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Mechanism of the Conversion of Inverted CB[6] to CB[6]

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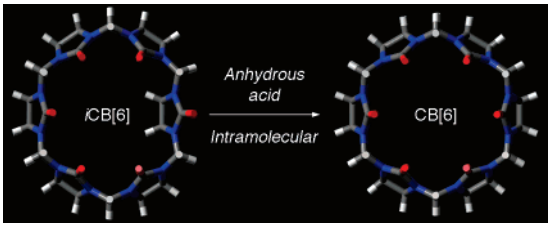

Inverted cucurbit[*n*]urils (*i*CB[*n*]) form as intermediates during the synthesis of cucurbit[*n*]urils from glycoluril and formaldehyde in HCl (85 °C). Product resubmission experiments establish that the diastereomeric *i*CB[6] and *i*CB[7] are kinetic products that are less stable thermodynamically than CB[6] or CB[7] (>2.8 kcal mol^{-1}). When *i*CB[6] or *i*CB[7] is heated under aqueous acidic conditions, a preference for ring contraction is noted in the formation of CB[5] and CB[6], respectively. Interestingly, under anhydrous acidic conditions ring size is preserved with *i*CB[6] delivering CB[6] cleanly. To establish the intramolecular nature of the *i*CB[6] to CB[6] conversion under anhydrous, but not aqueous, acidic conditions we performed crossover experiments involving mixtures of *i*CB[6] and its ^{13}C═O labeled isotopomer $^{13}C_{12}$-*i*CB[6]. An unusual diastereomeric CB[6] with a Möbius geometry (**13**) is proposed as a mechanistic intermediate in the conversion of *i*CB[6] to CB[6] under anhydrous acidic conditions. The improved mechanistic understanding provided by this study suggests improved routes to CB[*n*]-type compounds.

Introduction

The synthetic and supramolecular chemistry of the cucurbit[*n*]uril family[1] (CB[*n*]) of macrocycles has undergone rapid expansion in recent years with the development of a homologous series of macrocycles (CB[*n*], $n = 5, 6, 7, 8, 10$),[2,3] diastereomeric inverted CB[*n*] (*i*CB[*n*]),[4] and bis-nor-seco-CB[10], which contains two symmetry equivalent cavities.[5] The cavity volumes of these CB[*n*]-type compounds (82−870 Å^3) span and exceed those available with α-, *β*-, and *γ*-cyclodextrins and therefore a wide range of applications has been demonstrated including molecular shuttles and machines,[6] supramolecular dye lasers,[7] supramolecular dendrimers,[8] novel drug carriers,[9] peptide

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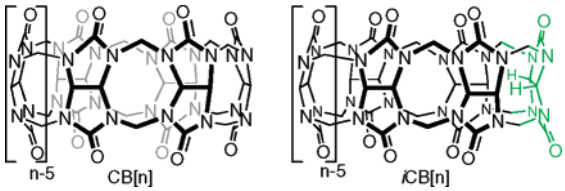
recognition,[10] DNA delivery and cleavage,[11] mediation of organic reactions,[12] chemical sensors,[13] and self-sorting systems.[14] Despite the fact that the recognition properties of CB[*n*] molecular containers surpass those of the cyclodextrins, their use in industrial applications has been limited by several factors. These include the following: (1) cyclodextrins—but not CB[*n*]—can be accessed in a size-selective manner by the inclusion of appropriate complexation agents in the cyclodextrin forming reaction mixture, (2) α-, *β*-, and *γ*-cyclodextrin have very good aqueous solubility whereas CB[*n*] ($n = 6, 8, 10$) compounds have low solubility in unbuffered water, and (3) strategies for the chemical derivatization of the cyclodextrins are better developed. We, and others, have been pursuing a synthetic and mechanistic approach toward the tailor-made synthesis of CB[*n*], CB[*n*] derivatives, and CB[*n*] analogues as part of our efforts to remove these impediments toward their industrial application.[15−18] For example, Kim's group has developed the direct functionalization of preformed CB[*n*] that yields $(HO)_{2n}$CB[*n*] compounds that undergo further covalent derivatization reactions.[19] These CB[*n*] derivatives have enhanced water and organic solubility, enable attachment to solid phases for separations and immobilization applications, and serve as multivalent building blocks in biology and materials science applications.[20] In this manner, significant progress has been made toward alleviating the limitations of the second and third factors. In this paper, we continue to tackle the first limitation by enhancing our knowledge of the mechanism of formation of the CB[*n*] family of macrocycles. Specifically, we show that *i*CB[6] and *i*CB[7] are kinetically controlled intermediates in the formation of CB[*n*] and investigate the mechanism of the conversion of *i*CB[6] to CB[6] under acidic conditions.

CHART 1. Chemical Structures of CB[*n*] and *i*CB[*n*]



Results and Discussion

This section begins with a brief presentation of the state-of-the-art regarding the mechanism of CB[*n*] formation, which serves as a basis for the subsequent discussion of the mechanism of conversion of *i*CB[6] to CB[6].

Previous Mechanistic Studies. Scheme 1 shows the fundamental steps of the mechanism of CB[*n*] formation that were presented by the groups of Isaacs and Day previously and modified here to include the presence of *i*CB[*n*] as kinetically controlled intermediates in the process.[3,21] In brief, glycoluril **1** undergoes dimerization in the presence of a source of formaldehyde under acidic conditions to yield a mixture of two diastereomers (**2**C and **2**S). These dimers are hypothesized to undergo further oligomerization to yield Behrend's polymer (**3**), which has a large number of possible diastereomers dependent on the relative orientation of the H-atoms on the convex face of the glycoluril oligomer. Behrend's polymer **3** then interconverts most or all of its S-shaped to C-shaped subunits—perhaps under the influence of templating groups[22]—to create an oligomer (e.g., **4** or **5**) that is poised to undergo macrocyclization to enter the CB[*n*] family manifold.

The majority of the previously reported mechanistic studies of CB[*n*] formation have been reported by the groups of Day and Isaacs.[3,18,21,22] For example, the Day group focused on the latter stages of the mechanism of CB[*n*] formation compounds by examining the influence of a variety of potential templates (e.g., ammonium ions and alkali metal cations) on the distribution of CB[*n*] compounds obtained. Day's group also reported the influence of acid type, acid concentration, and concentration of glycoluril on the outcome of the CB[*n*]-forming reaction. Although these variables do influence the outcome of the CB[*n*]-forming reaction, the impact of these variables on the

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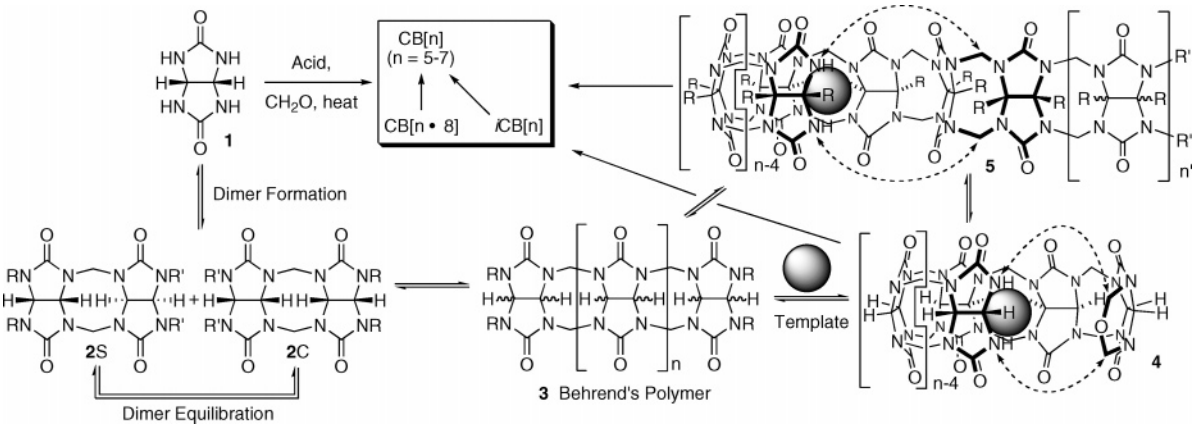
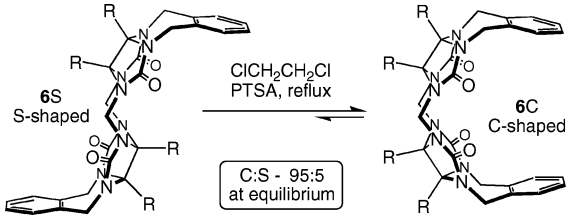
SCHEME 1. Proposed Mechanism of CB[*n*] Formation



SCHEME 2. Equilibrium between S- and C-Shaped Methylene-Bridged Glycoluril Dimers



individual steps of the mechanism of CB[*n*] formation has not yet been clarified. In an elegant product resubmission experiment Day also showed that CB[8] is converted into the smaller CB[*n*] homologues ($n = 5, 6, 7$) under the reaction conditions.

In contrast, the Isaacs group has focused on the formation and interconversion of C-shaped and S-shaped methylene-bridged glycoluril dimers (e.g., **2**S and **2**C) in the earliest stages of the mechanism of CB[*n*] formation. For this purpose, we previously prepared S- and C-shaped methylene-bridged glycoluril dimers bearing *o*-xylylene end-groups and CO_2Et solubilizing groups (**6**S and **6**C) by dimerization under *anhydrous acidic conditions* ($ClCH_2CH_2Cl$, *p*-toluenesulfonic acid (PTSA), reflux).[21,23] By product resubmission experiments, we established that the C-shaped compounds are thermodynamically more stable than their S-shaped diastereomers by factors of ca. 95:5 (Scheme 2). At the time, this high preference for the C-shaped form allowed us to rationalize the high yield (82%)[24] obtained in the synthesis of CB[6], which contains six independent C-shaped subunits (e.g., $0.95^6 = 74\%$). It also alerted us to the possibility of diastereomeric CB[*n*] where one or more glycoluril subunits adopt the S-shaped configuration with respect to the neighboring glycolurils (e.g., *i*CB[*n*]).

In the course of these studies we made several observations that led us to suspect that the S- to C-shaped equilibrium might be an intramolecular process. To test this hypothesis, we devised a crossover experiment based on doubly methoxy-labeled (±)-**7**S. When (±)-**7**S was heated under the anhydrous reaction conditions we observed the clean transposition of the OMe labels under the formation of (±)-**7**C. This observation and control experiments described previously establish the *intramolecular* nature of the isomerization process and lead us to formulate the isomerization mechanism shown in Scheme 3. In brief, the (±)-**7**S to (±)-**7**C interconversion proceeds by protonation and fragmentation of (±)-**7**S to yield iminium ion (±)-**8**. Iminium ion (±)-**8** undergoes intramolecular capture (under *anhydrous acidic conditions*) to yield spirocyclic intermediate (±)-**9** that can fragment in the opposite sense to yield iminium ion (±)-**10**, which then closes to yield (±)-**7**C.

The mechanistic insights gained from the studies of Day and Isaacs lead both groups to independently suggest that building block approaches would deliver tailor-made CB[*n*]-type compounds. These predictions were subsequently demonstrated experimentally by the directed synthesis of Me_6CB[6], Me_4-CB[6], and CB[*n*] analogues.[15,16,18] Despite the successful synthesis of CB[*n*] analogues based on our mechanistic reasoning derived from studies of *solubilized methylene bridged glycoluril dimers in anhydrous organic solvents* we wanted to assess whether those mechanistic insights carried over to true CB[*n*] systems based on unsubstituted glycoluril (**1**) where synthesis and study are typically performed under aqueous acidic conditions.

Isolation of *i*CB[*n*] ($n = 6$ and 7) Enables Further Mechanistic Studies. In 2005, the groups of Isaacs and Kim reported the isolation of two new members of the CB[*n*] family—*i*CB[6] and *i*CB[7]—that contain a single inverted glycoluril unit (Scheme 4).[4] Similar to **6**S and **7**S described above, the H-atoms on the convex face of the inverted glycoluril ring of *i*CB[6] and *i*CB[7] point in the opposite direction to those of the neighboring glycoluril rings. Hence, the *i*CB[6] and *i*CB[7] can be recognized as being the macrocyclic relatives of the S-shaped methylene bridged glycoluril dimers studied earlier. Since *i*CB[6] and *i*CB[7] are macrocyclic they can be viewed as containing two S-shaped methylene-bridged glycoluril dimer subunits. This realization immediately raised several questions in our mind and presented us with the opportunity to test the mechanistic conclusions of our model system studies within the real system. Are *i*CB[6] and *i*CB[7] kinetically controlled intermediates in the synthesis of CB[*n*]? What are the stabilities of *i*CB[6] and *i*CB[7] relative to CB[6] and CB[7]? Does the mechanism presented above for the transformation of (±)-**7**S to (±)-**7**C also apply to the conversion of *i*CB[6] to CB[6]?

Inverted CB[*n*] ($n = 6, 7$) Are Kinetic Intermediates in the Formation of CB[*n*]. To test whether *i*CB[*n*] ($n = 6, 7$), which contain S-shaped fragments similar to **6**S above, are kinetic intermediates in CB[*n*] synthesis, we performed product

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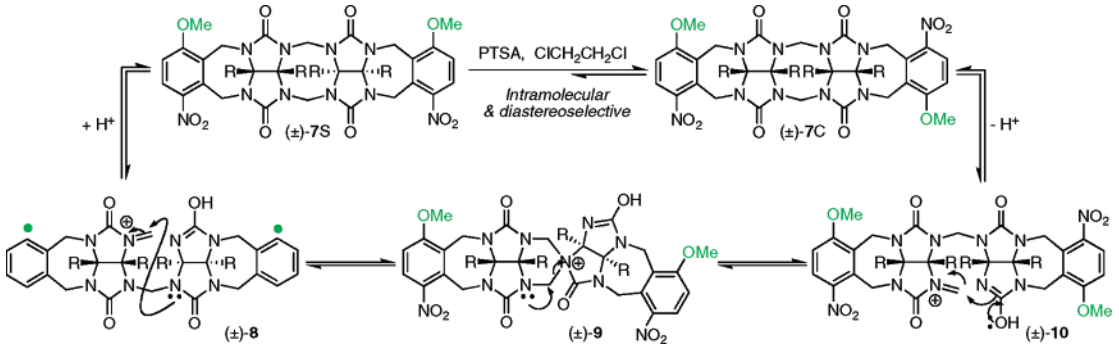
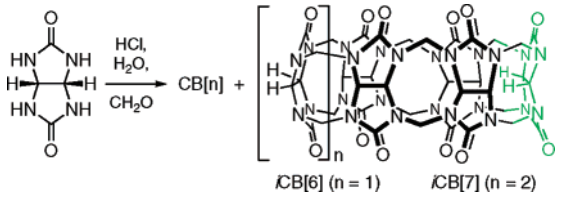
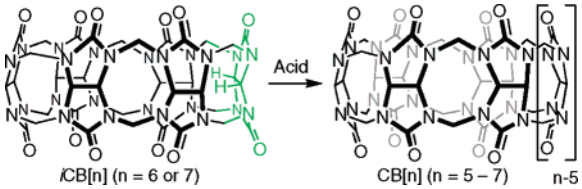
SCHEME 3. Proposed Mechanism for the Diastereoselective Conversion of S-Shaped (±)-7S to C-Shaped (±)-7C (R = CO_2Et)



SCHEME 4. Synthesis of *i*CB[*n*]



SCHEME 5. Conversion of *i*CB[6] to CB[*n*] under Acidic Conditions



resubmission experiments (Scheme 5). When *i*CB[6] and *i*CB[7] were separately heated in concentrated HCl complete consumption of *i*CB[*n*] starting material was observed with the formation of CB[*n*]. Inverted CB[6] yields a 24:13:1 mixture of CB[5], CB[6], and CB[7] whereas *i*CB[7] yields a 4:1 mixture of CB[6] and CB[7]. This result establishes that *i*CB[6] and *i*CB[7] are kinetic intermediates in the formation of CB[*n*] and that *i*CB[6] and *i*CB[7] are significantly less stable than CB[*n*] ($n = 5-7$). Although our model system studies suggest that *i*CB[6] and *i*CB[7] should be ca. 4 kcal mol^{-1} (2 × 2 kcal mol^{-1}/S-shaped fragment) less stable than the corresponding CB[*n*], the sensitivity of our ^1H NMR only allows us to provide a lower estimate on the difference in stability between CB[*n*] and *i*CB[*n*] (≥98:2, $\Delta G \geq 2.8$ kcal mol^{-1}). An interesting aspect of this set of experiments under aqueous acidic conditions was the propensity for ring contraction concomitant with the transformation of *i*CB[*n*] to CB[*n*], that is, *i*CB[6] yields mainly CB[5] whereas *i*CB[7] yields mainly CB[6]. This observation requires the extrusion of one glycoluril unit—most likely the inverted glycoluril unit—and dictates the cleavage of four adjacent N−CH_2 bonds.[25]

The Presence of H_2O Influences the Size Distribution Obtained in the Conversion of *i*CB[6] to CB[*n*]. The observation described above—the preference for ring contraction of *i*CB[6] and *i*CB[7] to CB[5] and CB[6], respectively—suggested that the *intramolecular nature* of the S-shaped to C-shaped conversion of our model studies[21] under anhydrous acidic conditions was not valid under the aqueous acidic CB[*n*]-forming reaction conditions. To determine whether this discrepancy was due to the change from anhydrous to aqueous acidic conditions or the presence of the CO_2Et solubilizing or *o*-xylylene capping groups of our model compounds (**6**S and **6**C) we performed the transformation of *i*CB[6] to CB[*n*] in anhydrous $MeSO_3H$ and also in mixtures of concd HCl and $MeSO_3H$ (Figure 1).[26] Interestingly, when anhydrous $MeSO_3H$ was used as the solvent CB[6] was the only product. No CB[5] or CB[7]—that would be the products of ring contraction or ring expansion—was observed under anhydrous acidic conditions. When mixtures of concd HCl and $MeSO_3H$ were used as solvent a smooth transition between these two extreme situations—ring contraction versus retention of ring size—was observed (Figure 1). This result strongly suggested—but did not prove—that the conversion of *i*CB[6] to CB[6] under anhydrous acidic conditions was an intramolecular process analogous to that observed for model system **7**S and **7**C (Scheme 3).

Mechanistic Extremes in the Transformation of *i*CB[6] to CB[6]. On the basis of the previously published mechanistic studies in the CB[*n*] area[3,21,22] and the results described above we can postulate the presence of two mechanistic extremes (aqueous versus anhydrous) in the conversion of *i*CB[6] into CB[*n*] (Scheme 6; blue: aqueous pathway; light blue: some H_2O present; green: anhydrous pathway). In both extremes, the initial steps in the proposed mechanism are similar, namely protonation of *i*CB[6] followed by bond cleavage to yield iminium ion **11**. Under anhydrous acidic conditions, iminium ion **11** is forced to react with an internal (e.g., intramolecular) nucleophile—namely the ureidyl N-atom—to yield spirocyclic N-acyl ammonium ion **12**. Spiro-intermediate **12** may then undergo cleavage in the opposite direction followed by ring

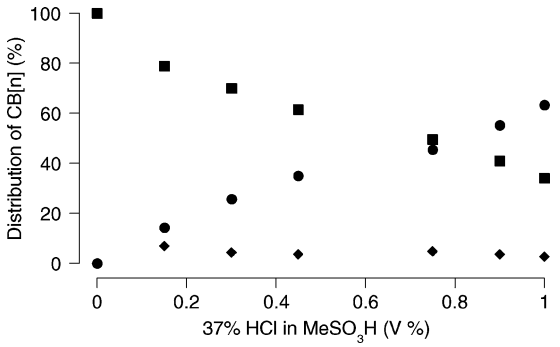
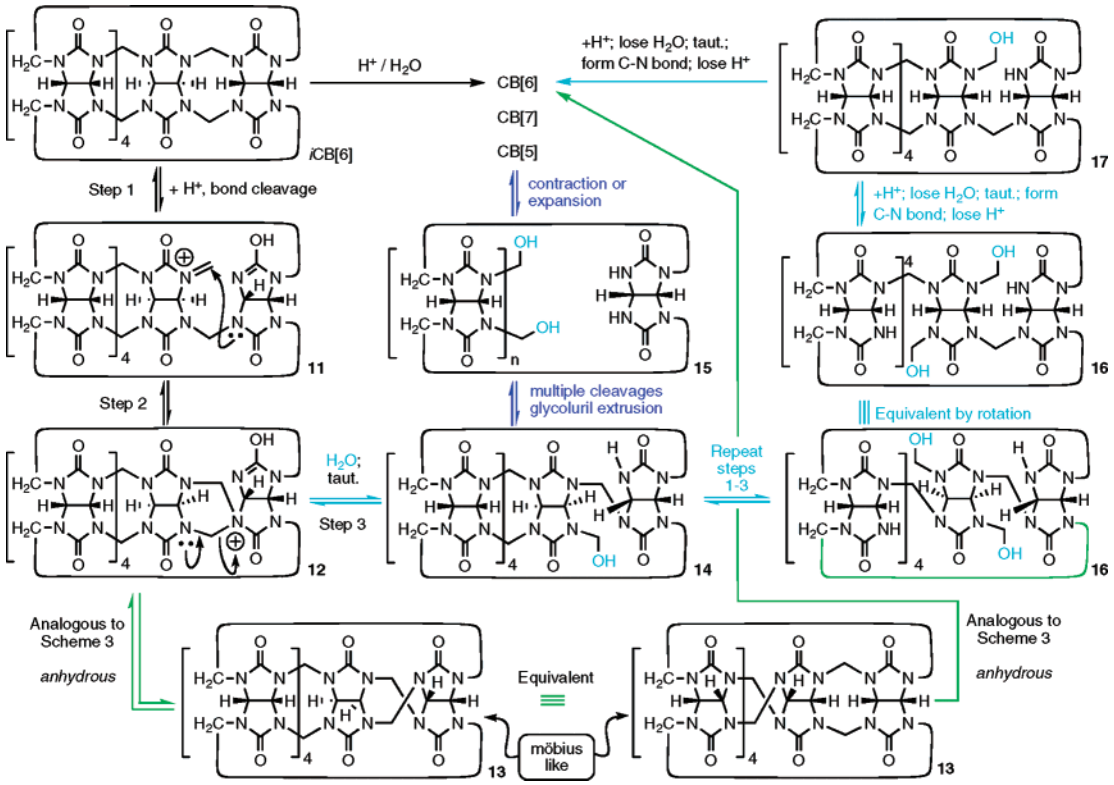


FIGURE 1. Influence of the composition of the concd HCl/$MeSO_3H$ solvent system on the distribution of CB[*n*] obtained from *i*CB[6]. Key: CB[6], ■; CB[7], ◆; CB[5], ●.

(25) To conclusively establish that the inverted glycoluril unit is extruded would require the availability of *i*CB[6] specifically isotopically labeled on the inverted glycoluril unit. Unfortunately, the statistical synthesis process makes such a compound inaccessible.

SCHEME 6. Mechanistic Possibilities for the Transformation of *i*CB[6] to CB[6] under Acidic Conditions[a]



[a] Color code: green equilibria, anhydrous conditions; light blue equilibria, some water present; blue equilibria, high concentrations of water.

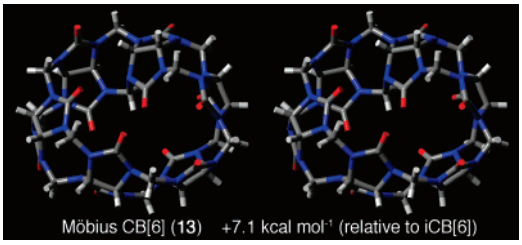


FIGURE 2. Cross-eyed stereoview of the PM3 minimized geometry of Möbius CB[6] (**13**).

closure to yield **13**. Intermediate **13** is quite unusual in that it has the connectivity of a Möbius strip. Although **13** may appear to be a prohibitively high energy intermediate, PM3 calculations suggest that **13** (Figure 2) is only 7.1 kcal mol^{-1} higher in energy than *i*CB[6]. Repetition of the protonation, cleavage, intramolecular N-atom capture, cleavage, and ring closure processes (analogous to Scheme 3) is then capable of producing CB[6] by a completely intramolecular process with no possibility for either ring contraction or ring expansion. Under aqueous acidic conditions, iminium ion **11** can undergo intramolecular N-atom capture to yield **12** followed by addition of H_2O to yield **14**. When the concentration of H_2O is high (e.g., concd HCl) we believe the dominant pathway leads to intermediate **15** by a process of multiple N−CH_2 bond cleavage events mediated by water. Intermediate **15** (n = 4 or 5) may then undergo ring closure to yield CB[5] or CB[6]. The small but reproducible amount of CB[7] observed suggests that intermediate **15** (n = 5) is also able to insert a glycoluril unit to undergo ring expansion.[27] When the concentration of H_2O is low, intermediate **14** can be transformed into **16** by a repetition of steps 1−3. Interestingly, intermediate **16** can be redrawn in a manner that makes it clear that it is now poised to undergo a series of intramolecular ring closures (via **17**) to yield CB[6].[28]

The data presented in Figure 1 suggest that the [H_2O] controls which of the three pathways dominates under a given set of conditions. It is tempting to suggest that the pathway via Möbius-intermediate **13** must operate to the exclusion of the water-mediated pathways in $MeSO_3H$. Unfortunately, all CB[n] compounds are quite hygroscopic and removal of residual H_2O from samples of *i*CB[6]—and similarly from $MeSO_3H$—is impractical. Hence, it is possible that small amounts of H_2O are present even under anhydrous conditions. To address whether small quantities of adventious water—either from solvent or *i*CB[6]—might influence the mechanistic pathway followed during the reaction, we followed the conversion of *i*CB[6] to CB[6] by ^{1}H NMR as a function of [*i*CB[6]] both without and with (2% v:v) added water (Figure 3). As can be readily seen, changing the concentration of *i*CB[6] by 50-fold has only a small effect on the relative rate of conversion to CB[6], which strongly suggests that the transformation of *i*CB[6] to CB[6] is a first-order process. Similarly, the presence of small amounts of water (2% v:v) does not significantly

(26) These experiments were performed with *i*CB[6] rather than *i*CB[7] because of its much greater abundance in the crude CB[n] forming reaction mixture and also its far greater ease of purification.

(27) We have attempted to insert functionalized glycolurils or glycoluril cyclic ethers via a ring expansion process but were not able to isolate a single pure compound from these reactions. We believe that differential reactivity between **1** and functionalized glycolurils as well as stoichiometric imbalance between glycoluril and formaldehyde is responsible.

(28) Intermediate **16** is C_2 symmetric and chiral. A related intermediate with C_s symmetry is also possible (Supporting Information) that leads to CB[6] in an analogous manner.

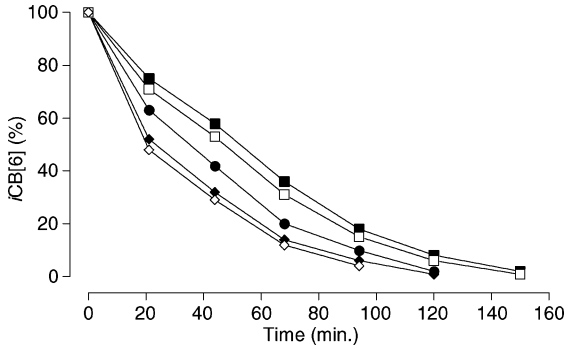


FIGURE 3. Influence of the concentration of *i*CB[6] and H_2O on the rate of transformation of *i*CB[6] to CB[6] in $MeSO_3H$ at 85 °C. Key: [*i*CB[6]] = 20 mM, ●; [*i*CB[6]] = 2 mM, ■; [*i*CB[6]] = 0.4 mM, ◆; [*i*CB[6]] = 2 mM, 2% H_2O, □; [*i*CB[6]] = 0.4 mM, 2% H_2O, ◇.

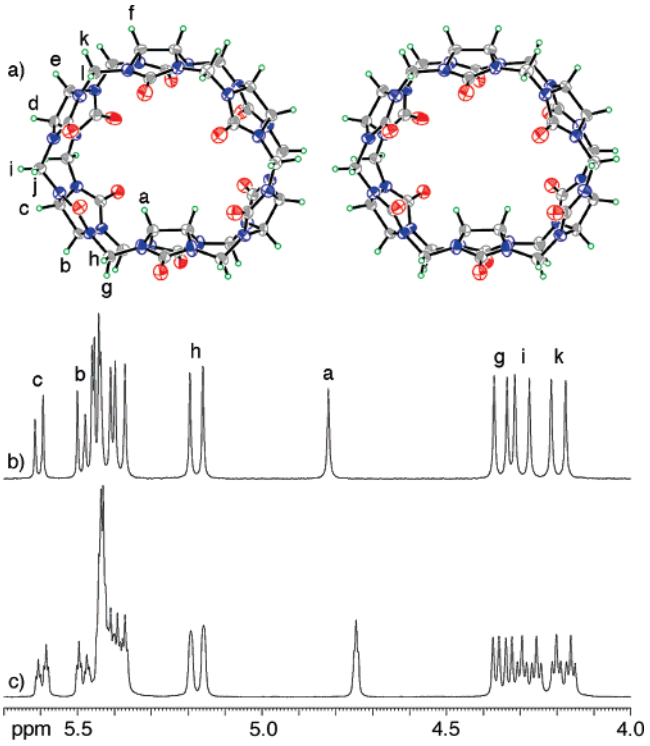


FIGURE 4. (a) Cross-eyed stereoview of the X-ray crystal structure of *i*CB[6] and ^1H NMR spectra (400 MHz, 20% DCl/D_2O, rt) for (b) *i*CB[6] and (c) $^{13}C_{12}$-*i*CB[6].

change the relative rate of the *i*CB[6] to CB[6] conversion, which suggests that the interconversion of *i*CB[6] to CB[6] proceeds via Möbius intermediate **13** under anhydrous conditions.

$^{13}C_{12}$-*i*CB[6] and *i*CB[6] Do Not Undergo Crossover During the Transformation to $^{13}C_{12}$-CB[6] and CB[6] in $MeSO_3H$. Although the experiments described above clearly suggest the mechanism of the transformation of *i*CB[6] to CB[6] is an intramolecular process under anhydrous acidic conditions and involves intermolecular (e.g., ring contraction or expansion) under aqueous acidic conditions we did not regard that as sufficient proof. Accordingly, we set out to prepare a labeled version of *i*CB[6] with the goal of transforming a mixture of labeled *i*CB[6] and *i*CB[6] into labeled CB[6] and CB[6] to observe whether any scrambling of the label occurs. For this purpose we chose to react ^{13}C-labeled urea with glyoxal

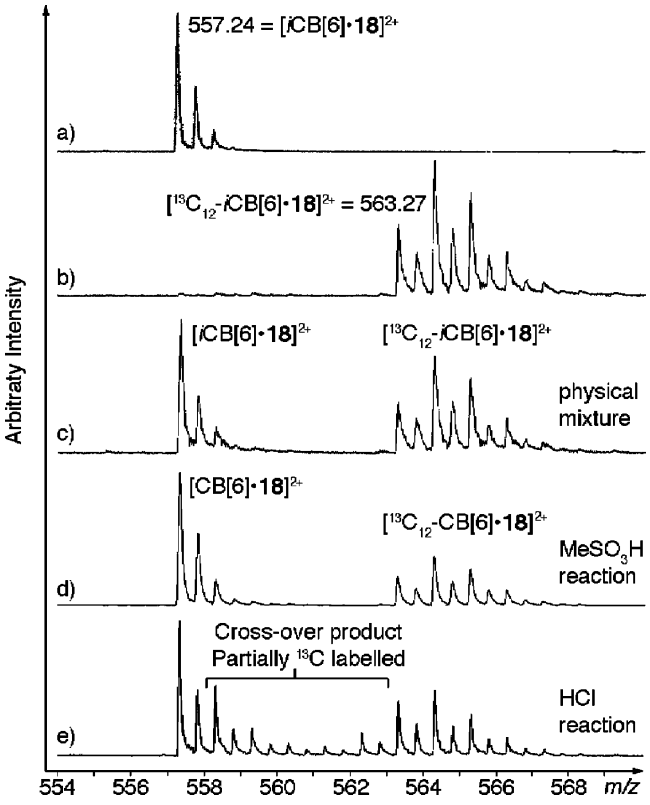


FIGURE 5. ES-MS spectra collected for(a) *i*CB[6]·**18**, (b) $^{13}C_{12}$-*i*CB-[6]·**18**, (c) an equimolar mixture of *i*CB[6]·**18** and $^{13}C_{12}$-*i*CB[6]·**18**, (d) a mixture of CB[6] and $^{13}C_{12}$-CB[6] obtained from the reaction of *i*CB[6] and $^{13}C_{12}$-*i*CB[6] in $MeSO_3H$ as their complexes with **18**, and (e) a mixture of CB[6], $^{13}C_{12}$-CB[6], and partially labeled $^{13}C_{<12}$-CB-[6] obtained from the reaction of *i*CB[6] and $^{13}C_{12}$-*i*CB[6] in concd HCl as their complexes with **18**.

to obtain $^{13}C_2$-glycoluril ($^{13}C_2$-**1**). Compound $^{13}C_2$-**1** was then reacted with formaldehyde in HCl to yield a mixture of fully ^{13}C-labeled CB[*n*] and *i*CB[*n*] from which we isolated $^{13}C_{12}$-CB[6] and $^{13}C_{12}$-*i*CB[6]. Figure 4 shows the ^1H NMR spectra recorded for *i*CB[6] and $^{13}C_{12}$-*i*CB[6]. As expected the resonances for the methine protons on the equator of $^{13}C_{12}$-*i*CB[6] are split (H_a, triplet; H_b and H_c, doublet of triplets) due to two equivalent $^3J_{C-H}$ couplings. The magnitude of these coupling constants ($^3J_{C-H}$ = 2.1−2.4 Hz) is consistent with the H−C−N−^{13}C dihedral angles (112−127°) observed in the X-ray structure of *i*CB[6]. Similarly, the doublet of triplets observed for H_i and H_k ($^3J_{C-H}$ = 4.7−5.0 Hz) is consistent with the range of H−C−N−^{13}C dihedral angles (125−142°) to the ^{13}C═O group on the two flanking glycoluril rings. More interesting is the doublet of doublets observed for the methylene proton H_g connected to the inverted glycoluril ring. In this case, there are two H−C−N−^{13}C dihedral angle ranges (145−159° and 84−96°) resulting in one modest ($^3J_{C-H}$ = 6.5 Hz) and one very small value of $^3J_{C-H}$ which in addition to the $^2J_{HCH}$ = 14.0 Hz for the diastereotopic CH_2-group results in the observed doublet of doublets.

Once we had prepared and characterized $^{13}C_{12}$-*i*CB[6] we set out to perform crossover experiments by transforming a mixture of *i*CB[6] and $^{13}C_{12}$-*i*CB[6] into labeled CB[*n*] under either anhydrous or aqueous acidic conditions. Spectra a and b of Figure 5 show the electrospray mass spectra obtained for *i*CB[6]·**18** (hexanediammonium ion, **18**) and $^{13}C_{12}$-*i*CB[6]·**18** which show parent ions at *m*/*z* 557.24 and 563.27, respectively,

for their doubly charged cations. We measure the electrospray mass spectra in the presence of **18** to avoid the possibility of interaction with adventitious alkali metal cations (Li^+, Na^+, or K^+) which would distort the results of the crossover experiment.[29] As a control experiment we measured the electrospray mass spectrum of a physical mixture (equimolar) of *i*CB[6]·**18** and $^{13}C_{12}$-*i*CB[6]·**18** (Figure 5c). We next heated an equimolar mixture of *i*CB[6] and $^{13}C_{12}$-*i*CB[6] in either anhydrous $MeSO_3H$ or concd HCl and obtained the electrospray mass spectra shown in Figure 5, spectra d and e. In anhydrous $MeSO_3H$, no crossover products (e.g., $^{13}C_{<12}$-CB[6]·**18**) were observed, which conclusively established the intramolecular nature of the transformation in this solvent. In contrast when the solvent was concd HCl, a substantial amount of crossover product ($^{13}C_{<12}$-CB[6]) was observed. This observation establishes that *i*CB[6] ejects glycoluril units into solution and reincorporates those glycoluril units during the transformation into CB[6]. Under aqueous acidic conditions, the pathway that proceeds via intermediate **14** is operative whereas under anhydrous acidic conditions the pathway that proceeds via Möbius intermediate **13** is dominant.

Conclusions

In summary, we have shown that diastereomeric *i*CB[6] and *i*CB[7] form as kinetic intermediates during CB[*n*] formation. Product resubmission experiments establish that *i*CB[*n*] are less stable than CB[*n*] by more than 2.8 kcal mol^{-1}. Interestingly, the transformation of *i*CB[6] and *i*CB[7] into CB[*n*] under aqueous acidic conditions occurs with substantial amounts of ring contraction. In contrast, under anhydrous acidic conditions *i*CB[6] is cleanly transformed into CB[6]. No crossover products were observed when mixtures of *i*CB[6] and $^{13}C_{12}$-*i*CB[6] were used, which establishes that this conversion is an intramolecular process. An unusual diastereomeric CB[6] with a Möbius geometry (**13**) is proposed as a critical intermediate in the mechanism of intramolecular conversion of *i*CB[6] to CB[6] under anhydrous acidic conditions. These studies shed light on the fate of one of the final kinetically controlled intermediates in the formation of CB[*n*].

Although we previously reached conclusions about the mechanism of the earliest steps in the formation of CB[*n*] (e.g., thermodynamics and mechanism of the **2S** to **2C** conversion) based on *o*-xylylene capped methylene-bridged glycoluril dimers bearing solubilizing CO_2Et groups, the applicability of those conclusions to systems based on unsubstituted glycoluril **1** was questionable. The elucidation of the mechanism of one of the final steps in CB[*n*] formation, namely, the *i*CB[6] to CB[*n*] conversion described here—based on and in complete accord with the predictions from the model studies—further reinforces the value and relevance of model studies in the CB[*n*] area. That enhanced confidence allows us to make a series of predictions: (1) Despite the thermodynamic instability of *i*CB[*n*] relative to CB[*n*], the *i*CB[*n*] have good kinetic stability. This kinetic stability suggests that inverted CB[*n*] compounds containing two or more inverted glycoluril rings will be accessible. (2) Although *i*CB[6] and *i*CB[7] are less stable than the diastereomeric CB[6] and CB[7] we believe that larger *i*CB[*n*] (e.g., $n > 8$) may exhibit enhanced thermodynamic stability relative to CB[*n*] ($n > 8$) due to reduced ring strain. (3) The larger *i*CB[*n*] and *i*CB[*n*] containing multiple (functionalized) inverted glycoluril units may be best accessed by using a building block approach[15,16,18] that employs preformed oligomers[16,18] containing S-shaped subunits. The use of low temperatures and acidic solvents containing no or only small amounts of H_2O—which preserve ring size during the reaction—is predicted to be particularly suitable. The enhanced understanding of the mechanism of CB[*n*] formation described herein suggests routes toward tailor-made CB[*n*] compounds, which promises to broaden the range of applications to which the CB[*n*] family may be applied.

Experimental Section

Preparation of $^{13}C_2$-glycoluril ($^{13}C_2$-1). To a solution of $H_2N^{13}CONH_2$ (5.0 g, 81.9 mmol) in water (10 mL) was added a 40% aq solution of glyoxal (4.6 g, 31.7 mmol) and concd HCl (0.6 mL). The resulting solution was heated at ca. 85−90 °C until a heavy precipitate had formed. The reaction mixture was allowed to cool to room temperature and filtered. The filter cake was washed with water (10 mL) and then acetone. The resulting white solid was dried under high vacuum to yield $^{13}C_2$-**1** as a white solid (3.50 g, 78%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.14 (d, $J = 2.4$, 4H), 5.20 (t, $J = 2.9$, 2H). ^{13}C (100 MHz, DMSO-d_6) δ 162.1, 65.4. MS (FAB, glycerol) m/z 145 ($[M + H]^+$).

Preparation of $^{13}C_{12}$-CB[6] and $^{13}C_{12}$-*i*CB[6]. $^{13}C_2$-**1** (3.44 g, 23.9 mmol) and powdered paraformaldehyde (1.50 g, 47.8 mmol) were thoroughly mixed. To this mixture was added concd HCl (4.0 mL) while the mixture was stirred rapidly. Gelation was observed in 2 min. The mixture was then heated to 100 °C for 6 h. After cooling to rt, the reaction mixture was poured into MeOH (80 mL) under stirring. The precipitate was collected by centrifugation and dried under high vacuum. $^{13}C_{10}$-CB[5] and $^{13}C_{14}$-CB[7] were removed from the mixture by washing with water three times (3 × 50 mL). $^{13}C_{16}$-CB[8] was removed as an insoluble solid by partially dissolving the mixture with 3 M HCl (60 mL). After the filtrate was concentrated (20 mL) it was poured into MeOH (120 mL) and the solid was isolated by filtration. $^{13}C_{12}$-CB[6] was obtained by recrystallization from concentrated HCl (1.50 g, 35%). Fractions containing mixtures of $^{13}C_{12}$-CB[6] and $^{13}C_{12}$-*i*CB[6] were collected and then purified by ion exchange chromatography (Dowex 50WX2). A mixture of $^{13}C_{12}$-CB[6] and $^{13}C_{12}$-*i*CB[6] (0.80 g) was dissolved in H_2O/88% formic acid (1:1, 15 mL). The column was eluted with 0.4 M HCl/88% formic acid (1:1). After monitoring the collected fractions by ^{1}H NMR, fractions were combined and dried under high vacuum overnight to give $^{13}C_{12}$-*i*CB[6] (32 mg, 1%) as a white solid. $^{13}C_{12}$-CB[6]: ^{1}H NMR (400 MHz, 20% DCl/D_2O) δ 5.38 (s, 12H), 5.34 (d, $J = 15.6$ Hz, 12H), 4.15 (dt, $J = 15.6$, 3 Hz, 12H). ^{13}C (400 MHz, 20% DCl/D_2O) δ 155.1, 68.9 (m), 50.3 (m). ES-MS 505 $(M + 2H)^{2+}$. $^{13}C_{12}$-*i*CB[6]: ^{1}H NMR (400 MHz, 20% DCl/D_2O) δ 5.59 (dt, $J =$ 8.6, 2.3 Hz, 2H), 5.48 (dt, $J = 8.6$, 2.3 Hz, 2H), 5.45−5.35 (m, 14H), 5.18 (d, $J = 13.9$ Hz, 4H), 4.75 (t, $J = 2.1$ Hz, 2H), 4.35 (dd, $J = 14.0$, 6.5 Hz, 4H), 4.28 (dt, $J = 15.7$, 5.0 Hz, 4H), 4.17 (dt, $J = 15.6$, 4.7 Hz, 4H). ^{13}C (400 MHz, 20% DCl/D_2O) δ 155.2, 155.1, 154.7, 154.6, 70−67 (m), 62−60 (m), 52−49 (m). ES-MS 505 $(M + 2H)^{2+}$.

Transformation of *i*CB[6] to CB[6] in a Mixture of $MeSO_3H$ and Concentrated HCl. A solution of *i*CB[6] (20 mg) in the indicated v:v mixture of $MeSO_3H$ and 37% HCl (0.6 mL) was heated at 85 °C for 4 h. The solution was pipetted into methanol (10 mL). The precipitate was collected by centrifugation and dried under high vacuum overnight to give a mixture of the various CB[*n*] compounds (17.4−18.4 mg, 87−92%). The distribution of the various CB[*n*] compounds was obtained by careful integration of the ^{1}H NMR spectrum of the mixture obtained with 20% DCl/D_2O as the solvent.

(29) The isotope pattern for $^{13}C_{12}$-*i*CB[6] is somewhat irregular, but reproducibly so, in that the peak intensities oscillate. We do not understand the cause of this effect.

Concentration Effect on the Transformation of *i*CB[6] to CB[6]. A solution of *i*CB[6] in $MeSO_3H$ (0.6 mL) or $MeSO_3H/H_2O$ (98:2 v:v, 0.6 mL) was heated at 85 °C. After the indicated time, the reaction was pipetted into MeOH to yield a precipitate, the precipitate was isolated by centrifugation, and the crude solid was dried under high vacuum. The content of *i*CB[6] and CB[6] was monitored by careful integration of the ^1H NMR spectrum (H_a singlet versus the 4.5−4.1 ppm region) obtained with 20% DCl/D_2O as the solvent.

Transformation of a Mixture of *i*CB[6] and $^{13}C_{12}$-*i*CB[6] into CB[6] and $^{13}C_{12}$-CB[6] in $MeSO_3H$. A solution of *i*CB[6] (10 mg) and $^{13}C_{12}$-*i*CB[6] (10 mg) in $MeSO_3H$ (0.6 mL) was heated at 85 °C for 4 h. The solution was pipetted into methanol (10 mL). The precipitate was collected by centrifugation and dried under high vacuum overnight to give CB[6] and $^{13}C_{12}$-CB[6] (18.2 mg, 91%) as a pale brown solid. No significant crossover (e.g., $^{13}C_{<12}$-CB[6]) products were observed by electrospray mass spectrometric analysis of the reaction mixture.

Intermolecular Isomerization of a Mixture of *i*CB[6] and $^{13}C_{12}$-*i*CB[6] in 37% HCl. A solution of *i*CB[6] (10 mg) and $^{13}C_{12}$-*i*CB[6] (10 mg) in 37% HCl (0.6 mL) was heated at 85 °C for 4 h. The solution was pipetted into methanol (10 mL). The precipitate was collected by centrifugation and dried under high vacuum overnight to give a mixture of partially labeled $^{13}C_{\leq 12}$-CB[6] (17.2 mg, 86%) as established by analysis of the electrospray mass spectrum.

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Supporting Information Available: Synthetic procedures and characterization data for new compounds and crystallographic information file (CIF) for *i*CB[6]. This material is available free of charge via the Internet at http://pubs.acs.org.

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