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Stuffed Pumpkins: Mechanochemical Synthesis of Host-Guest Complexes with Cucurbit[7]uril

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Solvent-free mechanochemical synthesis (ball-milling) was used to prepare inclusion complexes with cucurbit[7]uril and four model guest molecules (adamantane, adamantyl-1-amine hydrochloride, toluidine hydrochloride, and *p*-phenylenediamine dihydrochloride). Successful formation of individual inclusions was independently confirmed by one-dimensional (^{1}H and ^{13}C MAS) and two-dimensional (^{1}H homonuclear double-quantum – single-quantum correlation spectrum) solid-state NMR techniques and differential scanning calorimetry. Mechanochemical synthesis represents an alternative path towards new types of cucurbit[n]uril/guest inclusion complexes that are not accessible due to limited solubility of the individual components.

Cucurbit[*n*]uril macrocycles (CB[n]) are now well-established hosts for the encapsulation of guests in aqueous medium,[1-3] often with extreme binding affinities (up to 7.2 × 10^{17} M^{-1}).[4-6] Native CB[n]s[7-11] and their acyclic congeners[12-14] have been shown to greatly enhance the solubility of hydrophobic substrates in aqueous medium upon encapsulation. For example, CB[6] and CB[7] enhance the solubility of anti-parasitic Albendazole by 2000-fold,[15] and Calabadion, an acyclic CB[n] congener developed by the Isaacs group, enhances the solubility of anti-tumoral paclitaxel by 2750-fold.[14] Solubilities can then approach or even exceed that of the free hosts (typically in the low mM range). However, while this effect is an essential feature of CB[n] encapsulation, it is highly likely that researchers in the field have had to cope at some point with suspensions of a CB[n] host and a potent guest, and their inability to form the desired complex due to either (i) very low solubilities of either or both partners, or (ii) exceedingly slow deaggregation of either or both partners in aqueous medium.

To overcome this complication, we show here that mechanical force can be used to encapsulate guests into CB[n]s, by simple solvent-free ball-milling of both substrates. Mechanochemistry has been successfully applied in several studies.[16-20] We used this technique in earlier studies towards the synthesis of various 2D and 3D arrays of molecular machines in highly porous zeolite-like tris(*o*-phenylenedioxy)cyclotriphosphazene.[21-26] While CB[n]s have been used as solid excipients[8, 10] in the formulation of oral tablets,[27, 28] encapsulation of the guest molecules into the CB[n] cavities when both hosts and guests are mixed as powders has never been systematically investigated. To the best of our knowledge, this study provides such detailed evidence for the first time.

As a proof of concept, we present here the mechanical encapsulation of guests **1**–**4** into the cavity of CB[7] (Chart 1). All guests bind tightly to CB[7] in aqueous medium; Isaacs and coworkers extracted the binding affinity of adamantane in D_2O from phase-solubility diagrams (5.2 × 10^6 M^{-1}),[29] and obtained binding affinities of 4.2 × 10^{12}, 2.1 × 10^6 and 8.4 × 10^6 M^{-1} for guests **2**–**4**, respectively, by competitive nuclear magnetic resonance spectroscopy (NMR) experiments in a 50 mM sodium acetate buffer (pD 4.74).[30]

Free guests were characterized first as solids before and after ball-milling by ^{1}H and ^{13}C magic-angle-spinning solid-state nuclear magnetic resonance technique (MAS ssNMR). Inclusion complexes were then prepared by ball-milling both guest and host for 4 × 5 min at room temperature in three different guest/CB[7] ratios (2:1, 1:1 and 1:2), and were thoroughly characterized by ^{1}H and ^{13}C MAS ssNMR, as well as differential scanning calorimetry (DSC). Finally, all inclusion complexes were dissolved in D_2O to allow the typical host-guest recognition in water to take place, after which samples were evaporated to dryness, and characterized by ^{1}H and ^{13}C MAS ssNMR again. Identical spectra recorded before and after water treatment would confirm encapsulation by ball-milling.

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Electronic Supplementary Information (ESI) available: Experimental procedures, solution and solid-state NMR spectra of compounds **1**–**4** and their inclusion complexes with CB[7], DSC traces for compounds **2**–**4**, and ESI MS data for **1**–**4**⊂CB[7]. See DOI: 10.1039/x0xx00000x

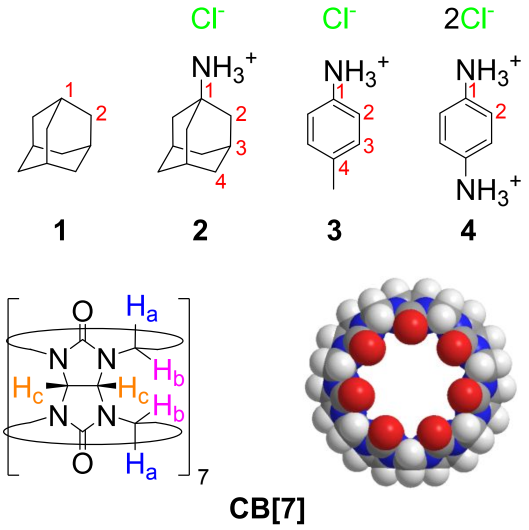

Chart 1. Guest molecules **1**–**4** with atom numbering; top view of a space filling model of CB[7] (structure from X-ray diffraction analysis).[31]

Importantly, CB[n]s as solid samples are notorious for trapping significant amounts of water and hydrochloric acid despite thorough drying.[1, 2, 32, 33] In one of the rare studies that highlights this property, Kaifer reported an apparent molecular weight of approximately 1500 g/mol for their CB[7] source (vs. 1163 g/mol in its anhydrous form), that increased to 1900 and 2400 g/mol after 1- and 13-day exposures to ambient air.[33] The apparent molecular weight of the CB[7] sample used in this study ranged from 1400 to 1700 g/mol, as determined by ^1H NMR titration with adamantylammonium (**2**) present in known quantities and elemental analysis (see ESI for details).

Regardless of the nature of the guest, ^1H and ^{13}C chemical shifts of the pseudo-axial (H_a), pseudo-equatorial (H_b) and equatorial hydrogens (H_c) of CB[7] are not significantly affected by encapsulation in solution (less than 0.08 ppm for hydrogen nuclei and 0.45 ppm for carbon atoms). As is typical, hydrogen nuclei of the guests undergo significant upfield shifts (towards lower chemical-shift values) when they reside inside the cavity of CB[7] (up to 0.8 ppm for one of the H4 signals of guest **2** and for the H2 signal of guest **4**). Carbon nuclei undergo either upfield or downfield shifts (−1.8 to 0.7 ppm).

While the solubility of adamantane (**1**) in water is exceedingly low (0.78 μM), stirring in the presence of CB[7] dramatically increases its solubility, albeit slowly (approximately 0.2, 0.8 and 2.4 mM after 1, 24 and 72 h, respectively, in the presence of 3.5 mM CB[7]; see Figure S2 in the ESI).

The host-guest assemblies were then characterized in the solid state by ^1H and ^{13}C MAS ssNMR spectroscopy with ultra-fast MAS speed of 70 kHz, which leads to a significant narrowing of proton NMR signals. This feature was also exploited in proton-detected two-dimensional (2D) ssNMR experiments, which were necessary for the detection of hydrogen signals of guest molecules overlapped with signals of the host and for an unequivocal confirmation of host-guest complex formation.

The ^1H ssNMR spectrum of CB[7] (Figure 1a) shows overlapping H_a and H_c signals (see Chart 1 for numbering) at 6.0 ppm that are separated from H_b at 4.9 ppm. The broad signal at 1.3 ppm probably corresponds to water molecules trapped inside CB[7], as it disappears upon guest inclusion (Figure 1 and Figure S25). The spectrum of neat ball-milled adamantane (**1**) is characterized by a peak centered at 1.6 ppm (both the CH and CH_2 groups overlap, Figure 1b). ^1H chemical shifts of guest **1** undergo clear upfield shifts (0.6 ppm) upon encapsulation into CB[7]. Signals of both free and bound adamantane (**1**) can be seen in the presence of a sub-stoichiometric amount of CB[7] (spectrum c), while only CB[7]-bound adamantane (**1**) is observed in the presence of 1.0 or 2.0 equiv. CB[7] (spectra d and e).

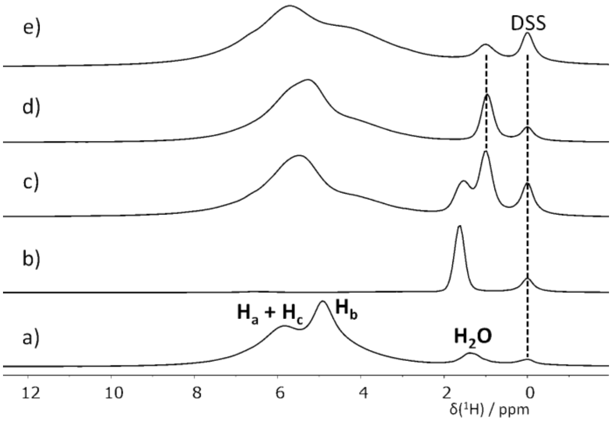

Figure 1. ^1H ssNMR spectra of ball-milled (a) CB[7], (b) adamantane (**1**), and **1** in the presence of (c) 0.5 equiv, (d) 1.0, and (e) 2.0 equiv CB[7]. Sodium trimethylsilylpropane-sulfonate (DSS) was used as an internal standard.

Similarly, ^{13}C chemical shifts of adamantane (**1**) undergo significant changes upon encapsulation (upfield by 1.1 ppm for C(1) and downfield by 0.6 ppm for C(2), see Figure 2 and Chart 1 for numbering). The chemical shifts of CB[7] (52.8, 71.3 and 156.5 ppm in the neat sample) are not affected by complexation.

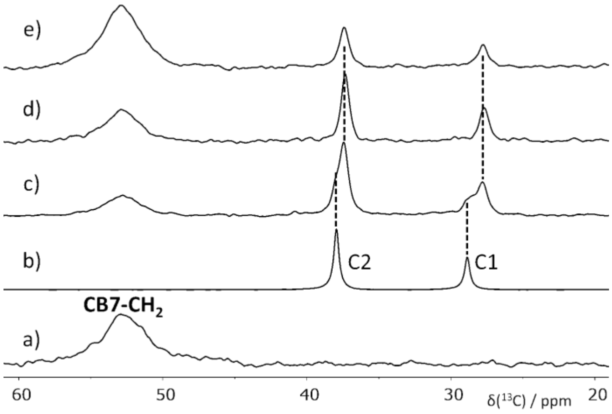

Figure 2. Part of ^{13}C CP-MAS ssNMR spectra of ball-milled (a) CB[7], (b) pure adamantane (**1**), and **1** in the presence of (c) 0.5 equiv, (d) 1.0, and (e) 2.0 equiv CB[7].

Intermolecular interactions, and hence spatial proximity between CB[7] and guest molecules can be observed in spectra that exploit dipolar (through-space) coupling. Figure 3 shows a ^1H homonuclear double-quantum (DQ) – single-quantum (SQ) correlation spectrum, where correlations between the adamantane (**1**) and CB[7] hydrogen atoms (overlapping H_a and H_c signals) are clearly visible. The experiment was carried out in the presence of 0.5 equiv CB[7], and clearly differentiates between the signals of free and CB[7]-bound adamantane (**1**). An additional strong autocorrelation signal of the adamantane nuclei indicates larger domains of neat **1** in this sample (Figure 3).

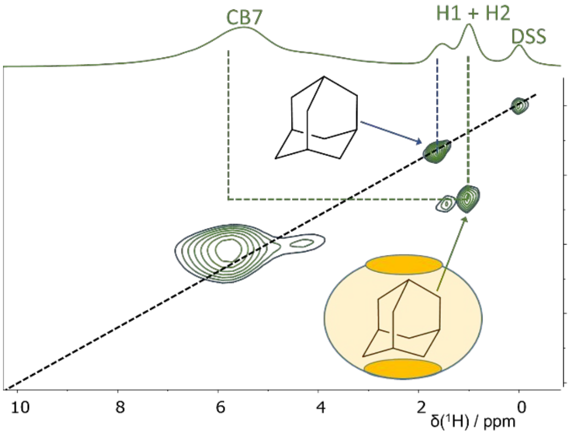

Figure 3. ^1H–^1H DQ–SQ MAS spectrum of a milled 2:1 mixture of adamantane (**1**) and CB[7] obtained using rotor-synchronized BABA (back-to-back) recoupling at a MAS rate of 70 kHz. DSS was used as an internal standard.

To confirm that ball milling indeed leads to inclusion complexes similar to those obtained in solution, and not merely to exclusion complexes, the ^1H ssNMR spectrum of the sample obtained after ball milling (Figure 4a) was compared with the spectrum of the mixture obtained after evaporation of a water solution of the guest and host (Figure 4b). Both spectra are indeed identical, except for a residual water signal present in the sample prepared from the aqueous solution (Figure 4b). Notably, its chemical shift (4.3 ppm) is very different from the one observed in free CB[7] (1.3 ppm, Figure 2a). We propose that the latter belongs to water inside the CB[7] cavity, while the former corresponds to water at the CB[7] portals.

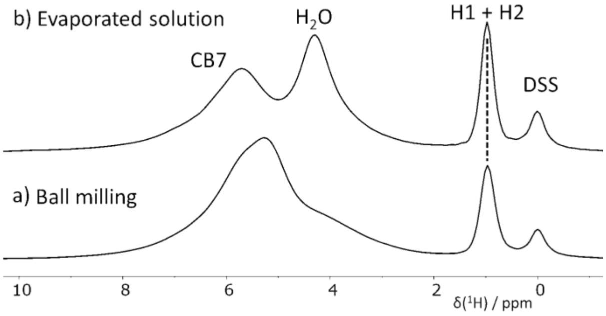

Figure 4. ^1H ssNMR spectra of a 1:1 mixture of adamantane (**1**) and CB[7] (a) after ball milling, and (b) prepared in water and evaporated to dryness. DSS is used as internal standard.

Guests **2**–**4** also form host-guest inclusion complexes with CB[7] upon ball milling (Figure 5 and Figures S13–S27), with similar upfield shifts in ^1H spectra upon encapsulation, with the exception of the methyl group in *p*-toluidine (**3**) where a 0.6 ppm downfield shift is observed. In the latter case, the significant difference in the chemical shift measured in aqueous solution (2.3 ppm) and in the solid state (1.0 ppm) suggests an unusual packing feature of the free guest in the solid state. However, after milling with CB[7], the methyl signal resonates at 1.6 ppm, i.e. close to the value observed in a water solution of the **1**⊂CB[7] complex (see ESI section for details). Symbol "⊂" is used for the inclusion complexes. We also observe that the aromatic hydrogen nuclei of guest **4** are non-equivalent in the solid state (8.1 and 6.8 ppm); this is in agreement with its known crystal structure.[34] The aromatic protons become equivalent and resonate at a lower frequency (6.6 ppm) upon encapsulation in CB[7].

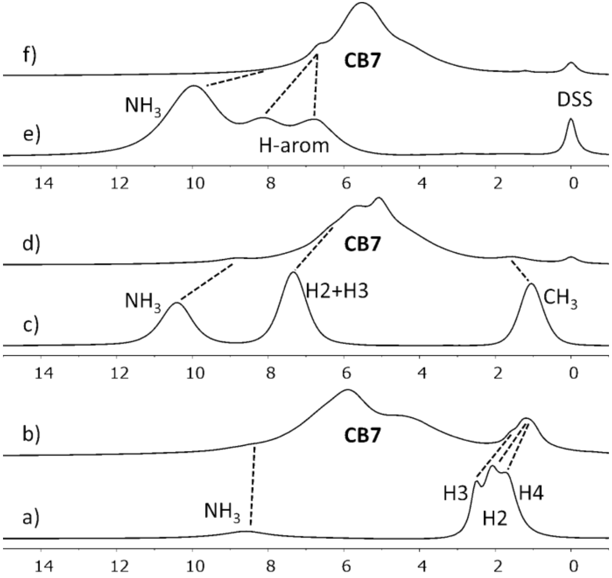

Figure 5. ^1H ssNMR spectra of: (a) free guest **2**, (b) complex **2**⊂CB[7], (c) free guest **3**, (d) complex **3**⊂CB[7], (e) free guest **4**. (f) complex **4**⊂CB[7]. DSS is used as internal standard, and all complexes obtained by ball-milling. See ESI for magnified spectra.

Differential scanning calorimetry (DSC) traces of free adamantane (**1**), free CB[7] and complex **1**⊂CB[7] (1:1 ratio) were recorded on ball-milled samples (Figure 6). In agreement with published data,[28, 35] the broad endotherm centered at □140 °C corresponds to the slow evaporation of water molecules from the cavity and portals of CB[7] while the second one at □380 °C refers to the decomposition of the macrocycle (Figure 6a). The DSC trace of adamantane (**1**)[36] is characterized by two sharp endotherms: a phase transition at −64 °C, and sublimation at □227 °C (Figure 6b). The DSC trace of complex **1**⊂CB[7] shows two broad endotherms (Figure 6c) and resembles the trace of neat CB[7]. The broad peak at □140 °C again corresponds to the loss of residual water molecules at the carbonyl rims, and the endotherm at □420 °C is attributed to the decomposition of the inclusion complex. Both endotherms characterizing neat adamantane (**1**) are absent in complex **1**⊂CB[7], thereby confirming guest encapsulation.

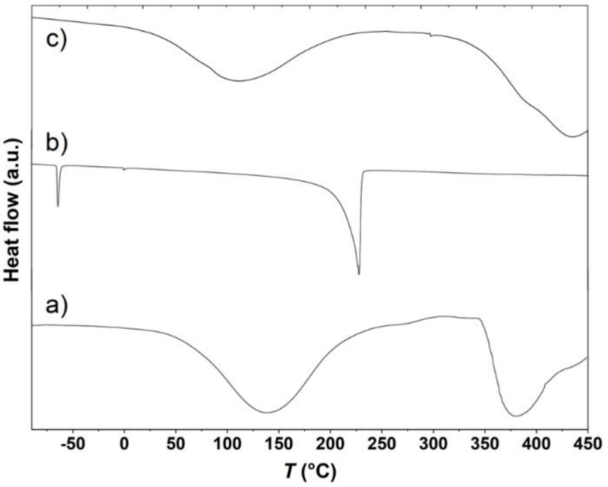

Figure 6. Differential scanning calorimetry traces from −90 °C to 450 °C: (a) neat CB[7], (b) neat adamantane (1), and (c) complex **1**$\subset$CB[7]. Endotherms are shown as negative peaks.

As reminded by Bardelang and coworkers,[37] free CB[7] is notoriously difficult to crystallize, with only two crystal structures reported in the literature by Kim and coworkers.[31, 38] While the carbonyl portals certainly interact with the positive outer-wall of neighboring CB[7] units (this herringbone pattern is immediately apparent in crystalline CB[7], as well as in CB[5], CB[6] and CB[8]),[38] the interaction is likely loose enough to allow the mechanical penetration of the guests (like CO$_2$ in CB[7][39] or acetylene in CB[6][40] for example) without major obstruction at the CB[7] rims. As noted above, solid CB[7] is hygroscopic, and contains residual water and hydrochloric acid despite thorough drying. The driest sample used in this study (apparent molecular weight 1471–1485 g/mol, as determined by elemental analysis and NMR titration, respectively) was found to contain approximately 11 water and 3 HCl molecules. Nau and coworkers showed that 7–8 of these water molecules can fit inside the cavity of the macrocycle.[41] We suspect that upon guest encapsulation, the ejected water molecules added to the interstitial ones already present in the solid could also provide local environments reminiscent of high concentrations in the solution phase, and again favor efficient guest encapsulation.

Using state-of-the-art ssNMR techniques, we showed unequivocally that ball milling leads to the same host-guest inclusion complexes of compounds **1**–**4** with CB[7] as those formed in aqueous solution. Successful formation of inclusion complexes was also independently confirmed by DSC. Mechanical encapsulation could thus serve as an alternative to mostly used “wet” approaches to the formation of supramolecular complexes, and could lead to new types of materials or architectures that are not accessible in solution due to limited solubility or unfavorable deaggregation of the individual components.

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There are no conflicts to declare.

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