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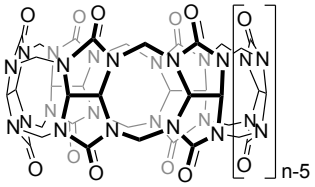
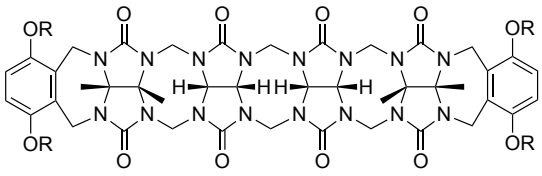
We report the synthesis of three new triazole functionalized acyclic CB[n]-type receptors (2 – 4) by click chemistry. The compounds have good solubility in water (≥ 8 mM) and do not undergo strong self-association (K_s ≤ 903 M^{-1}). We measured the binding constants of 2 – 4 toward guests 9 – 24 and compared the results to those obtained for the prototypical acyclic CB[n]-type receptor 1. The x-ray crystal structure of 4 is also described.

Introduction

Over the past two decades, we and others, have investigated the synthesis and molecular recognition properties of cucurbit[n]uril (CB[*n*]) type receptors.[1] As shown in Figure 1, CB[*n*] macrocycles are composed of *n* glycoluril subunits connected by *2n* CH_2-bridges which define a central hydrophobic cavity which is guarded by two symmetry equivalent ureidyl carbonyl lined portals. Accordingly, guest molecules that feature a central hydrophobic domain flanked by two cationic moieties – typically ammoniums – bind strongly toward CB[n] in aqueous solutions with K_a values commonly in the 10^6 – 10^9 M^{-1} range, sometimes exceeding 10^{12} M^{-1} and even exceeding 10^{17} M^{-1} in special cases.[1a, 2] CB[n]•guest complexes are responsive toward chemical (e.g. pH, guest transformation, or competing guest), electrochemical, and photochemical stimulation.[1f, 3a, 3b, 3c] Accordingly, CB[n] type receptors have been used in a variety of applications including the creation of (bio)sensing ensembles,[4] molecular machines,[3a] supramolecular catalysis,[1g, 5] and to create new supramolecular materials.[6] The development of methods to functionalize macrocyclic CB[n] compounds[1i, 7a-7g] has enabled their attachment to surfaces, polymers, nanoparticles, fluorophores, and targeting ligands and has broadened their applicability in particular toward biomedical applications.[8]



CB[n] n=5, 6, 7, 8, 10, 14



M1 R = $(CH_2)_3SO_3Na$

Figure 1 Chemical structures of CB[n] and acyclic CB[n]-type molecular container **M1.**

As part of our study of the mechanism of CB[n]-formation, we had reason to prepare acyclic CB[n]-type receptors (e.g. **M1**) that retained the essential molecular recognition properties of macrocyclic CB[n] and that undergo straightforward functionalization reactions.[1j, 9] Over the past decade, we and others,[10] have been investigating the use of acyclic CB[n] in biomedical applications.[11] For example, we found that **M1** functions as a solubilizing excipient for insoluble drugs *in vitro* and can be used to deliver albendazole to animals *in vivo*. **M1** and related compounds display excellent biocompatibility according to both *in vitro* (metabolic and cell death assays; no mutagenicity by Ames test; lack of hERG ion channel inhibition) and *in vivo* (e.g. maximum tolerated dose; blood pH and blood gas analysis; mean arterial pressure; urinary excretion) tests.[11a-11i, 12] Acyclic CB[n] compounds also function as components of sensing ensembles for biologically active substances like nitrosamines and over the counter drugs.[13] Given the high affinity of (acyclic) CB[n]-type receptors toward their guests, we envisioned they could also be used as *in vivo* reversal agents for appropriate targets similar to the use of Sugammadex to reverse neuromuscular block.[14] Indeed, we found that acyclic CB[n] dose dependently reversed the *in vivo* biological effect of the neuromuscular

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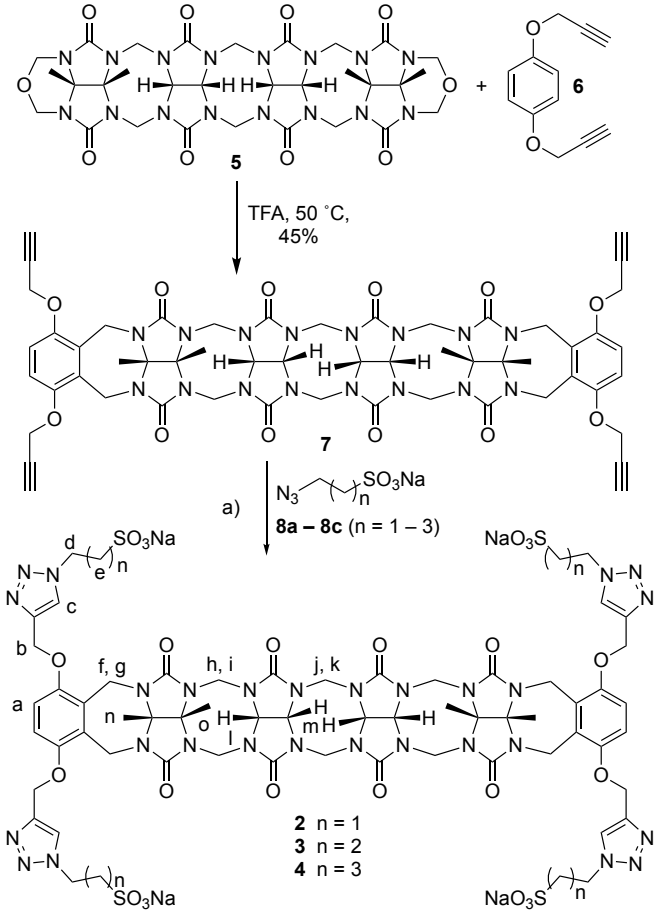
blockers rocuronium, vecuronium, and cisatracurium as well as the depth of anesthesia after treatment with ketamine.[11c-11f] Conceptually related sequestration and reversal applications using macrocyclic CB[n] have been investigated in the laboratory of Prof. Ruibing Wang.[15]

A class of compounds that are in urgent need of *in vivo* reversal agents are opioid and non-opioid drugs of abuse. Intoxication with drugs of abuse results in countless emergency room visits which are estimated to directly cost $11 billion per year with an additional $193 billion per year due to crime and lost work productivity.[16] Currently, naloxone is used to treat overdose with opioids (e.g. heroin, fentanyl) by competitively binding to the opioid receptor; multiple doses of naloxone is often needed for fentanyl and high potency fentanyl analogues. Naloxone is ineffective at treating non-opioid drugs of abuse like methamphetamine, cocaine, ketamine, and PCP. Accordingly, we recently quantified the sub-micromolar *in vitro* affinity of acyclic CB[n] (e.g. **M1**) toward a panel of drugs of abuse and demonstrated their ability to modulate the hyperlocomotion observed in rats treated with methamphetamine (0.3 mg kg^{-1}).[11i] In this paper, we prepare several analogues of **M1** with the goal of increasing their *in vitro* binding affinity toward methamphetamine and fentanyl.

Results and Discussion

This results and discussion section is organized as follows. First, we present the design, synthesis, and characterization of hosts **2** – **4**. Next, we demonstrate their low self-association in water and qualitatively examine their molecular recognition properties by ^{1}H NMR spectroscopy. Subsequently, we measure the binding constants of **M1** and **2** – **4** toward guests **9** – **24** by direct and competitive isothermal titration calorimetry (ITC). Finally, we discuss the trends in the binding constant data as a function of host and conclude.

Design, Synthesis, and Characterization of Hosts 2 – 4. Previously, our efforts to enhance the binding affinity of acyclic CB[n] compounds focused on the use of different aromatic walls, different glycoluril oligomer lengths, different linker length to the sulfonate solubilizing groups, and covalent capping groups.[1j, 11h, 17a-17d] In this paper, we prepared and studied **2** – **4** which are triazole containing analogues of **M1**; **2** – **4** differ from each other in the length of the $(CH_2)_n$ chain between the triazole and sulfonate groups. We hypothesized that the electron deficient triazole groups would serve as capping groups that would further define the hydrophobic cavity and provide additional π-surfaces to form non-covalent interactions with the guests. The preparation of triazole functionalized hosts **2** – **4** is based on our previously described building block approach (Scheme 1).[1j] As the central glycoluril oligomer building block we selected tetramer bis(cyclic ether) **5** because the derived hosts display higher affinity binding than those prepared from monomer – trimer building blocks.[17c] As the wall component, we choose benzene derivative **6** which bears two propargyloxy substituents which enable the planned double electrophilic aromatic substitution reaction and functionalization by click chemistry. The condensation reaction of **5** with **6** occurred smoothly in trifluoroacetic acid (TFA) as solvent at 50 ˚C by a double electrophilic aromatic substitution process to yield tetrapropargylated acyclic CB[n]-type host **7** in 45% yield after reprecipitation from aqueous HCl as previously disclosed in a patent.[18] Host **7** was subsequently functionalized by 4-fold click reactions with azido sulfonates **8a** – **8c** (ascorbic acid, NaOH, $CuSO_4$, EtOH/H_2O, 80 ˚C, microwave, 28-46% yield) to deliver the acyclic CB[n]-type hosts **2** – **4** that bear four triazolo sulfonate arms. Hosts **2** – **4** were purified by crystallization from EtOH-H_2O (3:1) mixtures and fully characterized by spectroscopic methods. For example, the ^{1}H NMR spectrum of **2** displays two resonances for the CH_3-substituents (H_n, H_o) on the glycoluril backbone, one triazolyl (H_c) resonance, and a single resonance for the aromatic wall protons (H_a) as required for the depicted C_{2v}-symmetric structure of **2**. Additionally, the resonances for the diastereotopic methylenes of the glycoluril backbone (H_f – H_k) appear as three pairs of resonances in the expected 4:4:2 integral ratios as required. Similarly, the ^{13}C NMR spectrum of **2** displays 19 resonances as expected based on the depicted C_{2v}-symmetry. The spectra for **3** and **4** are also in accord with their depicted structures. Host **2** – **4** are soluble up to at least 8, 10, and 8 mM, respectively.



Scheme 1 Synthesis of triazole hosts **2** – **4.**

Self-Association Properties of 2 – 4. It is important to investigate the self-association properties of any new host

before performing host•guest binding titrations to inform the selection of the fixed host concentrations where self-association is minimized.[19] For example, Figure S14 shows the ^{1}H NMR spectra recorded as the concentration of **4** is decreased from 8 mM to 0.1 mM we observe changes in the chemical shift of the aromatic wall protons (H_a). A plot of [**4**] against the chemical shift of H_a (Figure 3) could be fitted to a 2-fold self-association model implemented within Scientist™ (Supporting Information) which allowed us to determine the self-association constant K_s = 546 M^{-1}. Self-association constants for hosts **2** (K_s = 299 M^{-1}) and **3** (K_s = 903 M^{-1}) were determined similarly (Figures S8 – S11). Accordingly, we conclude that at the [host] = 100 μM required for ITC determination of the thermodynamic parameters of binding that the hosts are predominantly monomeric.

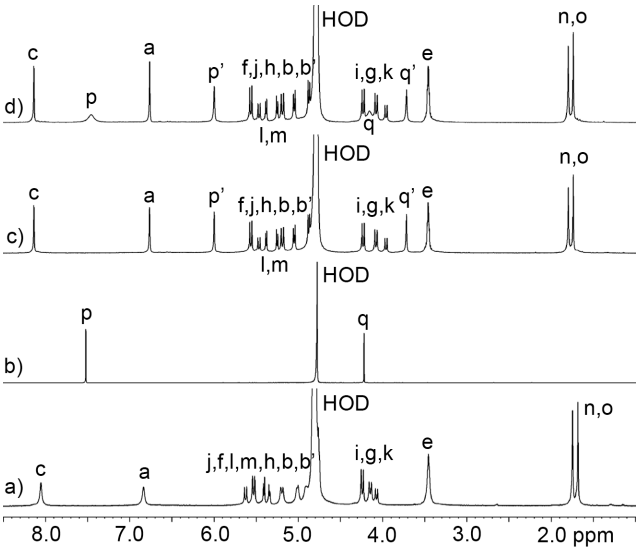


Figure 2 ^{1}H NMR spectra recorded (600 MHz, RT, 20 mM phosphate buffered D_2O, pH 7.4) for: a) receptor **2** (1 mM), b) guest **17** (1 mM), c) **2** (1 mM) and **17** (1 mM), c) **2** (1 mM) and **17** (2 mM).

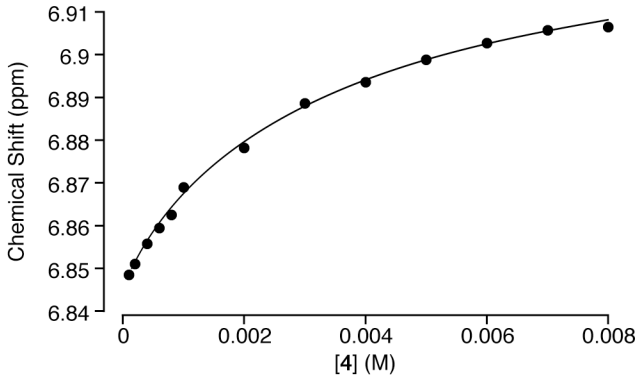


Figure 3. Plot of chemical shift of H_a versus [**4**] used to determine the self-association constant K_s = 546 M^{-1} for **4**.

Qualitative ^{1}H NMR Host•Guest Recognition Study. First, we qualitatively investigated the recognition properties of **2** – **4** toward the ammonium ion guests **9** – **21** by ^{1}H NMR spectroscopy (Figure 2, Figure 4, and Supporting Information). For example, Figure 2a and 2b shows the ^{1}H NMR spectra of uncomplexed **2** and **17** whereas Figure 2c and 2d show the spectra recorded at 1:1 and 1:2 host:guest ratios. Upon complexation, the resonances for host **2** sharpen and undergo minor changes in chemical shift which we attribute to the disruption of any self-associated form of **2** present at the 1 mM concentration used for NMR or to changes in the curvature along the methylene bridged glycoluril oligomer backbone induced by complexation. As expected based on the known binding preferences of macrocyclic and acyclic CB[n] hosts,[1e, 1j] the H_p resonance for guest **17** undergoes a substantial upfield chemical shift (≈ 1.5 ppm) upon complexation (Figure 2c) which indicates that the aromatic ring is located in the cavity of the host which constitutes an anisotropic shielding region by virtue of the aromatic and glycoluril walls of the host. The ammonium ions are located at the electrostatically negative C=O portals of the host. Somewhat interestingly, the H_q resonance for the methylene units of guest **17** also experience upfield shifts (≈ 0.5 ppm) upon complexation. We attribute this to the propensity of acyclic CB[n] hosts to undergo an out-of-plane helical distortion[11a] which brings the CH_2-group into closer proximity to the aromatic walls. At a 1:2 ratio of **2**:**17** we observe the presence of separate resonances for free **17** (e.g. H_p and H_q) and **2•17** (e.g. $H_{p'}$ and $H_{q'}$) which indicates that the complexation process is slow on the ^{1}H NMR chemical shift timescale. Analogous trends in changes in chemical shift upon complexation were observed for hosts **3** and **4** binding to **17** and also for host **2** – **4** binding to guests **9**, **15**, **19**, and **20**. These guests feature different sized hydrophobic moieties (e.g. hexane, cyclohexane, viologen, adamantane) which indicates that **2** – **4** have the ability to flex and expand their cavities to accommodate larger guests as observed for related acyclic CB[n]-type hosts previously.[13c, 20a - 20c] Intermediate kinetics of guest exchange on the chemical shift timescale are generally observed for guests **9**, **15**, **19**, and **20**.

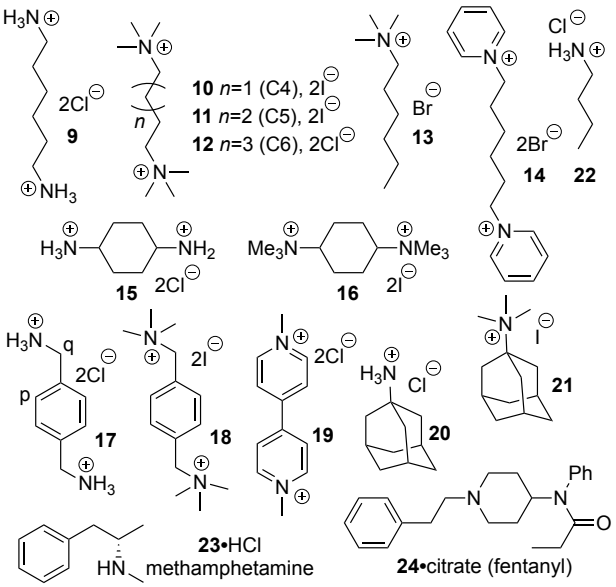


Figure 4 Structures of guests **9** – **24** used in this study.

Measurement and Discussion of the Thermodynamic Parameters of Complex Formation. With our qualitative ^{1}H NMR survey of host-guest binding completed, we focussed on the measurement of the thermodynamic parameters for the various host•guest complexes. Figure 4 shows the chemical structures of the full range of guests (**9** – **24**) studied. Compounds **9** – **22** were selected to probe the influence of guest size, guest charge, diammonium guest length, and the nature of head group (1˚ – 4˚ ammonium, pyridinium). Drugs **23** and **24** were selected to gauge their potential use as an *in vivo* reversal agent. We decided to use ITC to measure the thermodynamic parameters of binding because it is sensitive enough to detect complexation at [host] = 100 μM where the hosts are monomeric. As an example, Figure 5 presents the ITC thermogram recorded during the direct titration of host **3** (100 μM) with guest **13** (0 – 200 μM). The K_a and ΔH values for **3•13** complex were determined to be (4.00 ± 0.13) × 10^5 M^{-1} and -5.13 ± 0.032 kcal mol^{-1} using the single set of sites binding model within the MicroCal PEAQ-ITC analysis software. The K_a and ΔH values for the remaining complexes of host **2** – **4** with guests **9** – **24** were measured in the same way by direct ITC titrations (Supporting Information) and the results are presented in Table 1. When using ITC to determine K_a values, it is prudent not to exceed a c-value of 300.[21] When using a fixed concentration of host in the cell of 100 μM, this translates to a maximum K_a value of 3 x 10^6 M^{-1}. For **M1** whose K_a values are often larger, we turned to competitive ITC titrations to extend the dynamic range. For ITC competition experiments, the K_a and ΔH values of a weak binding competitor are first measured by ITC. In the ITC competition experiment, a fixed concentration of host and an excess of a weaker binding guest in the cell is titrated with the stronger binding guest and the data fitted to a competitive binding model in the PEAQ ITC analysis software using the previously measured K_a and ΔH values as inputs. In our experiments, we measured K_a and ΔH for **M1•22** and then used **22** as our weak binding competitor in ITC competition experiments. The results of the ITC competition experiments with host **M1** are presented in Table 1. In the following sections we discuss the trends in the binding data as a function of guest structure.

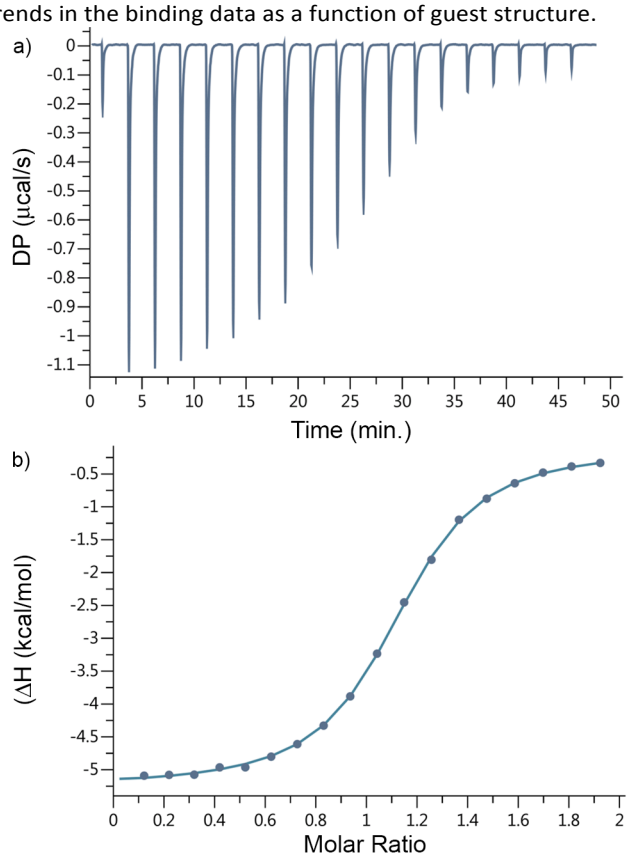


Figure 5 a) ITC thermogram recorded during the titration of host **3** (100 μM) in the cell with guest **13** (1.0 mM) in the syringe, b) Fitting of the data to a 1:1 binding model with K_a = (4.00 ± 0.13) × 10^5 M^{-1}.

Table 1 Binding constants (K_a, M^{-1}) and binding enthalpies (ΔH, kcal mol^{-1}) measured for the different container•guest complexes (298 K, 20 mM NaH_2PO_4 buffered water, pH 7.4).

Guest	**2**	**3**	**4**	**M1**
9	(2.05 ± 0.07) × 10^6 -5.97 ± 0.021	(1.72 ± 0.17) × 10^6 -6.03 ± 0.065	(1.13 ± 0.04) × 10^6 -5.64 ± 0.021	(5.05 ± 0.31) × $10^{7\,b}$ -6.23 ± 0.014
10	(1.39 ± 0.36) × 10^5 -8.76 ± 0.750	(1.26 ± 0.24) × 10^5 -8.10 ± 0.481	(8.55 ± 1.04) × 10^4 -6.22 ± 0.263	(1.82 ± 0.09) × 10^6 -7.61 ± 0.035
11	(1.79 ± 0.09) × 10^6 -9.09 ± 0.043	(1.83 ± 0.09) × 10^6 -8.95 ± 0.044	(6.33 ± 0.15) × 10^5 -7.85 ± 0.026	(1.73 ± 0.20) × $10^{7\,c}$ -7.59 ± 0.103
12	(2.51 ± 0.16) × 10^6 -9.30 ± 0.053	(2.86 ± 0.17) × 10^6 -9.23 ± 0.047	(1.24 ± 0.07) × 10^6 -8.03 ± 0.054	(8.93 ± 0.33) × $10^{7\,c}$ -9.35 ± 0.021
13	(3.73 ± 0.09) × 10^5 -4.98 ± 0.025	(4.00 ± 0.13) × 10^5 -5.13 ± 0.032	(2.15 ± 0.06) × 10^5 -4.89 ± 0.028	(1.24 ± 0.06) × 10^6 -5.67 ± 0.033
14	(1.51 ± 0.24) × 10^6 -9.89 ± 0.198	(2.53 ± 0.17) × 10^6 -8.95 ± 0.054	(1.27 ± 0.09) × 10^6 -8.44 ± 0.069	(2.02 ± 0.22) × $10^{8\,d}$ -8.01 ± 0.049
15	(8.62 ± 0.27) × 10^5 -6.87 ± 0.027	(8.20 ± 0.27) × 10^5 -6.54 ± 0.027	(2.99 ± 0.06) × 10^5 -5.82 ± 0.022	(1.95 ± 0.09) × 10^6 -5.70 ± 0.027
16	(1.12 ± 0.05) × 10^6 -7.43 ± 0.040	(9.52 ± 0.68) × 10^5 -7.44 ± 0.067	(4.37 ± 0.17) × 10^5 -6.79 ± 0.044	(2.25 ± 0.08) × $10^{7\,c}$ -8.37 ± 0.025
17	(3.34 ± 0.19) × 10^6 -9.74 ± 0.045	(2.01 ± 0.09) × 10^6 -6.26 ± 0.030	(2.03 ± 0.06) × 10^6 -8.74 ± 0.024	(1.67 ± 0.08) × $10^{8\,c}$ -8.09 ± 0.018
18	(3.72 ± 0.61) × 10^6	(3.24 ± 0.18) × 10^6	(2.05 ± 0.07) × 10^6	(1.78 ± 0.07) × $10^{8\,c}$

	-11.7 ± 0.167	-11.8 ± 0.054	-10.5 ± 0.035	-11.4 ± 0.022
19	(3.38 ± 0.33) × 10^6	(9.62 ± 0.38) × 10^5	(1.77 ± 0.07) × 10^6	(4.69 ± 0.22) × 10^8 [d]
	-10.0 ± 0.084	-10.0 ± 0.047	-9.07 ± 0.039	-12.3 ± 0.032
20	(1.63 ± 0.07) × 10^5	(1.57 ± 0.07) × 10^5	(8.47 ± 0.82) × 10^4	(9.62 ± 0.34) × 10^5
	-4.61 ± 0.047	-4.60 ± 0.051	-3.88 ± 0.124	-6.55 ± 0.029
21	(1.02 ± 0.04) × 10^6	(1.04 ± 0.03) × 10^6	(5.15 ± 0.58) × 10^5	(1.70 ± 0.05) × 10^7 [c]
	-8.13 ± 0.036	-8.14 ± 0.031	-7.24 ± 0.055	-9.09 ± 0.027
22	–	–	–	(1.75 ± 0.08) × 10^5
				-4.86 ± 0.048
23	(7.35 ± 0.43) × 10^5	(6.94 ± 0.32) × 10^5	(5.35 ± 0.18) × 10^5	(7.5 ± 2.9) × 10^6 [e]
(methamphetamine)	-7.99 ± 0.066	-8.03 ± 0.055	-7.56 ± 0.038	
24	(1.31 ± 0.09) × 10^6	(1.15 ± 0.07) × 10^6	(1.15 ± 0.08) × 10^6	(1.1 ± 0.04) × 10^7 [e]
(fentanyl)	-9.55 ± 0.080	-9.12 ± 0.076	-8.97 ± 0.076	

[a] Measured by direct ITC titration of host (100 μM) in the cell with guest (1 mM) in the syringe. [b] Measured by ITC competition assay using **22** (0.2 mM) as competitor included in the cell. [c] Measured by ITC competition assay using **22** (0.5 mM) as competitor included in the cell. [d] Measured by ITC competition assay using **22** (1.0 mM) as competitor included in the cell. [e] From reference 11i.

Magnitude of Binding Constants and Enthalpies. New hosts **2** – **4** exhibit strong binding toward guests **9** – **24** with K_a values in the 10^4 – 10^6 M^{-1} range. The formation of the host•guest complexes between **2** – **4** and guests **9** – **24** are enthapically driven with ΔH values in the -4 to -12 kcal mol^{-1} range. The substantial enthalpic driving forces are not surprising given the known presence of high energy waters in the cavity of CB[n] hosts which are released upon host•guest binding. Table 1 also presents the K_a values for **M1** which range from 10^6 – 10^8 M^{-1}. Once again, the complexes are strongly enthalpically driven with K_a values in the -5 to -12 kcal mol^{-1} range. Some host•guest binding data for **M1** has been presented previously using values derived from phase solubility diagram and optical methods.[11b, 11f, 17a, 20b] The data presented in Table 1 is uniformly measured by ITC which is the preferred method across fields which may render it useful as a reference for other researchers. A close inspection of Table 1 reveals that **M1** is uniformly the superior host toward **9** – **24** with K_a values typically 1-2 orders of magnitude stronger than **2** – **4.**

*Influence of Linking Chain Length on the Binding Affinity of **2** – **4** toward guests **9** – **24**.* Hosts **2** – **4** differ in the length of the $(CH_2)_n$ linking group between triazole and sulfonate. An examination of Table 1 reveals that **2** and **3** exhibit comparable affinity toward **9** – **24** whereas **4** generally binds more weakly (≈ 2-fold). Previously, for a naphthalene walled acyclic CB[n]-host, we observed that the n = 3 host was the best solubilizing agent for insoluble drugs.[11h] We propose two reasons for the fact that **4** is the weakest host: 1) the more hydrophobic butyl chains may undergo partial cavity inclusion which would cost energy to extrude upon guest binding, and 2) the sulfonate groups of **4** are more remote from the cavity than for **2** or **3** due to the shorter linking chain which should reduce the electrostatic contributions to binding. Given the well known importance of electrostatic interactions on CB[n]•guest binding[1a, 1c, 22] we prefer the second possibility.

Influence of Diammonium Ion Length. CB[n]-type receptors are well known to discriminate between alkane diammonium ion guests based on their length.[1a] We measured the binding constants of guests **10** – **12** which differ in length from C4 to C6 (Table 1). We find that the K_a values for **M1** and **2** – **4** toward **10** – **12** follow the order **12** > **11** > **10** with differences of 14 – 50-fold across the series. We attribute this to the higher hydrophobicity of the guest as additional CH_2-groups are added which increases the K_a value.[2e, 23]

Influence of Guest Charge. It is known that CB[n]•guest complexes are driven by the hydrophobic effect and by ion-dipole interactions at the ureidyl C=O portals.[1d] Ion dipole interactions at each portal in macrocyclic CB[n] hosts are worth roughly 10^3 in binding affinity. To gauge the influence of guest charge on K_a toward hosts **M1** – **4** we compared **13** (ammonium) and **12** (diammonium). As can be seen from Table 1, hosts **2** – **4** prefer diammonium **12** over **13** by factors of 5.8 – 7.2 fold. In contrast, **M1** prefers **12** over **13** by a factor of 72-fold. One possible explanation of this difference is that the sulfonate groups on **M1** are closer to the ureidyl C=O portal than for triazole extended compounds **2** – **4** which result in secondary sulfonate•••ammonium ion interactions for **M1** which result in a higher selectivity for **M1**.

Influence of the Cationic Headgroup. For CB[n]-type receptors, a preference has been observed for quaternary ammonium ion guests over primary ammonium ions for selected guest compounds.[2c, 24a, 24b] To determine if similar selectivity is seen for **M1** and **2** – **4** we compared the binding behavior of **9** versus **12** and **14**, **15** versus **16**, **17** versus **18**, and **20** versus **21**. Interestingly, hosts **2** and **3** exhibit only a small (< 1.7-fold) preference for quaternary ammonium ion guest **12** whereas **4** is not selective. We had hoped that **2** – **4** would exhibit enhanced affinity toward **14** due to π–π interactions between the pendant triazole arms and the pyridinium groups which protrude from the cavity; unfortunately, such an effect was not observed experimentally. Related trends are seen in the binding of **2** – **4** toward the **15**/**16**, **17**/**18** where only a small preference (< 1.5-fold) is seen for the quaternary ammonium ions. In contrast, a larger preference is seen in the binding of **2** – **4** (≈ 6-fold) and **M1** (18-fold) toward **20**/**21** where the quaternary ammonium ion is preferred. Similar to CB[n], the

degree of preference of acyclic CB[n] hosts for quaternary ammonium ions is dependent on the nature of the guest.

Influence of Guest Size. Macrocyclic CB[n] are relatively rigid and very selective hosts; guests whose hydrophobic moiety sterically interacts with the walls of the cavity are effectively rejected.[2a] In contrast, acyclic CB[n] are known to be able to flex their methylene bridged glycoluril oligomer backbone to accommodate larger guests. This trend can be seen for **M1** (Table 1) where the order of K_a values is **21** ≈ **16** < **12** < **18** < **19**. Expanding the cavity of **M1** to accommodate the larger cyclohexane and adamantane units of **16** and **21**, however, costs energy which reduces their K_a values. Narrower guests **12** and **18** exhibit higher affinity and **19** with its two aromatic rings exhibits highest affinity probably due to enhanced π–π interactions. Related trends in the binding affinity data are seen for hosts **2** – **4** modulated of course by their generally lower affinity and selectivity.

X-ray Crystal Structure of Host 4. We were fortunate to obtain single crystals of **4** by recrystallization from mixtures of EtOH and H_2O and to solve its structure by x-ray crystallography (CCDC-1911232). Figure 6 shows a stereoscopic representation of one molecule of **4** in the crystal. Several features of the structure are noteworthy. First, **4** displays an overall end-to-end helical twist; that is one aromatic wall is displaced up and one is displaced down with respect to the plane defined by the glycoluril methine C-atoms. The helical distortion was invoked earlier to explain the upfield shifting observed in the ^{1}H NMR spectra of the host•guest complexes for the protons adjacent to the cationic N-atoms. The cavity of the host becomes deeper as a result of this helical distortion. Interestingly, three of the pendant triazolosulfonate arms are directed away from the cavity and two of them appear to interact with one another by van der Waals or π–π interactions. These three sulfonates are remote from the cavity and C=O portals of the hosts. The fourth triazolosulfonate arm is folded back toward the carbonyl portal where it coordinates to a Na^+ ion which is itself bound to two ureidyl C=O groups. The cavity of the host is filled with disordered EtOH molecules (omitted in Figure 6) whose O-atom coordinates to the Na^+ at the portal. The remaining coordination sites on Na^+ are occupied by disordered waters. The ability of the side arm to fold back to the portal and neutralize its negative electrostatic potential may be partially responsible for the relatively poor affinity of **2** – **4** toward its guests.

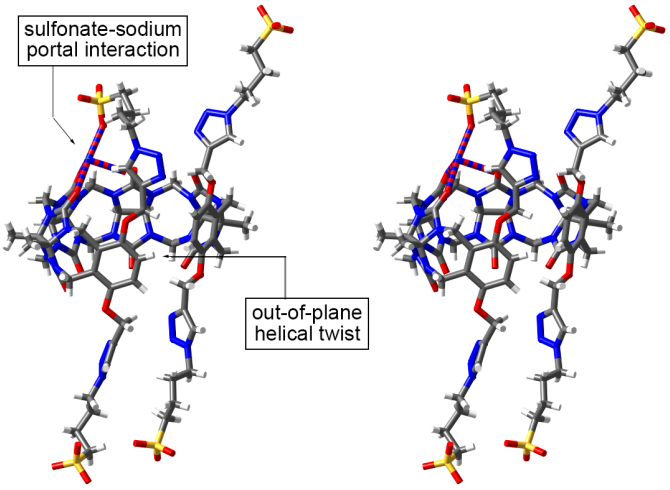


Figure 6 Cross-eyed stereoview of one molecule of **4** in the crystal. Color code: C, grey; H, white; N, blue; O, red, O-Na, blue-red striped.

Conclusions

In summary, we have prepared tetrapropargylated host **7** which acts as a clickable common intermediate toward three new acyclic CB[n]-type hosts **2** – **4** containing triazole arms. New hosts **2** – **4** have good solubility in aqueous solution (≥ 8 mM) and do not undergo strong self-association in water (K_s ≤ 903 M^{-1}). ^{1}H NMR spectroscopy and ITC were used to determine the geometrical features of the host•guest complexes and to determine the thermodynamics of complexation. We find that the new hosts retain the essential binding features of CB[n] hosts but do so with lower affinity and selectivity than the prototypical acyclic CB[n] (**M1**). We attribute the weaker binding to the remote location of the sulfonate groups which decrease secondary electrostatic interactions in the complex relative to **M1**. The x-ray crystal structure of **4** shows a backfolding of the butanesulfonated arm to the C=O portal which may also lower affinity by satisfying the electrostatically negative potential at the portal in concert with a Na^+ ion. Unfortunately, the binding affinity of the new hosts **2** – **4** toward our desired targets (methamphetamine **23** and fentanyl **24**) follow these trends and display lower affinity than **M1**. We conclude that modifications of the structure of acyclic CB[n] that are remote from their cavity (e.g. capping groups and pendant arms) are ineffective at increasing the binding affinity of acyclic CB[n] hosts.

Experimental.

General Experimental. Starting materials were purchased from commercial suppliers and used without further purification or were prepared by literature procedures.[11a, 25] 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}. NMR spectra were measured on commercial instruments operating at 400 or 600 MHz for ^{1}H and 100 or 125 MHz for ^{13}C using D_2O or DMSO-d_6 as solvents. Chemical shifts (δ) are referenced relative to the residual resonances for HOD (4.80 ppm) and DMSO-d_6 (2.50 ppm for ^{1}H, 39.51 ppm for ^{13}C). Mass

spectrometry was performed using a JEOL AccuTOF electrospray instrument (ESI). ITC data were collected on a Malvern Microcal PEAQ-ITC instrument.

Host 2. Ascorbic acid (28 mg, 0.16 mmol), NaOH (8 mg, 0.16 mmol) and $CuSO_4$ (8 mg, 0.04 mmol) were mixed and then dissolved in a mixture of H_2O and EtOH (4 mL, 1:1). Propargyl host **7** (104 mg, 0.096 mmol) and **8c** (155.4 mg, 0.773 mmol) were added as solids to the reaction mixture and heated in a microwave reactor at 80 ˚C for 30 minutes. Solid was removed by centrifugation and the liquid portion was treated with EtOH (5 mL) resulting in precipitation of the crude product. The crude product was recrystallized from a mixture of EtOH (3 mL) and H_2O (1 mL). A white solid was obtained after drying under high vacuum (52 mg, 28%). M.p. > 305 ˚C (decomposed). IR (ATR, cm^{-1}): 3407w, 1722s, 1469s, 1425m, 1379m, 1314m, 1230s, 1179s, 1085s, 1009m, 975m, 845m, 797s, 757m, 667m. ^{1}H NMR (600 MHz, D_2O): 7.97 (s, 4H), 6.84 (s, 4H), 5.67 (d, *J* = 15.6, 2H), 5.57 (d, *J* = 15.6, 4H), 5.44 (d, *J* = 8.4, 2H), 5.38 (d, *J* = 9, 2H), 5.22 (d, *J* = 16.2, 4H), 5.06 (d, *J* = 12, 4H), 4.99 (d, *J* = 12, 2H), 4.34 (m, 8H), 4.26 (d, *J* =15.6, 4H), 4.12 (d, *J* = 16.8, 6H), 2.87 (m, 8H), 1.91 (m, 8H), 1.77 (s, 6H), 1.69 (s, 6H), 1.66 (m, 8H). ^{13}C NMR (100 MHz, D_2O, 1, 4-dioxane as internal reference): δ 157.2, 156.6, 150.4, 144.1, 129.1, 125.8, 116.6, 79.3, 78.1, 72.1, 71.9, 64.2, 50.8, 50.4, 49.1, 35.7, 29.0, 21.8, 16.8, 15.7. HR-MS (ESI, negative): *m/z* 915.2599 ([M + 2H – 4Na]$^{2-}$), calculated 915.2632.

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Conflicts of interest

L.I. is an inventor on patents held by the University of Maryland on the use of acyclic CB[n]-type receptors in biomedical applications.

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