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Acyclic CB[n]-Type Molecular Containers: Effect of Solubilizing Group on their Function as Solubilizing Excipients

Ben Zhang, Peter Y. Zavalij, Lyle Isaacs*

We report the synthesis and x-ray crystal structures of three acyclic CB[n]-type molecular containers (2a, 2h, 2f) that differ in the charge on their solubilizing groups (SO\textsuperscript{−}, OH, NH\textsubscript{3}\textsuperscript{+}). The x-ray crystal structures of compounds 2h and 2f reveal a self-folding of the ArOCH\textsubscript{2}CH\textsubscript{2}X wall into the cavity driven by π–π interactions, H-bonds and ion-dipole interactions. The need to reverse this self-folding phenomenon upon guest binding decreases the affinity of 2h and 2f toward cationic guests in water relative to 2a as revealed by direct \textsuperscript{1}H NMR and UV/Vis titrations as well as UV/Vis competition experiments. We determined the pK\textsubscript{a} of 6-aminoacoumarin (pK\textsubscript{a} = 3.6) on its own and in the presence anionic, neutral, and cationic hosts (2a: pK\textsubscript{a} = 4.9; 2h: pK\textsubscript{a} = 4.1; 2f: pK\textsubscript{a} = 3.4) which reflect in part the relevance of direct ion-ion interactions between the arms of the host and the guest toward the recognition properties of acyclic CB[n]-type containers. Finally, we showed that the weaker binding affinities measured for neutral and positively charged hosts 2h and 2f compared to anionic 2a results in a decreased ability to act as solubilizing agents for either cationic (tamoxifen), neutral (17α-ethynylestradiol), or anionic (indomethacin) drugs in water. The results establish that acyclic CB[n] compounds that bear anionic solubilizing groups are most suitable for development as general purpose solubilizing excipients for insoluble pharmaceutical agents.

Introduction

A major thrust in the area of supramolecular chemistry is the development of macrocyclic compounds that act as molecular containers.\textsuperscript{1,2} Accordingly, the synthesis and basic molecular recognition properties of numerous classes of macrocycles including cyclodextrins, calixarenes, cyclophanes, crown ethers, self-assembled systems, and most recently pillararenes have been extensively studied.\textsuperscript{3,4,6} Importantly, the properties of guest compounds bound within molecular containers are distinct from those of the same compounds free in solution. For example, the lifetime of high energy molecules like cyclobutadiene can be extended,\textsuperscript{2} the photophysical properties of encapsulated dyes can be improved,\textsuperscript{5} the conformation of natural and non-natural molecules can be controlled,\textsuperscript{6} the pK\textsubscript{a} of included guests can be shifted,\textsuperscript{10} and the reactions of certain substrates can be catalyzed.\textsuperscript{5} Over the past decade, the supramolecular chemistry of the cucurbit[n]uril family (Figure 1) of molecular containers\textsuperscript{11} has developed rapidly due in large part to the remarkable affinity and selectivity displayed by CB[n] toward their guests in water\textsuperscript{12,13} and the stimuli responsiveness of the resultant CB[n]-guest complexes.\textsuperscript{14} Accordingly, CB[n] have been used as components of a large number of functional systems including molecular machines,\textsuperscript{14} sensing ensembles,\textsuperscript{15} supramolecular catalysts,\textsuperscript{16} supramolecular polymers and materials,\textsuperscript{17} supramolecular velcro,\textsuperscript{18} membrane protein fishing,\textsuperscript{19} and non-covalent inducers of dimerization.\textsuperscript{20}

A major problem facing the pharmaceutical industry over the past 20 years has been the increase in the percentage of new chemical entities with excellent biological activity but such poor solubility characteristics that they cannot be formulated on their own.\textsuperscript{21} Accordingly, the pharmaceutical industry has developed numerous techniques to increase the solubility of these poorly soluble drugs including solid dispersions,\textsuperscript{22} the generation of nanocrystal solid forms,\textsuperscript{23} the preparation of amorphous solid forms of the API,\textsuperscript{24} the application of co-solvents systems (e.g. EtOH / Cremophore), the formation of salts,\textsuperscript{25} higher solubility pro-drugs,\textsuperscript{26} co-crystals,\textsuperscript{27} the encapsulation within or attachment to the outside of a dendrimer construct,\textsuperscript{28} and complexation within cyclodextrin molecular containers (e.g. HP-β-CD and Captisol\textsuperscript{TM}).\textsuperscript{29,30} Accordingly, researchers in the CB[n] area have begun to investigate the in vitro and in vivo toxicology of macrocyclic CB[n] containers,\textsuperscript{30} their ability to increase the solubility of insoluble drugs (e.g. camptothecin, albendazole, chlorambucil),\textsuperscript{31,32} protect them against degradation,\textsuperscript{33} promote...
transformation into their biologically active form,\textsuperscript{34} and target them to specific cells.\textsuperscript{35-36}

Over the years, the Isaacs group has investigated the mechanism of CB[n] formation\textsuperscript{17,28} and used that mechanistic knowledge to prepare a variety of CB[n]-type receptors including CB[n] analogues,\textsuperscript{29} inverted CB[n],\textsuperscript{30} nor-seco-CB[n],\textsuperscript{41,42} and CB[n] derivatives.\textsuperscript{35,38,42-43} Most recently, we have synthesized acyclic CB[n]-type receptors comprising a central C-shaped glycoluril tetramer backbone, two terminal substituted aromatic rings derived from 1, and four arms bearing anionic sulfonate solubilizing groups.\textsuperscript{44,45,46} Previously we reported that 2b is highly water soluble (3.46 mM), increases the solubility of insoluble drugs in water by factors of up to 2750-fold, is not toxic in \textit{in vitro} and \textit{in vivo} tests, and that the 2b-paclitaxel complex efficiently kills HeLa cells.\textsuperscript{44} In complementary work we showed that related acyclic CB[n]-type receptors are capable of reversing the biological activity of the neuromuscular blocking agent rocuronium in rats.\textsuperscript{45} In this paper we examine the influence of the nature of the solubilizing group (e.g. anionic, neutral, cationic) and the linker connecting the solubilizing group to the aromatic walls on their ability to act as solubilizing agents.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Structures of molecular containers used previously as solubilizing agents for insoluble drugs: HP-β-CD, Captisol\textsuperscript{TM}, CB[n], and acyclic CB[n]-type container.}
\end{figure}

\section*{Results and discussion}

This results and discussion section is organized as follows. First, we discuss the design and synthesis of a series of acyclic CB[n]-type receptors 2a – 2h and \textit{x}-ray crystallographic determination of their solid state structures. Subsequently, we show that these containers do not self-associate and study their container-guest recognition properties by \textit{\textsuperscript{1}H} NMR and UV/Vis spectroscopy. Finally, we describe the use of these compounds as containers for insoluble drugs as a function of the charge on the solubilizing group employed.

\subsection*{Design and Synthesis of Acyclic CB[n]-Type Receptors 2a – 2h}

Previously, we have published the design and synthesis of compound 2b and its use as a solubilizing excipient for insoluble pharmaceutical agents.\textsuperscript{44} Acyclic CB[n]-type receptor 2b is composed of a central glycuril tetramer to which two aromatic walls have been attached. The central glycuril tetramer imparts an overall C-shape to compound 2b which allows it to preferentially bind to and solubilize hydrophobic and cationic drugs whereas the aromatic walls were incorporated to allow 2b to interact by π-π interactions with the wide variety of insoluble drugs which contain aromatic rings in their structures. Finally, container 2b features four anionic sulfonate (SO\textsubscript{3}\textsuperscript{-}) solubilizing groups which greatly enhance its solubility in water.\textsuperscript{44} In this paper, we prepare derivatives of 2b – compounds 2a – 2h – that contain different solubilizing groups and study the influence on their ability to act as a host and a solubilizing agent for drugs in water (Scheme 1).

Synthetically, the preparation of compound 2b involves the reaction of glycuril tetramer 3 with aromatic sidewall 1b by a double electrophilic aromatic substitution reaction as described previously.\textsuperscript{44} Accordingly, to prepare derivatives of 2b which differ in the nature of the solubilizing groups we needed to prepare a series of aromatic sidewalls. In analogy to the preparation of 1b, we allowed hydroquinone to react with butanesulfone under basic conditions (aq. NaOH) to deliver 1c in 80% yield. To prepare aromatic sidewall 1a with a shorter linker between the aromatic ring and the SO\textsubscript{3}\textsuperscript{-} groups we first reacted commercially available diol 1d with CBr\textsubscript{4} and PPh\textsubscript{3} to give 1e in 91% yield according to the literature report.\textsuperscript{47} Next, 1e was reacted with Na\textsubscript{2}SO\textsubscript{3} in DMF to give aromatic sidewall 1a in high yield (88%). Reaction of glycuril tetramer 3 with the new anionic sidewalls 1a and 1c in trifluoroacetic acid (TFA) yielded acyclic CB[n]-type receptors 2a and 2c in good yield (61 and 40\%), respectively. The series of hosts 2a – 2c differ only in the number of CH\textsubscript{2}-groups between the aromatic sidewall and the anionic SO\textsubscript{3}\textsuperscript{-} solubilizing groups.
To prepare acyclic CB[n]-type receptor 2f we first reacted glycoluril tetramer 3 with 1e in hot TFA for 3 hours to obtain tetra-bromo host 2d in good yield (79%). Transformation of 2d into the corresponding tetra-azide compound 2e proceeded smoothly with NaN₃ in DMSO. Reduction of the tetra-azide host 2e with PPh₃ in DMF/H₂O gave the corresponding tetramine host which was isolated in pure form as its tetrahydrochloride salt 2f in 39% yield. Lastly, we targeted the preparation of acyclic CB[n] type container 2h which contains uncharged solubilizing arms. For this purpose we reacted commercially available diol 1d with glycoluril tetramer 3 in a mixed solvent of TFA and Ac₂O (v/v = 1:1) which delivered tetraacetoxoy compound 2g in 90% yield. Hydrolysis of 2g with an aqueous solution of LiOH following by acidification with HCl gives host 2h in 67% yield. The solubility of host 2h in water is modest (< 2 mM) which provides practical limits on the titration and solubilization experiments described below.

**X-ray Crystal Structures of Hosts 2b, 2f, and 2h that differ in the charge on their solubilizing groups.** We were fortunate to obtain the crystal structures for host 2b, 2f, and 2h, which are the representatives of the negative, neutral and positive hosts (Figure 2). As we expected, all of the three structures assume a C-shaped conformation, which can be attributed to the polycyclic nature of the glycoluril tetramer backbone. In the crystal structure of 2b (Figure 2a), the substituted o-xylene tips interact with each other by CH···π interactions whereas the O(CH₂)₃SO₃⁻ arms are extended away from the cavity; the cavity is filled by a solvating CF₃CO₂H molecule. To quantify the size of the cavity we measure the distance between the opposing quaternary (MeC) carbon atoms (10.92 and 11.44 Å) on the glycoluril tetramer backbone of 2b. In the crystal the individual molecules of 2b form tapes along the c-axis. The formation of tapes is driven by π···π interactions between the o-xylene rings of 2b; the mean separation between the planes of the aromatic rings amounts to 3.49 Å. The tapes stack parallel to one another along the a-axis. For the cationic host 2f (Figure 2b) the distance between the opposing quaternary (MeC) carbon atoms amounts to 10.50 Å and 10.62 Å which is slightly smaller than that observed for 2b. We attribute this decreased dimension of 2f relative to 2b to the folding of one aromatic wall into the cavity of 2f. This self-complexation is driven by π···π interactions and the formation of N–H···O=C H-bonds / ion-dipole interactions between the OCH₂CH₂NH₃⁺ solubilizing arms and the carbonyl portal (N–H···O distance = 2.790 Å; N–H–O angle = 160°). The ¹H NMR chemical shift of the Ar–H protons of 2f (6.44 ppm) relative to those of 2a (6.93 ppm) support a similar π···π stacked geometry in solution. In order for 2f to act as a container for guests the self-complexation process would need to be reversed. The self-complexation also results in an out-of-plane twist which extends one OCH₂CH₂OH arm toward a neighboring molecule of 2f in the crystal which reciprocates and forms a dimeric motif driven by N–H···O=C H-bonds / ion-dipole interactions (N···O distance = 2.790 Å; N–H–O angle = 165°). The dimers pack in the ac-plane which stack along the b-axis separated by chloride counterions. A similar self-complexation phenomenon was observed in the crystal structure of 2h (Figure 2c). Once again, the folding of the o-xylene ring of 2h into the cavity results in a decreased distance between the opposing quaternary (MeC) carbon atoms which amounts to 10.88 Å and 11.00 Å. In this case the self-complexation is driven by O–H···O=C H-bonding interactions between on of the OCH₂CH₂OH arms and the carbonyl portal...
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Hosts 2a, 2f, and 2h Do Not Undergo Self-Association. A prerequisite for the use of negative, neutral and positive hosts as solubilizing agents for insoluble drugs is that they do not undergo strong self-association in water which would compete with the formation of the host-guest complexes. Previously, we have performed \textsuperscript{1}H NMR dilution experiments with negatively charged host 2b and determined a self-association constant $K_a = 47 \text{ M}^{-1}$ by fitting the change in observed chemical shift as a function of host concentration.\textsuperscript{44} The low value of $K_a$ ($47 \text{ M}^{-1}$) ensures that the majority of the host molecules are uncomplexed and ready to bind to drug molecules. Accordingly, we performed related \textsuperscript{1}H NMR dilution experiments\textsuperscript{51} with the newly prepared neutral (2h) and positively charged (2f) hosts. We did not observe any significant change in chemical shift of H$_6$ over the accessible concentration ranges (2h: 1.3 mM – 0.05 mM; 2f: 10.5 mM – 0.05 mM). These result establish that hosts 2f and 2h do not undergo significant self-association in the 20 mM sodium phosphate buffered D$_2$O (pH 7.4) used in the drug solubilization experiments described below.

Binding Studies Between Acyclic CB[n]-Type Receptors and Guests 4 – 8. This section describes our investigation of the binding between hosts 2a – 2c, 2f, and 2h toward guests 4 – 8 (Figure 3) by a combination of \textsuperscript{1}H NMR spectroscopy and direct and competition UV/Vis spectroscopic titrations.

\textbf{4 H NMR Investigations of the Binding Interactions.} In this section we use \textsuperscript{1}H NMR experiments to qualitatively and quantitatively study the geometrical features and association constants of the host-guest complexes. Initially, we performed a qualitative \textsuperscript{1}H NMR study of the difference in binding of guest 6 toward hosts 2a, 2f, and 2h. Figure 4a – c shows the \textsuperscript{1}H NMR spectra recorded for 6 (1.0 mM), and equimolar mixtures of 6 (1.0 mM) with hosts 2a (1.0 mM), 2h (1.0 mM) and 2f (1.0 mM). Interestingly, for an equimolar mixture of host 2f and guest 6 we do not observe any changes in chemical shift for protons H$_3$, H$_8$, and H$_6$ on guest 6 or protons H$_4$ on host 2f. We surmise that the interaction between host 2f and guest 6 is simply too weak to be detected at the 1 mM concentrations used. In contrast, however, we do observe significant upfield shifts of the protons H$_3$, H$_8$, and H$_6$ on guest 6 in the presence of neutral host 2h (Figure 4c) and negative host 2a (Figure 4d). The upfield nature of the changes in chemical shift is indicative of guest 6 binding within the cavity of 2h and 2a as observed

(OH···O=C distance = 2.799 Å; O-H···O angle = 164°). In the crystal the self-folded forms of 2h appear as dimeric pairs driven by $\pi-\pi$ interactions (mean interplanar separation = 3.5 Å). Quite interestingly, a second conformation of 2h is also observed in the crystal (Figure 2d). In this second conformation, the size of the cavity is increased as evidenced by the larger distance between the opposing quaternary (MeC) carbon atoms (12.23 and 13.70 Å) and the centroid – centroid distance between the two terminal aromatic rings (10.29 Å). This result is significant because it provides direct evidence for the highly flexible nature of methylene bridged glycoluril oligomers and acyclic CB[n]-type receptors in general which had previously been surmised based on their ability to solubilize drugs with a range of sizes and single walled carbon nanotubes.\textsuperscript{44,49} These expanded conformers of 2h occur in dimeric pairs within the crystal; the ArOCH$_2$CH$_2$OH wall and arm of one molecule fills the cavity of its partner and vice versa.\textsuperscript{52} Overall, the x-ray crystal structures point to a high level of conformational flexibility of the acyclic CB[n]-type receptors and highlight the possibility of both self-complexation and dimerization.
previously for (acyclic) CB[n]-type receptors. The larger upfield shift observed for protons H₈, H₆, and H₄ within the mixture of negative host 2a (Figure 2d) and guest 6 relative to neutral host 2h and guest 6 (Figure 4c) indicates that the negatively charged host 2a binds the dicaticonic guest significantly stronger than the neutral host 2h. It was also observed that the resonances for protons H₄ on the aromatic sidewalls of hosts 2h and 2a undergo a downfield shift upon complexation with guest 6. This observation can be explained by the fact that the neutral and positive hosts undergo π–π interactions between their aromatic walls within the uncomplexed host (Figure 2) which shifts the resonances for protons H₄ upfield (≈ 6.44 ppm). Binding to guest 6 breaks the π–π interactions and shifts the resonances for H₄ downfield (≈ 7.4 ppm).

![Figure 4](https://example.com/figure4.png)

Figure 4. ¹H NMR recorded (400 MHz, RT, 20 mM sodium phosphate buffered D₂O, pH 7.4) for: a) 6, b) an equimolar mixture of 2f (positive host) and 6, c) and equimolar mixture of 2h (neutral host) and 6, and (d) and equimolar mixture of 2a (negative host) and 6.

After performing these initial ¹H NMR experiments which showed substantial differences in the complexation behavior of negative, neutral, and positively charged hosts 2a, 2f, and 2h toward diammonium ion 6 we decided to determine the binding constants for these complexes by suitable titration experiments. To measure the binding constant for complex 2a•5c, we performed a direct ¹H NMR titration experiment. A solution containing a fixed concentration of host 2a (0.5 mM) in 20 mM sodium phosphate buffer (pH 7.4) was titrated with increasing concentrations of compound 5c (Supporting Information). We monitored the change in the ¹H NMR chemical shift of proton H₄ of host 2a as a function of [5c] and fitted the data to a 1:1 host:guest binding model which allowed us to determine the Kₐ value for 2a•5c (Kₐ = 3.33 × 10⁵ M⁻¹). In an analogous manner, we performed direct ¹H NMR titration experiments to obtain the Kₐ values (Table 1) for the complexes between host 2b and guests 5b, 5c, host 2h and guests 5b and 5c, and host 2f and guests 5c and 6 (Supporting Information).

**DIRECT UV/VIS TITRATIONS.** The ¹H NMR titration experiments described above were not applicable for the determination of the Kₐ values for the tighter host guest complexes and complexes with poor solubility characteristics. Accordingly, we decided to measure the Kₐ values for the remaining host-guest complexes by UV/Vis competition assays referenced to Kₐ values determined by direct UV/Vis titration. Dye 4 was used in displacement assays to determine the Kₐ values of negative host 2a towards different guests. However, the application of 4 in the detection of the Kₐ values of neutral host 2h was limited by the fact that the dye induces precipitation of the host in the displacement experiments. To avoid that problem, we chose dye 8 as the indicator for competition experiments involving neutral host 2h. Figure 3a shows the UV/Vis spectra recorded when a fixed concentration of dye 4 (10.0 µM) was titrated with negative host 2a (0 – 0.45 mM). We observed an isosbestic point at 533 nm which is indicative of the formation of a well defined 2a•4 complex. Figure 3b shows the best nonlinear least-squares fit of the absorbance at 550 nm versus concentration data to a 1:1 binding model which allowed us to determine the binding constant for complex 2a•4 (Kₐ = (1.83 ± 0.08) × 10⁵ M⁻¹). Similar experiments were carried out to determine the binding constant constants for complex 2h•8 (Kₐ = (1.32 ± 0.01) × 10³ M⁻¹, Supporting Information). With those binding constants in hand we were able to perform the indicator displacement assays to determine the Kₐ values for a larger variety of guests.

![Figure 5](https://example.com/figure5.png)

Figure 5. a) UV/vis spectra obtained during the titration of a fixed concentration of 4 (10.0 µM) with 2a (0 – 0.45 mM) and b) plot of absorbance versus [2a] used to determine the Kₐ value of the 2a•4 complex by nonlinear least-square fitting.

**UV/Vis COMPETITION ASSAYS.** To measure the values of Kₐ for guests whose binding affinity exceeds that measurable by direct ¹H NMR titrations (approx. 10⁴ M⁻¹) we turned to...
UV/Vis competition assays involving a colorimetric indicator as guest. In these UV/Vis competition assays a complex between host and indicator (of known K_a) is initially formed – which shows a UV/Vis change upon host•indicator formation – and then titrated with an increasing concentration of UV/Vis silent guest. Upon competitive formation of the host•guest complex the indicator is released and the UV/Vis change is reversed. Fitting of a plot of UV/Vis absorbance values versus [guest] to the competitive binding model (Supporting Information) as described previously yields the unknown K_a value for host•guest. For example, we performed a UV/Vis competition assay employing fixed concentrations of dye 4 (10.0 μM) and host 2a (9.15 μM) and increasing concentrations of 5a (0 to 65.0 μM). The absorbance of dye 4 was monitored and was then plotted against the concentration of guest 5a. Fitting the data to a competitive binding model, we determined the K_a value of 2a•5a to be (1.68 ± 0.09) × 10^6 M^-1. Similar experiments were also performed for hosts 2a, 2b, 2c and 2h with guest 5a – 5c, and 6 (Table 1, Supporting Information).

**Table 1.** Binding Constants (K_a, M^-1) obtained for the interaction between host 2a – 2f with various guests.

<table>
<thead>
<tr>
<th></th>
<th>2a</th>
<th>2b</th>
<th>2c</th>
<th>2h</th>
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<td>4</td>
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<tr>
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<tr>
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<td>4.47 × 10^6</td>
<td>1.67 × 10^7</td>
<td>5.54 × 10^7</td>
<td>2.36 × 10^7</td>
<td>–</td>
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<tr>
<td>5c</td>
<td>3.33 × 10^6</td>
<td>1.87 × 10^7</td>
<td>3.45 × 10^8</td>
<td>1.08 × 10^8</td>
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<td>8</td>
<td>n.d.</td>
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<td>1.32 × 10^8</td>
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a Measured by direct titration monitored by UV/Vis absorption spectroscopy.
b Measured by competition with guest 4 monitored by UV/Vis spectrometry.
c Measured by competition with guest 8 monitored by UV/Vis spectrometry.
d Measured by direct titration monitored by 1H NMR. n.d.: not determined. –, below detection limit of 1H NMR titration. ppt. = precipitate formed.

**Trends in the K_a Values Between Hosts 2a, 2h, and 2f and Guests 5a – 5c, and 6.** Hosts 2a, 2h, and 2f differ in the nature of the charge on the solubilizing arms with a constant OCH_2CH_3 linker connecting them to the aromatic sidewall. In previous work, we reported the x-ray crystal structure of host 2b which showed that the sulfonate solubilizing groups extend away from the cavity and portals of the acyclic CB[n]-type receptor. However, the x-ray crystal structures of hosts 2h and 2f reveal the presence of intramolecular H-bonds between the solubilizing arms and the ureidyl C=O portal of the host. In addition, the presence of intramolecular H-bonds prompts the attached substituted α-xylene sidewall to fold into the cavity to undergo offset π−π stacking. Accordingly, for hosts 2h and 2f to undergo guest binding these intramolecular H-bonds, ion-dipole interactions, and π−π interactions need to be disrupted which should result in lower binding strength relative to anionic host 2a. In accord with these expectations we note that adamantaneammonium ion 5a binds 461-fold more tightly to anionic host 2a than to neutral host 2h; binding of 5a to positively charged host 2f was too weak to be detected. Similarly, cyclohexanediammonium ion 6 binds 406-fold more tightly to 2a than it does to neutral host 2h which in turn binds 35-fold more tightly to 6 than cationic host 2f does. The effect of solubilizing group charge on the binding process toward neutral guests is somewhat different. For a neutral guest like adamantanol 2b the main driving force for complexation is the hydrophobic effect; the presence of a RO−H•••O=C H-bond is of no consequence to the binding because 2b is H-bonded in both water and the complex. We find that host 2a binds 5b only 19-fold more tightly than 2h which can be attributed to the loss of intrahost π−π interactions upon formation of the 2h•5b complex. Host 2f does not complex 5b at all because it is energetically unfavorable to sacrifice intrahost ammonium•O=C ion-dipole interactions. Interestingly, the influence of solubilizing group charge on the binding of negatively charged guests is different still. For example, negatively charged host 2a binds 30-fold more tightly to adamantane carboxylate 5c than neutral host 2h. We believe this difference is due to the loss of intrahost π−π interactions upon formation of the 2h•5c complex, although differences in the protonation state of 5c within the 2a•5c and 2h•5c complexes cannot be ruled out. However, host 2f forms a relatively stable complex with 5c (K_a = 645 M^-1) which is 6-fold stronger than 2h•5c. We suggest that this increase in K_a is due to the presence of direct ammonium-carboxylate (H(N^−)−−−−O=C) electrostatic interactions between the solubilizing arms of cationic host 2f and guest 5c. Apparently, these electrostatic interactions are sufficiently strong to compensate for the loss or reduction of ion-dipole interactions and π−π stacking interactions in uncomplexed host 2f. A related trend is noted in the recognition properties of anionic host 2a toward cationic (5a), neutral (5b), and negatively (5c) charged adamantane derivatives where 5a binds 38-fold more tightly than 5b and 505-fold more tightly than 5c. Overall, these results suggest that the charge on the solubilizing arms (e.g., anionic, neutral, cationic) has a major impact on the molecular recognition capabilities of the hosts.

We also studied the length of the linker (O(CH_2)_nSO_3^-; n = 2, 3, 4) between the aromatic wall and the anionic solubilizing group. For example, the binding affinities of 2a, 2h, and 2c toward a common guest (e.g., 5b) differ by only 4-fold from one another. Because the magnitude of the differences in K_a for hosts 2a, 2b, and 2c toward a given guest are small we will not speculate further on the reasons for any observed differences.

**Acyclic CB[n]-Type Receptors that Differ in Charge Induce pK_a Shifts of Bound Guests of Different Magnitude.** It is well known in the literature that the pK_a values for the guest within CB[n]-guest complexes can differ substantially from the pK_a for guest alone; the magnitude of these complexation induced pK_a shifts can exceed 4 pK_a units. The origin of these pK_a shifts can be traced to the strong ion-dipole interactions that occur between CB[n] host..."
and cationic guests that are not possible with the corresponding neutral guests. In this paper, we studied the influence of the charges on the solubilizing groups on the pKₐ shift of 6-aminocoumarin (7) when binding with acyclic CB[n] type receptors. UV/Vis spectroscopy was used to monitor the protonation and deprotonation process of 7. Figure 6 shows the plot of the percentage of the absorbance change of 7 at 345 nm versus pH; the pKₐ value (Table 2) was obtained by non-linear fitting of the data to the Equation 1 (Supporting Information). 

From Table 2, we can observe an increase in pKₐ values in the presence of neutral host 2h (pKₐ = 4.1) and negative host 2a (pKₐ = 4.9) compared with dye 7 alone (pKₐ = 3.6), while a small decrease was observed in the presence of positive host 2f (pKₐ = 3.4). These changes in pKₐ are consistent with our expectations based on the net charge of the host. For example, protonation of guest 7 to give 7H⁺ is more favorable in the presence of neutral host 2h because 2h establishes ion-dipole interactions in the 2h+7H⁺ that are not formed in the 2h+7 complex. Protonation of guest 7 to give 7H⁺ is even more favorable (larger pKₐ shift to 4.9) in the presence of anionic host 2a not only because of ion-dipole interactions in 2a+7H⁺ complex but also because of the favorable ion-ion electrostatic interactions between the SO₃ groups and 7H⁺. Finally, the pKₐ of the 7H⁺ in the presence of cationic host 2f is comparable to that of 7H⁺ which probably reflects the weak binding between 2f and 7H⁺ due to binding of the OCH₂CH₂NH₃⁺ arms of 2f to its C=O portals and unfavorable ion-ion electrostatic interactions in the putative 2f+7H⁺ complex.

\[ A_{obs} = \frac{A_{7H^+}}{(1 + 10^{\text{pH} - \text{pK}_a})} + \frac{A_7}{(1 + 10^{\text{pK}_a - \text{pH}})} \]  

(1)

\[ \Delta A_{50\%} \text{ vs pH} \]

\[ 2, 3, 4, 5, 6, 7 \]

\[ A_{50\%} \text{ vs pH} \]

\[ 2, 3, 4, 5, 6, 7 \]

Figure 6. Plot of absorbance change (%) versus pH to determine the pKₐ values of 6-aminocoumarin (7, 35.6 µM) by itself (●), and with 2f (cationic host, 1.5 mm, ▲), 2h (neutral host, 1.2 mM, ●), and 2a (anionic host, 1.5 mM, ▼).

<table>
<thead>
<tr>
<th>pKₐ</th>
<th>7</th>
<th>2a+7</th>
<th>2b+7</th>
<th>2f+7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.6</td>
<td>4.9</td>
<td>4.1</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Kₐ (M⁻¹)  n.a.  2.74 × 10⁶  9.59 × 10⁴  678

n.a. = not applicable. – = no changes in ¹H NMR chemical shift observed. a) Conditions: 20 mM sodium phosphate buffer, pH 7.4, RT.

**Phase Solubility Diagrams for Acyclic CB[n] Type Receptors with Insoluble Drugs of Different Charges.** Our purpose in preparing and studying hosts 2a, 2h, and 2f was to determine whether the charge on the solubilizing arms of the acyclic CB[n]-type receptor effects their ability to act as a solubilizing excipient for insoluble drugs. Given that we observed significantly weaker binding of neutral (2h) and positively charged (2f) hosts toward most soluble guests as described above we anticipated that the anionic host 2a would be the superior solubilizing agent for insoluble drugs. Accordingly, we decided to test the ability of hosts 2a, 2h, and 2f to enhance the solubility of three insoluble drugs: tamoxifen, 17α-ethynylestradiol, and indomethacin (Figure 7). We selected these three drugs because they differ in their net charge in neutral aqueous solution (tamoxifen, positive; 17α-ethynylestradiol, neutral; indomethacin, negative). For this purpose, we constructed phase solubility diagrams (plots of [drug] versus [host]) for each of the three hosts with each of the three water insoluble drugs (Figure 8). Experimentally, a series of solutions containing known concentrations of host 2a (or 2h or 2f) in sodium phosphate buffer (20 mM, pH 7.4) were stirred with excess of solid insoluble drug (e.g. tamoxifen, 17α-ethynylestradiol, or indomethacin) at RT until equilibrium was established. The mixture was then filtered and the supernatant was collected. A known concentration of benzene-1,3,5-tricarboxylic acid was added into the supernatant as an internal standard. The concentration of the solubilized drug was then determined by ¹H NMR spectroscopy using the integrals of the resonances of the known concentration of internal standard versus those of solubilized drug. Figure 8a-c shows the phase solubility diagrams constructed for tamoxifen, 17α-ethynylestradiol, and indomethacin with hosts 2a, 2h, and 2f.

As is readily apparent, negatively charged host 2a is able to solubilize substantially more drug than neutral or positively charged hosts 2h and 2f. This behavior can be further rationalized based on an analysis of the phase solubility diagrams. For linear (Aₐ) phase solubility diagrams, the initial slope of the PSD obeys equation 2 where S₀ is the intrinsic solubility of the drug, slope is the slope of the PSD, and Kₛ is the binding constant for the host-drug complex.

In this manner, we calculated the binding constant for host 2a towards all three drugs (1.83 × 10⁵ M⁻¹ for tamoxifen, 1.73 × 10⁴ M⁻¹ for 17α-ethynylestradiol, and 6.07 × 10⁴ M⁻¹ for indomethacin). It is also possible to use the phase solubility diagram to compare the behavior for a given drug (with common S₀) with different hosts. In this situation, the relative slopes of the phase solubility diagrams reflect the relative...
binding affinities of the different host-drug complexes. Accordingly, the inability of hosts 2h and 2f to solubilize any of the three drugs can be traced to their poor abilities as hosts (e.g. low K_a values). In turn, this may be attributed to the blockade of the host cavity in 2h and 2f which was induced by intramolecular H-bonds, ion-dipole interactions, and π−π stacking.

**Figure 7.** Chemical structures of water-insoluble drugs used in this study.

<table>
<thead>
<tr>
<th></th>
<th>tamoxifen</th>
<th>17α-ethynylestradiol</th>
<th>indomethacin</th>
</tr>
</thead>
</table>

**Figure 8.** Phase solubility diagrams constructed using solutions of hosts 2a (●), 2h (○) and 2f (▲) of known concentrations and an excess of solid drug: a) tamoxifen, b) 17α-ethynylestradiol, and c) indomethacin. Conditions: 20 mM sodium phosphate buffered D_2O (pH = 7.4, RT).

\[ K_a = \text{slope} / [S_m \text{ (1-slope)}] \quad (2) \]

**Experimental**

**General Experimental.** Starting materials were purchased from commercial suppliers and were used without further purification or were prepared by literature procedures. Compound 1b, 1e, 2h and 3 were prepared according to literature procedures. Melting points were measured on a Meltcomp apparatus in open capillary tubes and are uncorrected. IR spectra were measured on a JASCO FT/IR 4100 spectrometer and are reported in cm\(^{-1}\). NMR spectra were measured at 400 MHz or 600 MHz for \(^1\)H and 125 MHz for \(^13\)C. Mass spectrometry was performed using a JEOL AccuTOF electrospray instrument using the electrospray ionization (ESI) technique. UV/Vis spectra were measured on a Varian Cary 100 UV/Visible spectrophotometer.

Compound 2a. Compound 1a (0.285 g, 0.23 mmol) was added into a solution of 3 (0.181 g, 0.77 mmol) in TFA (2 mL). The mixture was stirred and heated at 70 °C for 4 h. The solvent was removed under reduced pressure and the solid was further dried under high vacuum. The solid was washed with the mixture of water and acetone (1:2, v/v, 30 mL) twice and then dissolved in water and adjusted to pH = 7 by adding 1 M aqueous NaOH. The solvent was removed under reduced pressure and then the solid was further dried under high vacuum to yield 2a as a white solid (0.208 g, 61%). M.p. > 300 °C. IR (ATR, cm\(^{-1}\)): 2990w, 1726s, 1480s, 1381m, 1318m, 1182, 1087s, 968m, 938m, 799s, 759m, 526m. \(^1\)H NMR (400 MHz, D_2O): 6.93 (s, 4H), 5.67 (d, \(J = 15.5\), 2H), 5.56 (d, \(J = 16.0\), 4H), 5.44 (d, \(J = 7.6\), 2H), 3.58 (d, \(J = 7.6\), 2H), 5.35 (d, \(J = 16.3\), 4H) 4.45 - 4.25 (m, 8H), 4.24 (d, \(J = 16.0\), 4H), 4.21 (d, \(J = 16.3\), 4H) 4.10 (d, \(J = 15.5\), 2H), 3.55 - 3.40 (m, 4H), 3.35-3.20 (m, 4H), 1.79 (s, 6H), 1.75 (s, 6H). \(^13\)C NMR (125 MHz, D_2O, 1,4-dioxane as internal reference): δ 168.3, 167.8, 161.5, 139.5, 126.3, 90.3, 89.0, 82.8, 82.7, 77.4, 64.2, 62.0, 59.9, 46.7, 27.5, 26.5. High-Res MS (ESI): m/z 708.1271 ([M - 3Na + H]^+) calculated 708.1256.

**Conclusions**

In summary, we have synthesized a series of acyclic CB[n]-type molecular containers (2a – 2h) with different solubilizing groups bearing different charges for evaluation as potential drug solubilizing agents. The X-ray crystal structures of the negative, positive and neutral hosts (host 2b, 2f, and 2h) show us that all of these acyclic hosts assume a C-shaped conformation. However, for neutral (2h) and positively charged (2f) hosts, we observed intramolecular H-bonds and ion-dipole interactions between the solubilizing arms and the ureidyl C=O portals as well as intrahost π−π stacking interactions which result in a self-filling of the cavity. We used \(^1\)H NMR and UV/Vis spectroscopy to measure the K_a values of hosts 2a, 2h, and 2f toward guests with different charge and noted significant decrease in binding affinities of the neutral (2h) and positive (2f) hosts towards most guests. There are exceptions, however, with adamantane carboxylate 5e binding more tightly to positively charged host 2f than to neutral host 2h probably due to ion-ion electrostatic interactions. We measured the pK_a of 7H^- alone and in the presence of 2a, 2h,
and 2f and noted that the 2a induces the largest pKa shift which we attribute to the presence of ion-ion electrostatic interactions in the 2a-7H+ complex. Both the Ks and pKs measurements indicate that the solubilizing groups are not innocent bystanders. The poor recognition properties of hosts 2h and 2f are reflected in their phase-solubility diagrams with insoluble drugs (tamoxifen, 17α-ethylxylestradiol, and indomethacin). In all cases, the anionic host 2a functions more efficiently as a solubilizing agent that either neutral 2h, or cationic host 2f.

In conclusion, we have established that host 2a which bears anionic sulfonate solubilizing groups is far more efficient as a solubilizing agent than either 2h or 2f. The work reinforces the need to employ solubilizing groups that do not impinge upon the innate recognition abilities of the host cavity by either self-association or self-folding due to H-bonds; ion-dipole interaction, or π–π interactions. Accordingly, further development of acyclic CB[n]-type receptors as solubilizing excipients for insoluble drugs will focus on derivatives with sulfonate solubilizing groups. Because the synthesis of acyclic CB[n]-type receptors is modular, we are able to attach different aromatic sidewalls (e.g. naphthalene) to create tailor made analogues of 2. Ongoing work targets an understanding of the role of aromatic walls on the performance of analogues of 2 as solubilizing excipients.

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Notes and references


