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Cucurbiturils Monofunctionalized on the Methylene Bridge and their Host-Guest Properties

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Abstract: Monofunctionalization of cucurbiturils is essential for the transfer of these potent supramolecular macrocyclic hosts into a real-world application. Here, we present the synthesis of cucurbit[6]urils **1** and **2** in which one methylene bridge is modified by a single substituent containing a nitro or an ammonium group. We investigated host-guest properties in water and 0.2 M NaCl using ^{1}H NMR and isothermal titration calorimetry, particularly for **2**. The macrocycle **2** self-associated into dimeric aggregates in pure water, but readily disassembled in the presence of NaCl or organic cations. Cucurbit[7]uril was able to encapsulate the ammonium substituent of **2** inside its cavity resulting in a complex of 1:1 stoichiometry with an association constant of 3.1 × 10^5 M^{-1}. The presented host-guest properties together with further possible derivatization showcase the potential of cucurbiturils modified in the methylene position such as **1** and **2** for the development of advanced supramolecular systems.

Cucurbiturils are macrocycles composed of glycoluril building blocks, which are connected via two rows of methylene bridges.[1,2] The resulting macrocycles are very rigid with well-defined cavities, soluble in water, and are chemically stable. These properties make cucurbiturils suitable supramolecular hosts for biologically relevant neutral and cationic organic guests. Supramolecular complexes of cucurbiturils are appreciated for their high stability, as well their high selectivity toward particular guests.[3] For example, cucurbit[7]uril (CB7) is able to bind some ferrocene and adamantane ammonium salts with binding affinity ranging from 10^{12} to 10^{18} M^{-1}.[4–6] The ultra-stable complexes were utilized in protein fishing[7] and imaging,[8] biorthogonal catalysis, complexed therapeutic systems,[9] under-water adhesive materials,[10] optical sensing[11] and others. All of the above-mentioned applications require the use of monofunctionalized cucurbiturils as it enables selective attachment of the macrocycle to the place of interest. Monofunctionalized cucurbiturils can be prepared by monohydroxylation of a single methine proton in a cucurbituril molecule.[12–16] They can be also achieved by merging the glycoluril hexamer with a monofunctionalized glycoluril,[17] or with monofunctionalized phthalaldehydes.[18,19] Our group showed that cucurbiturils can also be substituted at the methylene bridge position by reacting glycoluril with formaldehyde in the presence of other aldehyde.[20] Using this approach, we prepared cucurbit[6]uril (CB6) bearing one 2-phenylethyl group. In this paper, we used similar synthetic strategy for the preparation of macrocycles **1** and **2** (Figure 1), having nitro and amino substituents, respectively. The self-assembly of these monofunctionalized cucurbiturils was investigated, as well as their ability to serve as both hosts and guests.

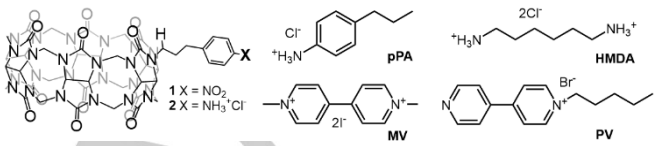
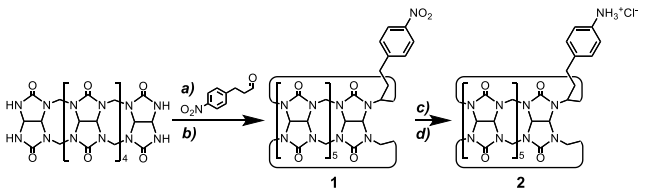


Figure 1. Structure of macrocycle 1 and 2 and supramolecular guests used in this study.

Cucurbituril **1** was prepared by the condensation reaction of acyclic glycoluril hexamer with formaldehyde and 3-(*p*-nitrophenyl)propionaldehyde in conc. HCl at 90 °C for 3 h (Scheme 1). Crude product was composed of **1** and the unsubstituted cucurbit[6]uril, which were successfully separated by column chromatography. Pure **1** was obtained after recrystallization from 4.8 M HCl in 16% yield. Subsequently, the nitro group in **1** was converted into the amino group. **1** was dispersed in aqueous acetic acid and stirred with 5 wt% palladium under hydrogen atmosphere overnight (Scheme 1), yielding macrocycle **2** as an acetate salt, which was subsequently transformed to a hydrochloric salt by recrystallization from 4.8 M HCl. The yield of the hydrogenation reaction was 72 %, where the loss of the product was probably caused by the interaction of cucurbituril moiety with palladium catalyst.



Scheme 1. Synthesis of monofunctionalized cucurbit[6]urils 1 and 2; a: concd. HCl, 90°C, 3 h; b: column chromatography HCO_2H/ CH_3CO_2H (1:1); c: H_2, Pd/C cat., dilut. CH_3CO_2H, RT, overnight; d: recrystallization from 4.8 M HCl.

The macrocycles **1** and **2** were characterized by MALDI-TOF MS and NMR spectroscopy. These measurements were performed in H_2O and D_2O in the presence of hexane-1,6-diammonium dichloride guest (**HMDA**, for structure, see Figure 1), which served as a solubilizing agent as well to prevent possible self-association interactions. Peaks at *m/z* of 1146.351 and 1116.377 in MS spectra of **1** (Figure S6) and **2**

(Figure S7) correspond to the desired monofunctionalized macrocycles. The monofunctionalization was further evident from the ^{1}H NMR spectra of **1** and **2** (Figure 2a,b) by comparing the intensities of the signals of 2-(*p*-nitrophenyl)ethyl substituents and the 2-(*p*-aminophenyl)ethyl hydrogenchloride (APE) substituents with those of the macrocycle repeating units. Other evidence of the monofunctionalized cucurbiturils can be drawn from the triplet belonging to the proton E of the methylene carbon atoms bearing the substitution, which was also observed previously on the first monosubstituted cucurbituril.[20] Downfield shift of proton E indicates its apical position along the carbonyl oxygen atoms, for both **1** and **2**. Transformation of nitro group in **1** into the amino group in **2** is clearly evident from an upfield shift of aromatic protons A from 8.29 to 7.41 ppm.

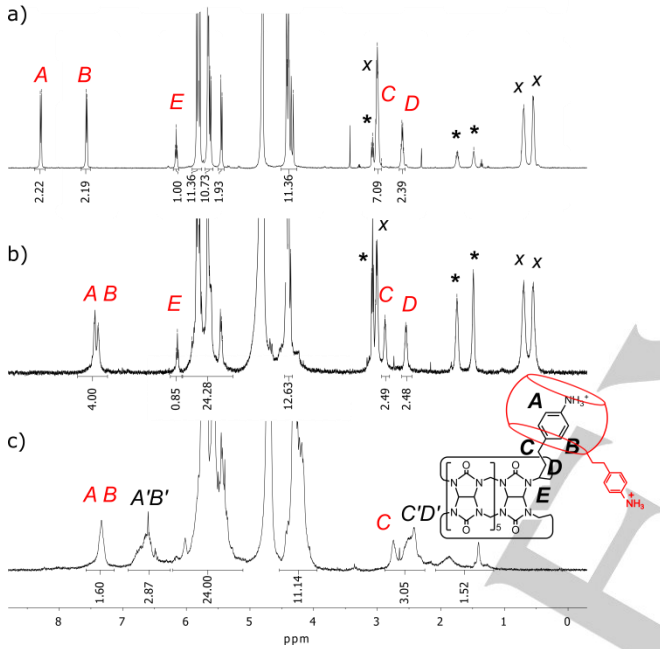


Figure 2. 1H NMR spectra of a) 1 and b) 2 in the presence of HMDA, and c) 2 in the absence of HMDA. All spectra were recorded in D2O at 30 °C. *Signals of free HMDA. xSignals of bound HMDA.

NMR spectra of the macrocycle **2** in D_2O in the absence of any guest (Figure 2c) showed additional signals when compared to the spectra recorded in the presence of **HMDA** (Figure 2b,c). These broad signals at 6.99-6.30 ppm and 2.69-2.46 ppm were recognized as aromatic (A', B') and aliphatic (C', D') signals of the APE substituent, which is included inside the cavity of another molecule of **2**. The exchange between free and bound substituents was slow on the NMR time scale, allowing assessment of the degree of aggregation using diffusion ordered spectroscopy (DOSY) (Figure S8). Diffusion coefficient of the free macrocycle, (2.618 ± 0.072) × 10^{-10} m^2 s^{-1}, was approximately 1.23 times higher than for the aggregates, (2.139 ± 0.026) × 10^{-10} m^2 s^{-1}, which is very close to the value of 1.26 obtained by theoretical calculation for a cucurbit[6]uril dimer.[19,21] Dimeric aggregates were also observed by MALDI-MS by signal at *m/z* of 2231.733 (Figure S7). Further NMR investigations (Figure S19) revealed, that ratios of the free and bound forms of the APE substituent do not change with increasing concentration of **2** (0.5 to 10 mM). This indicates that dimeric species are stable at least at millimolar concentrations in D_2O and that binding mode of the dimer corresponds to that one depicted on Figure 2c. The same experiment rules out the formation of supramolecular polymer or dimer in which both macrocycles are occupied by the substituents from the adjacent molecule (more discussion about possible binding modes is given in ESI below Figure S19).

As previously demonstrated, the binding affinity of host-guest complexes between cucurbiturils and aromatic guests can be significantly weakened by the addition of an inorganic salt.[22,23] Indeed, we found out that in the presence of 0.2 M NaCl, signals of self-assembled aggregates of macrocycle **2** are strongly reduced in the NMR spectra (Figure S5). Thus, we decided to explore further host-guest properties of **2** in 0.2 M NaCl. First, we turned our attention to the possibility of using macrocycle **2** as a guest for cucurbit[7]urils (CB7). NMR titration revealed that the addition of CB7 into the solution of **2** resulted in the appearance of new upfield shifted aromatic (A' and B') and aliphatic (C' and D') signals of the APE substituent (Figure 3).

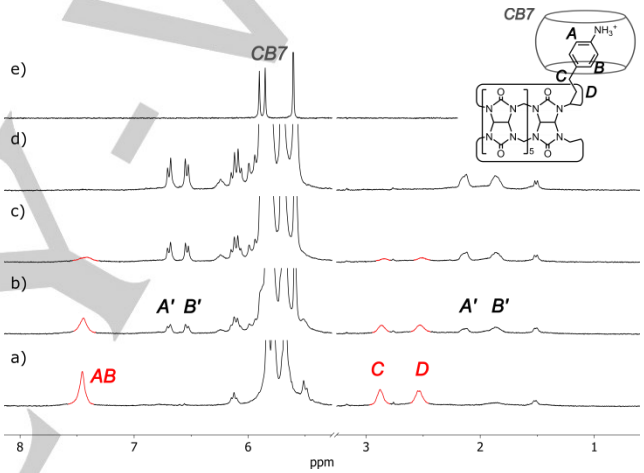


Figure 3. Cut of stacked ^{1}H NMR spectra (300 MHz, 0.2 M NaCl/D_2O) of a) pure 2, and after the addition of b) 0.3 equiv, c) 0.6 equiv and d) 1.1 equiv of CB7, and e) pure CB7.

Complete disappearance of the original signals upon the addition of 1 equivalent of CB7 is in agreement with the formation of a stable complex between **2** and CB7 at 1:1 ratio. The association constant of the **2**·CB7 complex of 2.9 × 10^5 M^{-1} was determined by ^{1}H NMR competition experiment using methylviologen diiodide (**MV**, for structure see Figure 1) as a competitor in 0.2 M NaCl (Figure S10). The host-guest exchange during this competition experiment was slow on the NMR time scale. Therefore, the association constant was determined based on the integration of signals belonging to the free and bound forms of the guests (for calculation see SI). We also performed direct titration using isothermal titration calorimetry (ITC), which resulted in a very similar association constant of 3.1 × 10^5 M^{-1} for the **2**·CB7 complex (Figure S11). Formation of this complex between CB7 and **2** was confirmed by MALDI-MS spectrum by a signal at *m/z* of 2300.712 (Figure S14).

Stability of the **2**·CB7 complex could be influenced by possible mutual interaction of both host and guest

macrocycles.To investigate such a possibility, we used *p*-propylanilinium chloride (**pPA**, for structure, see Figure 1) as a model compound for APE substituent of **2** and investigated its interaction with CB7. We found that **pPA** formed inclusion complex with CB7 (Figure S13) with binding affinity of 2.5 × 10^5 M^{-1} (determined by ITC, Figure S15) and 2.3 × 10^5 M^{-1} (determined by NMR competitive titration, Figure S14) in 0.2 M NaCl. These values did not differ significantly from those obtained for the **2**·CB7 complex, indicating a marginal influence of the macrocyclic core of **2** on the binding with CB7.

Two portals of cucurbiturils **1** and **2** are unequal as the substituent at the methylene bridge position is closer to one of the two macrocyclic portals. Possible orientation of the asymmetric guest after its inclusion inside the cucurbituril cavity can be thus influenced by the presence of the APE substituent, particularly in the case when part of the guest remains outside the macrocycle. Such host-guest complexation was tested in the case of N-pentyl-4-(4'-pyridyl)pyridinium (**PV**, for structure see Figure 1) and **1** using NMR in 0.2M NaCl/D_2O (Figure 4 and S16). As expected, **1** and **PV** formed an inclusion complex in a 1:1 ratio in which the aliphatic part of the guest is included inside the macrocycle. The binding mode is clearly indicated by the upfield shift of aliphatic protons of **PV** (H6-H9) upon complexation with **1**. On the other hand, the signals of the aromatic part of the guest (H1) shift downfield, which is consistent with its location outside of the macrocycle. Binding mode is consistent with those previously reported for viologen guests and CB6.[24] Closer investigation revealed that, upon complexation, the signals H3 and H4 of guest **PV** not only shift but also double (Figures 4 and S16). We believe that the splitting is the result of the formation of two diastereomeric complexes, which differ in the orientation of the guest molecule with respect to the substituent of **1** (as depicted in Figure 4) According to the integration of the split signals, we determine that the diastereomers are present in the solution in 1:0.7 ratio. Unfortunately, ROESY experiments did not reveal which of the two diastereomers dominates as we did not observe any cross-peaks between host and guest protons (Figure S17).

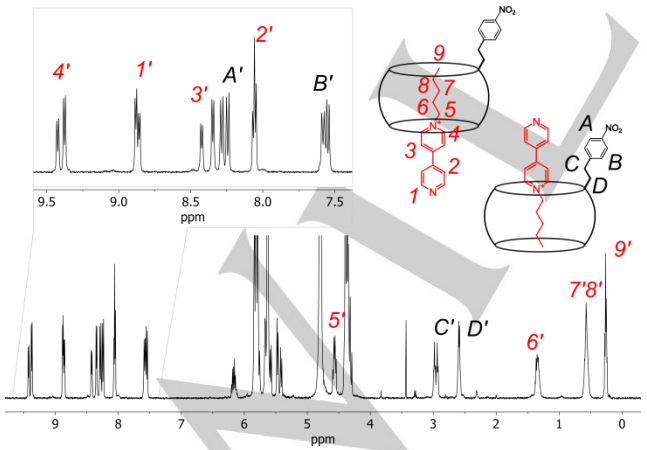


Figure 4. ^{1}H NMR spectra of PV (0.2 M NaCl/D_2O) and the complex of 1·PV with magnification of split aromatic signals of both host and guest. The picture shows two possible orientations of the guest upon its interaction with the host.

In conclusion, we prepared monofunctionalized cucurbit[6]urils **1** and **2**, in which substituents bearing nitro and amino groups were attached to one methylene bridge of the macrocycle. The macrocycle **1** was obtained by the macrocyclization reaction in 16 % yield, and its subsequent hydrogenation led to **2** in 72 % yield. The macrocycle **2** containing ammonium function underwent self-association into a dimer as indicated by DOSY measurements in D_2O. The dimerization could be suppressed in the presence of 0.2 M NaCl. The macrocycle **2** formed a supramolecular complex with CB7 in which its ammonium substituent was included inside the CB7 cavity. Association constants of 2.9 × 10^5 M^{-1} and 3.1 × 10^5 M^{-1} were determined by ^{1}H NMR and ITC, respectively. These values were similar to the one determined for the complex of the model compounds *p*-propylanilinium chloride and CB7, indicating a minor influence of CB6 macrocycle of **1** on the stability of the **1**·CB7 complex. Finally, NMR study enabled the observation of two diastereomers of the **1**·**PV** inclusion complex, which differed in the orientation of this unsymmetrical guest with respect to the inequivalent portals of the macrocycle.

Acknowledgements

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Keywords: Macrocycles • Cucurbiturils • Monofunctionalization • Self-assembly • Host-guest complexes

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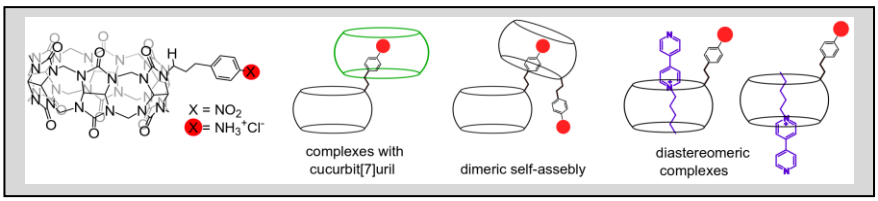

Monofunctionalized bambusurils bearing a nitro or an ammonium group were prepared. The macrocycles formed dimeric self-assembly, inclusion complexes with cucurbit[7]urils, and diastereomeric complexes with methylviologen.