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

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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_3^-, OH, NH_3^+). The x-ray crystal structures of compounds **2h** and **2f** reveal a self-folding of the $ArOCH_2CH_2X$ 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 ^{1}H NMR and UV/Vis titrations as well as UV/Vis competition experiments. We determined the pK_a of 6-aminocoumarin **7** (pK_a = 3.6) on its own and in the presence anionic, neutral, and cationic hosts (**2a**: pK_a = 4.9; **2h**: pK_a = 4.1; **2f**, pK_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.[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.[1,3,4-6] 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,[7] the photophysical properties of encapsulated dyes can be improved,[8] the conformation of natural and non-natural molecules can be controlled,[5,9] the pK_a of included guests can be shifted,[10] and the reactions of certain substrates can be catalyzed.[6] Over the past decade, the supramolecular chemistry of the cucurbit[n]uril family (Figure 1) of molecular containers[11] has developed rapidly due in large part to the remarkable affinity and selectivity displayed by CB[n] toward their guests in water[12,13] and the stimuli responsiveness of the resultant CB[n]•guest complexes.[14] Accordingly, CB[n] have been used as components of a large number of functional systems including molecular machines,[14] sensing ensembles,[15] supramolecular catalysts,[16] supramolecular polymers and materials,[17] supramolecular velcro,[18] membrane protein fishing,[19] and non-covalent inducers of dimerization.[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.[21] Accordingly, the pharmaceutical industry has developed numerous techniques to increase the solubility of these poorly soluble drugs including solid dispersions,[22] the generation of nanocrystal solid forms,[23] the preparation of amorphous solid forms of the API,[24] the application of co-solvents systems (e.g. EtOH / Cremophore), the formation of salts,[25] higher solubility pro-drugs,[26] co-crystals,[27] the encapsulation within or attachment to the outside of a dendrimer construct,[28] and complexation within cyclodextrin molecular containers (e.g. HP-β-CD and Captisol™).[4,29] Accordingly, researchers in the CB[n] area have begun to investigate the *in vitro* and *in vivo* toxicology of *macrocyclic* CB[n] containers,[30] their ability to increase the solubility of insoluble drugs (e.g. camptothecin, albendazole, chlorambucil),[31,32] protect them against degradation,[33] promote

transformation into their biologically active form,[34] and target them to specific cells.[35,36]

Over the years, the Isaacs group has investigated the mechanism of CB[n] formation[37,38] and used that mechanistic knowledge to prepare a variety of CB[n]-type receptors including CB[n] analogues,[39] inverted CB[n],[40] nor-seco-CB[n],[41,42] and CB[n] derivatives.[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.[44,45,46] Previously we reported that **2b** is highly water soluble (346 mM), increases the solubility of insoluble drugs in water by factors of up to 2750-fold, is not toxic in *in vitro* and *in vivo* tests, and that the **2b**•paclitaxel complex efficiently kills HeLa cells.[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.[46] 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.

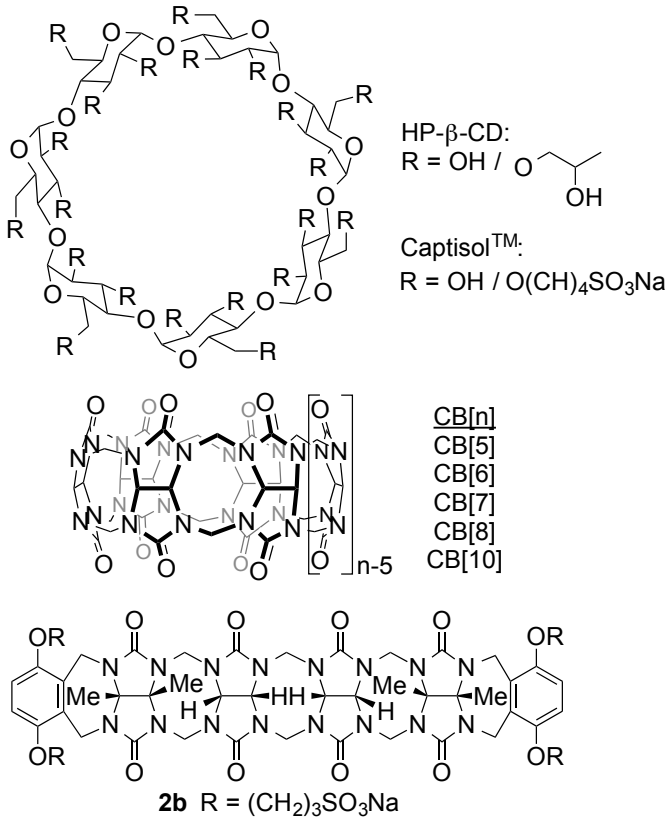

Figure 1. Structures of molecular containers used previously as solubilizing agents for insoluble drugs: HP-β-CD, Captisol™, CB[n], and acyclic CB[n]-type container.

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 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 ^{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.

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.[44] Acyclic CB[n]-type receptor **2b** is composed of a central glycoluril tetramer to which two aromatic walls have been attached. The central glycoluril 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_3^-) solubilizing groups which greatly enhance its solubility in water.[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 glycoluril tetramer **3** with aromatic sidewall **1b** by a double electrophilic aromatic substitution reaction as described previously.[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 butanesultone 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_3^- groups we first reacted commercially available diol **1d** with CBr_4 and PPh_3 to give **1e** in 91% yield according to the literature report.[47] Next, **1e** was reacted with Na_2SO_3 in DMF to give aromatic sidewall **1a** in high yield (88%). Reaction of glycouril 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_2-groups between the aromatic sidewall and the anionic SO_3^- solubilizing groups.

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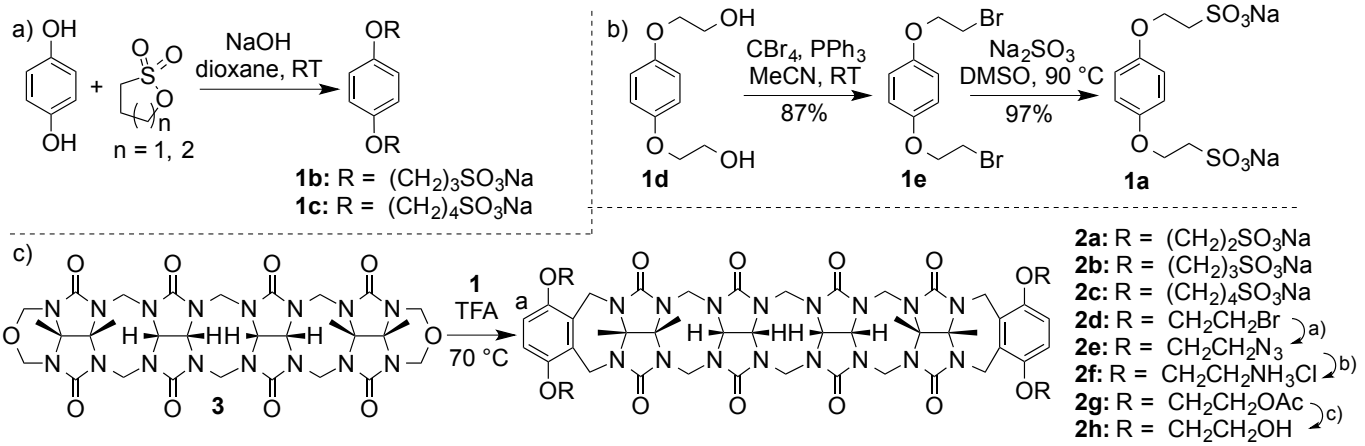

Scheme 1. Synthesis of acyclic CB[n] solubilizing excipients **2a** – **2h**. Conditions: a) NaN_3, DMSO, 90 °C, 95% yield, b) PPh_3, H_2O, DMF, 50 °C, 39% yield, c) LiOH, then HCl, 67% yield.

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 NaN_3 in DMSO. Reduction of the tetra-azide host **2e** with PPh_3 in DMF/H_2O gave the corresponding tetra-amine 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 Ac_2O (v/v = 1:1)[48] which delivered tetraacetoxy compound **2g** in 90% yield. Hydrolysis of **2g** with an aqueous solution of LiOH followed 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**,[44] **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 glycouril tetramer backbone. In the crystal structure of **2b** (Figure 2a), the substituted *o*-xylylene tips interact with each other by CH•••π interactions whereas the $O(CH_2)_3SO_3^-$ arms are extended away from the cavity; the cavity is filled by a solvating CF_3CO_2H 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*-xylylene 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 $OCH_2CH_2NH_3^+$ solubilizing arms and the carbonyl portal (N•••O distance = 2.790 Å; N-H•••O angle = 160˚). The ^{1}H 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 $OCH_2CH_2NH_3$ 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*-xylylene 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 OCH_2CH_2OH arms and the carbonyl portal

(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 π–π 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.[44,49] These expanded conformers of **2h** occur in dimeric pairs within the crystal; the $ArOCH_2CH_2OH$ wall and arm of one molecule fills the cavity of its partner and vice versa.[50] 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.

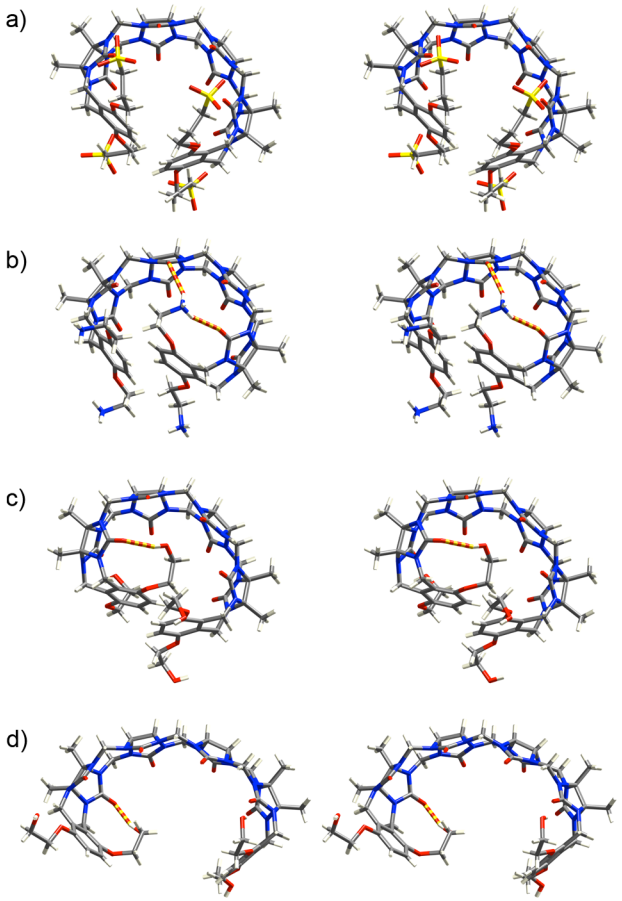

Figure 2. Cross-eyed stereoviews of the X-ray crystal structures of: a) **2b**, b) **2f**, and c&d) two different conformations of **2h** in the crystal. Color code: C, gray; H, white; N, blue; O, red; H-bonds, red-yellow striped.

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•drug complexes. Previously, we have performed ^{1}H NMR dilution experiments with negatively charged host **2b** and determined a self-association constant K_s = 47 M^{-1} by fitting the change in observed chemical shift as a function of host concentration.[44] The low value of K_s (47 M^{-1}) ensures that the majority of the host molecules are uncomplexed and ready to bind to drug molecules. Accordingly, we performed related ^{1}H NMR dilution experiments[51] with the newly prepared neutral (**2h**) and positively charged (**2f**) hosts. We did not observe any significant change in chemical shift of H_a 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_2O (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 ^{1}H NMR spectroscopy and direct and competition UV/Vis spectroscopic titrations.

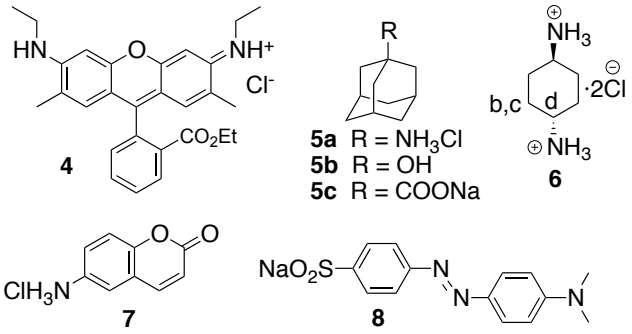

Figure 3. Chemical structures of guests used in this study.

^{1}H NMR Investigations of the Binding Interactions. In this section we use ^{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 ^{1}H NMR study of the difference in binding of guest **6** toward hosts **2a**, **2f**, and **2h**. Figure 4a – c shows the ^{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_b, H_c and H_d on guest **6** or protons H_a 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_b, H_c and H_d 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

previously for (acyclic) CB[n]-type receptors.[13,44,46,52,53] The larger upfield shift observed for protons H_b, H_c and H_d 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 dicationic guest significantly stronger than the neutral host **2h**. It was also observed that the resonances for protons H_a 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 H_a upfield (≈ 6.44 ppm). Binding to guest **6** breaks the π–π interactions and shifts the resonances for H_a downfield (≈ 7.4 ppm).

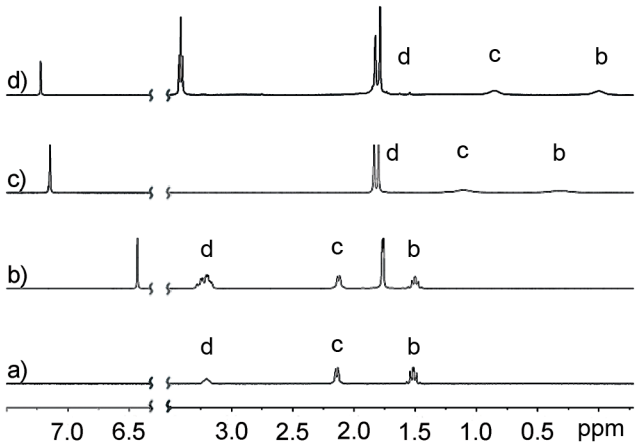

Figure 4. ^{1}H NMR recorded (400 MHz, RT, 20 mM sodium phosphate buffered D_2O, pH 7.4) for: a) **6**, b) an equimolar mixture of **2f** (positive host) and **6**, (c) and equimolar mixture of **2h** (neutral host) and **6**, and (d) and equimolar mixture of **2a** (negative host) and **6**.

After performing these initial ^{1}H 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 ^{1}H 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 ^{1}H NMR chemical shift of proton H_a 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 K_a value for **2a•5c** ($K_a = 3.33 \times 10^3\ M^{-1}$). In an analogous manner, we performed direct ^{1}H NMR titration experiments to obtain the K_a 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 ^{1}H NMR titration experiments described above were not applicable for the determination of the K_a values for the tighter host guest complexes and complexes with poor solubility characteristics. Accordingly, we decided to measure the K_a values for the remaining host-guest complexes by UV/Vis competition assays referenced to K_a values determined by direct UV/Vis titration. Dye **4** was used in displacement assays to determine the K_a values of negative host **2a** towards different guests. However, the application of **4** in the detection of the K_a 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** ($K_a = (1.83 \pm 0.08) \times 10^5\ M^{-1}$). Similar experiments were carried out to determine the binding constant constants for complex **2h•8** ($K_a = (1.32 \pm 0.01) \times 10^3\ M^{-1}$, Supporting Information). With those binding constants in hand we were able to perform the indicator displacement assays[54] to determine the K_a values for a larger variety of guests.

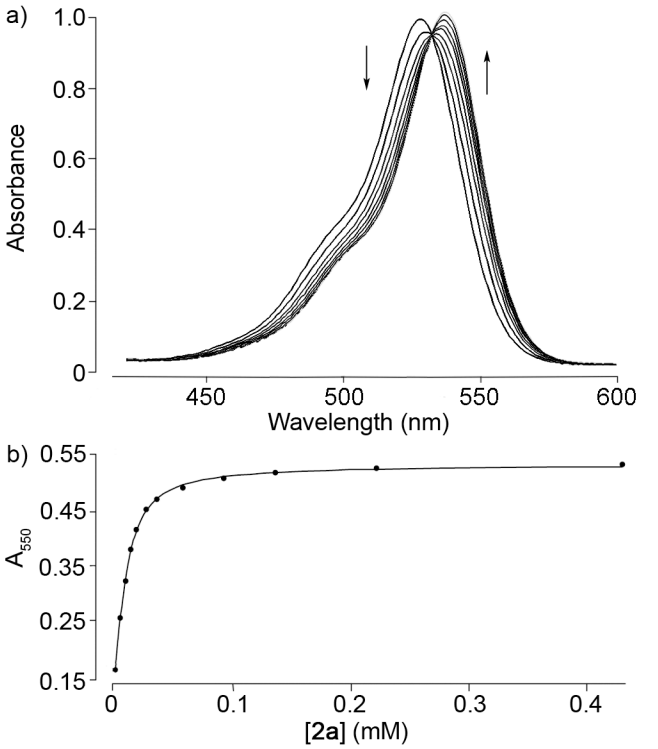

Figure 5. a) UV/vis spectra obtained during the titration of a fixed concentration of **4** (10.0 μM) with **2a** (0 – 0.45 mM) and b) plot of absorbance versus [**2a**] used to determine the K_a value of the **2a•4** complex by nonlinear least-square fitting.

UV/VIS COMPETITION ASSAYS. To measure the values of K_a for guests whose binding affinity exceeds that measurable by direct ^{1}H NMR titrations (approx. $10^4\ M^{-1}$) we turned to

UV/Vis competition assays[54] 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[46,52] then 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 – 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 $(1.68 \pm 0.09) \times 10^6$ 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.

	2a	2b	2c	2h	2f
4	1.83×10^5 [a]	4.23×10^5 [a]	1.29×10^5 [a]	ppt.	n.d.
5a	1.68×10^6 [b]	1.78×10^6 [b]	1.94×10^5 [b]	3.64×10^3 [d]	–
5b	4.47×10^4 [b]	1.67×10^5 [d]	5.54×10^4 [b]	2.36×10^3 [d]	–
5c	3.33×10^3 [d]	1.87×10^3 [d]	345[d]	108 [d]	645[d]
6	4.59×10^6 [b]	4.37×10^6 [b]	1.12×10^6 [b]	1.13×10^4 [c]	327[d]
8	n.d.	n.d.	n.d.	1.32×10^3 [a]	n.d.

[a]Measured by direct titration monitored by UV/Vis absorption spectroscopy. [b]Measured by competition with guest **4** monitored by UV/Vis spectroscopy. [c]Measured by competition with guest **8** monitored by UV/Vis spectroscopy. [d]Measured by direct titration monitored by ^{1}H NMR. n.d.: not determined. –, below detection limit of ^{1}H 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_2 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.[44] 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 o-xylylene 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 **5b** 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 **5b** is H-bonded in both water and the complex.[13] 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_3N^+•••^-O_2C) 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**, **2b**, 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.[31,55] 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 pK_a 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 pK_a value (Table 2) was obtained by non-linear fitting of the data to the Equation 1 (Supporting Information).[56] From Table 2, we can observe an increase in pK_a values in the presence of neutral host **2h** (pK_a = 4.1) and negative host **2a** (pK_a = 4.9) compared with dye **7** alone (pK_a = 3.6), while a small decrease was observed in the presence of positive host **2f** (pK_a = 3.4). These changes in pK_a are consistent with our expectations based on the net charge of the host. For example, protonation of guest **7** to give $\mathbf{7}H^+$ is more favorable in the presence of neutral host **2h** because **2h** establishes ion-dipole interactions in the $\mathbf{2h}\bullet\mathbf{7}H^+$ that are not formed in the **2h•7** complex. Protonation of guest **7** to give $\mathbf{7}H^+$ is even more favorable (larger pK_a shift to 4.9) in the presence of anionic host **2a** not only because of ion-dipole interactions in $\mathbf{2a}\bullet\mathbf{7}H^+$ complex but also because of the favorable ion-ion electrostatic interactions between the SO_3^- groups and $\mathbf{7}H^+$. Finally, the pK_a of the $\mathbf{7}H^+$ in the presence of cationic host **2f** is comparable to that of $\mathbf{7}H^+$ which probably reflects the weak binding between **2f** and $\mathbf{7}H^+$ due to binding of the $OCH_2CH_2NH_3^+$ arms of **2f** to its C=O portals and unfavorable ion-ion electrostatic interactions in the putative $\mathbf{2f}\bullet\mathbf{7}H^+$ complex.

$$A_{obs} = \frac{A_{7H+}}{(1 + 10^{pH\text{-}pKa})} + \frac{A_7}{(1 + 10^{pKa\text{-}pH})} \quad (1)$$

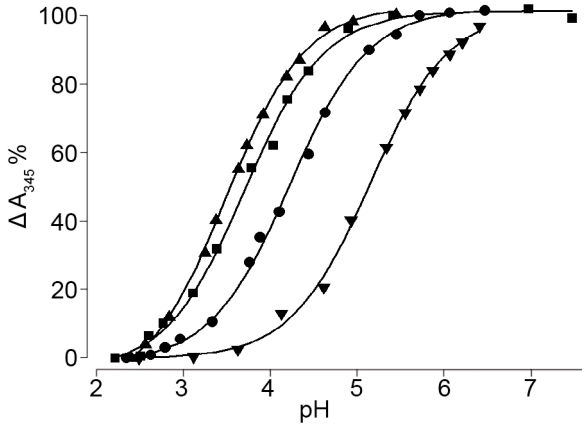

Figure 6. Plot of absorbance change (%) versus pH to determine the pK_a values of 6-aminocoumarin (**7**, 35.6 μM) by itself (■), and with **2f** (cationic host, 1.5 mm, ▲), **2h** (neutral host, 1.2 mM, ●), and **2a** (anionic host, 1.5 mM, ▼).

Table 2. pK_a values and binding constants (K_a) obtained for compound **7** with host **2a**, **2h** and **2f**.

	7	**2a•7**	**2h•7**	**2f•7**
pK_a	3.6	4.9	4.1	3.4
K_a (M^{-1})[a]	n.a.	2.74×10^5	9.59×10^3	678

n.a. = not applicable. – = no changes in ^{1}H 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])[51] for the 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 ^{1}H 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.[51] For linear (A_L) phase solubility diagrams, the initial slope of the PSD obeys equation 2 where S_0 is the intrinsic solubility of the drug, slope is the slope of the PSD, and K_a is the binding constant for the host•drug complex.[51] In this manner, we calculated the binding constant for host **2a** towards all three drugs (1.83×10^3 M^{-1} for tamoxifen, 1.73×10^4 M^{-1} for 17α - ethynylestradiol, and 6.07×10^3 M^{-1} for indomethacin). It is also possible to use the phase solubility diagram to compare the behavior for a given drug (with common S_0) 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.

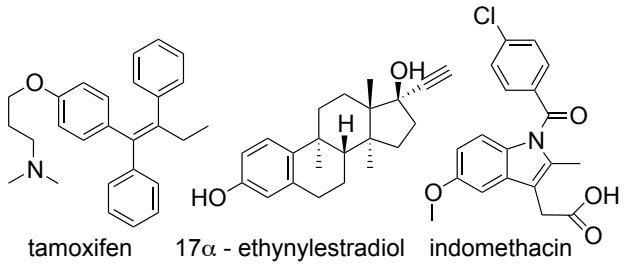

Figure 7. Chemical structures of water-insoluble drugs used in this study.

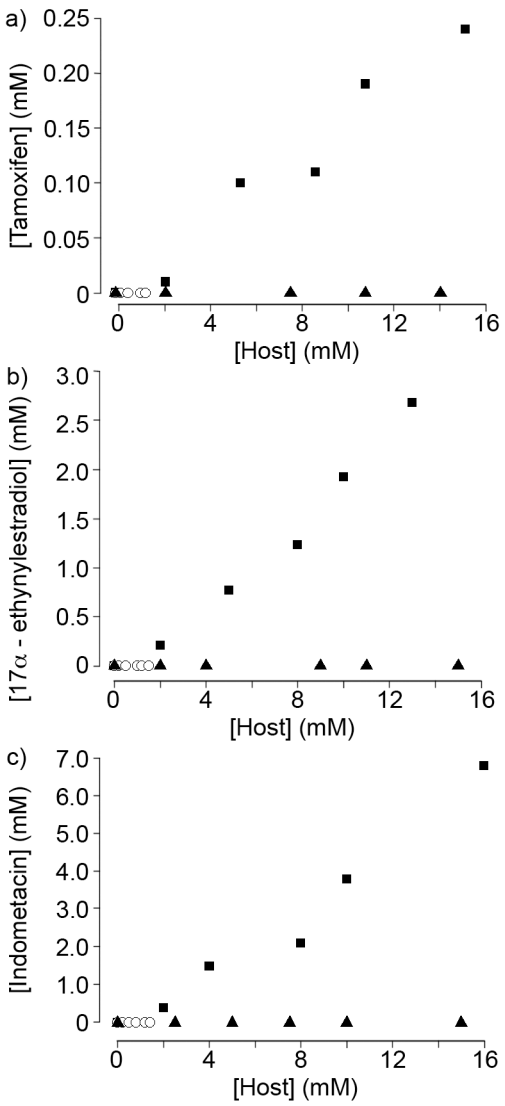

Figure 8. Phase solubility diagrams constructed using solutions of hosts **2a** (■), **2h** (○) and **2f** (▲) of known concentrations and an excess of solid drug: a) tamoxifen, b) 17α-ethynylestradiol, and c) indomethacin. Conditions: 20 mM sodium phosphate buffered D_2O (pH = 7.4, RT).

$$K_a = \text{slope} / [S_0(1\text{-slope})] \quad (2)$$

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**, **2b** and **3** were prepared according to literature procedures.[44,47,52] Melting points were measured on a Meltemp 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 ^1H 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, 822m, 799s, 759m, 526m. ^1H 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), 5.38 (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 ($[M - 3Na + H]^{2-}$), 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 ^1H 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 **5c** binding more tightly to positively changed host **2f** than to neutral host **2h** probably due to ion-ion electrostatic interactions. We measured the pK_a of $\mathbf{7}H^+$ alone and in the presence of **2a, 2h**,

and **2f** and noted that the **2a** induces the largest pK_a shift which we attribute to the presence of ion-ion electrostatic interactions in the **2a**•**7**H^+ complex. Both the K_a and pK_a 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–ethynylestradiol, 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

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†Electronic Supplementary Information (ESI) available: Synthetic procedures and characterization data for **1a, 1c**, and **2a** – **2h**, direct and competitive binding titrations for determination of K_a values, ^1H and ^{13}C NMR spectra for all new compounds (.pdf), ^1H NMR spectra of selected drugs solubilized by host **2a**; crystallographic information files (.cif) for **2f** (CCDC978646) and **2h** (CCDC 978645). See DOI: 10.1039/b000000x/

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