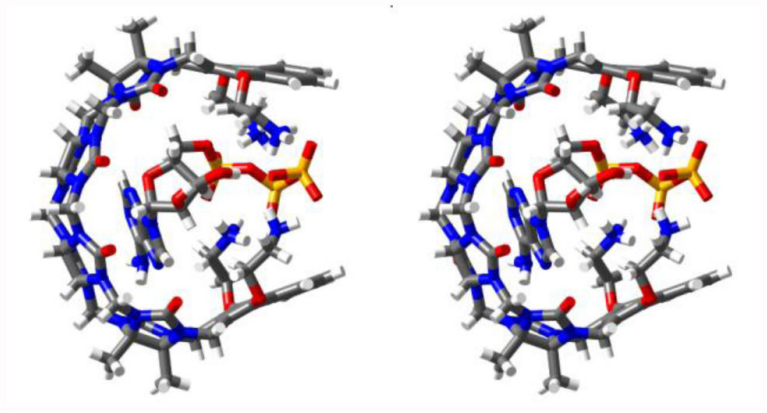


Figure 8.
Cross-eyed stereoview of an MMFF minimized geometry for **M2NH3•**ATP. Color code: C, gray; H, white; N, blue; O, red; P, orange.

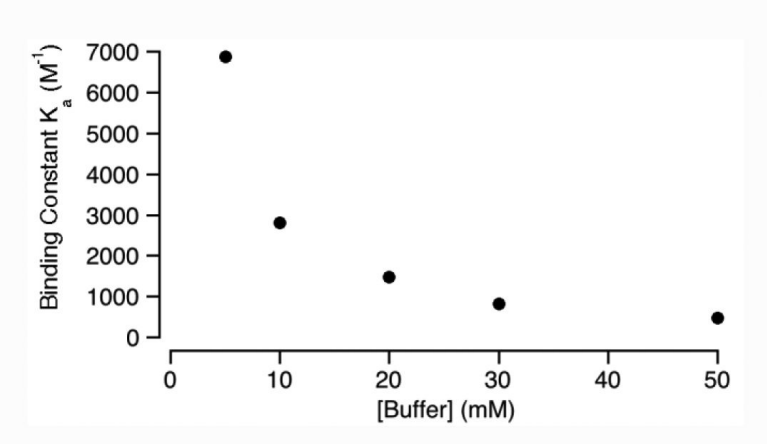
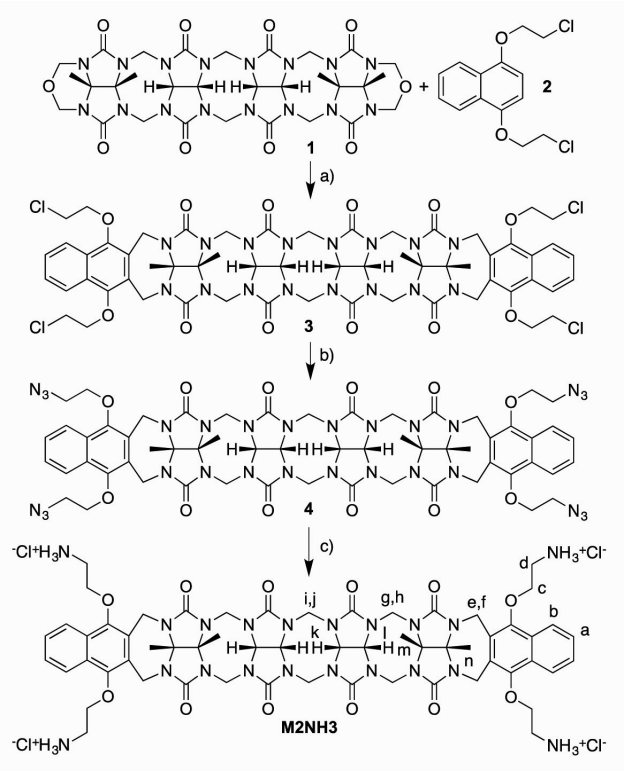


Figure 9.

Plot of Ka versus [Buffer] for the **M2NH3•**ATP complex. Conditions: sodium phosphate (5 – 50 mM) buffered D_2O, pD = 7.4, RT.



Scheme 1.

Synthesis of cationic acyclic CB[n]-type container **M2NH3**. Conditions: a) CF_3CO_2H / Ac_2O, 70 °C, 3 h, 36%, b) DMSO, NaN_3, 80 °C, 12 h, 88%, c) DMSO, H_2O, PPh_3, 80 °C, 4h then HCl, 81%.

Table 1

Binding constants (K_a, M^{-1}) measured for the complexes between **M1NH3** and **M2NH3** and the nucleotide guests.

	M1NH3	**M2NH3**
ATP	937 ± 75	(1.47 ± 0.16) × 10^3
UTP	461 ± 40	(1.10 ± 0.12) × 10^3
GTP	234 ± 20	648 ± 114
CTP	564 ± 49	556 ± 37
ADP	507 ± 80	(1.25 ± 0.13) × 10^3
AMP	199 ± 20	853 ± 173
cAMP	198 ± 68	82 ± 15

Table 2

Complexation induced changes in chemical shift (A5) for the various protons of the nucleotides for their complexes with **M1NH3** and **M2NH3**.

	M1NH3			**M2NH3**		
	H_p	**H_o**	**H_q**	**H_p**	**H_o**	**H_q**
ATP	−0.17	−0.05	−0.18	−0.26	−0.18	−0.26
UTP	−0.18	−0.14	−0.14	−0.20	−0.17	−0.18
GTP	−0.03	−0.07	−0.14	−0.04	−0.04 (H_s)	−0.09
CTP	−0.19	−0.17	−0.20	−0.31	−0.29	−0.31
ADP	−0.15	−0.05	−0.15	−0.21	−0.13	−0.20
AMP	−0.13	−0.03	−0.15	−0.07	−0.04	−0.07
cAMP	−0.01	−0.02	−0.04	−0.16	−0.19	−0.20