Molecular Recognition

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Cucurbit[7]uril: A High-Affinity Host for Encapsulation of Amino Saccharides and Supramolecular Stabilization of Their α-Anomers in Water**

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Abstract: *Cucurbit[7]uril (CB[7]), an uncharged and water-soluble macrocyclic host, binds protonated amino saccharides (*D*-glucosamine,* D*-galactosamine,* D*-mannosamine and 6-amino-6-deoxy-*D*-glucose) with excellent affinity ($K_a = 10^3$ to $10^4\ M^{-1}$). The host–guest complexation was confirmed by NMR spectroscopy, isothermal titration calorimetry (ITC), and MALDI-TOF mass spectral analyses. NMR analyses revealed that the amino saccharides, except* D*-mannosamine, are bound as α-anomers within the CB[7] cavity. ITC analyses reveal that CB[7] has excellent affinity for binding amino saccharides in water. The maximum affinity was observed for* D*-galactosamine hydrochloride ($K_a = 1.6 \times 10^4\ M^{-1}$). Such a strong affinity for any saccharide in water using a synthetic receptor is unprecedented, as is the supramolecular stabilization of an α-anomer by the host.*

Artificial saccharide receptors, operating in water through noncovalent interactions, are of great interest in chemical biology and supramolecular chemistry.[1] They are promising candidates for practical applications in sensing, drug discovery, and other areas.[2,3] The challenge for the receptor is to discriminate water and the saccharide, and selectively bind the latter. Unlike boronic acids which rely on covalent bonding,[4] synthetic receptors utilize their hydrophobic pocket and hydrogen-bonding functional groups by mimicking carbohydrate-binding proteins (lectins), and there are only a few examples.[5–11] Additionally, the structural/functional diversity of the saccharides they can target is highly limited compared to their organic-soluble analogues.[12,13]

Amino saccharides, prevalent in the cell wall as N-acyl derivatives,[14] and seldomly present in unprotected form,[15] are biomarkers for many biological processes and diseases.[14] In addition, they are components of aminoglycoside antibiotics.[16] Despite the range of potential applications[17] for artificial receptors recognizing amino saccharides, the design of such receptors is correspondingly more challenging. Synthetic receptors for complexing amino saccharides are quite rare even in organic solvents,[18–20] and none exist to recognize them in water through noncovalent interactions. A few boronic acid based nonbiomimetic receptors are known, and they show very weak to moderate affinity.[21,22] Metal ions bind amino saccharides, but their application is limited to sensors.[23]

Saccharide receptors can be valuable in studying the anomeric effect in water as well. Stereoelectronic effects favor stabilization of the α-anomer, whereas hydration promotes mutarotation to the β-anomer.[24] We anticipated that a water-soluble macrocyclic cage receptor might confine a saccharide to its hydrophobic cavity and stabilize the α-anomer, thus preventing mutarotation. However, such a supramolecular stabilization of the α-anomer of a saccharide in water using a synthetic receptor has never been demonstrated, although a few receptors are known to preferentially bind one anomer over the other.[7,18,25] Herein, we report that cucurbit[7]uril (CB[7]) has excellent selectivity and high affinity ($K_a = 10^3$ to $10^4\ M^{-1}$) for protonated amino saccharides in water (Figure 1). We also discovered that CB[7] provides extraordinary stabilization of the α-anomers of certain amino saccharides, thus preventing mutarotation in water.

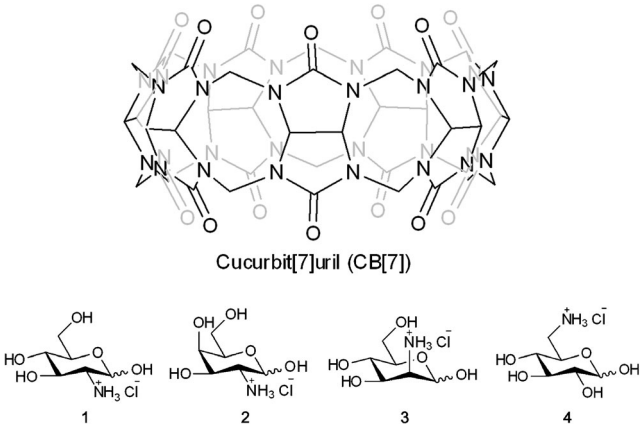

Figure 1. Molecular structures of cucurbit[7]uril (CB[7]) (top) and amino saccharides (**1**–**4**) examined for binding.

CB[7], a member of the cucurbit[*n*]uril family (CB[*n*], $n =$ 5–8, 10 and 14),[26,27] is an uncharged and conformationally

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rigid water-soluble (20–30 mM) macrocycle with a hydrophobic cavity (ca. 280 Å^3). The cavity and carbonyl portals[28,29] of CB[7] mediate encapsulation of organic ammonium ions through hydrophobic and ion–dipole interactions. The association constant (K_a) reaches a maximum of 10^{15} M^{-1} for certain guests in water,[30,31] and is on par with biotin–avidin complexes. We envisaged that CB[7] might encapsulate protonated amino saccharides (Figure 1) in its hydrophobic cavity with high affinity. Below are our findings based on extensive NMR spectroscopy, as well as MALDI-TOF and ITC analyses, which are supported by molecular modeling.

A freshly dissolved D-glucosamine hydrochloride (**1**), exists in D_2O as a mixture of anomers (α/β = 93:7), and undergoes mutarotation over a 24 hour period to reach an α/β ratio of 65:35. The ^{1}H NMR spectrum (298 K) of an equimolar (5 mM) solution of CB[7] and **1** in D_2O (Figure 2a) shows the resonances corresponding to **1** in the complex. The observed upfield shifts are consistent with the chemical shielding effect of the CB[7] cavity, thereby suggesting **1** is bound inside the CB[7] to form the CB[7]·**1** complex. The broadness of the resonances indicates fast exchange between the free and encapsulated **1**. The formation of the 1:1 complex was also confirmed by MALDI-TOF mass spectrometry (see Figure S1 in the Supporting Information). In the presence of 2 equivalents of CB[7], which forces **1** to be completely encapsulated within the CB[7] cavity, the fast exchange of **1** is suppressed on the NMR time scale and the resonances of **1** in the complex (Figure 2a) become sharper and reasonably well resolved.

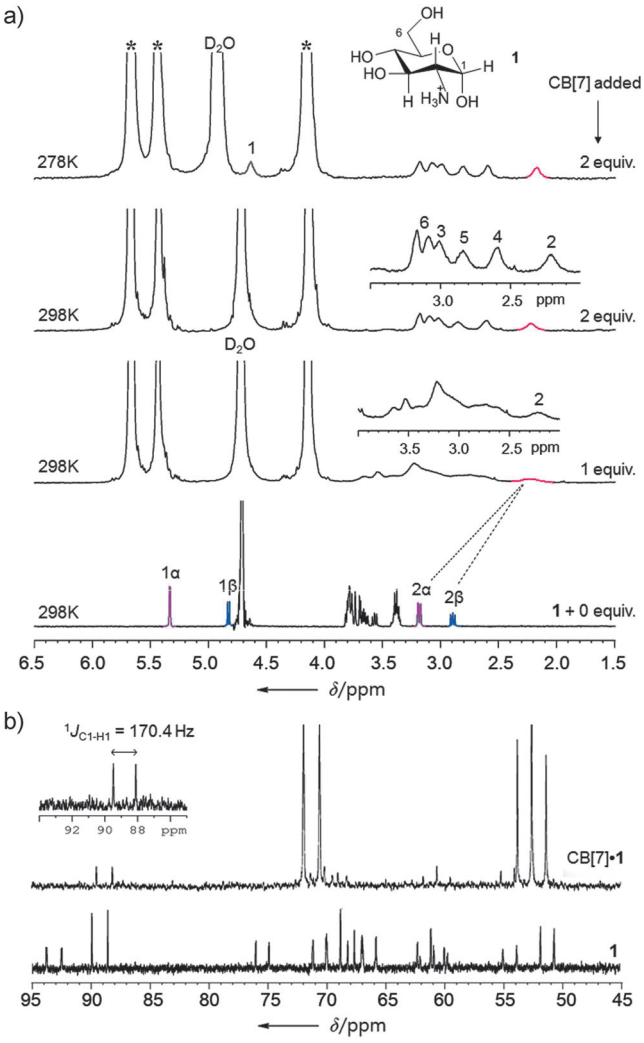

Figure 2. NMR spectra of CB[7]·**1** complex. a) ^{1}H NMR spectra of CB[7]·**1** complex: free saccharide **1** (bottom), **1** complexed with 1 equiv (above) and 2 equiv of CB[7] (top two) recorded at 298 and 278 K. The signals indicated with asterisks belong to CB[7]. b) Proton–coupled ^{13}C NMR spectra of **1** (bottom) and CB[7]·**1** complex.

A complete assignment of ^{1}H and ^{13}C NMR resonances of **1** in CB[7]·**1**, with 2 equivalents of CB[7], was possible with the aid of DQF-COSY, HSQC, and ROESY analyses (278 and 298 K). The signals of **1**, which appear as a single set of peaks, are shifted to higher field by 0.6–1.0 ppm relative to those of free **1** (see Table S1 in the Supporting Information). For example, at 278 K, there is only one anomeric peak appearing at $\delta = 4.68$ ppm which is hidden under residual H_2O signal at 298 K, and is relatively upfield shifted compared with that of free **1**. The resonances corresponding to H2α ($\delta = 3.2$ ppm) and H2β ($\delta = 2.9$ ppm) of free **1** appear as a single broad peak at $\delta = 2.2$ ppm in CB[7]·**1**. The proton-coupled ^{13}C NMR spectrum of the complex clearly shows a single doublet at $\delta = 88.8$ ppm with a coupling constant $^1J_{C1\text{-}H1} = 170.4$ Hz (Figure 2b). The chemical shift and coupling constant of the doublet are similar to that of the α-anomer of free **1** ($\delta = 88.5$ ppm, $^1J_{C1\text{-}H1} = 170.5$ Hz), thus suggesting that **1** is encapsulated as the α-anomer within the CB[7] cavity. ROESY spectrum (278 K; see Figure S4 in the Supporting Information) of the complex shows the presence of a H1*e*–H2*a* (*a* = axial, *e* = equatorial) correlation, and at the same time, the absence of any crosspeaks between H1*a* and both of H3*a* and H5*a* strongly support that C1-OH (**1**) is axially oriented (i.e., H1*e*), thus corresponding to α-anomer of **1**. Interestingly, the ROESY spectrum further reveals a weak intermolecular crosspeak between the CB[7] protons ($\delta =$ 5.5 ppm) and H1*e* of **1**. Overall, NMR analyses suggest that CB[7] stabilizes the α-anomer of **1**.

A quantitative measurement of the binding affinity between CB[7] and **1** by ^{1}H NMR titration was hampered by signal broadening of **1** upon complexation. Subsequently, we resorted to isothermal titration calorimetry (ITC) for thermodynamic analysis of the complexation. The ITC experiment (Table 1; see Figure S5 in the Supporting Information) confirmed the high affinity of CB[7] to **1** [$K_a = (4.4 \pm$

Table 1: Association constants (K_a) and thermodynamic parameters for 1:1 host-guest binding between CB[7] and amino saccharides **1**–**4** obtained from ITC analyses.

Substrate	K_a [M^{-1}]	$\Delta H°$ [kJ mol^{-1}]	$T\Delta S°$ [kJ mol^{-1}]
1	$(4.4\pm1.0)\times10^3$	-14.1 ± 1.0	6.2 ± 1.0
2	$(1.6\pm0.1)\times10^4$	-13.8 ± 1.0	10.1 ± 1.0
3	$(1.9\pm0.3)\times10^3$	-14.1 ± 1.0	2.8 ± 0.1
4	$(1.6\pm0.7)\times10^3$	-10.1 ± 1.0	2.8 ± 0.1

Standard deviations are obtained from three independent ITC titrations.

1.0) $\times 10^3\,M^{-1}$] to form a 1:1 stoichiometric complex. The binding is driven by both favorable enthalpy and entropy changes ($\Delta H° = -14.0\,kJ\,mol^{-1}$ and $T\Delta S° = 6.6\,kJ\,mol^{-1}$). The implications of these changes are discussed below.

D-Galactosamine hydrochloride (**2**), the C4 epimer of **1** with an axially oriented OH group at C4, undergoes mutarotation in D_2O to reach an equilibrium α-/β-anomer ratio of 63:37 from an initial ratio of 26:74 over 24 hours. The ^1H NMR spectrum (298 K) of a solution of CB[7] and **2** indicates the formation of the CB[7]·**2** complex with significant upfield shifts of the resonances of **2**, and complexation is also confirmed by MALDI-TOF mass spectrometry (see Figure S6 in the Supporting Information). The resonances are relatively sharper (Figure 3a) in comparison with those of **1** in CB[7]·**1**. Similar to the situation with **1**, the resonances of **2** appear well-resolved with 2 equivalents of the host. The presence of single anomer proton signal suggested that CB[7] encapsulates a single anomer of **2**. Additionally, proton-coupled ^{13}C NMR data suggests that **2** is bound within the CB[7] cavity as the α-anomer with a single characteristic doublet at $\delta = 88.8$ ppm with $^1J_{C1\text{-}H1} = 170.7$ Hz, which is similar to that of α-anomer of uncomplexed **2**. Furthermore, DQF-COSY spectroscopy provides direct evidence for the equatorially oriented H1 (H1*e*) for the α-anomer[32] (see Figure S8 in the Supporting Information): $^3J_{H1\text{-}H2a} = 4.7$ Hz, whereas $^3J_{H2a\text{-}H3a} = 10.7$ Hz. As expected, the ROESY spectrum at 278 K (see Figure S9 in the Supporting Information) shows the H1e–H2a correlation, whereas no other through-space crosspeak between H1*a* and both of H3*a* and H5*a* is observed. Taken together, NMR analyses unequivocally confirm that **2** is bound as the α-anomer inside the CB[7] cavity.

Although both methylene protons of CB[7] do not undergo any shift in peak position upon guest addition in general, the endocyclic methylene protons aligned along the carbonyl portal would be in close proximity with certain guest protons and their through space correlation (less than about 5 Å) could be readily identified in ROESY spectrum. Indeed, the ROESY spectrum (Figure S9) shows through-space cross-peaks between the proton of CB[7], resonating downfield at $\delta = 5.4$ ppm, and H1*e* and H6 of **2**. Further, the large upfield shifts, up to 1.0 ppm, of H3 and H5 indicate that they are positioned deep inside the CB[7] cavity (see Table S1). These shifts suggests that **2** might be positioned within CB[7] cavity at an angle with an imaginary long axis running through C2 and C6 of the hexameric ring. In other words, the -NH_3^+(C2) and -OH(C1) groups of **2** are pointing to a carbonyl portal of the CB[7] whereas -OH(C6) is pointing to the opposite carbonyl portal of CB[7]. The disposition of the α-anomer of **2** confined within the CB[7] cavity is also consistent with a molecular mechanics modeling study,[33] wherein H1*e* and H6 of **2** are in close proximity(ca. 3.9 to 4.1 Å) to the endocyclic methylene proton of CB[7] (Figure 3c). The model shows that the ring oxygen atom is buried within the CB[7] cavity. It also suggests possible hydrogen-bonding stabilization of the hydroxy groups (C1 and C6) of **2**, along with stabilization of ammonium ion from the CB[7] carbonyl portal.

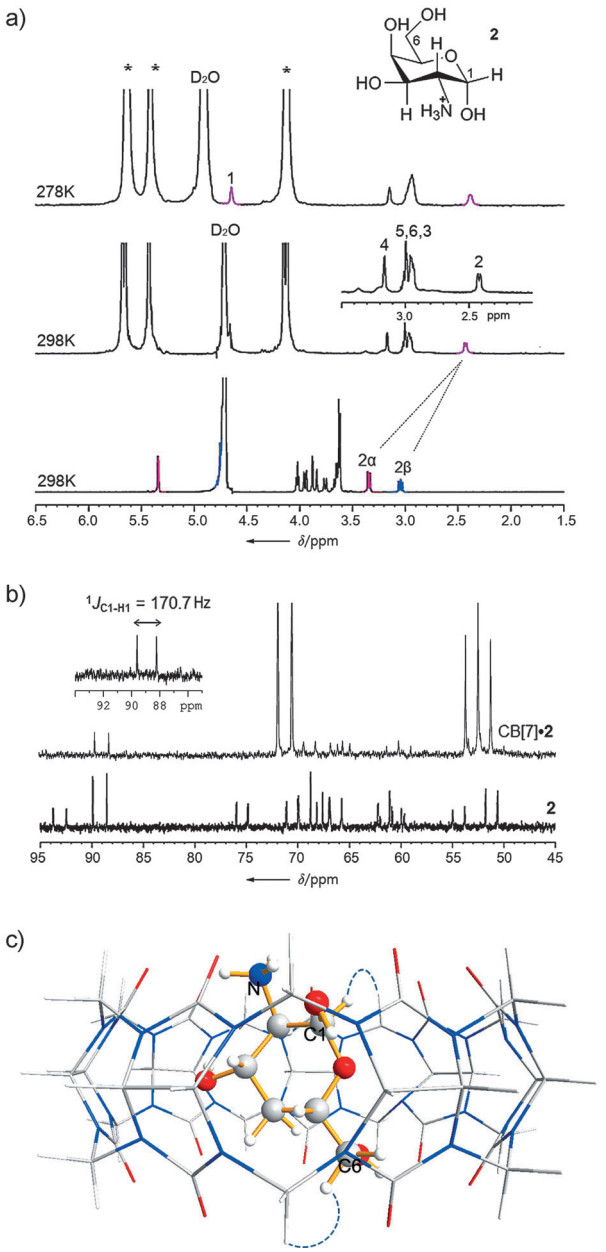

Figure 3. NMR spectra and proposed model for the CB[7]·**2** complex. a) ^1H NMR spectra of CB[7]·**2** complex: free saccharide **2** (bottom), **2** complexed with 2 equiv of CB[7] (top two) recorded at 298 and 278 K. The signals marked with asterisks belong to CB[7]. b) Proton–coupled ^{13}C NMR spectra of **2** (bottom) and CB[7]·**2** complex. c) Optimized structure of the CB[7]·**2** complex, based on molecular mechanics modeling, indicating favorable interaction between the ammonium ion and hydroxy groups at C1 and C6 of **2** with CB[7]. The dotted lines indicate ROESY crosspeaks (Figure S9) observed between CB[7] and **2**.

Once again, attempts to measure the binding affinity between CB[7] and **2** by ^1H NMR titration were impeded by peak broadening and mutarotation (see Figure S12 in the Supporting Information). However, ITC analysis (Table 1; see Figure S13 in the Supporting Information) revealed that CB[7] has excellent affinity for **2** [$K_a = (1.6 \pm 0.1) \times 10^4\,M^{-1}$]. Such a strong affinity is unprecedented for any synthetic

receptor binding either neutral or charged saccharides in water. The binding is associated with favorable changes in both enthalpy and entropy ($\Delta H° = -13.8\ kJ\,mol^{-1}$ and $T\Delta S° = 10.1\ kJ\,mol^{-1}$) as in the case of CB[7] and **1**. We note that while the enthalpy contribution to the binding affinity is almost the same for **1** and **2**, there is a much higher entropy contribution for **2**, which might in part be due to the greater hydration shell around **2** and the release of it upon binding,[34] thus resulting in the higher binding affinity of CB[7] for **2**.

The host–guest binding is mediated by the ion–dipole interactions between the carbonyl portal of CB[7] and the ammonium ions of the α-anomers of the amino saccharides **1** and **2**, as indicated by the relatively large negative enthalpy change. Further, the hydrophobic effect also seems to play a crucial role, as indicated by the positive entropy change. The CB[7] host cavity is occupied with high energy water molecules, which are readily released upon inclusion of the guest molecules (**1** or **2**). They may contribute not only to favorable entropy changes but also to favorable enthalpy changes, as suggested recently.[35] Such water-mediated binding of saccharides with lectins are often observed under biological conditions.[36] The stabilization of the α-anomer of a monosaccharide through supramolecular confinement within the hydrophobic cavity of a water-soluble host, as demonstrated herein, is unprecedented.

Subsequently, we also studied the ability of CB[7] to recognize D-mannosamine hydrochloride (**3**), with an axially oriented ammonium ion at C2. Compound **3** undergoes mutarotation in D_2O and the ratio of α/β anomers shifts from 29:71 to 38:62 over 24 hours. The ^1H NMR spectrum (see Figure S14 of the Supporting Information) of a 2:1 mixture of CB[7] and **3** indicates the formation of the CB[7]·**3** complex with substantial upfield shifts for all resonances of **3**. Unlike the situation with **1** or **2**, however, the signals are quite complex, and caused by the presence of both anomers. Detailed NMR analyses suggest that the anomeric ratio shifts to 65:35 in favor of the α-anomer upon complexation. An ITC experiment (Table 1; see Figure S15 in the Supporting Information) reveals that the complexation is also driven by both enthalpy and entropy ($\Delta H° = -13.0\ kJ\,mol^{-1}$ and $T\Delta S° = 5.7\ kJ\,mol^{-1}$) with a K_a value of $(1.9 \pm 0.3) \times 10^3\ M^{-1}$. However, a satisfactory fitting of the measured data was obtained for a 1:1 host–guest binding model only with a constrained N value of about 0.75. We presume that, as a result of the axial $-NH_3^+$ at C2, the orientation of **3** within the CB[7] cavity might be such that the ring oxygen atom is still exposed to the surrounding water, thus mutarotation is still feasible.

Finally, we turned our attention to a pyranose with a primary ammonium ion, namely 6-amino-6-deoxy-D-glucose (**4**). ^1H NMR spectrum (see Figure S16 of the Supporting Information) of an equimolar mixture of CB[7] and **4** shows that complexation induced upfield shifts for the guest resonances and the resonances of **4** become well-resolved in presence of 2 equivalents of CB[7]. Detailed NMR analyses reveal only the α-anomer of **4** is encapsulated within CB[7] (see Figures S18 and S19 in the Supporting Information). Moreover, an ITC experiment revealed that CB[7] has significant affinity towards **4**: $K_a = (1.6 \pm 0.7) \times 10^3\ M^{-1}$ (Table 1; see Figure S20 in the Supporting Information).

The present results suggest that CB[7] may also bind aminoglycoside-based antibiotics. Indeed, preliminary results indicate that CB[7] binds both neomycin and paromomycin with high affinity as confirmed by ITC titration (see Table S2 in the Supporting Information). A detailed examination is underway in our laboratory.

In conclusion, we have shown that CB[7] binds protonated amino saccharides in water with great selectivity and excellent affinity with a maximum K_a value of $(1.6 \pm 0.1) \times 10^4\ M^{-1}$ for D-galactosamine hydrochloride (**2**). We have also discovered that CB[7] provides supramolecular stabilization for the α-anomers of D-glucosamine hydrochloride (**1**), D-galactosamine hydrochloride (**2**), and 6-amino-6-deoxy-D-glucose (**4**) in water. Confinement of the saccharides within the hydrophobic cavity of CB[7] prevents the α-anomers from undergoing any mutarotaion, although the complexes are dissolved in water. Such stabilization of the α-anomer in water is unprecedented. The affinity of CB[7] for amino saccharides combined with the ready exocyclic functionalization of CB[7][37,38] might offer new opportunities, for example, as artificial synthetic lectin arrays for glycan analysis,[17] or as lipid/CB[7] conjugates to explore cell–cell interactions and others. Furthermore, CB[7] might be useful in the delivery and sensing of aminoglucoside antibiotic drugs, as indicated by promising preliminary results.

Experimental Section

A stock solution 5.0 mM and 0.5 mM of cucurbit[7]uril (CB[7]) was prepared in D_2O and deionized water for NMR and ITC experiments, respectively. Amino saccharides (**1**–**4**) were purchased from Sigma–Aldrich and used without purification. A stock solution 5 mM and 10 mM of each saccharide was prepared in D_2O and deionized water and equilibrated for 24 h for NMR and ITC experiments, respectively. All solutions were degassed and thermostated using ThermoVac accessory before ITC titration.

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