Published in final edited form as:

Supramol Chem. 2015 May 1; 27(5-6): 288–297. doi:10.1080/10610278.2014.940952.

Synthesis of a Disulfonated Derivative of Cucurbit[7]uril and Investigations of its Ability to Solubilize Insoluble Drugs

Elizabeth L. Robinson, Peter Y. Zavalij, and Lyle Isaacs*

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

Abstract

Cucurbit[7]uril (CB[7]) is currently being investigated as a solubilizing agent for insoluble drugs. We recently found that acyclic CB[n]-type receptors that bear sulfonate solubilizing groups are well suited for this application. Herein, we report cucurbit[7]uril derivative (**1**) that bears two sulfonate groups on its convex face that we hypothesized would be a superior solubilizing excipient for insoluble drugs. Before using **1** for drug solubilization experiments we showed that **1** does not self-associate and that it retained its ability to bind to diammonium compounds as common guests for CB[7] sized cavities. X-ray crystallography shows that **1** maintains the key structural features of CB[7] with only minor ellipsoidal deformations at the equator and carbonyl portals of **1**. Unfortunately, the aqueous solubility of **1** (20 mM) is slightly lower than CB[7] (20–30 mM) which limits its potential as a solubilizing excipient for insoluble drugs. We created phase solubility diagrams for the solubilization of three drugs (camptothecin, albendazole, cinnarizine) with two different containers (**1** and CB[7]). CB[7] and **1** exhibit comparable solubilization abilities (e.g. K_a and maximum solubility) toward camptothecin and albendazole but **1** is an inferior solubilizing agent for cinnarizine because of the low solubility exhibited by the **1**•cinnarizine complex.

Introduction

The preparation of molecular container compounds and studies of their unique supramolecular chemistry phenomena have long been a focal point for the field. For example, the past several decades has witnessed the development of the host-guest chemistry of molecular containers including cyclodextrins, calixarenes, cyclophanes, and crown ethers which have been formed either by covalent bond forming reactions or by non-covalent self assembly processes.¹ Encapsulation of a guest inside a molecular container by formation of the container•guest complex often confers new properties or reactivity upon the guest. For example, molecular containers have been used to tame otherwise unstable species like cyclobutadiene, P₄, and *o*-benzyne,² to protect π -conjugated chromophores for molecular electronic and imaging applications,³ to promote and control supramolecular polymerization processes,⁴ to catalyze bimolecular reactions,⁵ and to control the conformational properties of the guest. We, and others, have been very interested in an alternate class of molecular containers known as cucurbit[n]urils (CB[n], n = 5, 6, 7, 8, 10,

*To whom correspondence should be addressed..

14; Figure 1)^{6,7} which are composed of n glycoluril units connected by $2n$ CH₂-bridges and that are formed in high yield in a single condensation reaction under hot concentrated aqueous acidic conditions.^{8,9,10} The distinguishing features of the CB[n] family of molecular containers are the exceptionally high binding affinity (K_a up to 10^{17} M⁻¹) and selectivity that they exhibit toward their guests in aqueous solution.¹¹⁻¹³ Because of their high affinity and selectivity, CB[n]•guest complexes also respond sensitively to appropriate stimuli (e.g. pH, electrochemical, photochemical, exogenous guest addition) and can be used to switch CB[n] derived systems between two or more distinct states.^{9,14,15} For all these reasons, CB[n] have become popular components of chemically, biologically, and technologically oriented supramolecular systems including catalysis,^{16,17} gas sensing and purification,¹⁸ protein and peptide recognition and sensing,¹⁹ supramolecular materials,^{15,17,20} affinity capture materials,²¹ and non-covalent promoters of biological dimerization.²²

By some estimates, 40-70% of newly synthesized active pharmaceutical ingredients (API) are so poorly soluble in water that they cannot be formulated directly.²³ To overcome this issue, the pharmaceutical industry employs numerous tricks including the formation of higher solubility salts of the API, better soluble prodrugs, and kinetic trapping of the API in higher energy and therefore better soluble forms (e.g. nanocrystalline solids, amorphous dispersions).²⁴ To the supramolecular chemist, two strategies hold appeal: 1) the crystal engineering approach which targets co-crystalline forms of the API that display enhanced solubility,²⁵ and 2) the encapsulation of the API inside of a molecular container. Most notably, the β -cyclodextrin derivatives hydroxypropyl- β -CD and Captisol™ (Figure 1) are currently used to formulate several APIs that are administered to humans.²⁶ For these reasons, many researchers have been interested in exploring the use of CB[n] compounds in the context of drug formulation and delivery.²⁷⁻²⁹ For example, CB[n] have been used to enhance the solubility of many drugs (e.g. camptothecin, albendazole, chlorambucil),²⁸⁻³⁰ prevent degradation reactions,³¹ to promote the formation of the pharmaceutically active form,³² and for targeted therapy.³³ Other researchers have reported the use of CB[n] in the formation of pharmaceutical tablets and topical creams.³⁴ Basic *in vitro* and *in vivo* toxicology has been performed for CB[7] which establishes the biocompatibility of CB[n] compounds.³⁵ One of the unsolved issues surrounding the use of CB[7] as an API solubilizing agent is its modest solubility (lit.:³⁶ 20-30 mM) in water and the similarly modest solubility of the CB[7]•API complex.

Our group has been studying the mechanism of CB[n] formation^{37,38} with the aim of using this mechanistic knowledge to prepare new CB[n]-type receptors with enhanced properties.^{39,40} For example, we recently reported the templated synthesis of methylene bridged glycoluril hexamer which could be transformed into fluorescent CB[6], a cellularly targeted Biotin–CB[7] derivative, and Me CB[7] which displayed extraordinary solubility in water (264 mM).^{38,40,41} Encouraged by the high solubility of Me₂CB[7], we explored its use as a drug solubilizing agent toward albendazole and camptothecin, but found that the Me₂CB[7]•albendazole and Me₂CB[7]•camptothecin exhibit lower solubility than the corresponding CB[7]•albendazole and CB[7]•camptothecin complexes.⁴¹ Although the two Me-groups enhance the solubility of the uncomplexed Me₂CB[7] host, they are detrimental

to the solubility of the Me₂CB[7]•drug complexes. Concurrently, we have been exploring the biomedical applications of *sulfonated* acyclic CB[n]-type receptors (e.g. **M1**, Figure 1) and found that **M1** exhibits extremely high solubility (346 mM) in water and solubilizes numerous insoluble drugs (e.g. Paclitaxel) by factors up to 2750-fold.⁴² Accordingly, we hypothesized that CB[7] derivative bearing sulfonate groups (**1**) might display enhanced solubility in both its uncomplexed and complexed (e.g. **1**•drug) forms and would therefore surpass the abilities of CB[7]. This paper reports our work along this line of inquiry.

Results and Discussion

This results and discussion section is organized as follows. First, we discuss the synthesis of glycoluril bis(cyclic ether) **2** and its transformation into difunctionalized CB[7] derivative **1**. Subsequently, we discuss the x-ray crystal structure of **1**, determine its solubility in water, show that **1** does not undergo self-association, and establish its basic host•guest recognition properties. Finally, we explore the use of **1** as a solubilizing agent for camptothecin, albendazole, and cinnarizine.

Synthesis of Sulfonated Glycoluril Bis Cyclic Ether **2**

For the preparation of a CB[7] derivative bearing sulfonate groups we first needed to synthesize glycoluril bis(cyclic ether) derivative **2**. We adapted the chemistry developed by us earlier for the preparation of monofunctionalized CB[7].⁴¹ Accordingly, we reacted butanedione **3** with isopropylamine **4** in Et₂O in the presence of TiCl₄ as acid catalyst to give diimine **5** (Scheme 1). Diimine **5** was treated with 2.3 equivalents of LDA in THF at –78 °C, alkylated with Cl(CH₂)₃I, and subjected to hydrolytic workup to give dichlorodione **6** in 79% yield after purification by distillation. Dichlorodione was transformed into glycoluril derivative **7** in 30% yield by treatment with urea and TFA in refluxing benzene. Reaction of dichloroglycoluril **7** with formalin in 9M HCl gave bis(cyclic ether) **8** in high (90%) yield. Finally, reaction of **8** with Na₂SO₃ in water at reflux gave disulfonated glycoluril bis(cyclic ether) **2** in 50% yield.

Synthesis of Sulfonated CB[7] Derivative **1**

With gram scale quantities of sulfonated bis(cyclic ether) **2** in hand we turned our attention to the incorporation of this glycoluril derivative into a CB[7] derivative. For this purpose we investigated the reaction of **2** with methylene bridged glycoluril hexamer **9** under a variety of conditions (metal salts additives, acid identity and concentration, temperature) and monitored the CB[n] content of the reaction mixtures using *p*-xylylenediammonium ion as a ¹H NMR probe as described previously.^{38,41} The best reaction conditions involved heating **9** and **2** (2 equiv.) with KCl (2 equiv.) in conc. HCl at 100 °C for 30 minutes gave a crude solid that contained 46% of **1** (Scheme 2). Purification of the crude solid was achieved by Dowex ion exchange chromatography followed by treatment with NaOH solution to give disulfonate **1** in 28% yield. Compound **1** was fully characterized by ¹H NMR, ¹³C NMR, electrospray mass spectrometry, infrared spectroscopy (Supporting Information), and X-ray crystallography (*vide infra*). Figure 2a shows the ¹H NMR spectra of **1** alone which shows resonances in the 5.4–5.8 (integral 26H) and 4.1–4.4 (integral 14H) ppm region of the spectrum corresponding to the methine and diastereotopic methylene protons on the convex

face of the CB[7] ring system along with four resonances for the (CH₂)₄SO₃Na arms with the expected 4H:4H:4H:4H integrals. The ¹H NMR spectrum of **1** was also recorded in the presence of an excess of *p*-xylylenediammonium ion **10** as a probe guest that makes the spectrum more easily interpretable. For example, resonances are observed at 7.46 and 6.60 ppm which correspond to H_z of unbound **10** and of the **1**•**10** complex, respectively; similarly resonances for the methylene group (H_y) of unbound **10** and **1**•**10** appear as singlets at 4.12 and 3.91 ppm, respectively. These resonances appear as sharp singlets because of the commonly observed slow exchange of CB[7]•guest complexes relative to the ¹H NMR chemical shift timescale. Most diagnostic, however, is the presence of four doublets in a 2:4:4:4 integral ratio in the 4.35-4.10 ppm region which correspond to the four symmetry non-equivalent diastereotopic methylene protons (H_f, H_g, H_h, H_i) on the convex face of **1**. The ¹³C NMR spectrum recorded for **1**•**10** showed the expected 4 C=O resonances including one of approximately ½ intensity, 7 resonances for the equatorial C-atoms including C_e which is downfield shifted to 79.8 ppm due to the (CH₂)₄SO₃Na substituent, four resonances for the CH₂-groups on the CB[7] ring including one of approximately ½ intensity, and 4 CH₂-groups of the (CH₂)₄SO₃Na arms. The number and intensity of lines in the ¹³C NMR spectrum is consistent with the depicted C_{2v}-symmetric structure of **1**. The high resolution electrospray ionization mass spectrum displayed a parent ion at *m/z* 786.2488 which corresponds to the [**1**•**10**+2H-2Na]²⁺ complex.

X-ray Crystal Structure of **1**

We were fortunate to obtain x-ray quality single crystals of **1** as its *p*-xylylenediammonium ion complex (**1**•**10**, CCDC-1003470). Figure 3a shows a stereoscopic representation of **1**•**10** in the crystal. As expected, the guest **10** is bound within the cavity of **1** and displays ⁺NH•••O H-bonds to the ureidyl carbonyl portals of **1**. Interestingly, the two sulfonate groups of **1** act as the counterions to the two ammonium groups of **10** such that no additional counterions are present in the crystal of **1**•**10**. The structural features of the CB[7] unit of **1** are similar to that of CB[7] itself. For example, the distance between the ureidyl C=O O-atoms of a single glycoluril unit of **1** within **1**•**10** average 6.066 Å (range: 5.884-6.188 Å) which is similar to that of CB[7] (6.050 Å; range 5.913-6.114).^{7,41} As a measure of the ellipticity of the CB[7] cavity of **1** along its equator we report the distance between the opposing methine C-atoms on every fourth glycoluril unit: **1**•**10** (11.516 Å; range: 11.179-11.918 Å) and CB[7] (11.398 Å; range: 11.247-11.516). Similarly, we can provide an estimate of the ellipticity of the ureidyl C=O portals by measuring the distance between the ureidyl C=O O-atoms on every fourth glycoluril on a given portal: **1**•**10** (8.128 Å; 7.152-9.042 Å) and CB[7] (8.139 Å; range: 7.553-8.718 Å). The larger spread of distances both along the equator and at the portals of **1**•**10** relative to CB[7] arises in part because the glycoluril units pivot slightly whereby one O-atom shifts inward with the other shifts outward in order to accommodate the guest **10**. The presence of the (CH₂)₄SO₃ substituents on the convex face of **1** also influence the geometry of **1**•**10** in the crystal. The individual complexes **1**•**10** display an interesting packing motif in the extended crystal. Figure 3b shows that in the xz-plane individual **1**•**10** units pack with their (CH₂)₄SO₃⁻ arms pointing toward each other to create dimeric units that stack along the x-axis. These dimeric stacks pack with their long axes parallel to one another. When viewed in the xy-plane the

basic building units of the crystal structure can be recognized wherein four **1•10** complexes arrange themselves at the corners of a parallelogram driven by interactions between the electrostatically positive convex face of the CB[7] unit and the electrostatically negative ureidyl C=O portal of the neighboring CB[7] unit. These parallelogram units extend along the x-axis. Finally, when viewed along the x-axis (Supporting Information, Figure S31) it is obvious that the cavities of **1•10** arrange themselves in a collinear fashion.

Solubility of **1** in Water

The planned use of **1** as a solubilizing agent for the formulation of insoluble drugs would benefit from the ability to prepare highly concentrated solutions of **1**. Accordingly, we decided to measure the solubility of **1** in D₂O. Experimentally, we stir an excess of **1** in D₂O until equilibrium is reached, remove solid **1** by centrifugation, and then measure the concentration of **1** in solution by integrating the ¹H NMR resonances for **1** versus MeSO₃H as a non-binding internal standard of known concentration. In this manner, we measured the concentration of **1** (20.2 mM). Disappointingly, the addition of the sulfonate groups did not enhance the aqueous solubility of **1** beyond that of CB[7] (20-30 mM) as expected. We surmise that the lack of aqueous solubility enhancement is likely due to the hydrophobic nature of the eight CH₂-groups that are added along with the SO₃Na groups. Even though the (CH₂)₄SO₃Na groups were not able to enhance the solubility of uncomplexed **1** it is possible that they could enhance the solubility of the **1•drug** complexes relative to the corresponding CB[7]•drug complexes which would be advantageous. Accordingly, we continued our testing of **1** as a solubilizing agent for insoluble drugs as described below.

Container **1** Does Not Self-Associate

We were mindful of the fact that the (CH₂)₄SO₃Na arms of **1** could, in theory, become bound within the cavity of another molecule of **1** and thereby reduce its ability to bind to and solubilize insoluble drugs. Accordingly, we decided to perform ¹H NMR dilution experiments on solutions of **1** over the 16 mM – 130 μM range (Supporting Information). If self-association were to occur, we would expect to see upfield shifts in the ¹H NMR chemical shifts of the (CH₂)₄SO₃Na arms as [**1**] increases due to shielding nature of the CB[7] cavity.^{10,11} Experimentally, we do not observe any changes in chemical shift of H_a – H_d over this concentration range which indicates that **1** does not undergo significant self-association in water.

Container **1** Maintains the Innate Recognition Properties of CB[7]

Given that **1** does not self-associate we considered it very likely that **1** would display host-guest recognition properties that were comparable to those of CB[7]. Accordingly, we measured the ¹H NMR spectrum for 1:1 and 1:2 mixtures of **1** with a panel of guests (**10** – **17**, Figure 4) which are typical for CB[7]. Figure 5a-b shows the ¹H NMR spectra obtained for guest **11** alone and the **1•11** complex. We observe significant upfield shifts for protons H_q and H_r of guest **11** which are bound within the shielding region of the cavity of **1** in the **1•11** complex and a smaller upfield shift for H_p which are located closer to the C=O portals of **1**. Figure 5c shows the ¹H NMR spectrum of a 1:2 mixture of **1** and guest **11** which shows the presence of resonances for free **11** and bound **11** which indicates slow kinetics of

exchange on the ^1H NMR chemical shift timescale as is commonly seen with CB[7]•guest complexes.¹² Analogous spectra were obtained for the complexes between **1** and guests **12** – **17** (Supporting Information) which establish that the cavity of **1** maintains the essential molecular recognition properties of the unfunctionalized CB[7] container.

Drug Solubilization Using Host **1**

Even though the innate solubility of **1** was not higher than that of CB[7] we hoped that the $(\text{CH}_2)_4\text{SO}_3\text{Na}$ arms would enhance the solubility of the **1**•drug complexes beyond that achievable with CB[7] itself. Accordingly, we decided to construct phase solubility diagrams⁴³ for mixtures of insoluble drugs (Figure 6 and Figure 7) and soluble container. Experimentally, a series of samples of known concentration of container **1** or CB[7] are mixed with an excess of insoluble drug in either 50 mM NaOAc buffer (pD = 4.74) or in 10 mM aq. DCl (pD 2) solution and the mixture is stirred until equilibrium is reached. After removal of insoluble material by centrifugation, the concentration of container and drug in the supernatant is determined by ^1H NMR in the presence of added MeSO_3H or benzene-1,3,5-tricarboxylate sodium salt as non-binding internal standards of known concentration. For ideal 1:1 host•guest complexes of high solubility, the phase solubility should be linear and obey equation 1.⁴³ If the intrinsic solubility of the insoluble drug (s_0) is known accurately, then the slope of the phase solubility diagram can be used to calculate the binding constant (K_a , M^{-1}) for the container•drug complex.

$$K_a = \frac{\text{slope}}{s_0 (1 - \text{slope})} \quad (\text{eq. 1})$$

Figure 7a shows the phase solubility diagrams created for containers CB[7] and **1** with the insoluble drug albendazole in 50 mM NaOAc buffer (pD 4.74). As can be readily seen, the phase solubility diagrams are both linear throughout the accessible [container] range. The slopes are nearly the same which means that affinity of CB[7] and **1** toward albendazole are comparable. Attempts to obtain solutions of CB[7]•albendazole or **1**•albendazole with concentrations above that of CB[7] or **1** alone by adding an excess of solid CB[7] or **1** were not successful which indicates that the pendant $(\text{CH}_2)_4\text{SO}_3\text{Na}$ groups of **1** do not enhance the solubility of the **1**•albendazole complex as hypothesized. Figure 7b shows the phase solubility diagrams created for hosts CB[7] and **1** with the insoluble anti-cancer drug camptothecin. Camptothecin exists as either the lactone form (pH < 4) or the hydroxyl carboxylate form (pH > 7.2) in aqueous solution in a pH dependent manner.²⁹ We choose to solubilize camptothecin at pH 2 because it was expected that the neutral lactone would complex better with the containers than the anionic carboxylate. The phase solubility diagrams exhibited by CB[7] and **1** toward camptothecin were quite similar. Both phase solubility diagrams are linear across the accessible [container] range which indicates a 1:1 container:camptothecin binding mode as expected. The comparable slopes of the two lines in Figure 7b indicate that CB[7] and **1** show comparable affinity toward camptothecin in the pD 2.0 solution used. Once again, the $(\text{CH}_2)_4\text{SO}_3\text{Na}$ groups are unable to enhance the solubility of the **1**•camptothecin complex beyond that of CB[7]•camptothecin. Lastly, we created phase solubility diagrams for hosts CB[7] and **1** with the poorly soluble

antihistamine cinnarizine in 50 mM NaOAc buffer (pD 4.74) as shown in Figure 7c. In this case we observe linear phase solubility diagrams for both CB[7] and **1** which is indicative of a well defined 1:1 container•cinnarizine complex. However, the maximum [cinnarizine] obtained with CB[7] is 13.7 mM whereas **1** plateaus at 0.26 mM. When high concentrations of **1** are used (e.g. 20 mM), the final solution only contains 0.76 mM **1** and 0.26 mM cinnarizine. The remainder of **1** becomes insoluble due to the presence of cinnarizine. Unfortunately, this result indicates that the **1**•cinnarizine complex is significantly less soluble than CB[7]•cinnarizine. In this case, the (CH₂)₄SO₃Na arms of **1** are detrimental to the solubility of **1**•cinnarizine relative to CB[7]•cinnarizine.

Conclusion

In summary, we have reported the synthesis of disulfonate CB[7] derivative **1** by the reaction between glycoluril hexamer **9** and newly prepared glycoluril bis(cyclic ether) **2** in 28% yield. Container **1** is only modestly soluble in water (20 mM) but does not undergo self-association and therefore maintains the innate recognition properties of its CB[7] cavity. The x-ray crystal structure of **1**•**10** shows that **1** maintains the key structural features of the CB[7] cavity with minor distortions and packs in the solid state based on a repeating unit comprising four **1**•**10** complexes arranged in parallelograms. Finally, we created phase solubility diagrams for **1** and CB[7] with the insoluble drugs albendazole, camptothecin, and cinnarizine. We found that disulfonated host **1** is no better than CB[7] as a solubilizing agent for these drugs. On the contrary, the (CH₂)₄SO₃Na arms of **1** reduce the solubility of the **1**•cinnarizine complex relative to CB[7]•cinnarizine. The work highlights the challenges inherent in designing derivatives of molecular containers that simultaneously are highly soluble, maintain their recognition properties, and promote the solubility of the container•drug complexes.

Experimental Details

Starting materials were purchased from commercial suppliers and were used without further purification. Compounds **5** and **9** were synthesized following the known literature procedures.^{38,40,41} 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. ¹H and ¹³C NMR spectra were measured on 400 MHz and 500 MHz instruments (100 MHz and 125 MHz for ¹³C NMR, respectively). Mass spectrometry was performed using a JEOL AccuTOF electrospray instrument (ESI).

Compound 6

A solution of LDA in THF/heptane/ethylbenzene (2.0 M, 137 mL, 274 mmol) was added slowly over 90 min. to a stirring solution of N,N'-diisopropyl-2,3-butanediimine (20.0 g, 119 mmol) in distilled THF (180 mL) under N₂, maintained at -78 °C and stirred for an additional 5 h. A solution of 1-chloro-3-iodopropane (57.4 g, 280 mmol) in distilled THF (20 mL) was added over 15 min. and the mixture was stirred at -78 °C under N₂. After 15 h, aqueous HCl (1 M, 600 mL) was added and the mixture was allowed to stir at RT for 5 h. THF was removed under reduced pressure, and CH₂Cl₂ (160 mL × 3) was used to extract the product from the remaining aqueous layer. The organic layers were combined and

washed with HCl (1 M, 60 mL), H₂O (120 mL), and sat. NaHCO₃ (120 mL), and the organic layer was then dried over MgSO₄. The mixture was filtered and the filtrate was concentrated under reduced pressure to yield a crude orange oil. The oil was purified by distillation (133-137 °C/0.08 mm Hg) to give **6** (22.5 g, 94.1 mmol, 79%) as a yellow oil. ¹H NMR (500 MHz, CDCl₃): 3.55 (t, *J* = 6.3, 4H), 2.79 (t, *J* = 7.1, 4H), 1.85-1.70 (m, 8H). ¹³C NMR (125 MHz, CDCl₃): 198.9, 44.4, 35.1, 31.7, 20.2. ESI-MS: *m/z* 241 ([M + H]⁺, calcd. for C₁₀H₁₇³⁵Cl³⁷ClO₂⁺, 241.17554).

Compound 7

Benzene (70 mL) was added to a round bottom flask that was equipped with a Dean-Stark apparatus and heated to reflux while stirring under N₂. After 1 h, **6** (8.00 g, 33.5 mmol), urea (10.0 g, 167 mmol), and TFA (3.00 mL, 39.2 mmol) were added to the round bottom flask. The mixture was refluxed while stirring under N₂ with the Dean-Stark apparatus for 4 h, and then allowed to cool to RT. EtOH (200 mL) was added to the round bottom flask, which caused a precipitate to form. The white solid was collected by filtration and washed with EtOH (100 mL × 3), and dried under high vacuum overnight to yield **7** as a white powder (3.21 g, 9.94 mmol, 30%). M.p. 220-222 °C. IR (ATR, cm⁻¹): 3206m, 2955w, 1669s, 1497m, 1459m, 1306w, 1174m, 1108m, 1063m, 1007w. ¹H NMR (400 MHz, DMSO-*d*₆): 7.16 (s, 4H), 3.63 (t, *J* = 6.7, 4H), 1.73 (q, *J* = 6.7, 4H) 1.70-1.45 (m, 8H). ¹³C NMR (100 MHz, DMSO-*d*₆): 159.8, 77.6, 45.1, 34.0, 32.4, 20.1. ESI-MS: *m/z* 323 ([M + H]⁺). HR-MS: *m/z* 323.1056 ([M + H]⁺, calcd. for C₁₂H₂₁³⁵Cl₂N₄O₂⁺, 323.10416).

Compound 8

Compound **7** (8.80 g, 27.2 mmol), formalin (37% aqueous solution, 11.0 mL, 147 mmol), and HCl (9 M, 26.0 mL) were added to a round bottom flask which was capped with a polyethylene stopper. The mixture was allowed to stir at RT for 20 h and then H₂O (150 mL) was added. After an additional day of stirring, the precipitate was collected by filtration, washed with H₂O (100 mL × 3) and dried under high vacuum overnight to yield **8** as a gray solid (10.4 g, 25.8 mmol, 94%). M.p. 134-137°C. IR (ATR, cm⁻¹): 2950w, 1718s, 1419s, 1236m, 1173s, 1011s, 891s, 722s. ¹H NMR (400 MHz, CDCl₃): 5.52 (d, *J* = 11.4, 4H), 4.75 (d, *J* = 11.4, 4H), 3.60 (t, *J* = 6.2, 4H), 2.30-2.20 (m, 4H), 1.95-1.85 (m, 4H), 1.65-1.50 (m, 4H). ¹³C NMR (100 MHz, CDCl₃): 158.1, 75.7, 71.3, 44.4, 32.1, 28.7, 21.6. ESI-MS: *m/z* 407 ([M + H]⁺). HR-MS: *m/z* 407.1233 ([M + H]⁺, calcd. for C₁₆H₂₅³⁵Cl₂N₄O₄⁺, 407.12528).

Compound 2

Compound **8** (3.00 g, 7.37 mmol) and Na₂SO₃ (4.60 g, 36.8 mmol) were mixed in a round bottom flask. H₂O (60 mL) was added and the mixture was refluxed while stirring for 3 d. After cooling to RT the solvent was removed under reduced pressure and the solid was stirred in MeOH (100 mL × 3) for 1 d. The mixture was filtered and reduced pressure was used to remove MeOH from the combined filtrates to yield **2** as a white solid (2.00 g, 3.68 mmol, 50%). M.p. >300°C. IR (ATR, cm⁻¹): 2933w, 1743m, 1419m, 1171s, 1034s, 983m, 916m, 735m. ¹H NMR (500 MHz, D₂O): 5.41 (d, *J* = 10.9, 4H), 5.06 (d, *J* = 10.9, 4H), 2.96 (t, *J* = 7.1, 4H), 2.42 (t, *J* = 8.0 4H), 1.87 (q, *J* = 7.4, 4H), 1.55 (q, *J* = 7.4, 4H). ¹³C NMR

(125 MHz, D₂O, 1,4-dioxane as internal reference): 159.0, 76.2, 70.4, 49.9, 27.1, 23.5, 21.6. ESI-MS: m/z 248 ([M – 2Na]²⁻). HR-MS: m/z 248.0453 ([M – 2Na]²⁻, calcd. for C₁₆H₂₄N₄O₁₀S²²⁻, 248.04669).

CB[7] Derivative 1

Compound **9** (1.50 g, 1.54 mmol), KCl (0.229 g, 3.09 mmol), and conc. HCl (7.5 mL) were added to a round bottom flask. The mixture was stirred at RT for approximately 1 min. until all components dissolved, at which time **2** (1.68 g, 3.09 mmol) was added. The flask was then sealed with a rubber septum and stirred at 100 °C for 30 min. The reaction mixture was then poured into a 50 mL centrifuge tube containing MeOH (40 mL), which resulted in a red-brown precipitate. The mixture was centrifuged at 7700 rpm for 7 min. The supernatant was decanted, and an additional portion of MeOH (40 mL) was added, followed by sonication for 10 min. and then centrifuged at 7700 rpm for 7 min. This process was repeated with two additional portions of MeOH (40 mL). The precipitate was dried under high vacuum to give a crude red-brown powder (2.34 g). ¹H NMR with **10** as probe was used to determine the purity by integration of the Host **1•10** Ar-H hydrogens of **10** at 6.60 (s, 4H) versus that of the methylene/methine C-H hydrogens of Host **1** at 5.35-5.80 (m, 26H). This procedure allowed us to calculate that Host **1** comprised 46% of the crude solid. The crude solid was dissolved in H₂O (5 mL). This solution was loaded onto a column (3 cm diameter × 50 cm long) containing 35 cm Dowex 50WX2 ion-exchange resin pretreated with H₂O. The column was eluted with H₂O (600 mL). The purity of the fractions was assessed by ¹H NMR in the presence of **10** and the appropriate fractions were combined. The solvent was removed under reduced pressure. H₂O (10 mL) was used to dissolve the solid, which was then precipitated in MeOH (40 mL). The solid was collected by centrifugation and suspended in H₂O (5.0 mL). The solution was adjusted to pH = 7.0 using aq. sodium hydroxide solution (0.5 M). The solvent was removed under reduced pressure and then dried further under high vacuum overnight to yield the product **1** as a white solid (0.634 g, 0.429 mmol, 28%). M.p. >300 °C. IR (ATR, cm⁻¹): 1728m, 1465m, 1322m, 1231m, 1188m, 1035m, 963m, 802s, 759m, 671m. ¹H NMR (400 MHz, D₂O, >2 equiv. **10**, RT): 7.46 (s, unbound **10**), 6.60 (s, bound **10**, 4H), 5.77 (d, J = 15.1, 2H), 5.73 (d, J = 15.1, 4H), 5.70 (d, J = 15.3, 4H), 5.64 (d, J = 15.3, 4H), 5.57 (d, J = 8.6, 2H), 5.53 (d, J = 8.6, 2H), 5.50-5.35 (m, 8H), 4.29 (d, J = 15.6, 6H), 4.21 (d, J = 15.6, 4H), 4.13 (d, J = 15.6 4H), 3.91 (s, 4H), 2.92 (t, J = 7.2, 4H), 2.50-2.30 (m, 4H), 1.90-1.75 (m, 4H), 1.50-1.30 (m, 4H). ¹³C NMR (125 MHz, D₂O, 1,4-dioxane as internal reference, >1 equiv **10**, RT): 156.2, 156.1, 155.9, 155.9, 133.0, 128.9, 127.3, 79.8, 71.3, 71.0, 70.9, 70.8, 70.6, 70.4, 52.6, 52.0, 51.7, 49.7, 48.7, 42.2, 42.1, 41.9, 41.8, 26.7, 23.4, 20.3, 19.2. ESI-MS: m/z 786 ([M•**10** – 2Na + 4H]²⁺). HR-MS: m/z 786.2488 ([M – 2Na + PXDA + 4H]²⁺, calcd. for [C₅₈H₇₂N₃₀O₂₀S₂]²⁺, 786.24903). X-ray crystal structure (CCDC-1003470).

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.) and the University of Maryland for financial support. We thank Ms. Brittany Vinciguerra for intellectual contributions to the retrosynthesis of **1**.

References

- 1). Gutsche CD. Acc. Chem. Res. 1983; 16:161–170. Cram DJ. Angew. Chem., Int. Ed. Engl. 1988; 27:1009–1020. Diederich F. Angew. Chem., Int. Ed. Engl. 1988; 27:362–386. Lehn J-M. Angew. Chem., Int. Ed. Engl. 1988; 27:89–112. Rekharsky MV, Inoue Y. Chem. Rev. 1998; 98:1875–1917. [PubMed: 11848952] Rebek J. Acc. Chem. Res. 2009; 42:1660–1668. [PubMed: 19603810] Fiedler D, Leung DH, Bergman RG, Raymond KN. Acc. Chem. Res. 2005; 38:349–358. [PubMed: 15835881] Yoshizawa M, Klosterman J, Fujita M. Angew. Chem., Int. Ed. 2009; 48:3418–3438. Northrop BH, Zheng Y-R, Chi K-W, Stang PJ. Acc. Chem. Res. 2009; 42:1554–1563. [PubMed: 19555073]
- 2). Cram DJ, Tanner ME, Thomas R. Angew. Chem., Int. Ed. 1991; 30:1024–1027. Mal P, Breiner B, Rissanen K, Nitschke JR. Science. 2009; 324:1697–1699. [PubMed: 19556504] Warmuth R. Angew. Chem., Int. Ed. Engl. 1997; 36:1347–1350.
- 3). Frampton MJ, Anderson HL. Angew. Chem., Int. Ed. 2007; 46:1028–1064. Harmatys KM, Cole EL, Smith BD. Mol. Pharmaceutics. 2013; 10:4263–4271.
- 4). Harada A, Hashidzume A, Yamaguchi H, Takashima Y. Chem. Rev. 2009; 109:5974–6023. [PubMed: 19736930]
- 5). Nakamura A, Inoue Y. J. Am. Chem. Soc. 2005; 127:5338–5339. [PubMed: 15826169] Shimizu LS, Salpage SR, Koros AA. Acc. Chem. Res. 2014 Ahead of Print. J. Am. Chem. Soc. 2014; 136:2966–2969. [PubMed: 24512535]
- 6). Freeman WA, Mock WL, Shih N-Y. J. Am. Chem. Soc. 1981; 103:7367–7368. Day A, Arnold AP, Blanch RJ, Snushall B. J. Org. Chem. 2001; 66:8094–8100. [PubMed: 11722210] Liu S, Zavalij PY, Isaacs L. J. Am. Chem. Soc. 2005; 127:16798–16799. [PubMed: 16316221] Day AI, Blanch RJ, Arnold AP, Lorenzo S, Lewis GR, Dance I. Angew. Chem., Int. Ed. 2002; 41:275–277. Cheng XJ, 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; 52:7252–7255.
- 7). Kim J, Jung I-S, Kim S-Y, Lee E, Kang J-K, Sakamoto S, Yamaguchi K, Kim K. J. Am. Chem. Soc. 2000; 122:540–541.
- 8). Lagona J, Mukhopadhyay P, Chakrabarti S, Isaacs L. Angew. Chem., Int. Ed. 2005; 44:4844–4870. Nau WM, Florea M, Assaf KI. Isr. J. Chem. 2011; 51:559–577.
- 9). Ko YH, Kim E, Hwang I, Kim K. Chem. Commun. 2007:1305–1315.
- 10). Masson E, Ling X, Joseph R, Kyeremeh-Mensah L, Lu X. RSC Adv. 2012; 2:1213–1247.
- 11). Mock WL, Shih N-Y. J. Org. Chem. 1986; 51:4440–4446.
- 12). Liu S, Ruspic C, Mukhopadhyay P, Chakrabarti S, Zavalij PY, Isaacs L. J. Am. Chem. Soc. 2005; 127:15959–15967. [PubMed: 16277540]
- 13). 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; 104:20737–20742. [PubMed: 18093926] Cao L, Sekutor M, Zavalij PY, Mlinaric-Majerski K, Glaser R, Isaacs L. Angew. Chem. Int. Ed. 2014; 53:988–993. Moghaddam S, Yang C, Rekharsky M, Ko YH, Kim K, Inoue Y, Gilson MK. J. Am. Chem. Soc. 2011; 133:3570–3581. [PubMed: 21341773] Moghaddam S, Inoue Y, Gilson MK. J. Am. Chem. Soc. 2009; 131:4012–4021. [PubMed: 19133781] Biedermann F, Uzunova VD, Scherman OA, Nau WM, De Simone A. J. Am. Chem. Soc. 2012; 134:15318–15323. [PubMed: 22881280] Biedermann F, Vendruscolo M, Scherman O, De Simone A, Nau W. J. Am. Chem. Soc. 2013; 135:14879–14888. [PubMed: 23991836]
- 14). Isaacs L. Acc. Chem. Res. 2014; 47 ASAP. Stoffelen C, Voskuhl J, Jonkheijm P, Huskens J. Angew. Chem., Int. Ed. 2014; 53:3400–3404.
- 15). Yang H, Yuan B, Zhang X, Scherman OA. Acc. Chem. Res. 2014 Ahead of Print.

- 16). Pemberton BC, Raghunathan R, Volla S, Sivaguru J. *Chem. Eur. J.* 2012; 18:12178–12190. [PubMed: 22945866] Mock WL, Irra TA, Wepsiec JP, Adhya M. *J. Org. Chem.* 1989; 54:5302–5308. Kloeck C, Dsouza RN, Nau WM. *Org. Lett.* 2009; 11:2595–2598. [PubMed: 19462966] Lu X, Masson E. *Org. Lett.* 2010; 12:2310–2313. [PubMed: 20420390] Joseph R, Masson E. *Eur. J. Org. Chem.* 2014; 2014:105–110. Wang R, Yuan L, Macartney DH. *J. Org. Chem.* 2006; 71:1237–1239. [PubMed: 16438544] Jon SY, Ko YH, Park SH, Kim H-J, Kim K. *Chem. Commun.* 2001:1938–1939.
- 17). Ghosh S, Isaacs L. *J. Am. Chem. Soc.* 2010; 132:4445–4454. [PubMed: 20210325]
- 18). Miyahara Y, Abe K, Inazu T. *Angew. Chem., Int. Ed.* 2002; 41:3020–3023. Florea M, Nau WM. *Angew. Chem., Int. Ed.* 2011; 50:9338–9342.
- 19). Ghale G, Nau WM. *Acc. Chem. Res.* 2014 Ahead of Print. Bush ME, Bouley ND, Urbach AR. *J. Am. Chem. Soc.* 2005; 127:14511–14517. [PubMed: 16218648] Chinai JM, Taylor AB, Ryno LM, Hargreaves ND, Morris CA, Hart PJ, Urbach AR. *J. Am. Chem. Soc.* 2011; 133:8810–8813. [PubMed: 21473587] Logsdon LA, Schardon CL, Ramalingam V, Kwee SK, Urbach AR. *J. Am. Chem. Soc.* 2011; 133:17087–17092. [PubMed: 21967539] Logsdon LA, Urbach AR. *J. Am. Chem. Soc.* 2013; 135:11414–11416. [PubMed: 23883194]
- 20). Appel E, del Barrio J, Loh X, Scherman O. *Chem. Soc. Rev.* 2012; 41:6195–6214. [PubMed: 22890548] Baek K, Yun G, Kim Y, Kim D, Hota R, Hwang I, Xu D, Ko YH, Gu GH, Suh JH, Park CG, Sung BJ, Kim K. *J. Am. Chem. Soc.* 2013; 135:6523–6528. [PubMed: 23574044] Lee J, Baek K, Kim M, Yun G, Ko YH, Lee N-S, Hwang I, Kim J, Natarajan R, Park CG, Sung W, Kim K. *Nat. Chem.* 2014; 6:97–103. [PubMed: 24451584]
- 21). 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; 3:154–159. [PubMed: 21258389] Ahn Y, Jang Y, Selvapalam N, Yun G, Kim K. *Angew. Chem., Int. Ed.* 2013; 52:3140–3144.
- 22). Reczek JJ, Kennedy AA, Halbert BT, Urbach AR. *J. Am. Chem. Soc.* 2009; 131:2408–2415. [PubMed: 19199617] Nguyen HD, Dang DT, van Dongen JLJ, Brunsveld L. *Angew. Chem., Int. Ed.* 2010; 49:895–898. Dang DT, Schill J, Brunsveld L. *Chem. Sci.* 2012; 3:2679–2684. Dang D, Nguyen H, Merckx M, Brunsveld L. *Angew. Chem., Int. Ed.* 2013; 52:2915–2919.
- 23). Hauss DJ. *Adv. Drug Delivery Rev.* 2007; 59:667–676. Lipinski CA. *J. Pharmacol. Toxicol. Methods.* 2000; 44:235–249. [PubMed: 11274893]
- 24). Leuner C, Dressman J. *Eur. J. Pharmaceut. Biopharmaceut.* 2000; 50:47–60. Muller RH, Keck CM. *J. Biotechnol.* 2004; 113:151–170. [PubMed: 15380654] Serajuddin ATM. *Adv. Drug Delivery Rev.* 2007; 59:603–616. Stella VJ, Nti-Addae KW. *Adv. Drug Delivery Rev.* 2007; 59:677–694.
- 25). Blagden N, de Matas M, Gavan PT, York P. *Adv. Drug Delivery Rev.* 2007; 59:617–630.
- 26). Rajewski RA, Stella VJ. *J. Pharm. Sci.* 1996; 85:1142–1169. [PubMed: 8923319]
- 27). Walker S, Oun R, McInnes FJ, Wheate NJ. *Isr. J. Chem.* 2011; 51:616–624.
- 28). Zhao Y, Buck DP, Morris DL, Pourgholami MH, Day AI, Collins JG. *Org. Biomol. Chem.* 2008; 6:4509–4515. [PubMed: 19039358]
- 29). Dong N, Xue S-F, Zhu Q-J, Tao Z, Zhao Y, Yang L-X. *Supramol. Chem.* 2008; 20:659–665.
- 30). Dong N, Wang X, Pan J, Tao Z. *Acta Chim. Sinica.* 2011; 69:1431–1437.
- 31). Appel EA, Rowland MJ, Loh XJ, Heywood RM, Watts C, Scherman OA. *Chem. Commun.* 2012; 48:9843–9845. McInnes FJ, Anthony NG, Kennedy AR, Wheate NJ. *Org. Biomol. Chem.* 2010; 8:765–773. [PubMed: 20135032]
- 32). Saleh N, Koner AL, Nau WM. *Angew. Chem. Int. Ed.* 2008; 47:5398–5401.
- 33). 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; 49:4405–4408.
- 34). Walker S, Kaur R, McInnes FJ, Wheate NJ. *Mol. Pharmaceutics.* 2010; 7:2166–2172. Seif M, Impelido ML, Apps MG, Wheate NJ. *PLoS One.* 2014; 9:e85361/1–e85361/8. 8 pp. [PubMed: 24454850]
- 35). Uzunova VD, Cullinane C, Brix K, Nau WM, Day AI. *Org. Biomol. Chem.* 2010; 8:2037–2042. [PubMed: 20401379] Hettiarachchi G, Nguyen D, Wu J, Lucas D, Ma D, Isaacs L, Briken V. *PLoS One.* 2010; 5:e10514. [PubMed: 20463906]

- 36). Lee JW, Samal S, Selvapalam N, Kim H-J, Kim K. *Acc. Chem. Res.* 2003; 36:621–630. [PubMed: 12924959]
- 37). Chakraborty A, Wu A, Witt D, Lagona J, Fettingner JC, Isaacs L. *J. Am. Chem. Soc.* 2002; 124:8297–8306. [PubMed: 12105910] Huang W-H, Zavalij PY, Isaacs L. *J. Am. Chem. Soc.* 2008; 130:8446–8454. [PubMed: 18529059]
- 38). Lucas D, Minami T, Iannuzzi G, Cao L, Wittenberg JB, Anzenbacher P, Isaacs L. *J. Am. Chem. Soc.* 2011; 133:17966–17976. [PubMed: 21970313]
- 39). Huang W-H, Liu S, Zavalij PY, Isaacs L. *J. Am. Chem. Soc.* 2006; 128:14744–14745. [PubMed: 17105250] Huang W-H, Zavalij PY, Isaacs L. *Angew. Chem., Int. Ed.* 2007; 46:7425–7427. Huang W-H, Zavalij PY, Isaacs L. *Org. Lett.* 2008; 10:2577–2580. [PubMed: 18503277] Huang W-H, Zavalij PY, Isaacs L. *Org. Lett.* 2009; 11:3918–3921. [PubMed: 19655803]
- 40). Cao L, Hettiarachchi G, Briken V, Isaacs L. *Angew. Chem., Int. Ed.* 2013; 52:12033–12037.
- 41). Vinciguerra B, Cao L, Cannon JR, Zavalij PY, Fenselau C, Isaacs L. *J. Am. Chem. Soc.* 2012; 134:13133–13140. [PubMed: 22799491]
- 42). Ma D, Hettiarachchi G, Nguyen D, Zhang B, Wittenberg JB, Zavalij PY, Briken V, Isaacs L. *Nat. Chem.* 2012; 4:503–510. [PubMed: 22614387] Ma D, Zhang B, Hoffmann U, Sundrup MG, Eikermann M, Isaacs L. *Angew. Chem., Int. Ed.* 2012; 51:11358–11362. Minami T, Esipenko NA, Zhang B, Isaacs L, Nishiyabu R, Kubo Y, Anzenbacher P. *J. Am. Chem. Soc.* 2012; 134:20021–20024. [PubMed: 23194337] Shen C, Ma D, Meany B, Isaacs L, Wang Y. *J. Am. Chem. Soc.* 2012; 134:7254–7257. [PubMed: 22512431] Minami T, Esipenko N, Akdeniz A, Zhang B, Isaacs L, Anzenbacher P. *J. Am. Chem. Soc.* 2013; 135:15238–15243. [PubMed: 24000805]
- 43). Connors, KA. *Binding Constants*. John Wiley & Sons; New York: 1987.

Figure 1.
 Chemical structures of selected molecular containers used for the solubilization of insoluble pharmaceutical agents.

Scheme 1.

Synthesis of glycoluril bis(cyclic ether) **2**. Conditions: a) Et₂O, TiCl₄, b) LDA, THF, Cl(CH₂)₃I, 79%, c) urea, TFA, benzene, reflux, 30%, d) formalin, HCl, 90%, e) Na₂SO₃, H₂O, 50%.

Scheme 2.

Synthesis of **1**. Conditions: a) KCl, conc. HCl, 30 min., 100 °C.

Figure 2.

^1H NMR spectra recorded (400 MHz, D_2O , RT) for: a) **1** (1 mM), and b) a mixture of **1** (1 mM) and **10** (2 mM).

Figure 3.

X-ray crystal structure of **1•10**. a) Stereoview of one **1•10** complex, b) packing of **1•10** complexes in the xz-plane, c) packing of **1•10** complexes in the xy-plane. Color code: C, grey; H, white; N, blue; O, red; H-bonds, red-yellow striped.

Figure 4.
Guests **10** – **17** studied for binding with host **1**.

Figure 5.

^1H NMR spectra (400 MHz, D_2O , RT) recorded for: a) **11** (1 mM), b) a mixture of **1** (1 mM) and **11** (1 mM), and c) a mixture of **1** (1 mM) and **11** (2 mM). Resonances for unbound guest **11** are unprimed whereas those for bound guest (**1**•**11**) are annotated with a prime symbol (').

Figure 6.
Insoluble drugs used in this study.

Figure 7.

Phase solubility diagrams created for containers CB[7] (•) and **1** (○) with drugs: a) albendazole (50 mM NaOAc, pD 4.74), b) camptothecin (0.01 M DCl, pD 2), and c) cinnarizine (50 mM NaOAc, pD 4.74).