Carborane guests for cucurbit[7]uril facilitate strong binding and on demand removal

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ABSTRACT: High affinity guests have been reported for the macrocyclic host cucurbit[7]uril (CB[7]), enabling widespread applications, but preventing CB[7] materials from being returned to their guest-free state for reuse. Here we present polyhedral boron clusters (carboranes) as strongly-binding, yet easily removable, guests for CB[7]. Aided by a Pd-catalyzed coupling of an azide anion, we prepared boron-functionalized 9-amino and 9-ammonium modified *ortho*-carboranes that bind to CB[7] with a K_a=10^{10} M^{-1}. Upon treatment with base, the *ortho*-carboranes readily undergo deboronation to yield anionic *nido*-carborane, a poor guest of CB[7], facilitating recovery of guest-free CB[7]. We showcase the utility of the modified *ortho*-carborane guest by recycling a CB[7]-functionalized resin. With this report, we introduce stimuli-responsive decomplexation as an additional consideration in the design of high affinity host-guest complexes.

Molecular recognition is ubiquitous within the natural world with the genetic code, enzymes, and immune systems all reliant on non-covalent interactions between biomolecules.[1,2] These phenomena have inspired chemists to develop host-guest complexes in hopes of achieving comparable degrees of specificity for applications in functional materials,[3] sensors,[4] biological assays,[5] and therapeutics.[6–9] Much of the early molecular recognition work involved crown ether, cyclophane, and cyclodextrin hosts that exhibit modest binding affinities ($K_a \leq 10^5$ M^{-1}).[10] More recently, there has been a focus on the development of high affinity ($K_a \geq 10^{10}$ M^{-1}) host-guest complexes that rival the binding affinities of Nature's best molecular recognition systems: antibody-antigens and (strept)avidin-biotin.[11] High binding affinity complexes facilitate the expansion of host-guest chemistry to applications in dilute, complex settings and decrease the need to exploit multivalency.[12] However, large K_a values also lead to difficulties in dissociating the pair on-demand, limiting the reversibility, and therefore the flexibility of the system (Figure 1A).[13] Here we present "decomplexation", the ability to remove a guest on demand, as an important feature in the design of high affinity host-guest pairs. (Figure 1B).

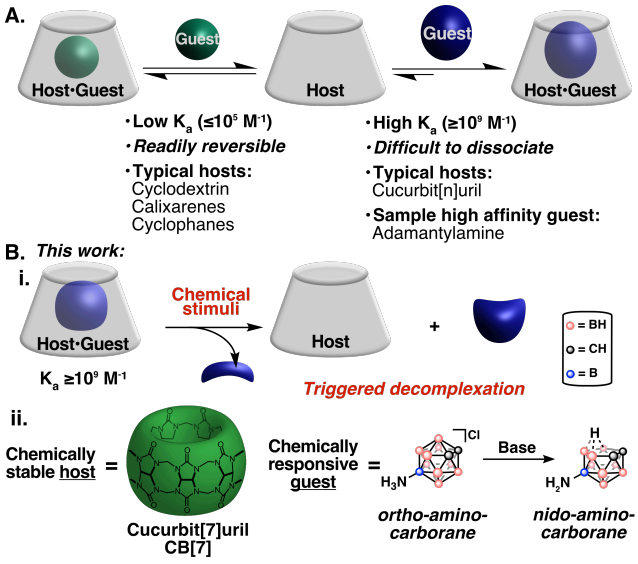


Figure 1. (**A**) Synthetic host-guest pairs are typically characterized either by low binding affinities and reversible association or high binding affinities with limited dissociation. (**B**) (i) This work presents triggered decomplexation as an advantageous property for high-affinity host-guest complexes. (ii) We demonstrate this concept with a cucurbit[7]uril (CB[7]) and *9*-amino-*ortho*-carborane host-guest pair that undergoes decomplexation via a mild chemical trigger.

Cucurbiturils, cyclic oligomers of glycoluril linked by methylene units, have become the host of choice for high-affinity complexes.[14,15] The heptamer, cucurbit[7]uril (CB[7]), displays the largest binding affinities, with the highest reported K_a being 10^{15} M^{-1} for complexation with 1,6-*N*-trimethylammonium diamantine at pH 4.75.[16] The high binding affinity was achieved obtaining complete displacement of the encapsulated water molecules and optimizing the alignment of cationic functionality with the carbonyl rings.[17] Other high affinity guests for CB[7] are adamantylamine (K_a=10^{12} M^{-1}) and derivatives (K_a=$10^{4\text{-}15}$ M^{-1})[16,18] as well as functionalized ferrocenes (K_a=$10^{9\text{-}15}$ M^{-1}).[15]

Due to the high binding affinities presented by various cationic guests, the CB[7] scaffold has been extensively studied as a biotin-(strept)avidin mimic.[19–23] CB[7] has also found use in small molecule separation,[24] dynamically crosslinked polymers,[3,25] surface patterning[26,27] and sensor development.[28] In many of these applications, CB[7] is immobilized on solid-supports and the high binding affinity of guests with CB[7] render these materials one-time use. If compounds captured by the immobilized CB[7] need to be released, a higher affinity guest is introduced to displace the captured material. The difficulty in returning surfaces and devices containing CB[7] to their initial guest-free state limits the use of CB[7] to highly specialized applications where cost and scale are not significant factors.

To enable recycling of CB[7]-containing materials, we designed a high affinity guest for CB[7] that upon chemical treatment could be transformed into a weak guest for easy removal. A guest that can undergo decomplexation could either be employed as the primary guest in the experiment or could be used in a separate step to displace an application-specific guest and then undergo decomplexation to allow the CB[7] material to return to its original guest-free state. To date, methods for high-affinity guest removal from the CB[7] cavity are limited to excessive salt treatment that suffer the same limitations as biotin-(strept)avidin systems. [23,29]

In our search for ideal guests to fit the criteria of both high binding affinity to CB[7] and the ability to be removed by triggered decomplexation, we looked to guests with shape complementarity to the cavity of CB[7] that could be readily transformed into a fragment that is poorly encapsulated by CB[7].[18,30–32] Icosahedral carboranes of the type $C_2B_{10}H_{12}$ appeared primed to meet these requirements. These 3D aromatic clusters[33–35] bear a close topological similarity to adamantane[36,37] and one of the three isomers, *ortho*-carborane, has previously been employed as a guest for CB[7], although no K_a was reported.[38] Importantly, carboranes can undergo Lewis base-mediated boron vertex removal (termed "deboronation") to generate the *nido*-7,8-$C_2B_9H_{11}$ anion (Figure 1B).[39–41] *Ortho*- and *meta*-carborane have significantly different rates of deboronation, as well as distinct dipole moments.[36,42] We set out to explore the binding affinities of derivatives of these two carborane isomers with CB[7] and their potential for removal upon deboronation to create a reliable recycling system for the CB[7] . We hypothesized that *ortho*-carboranes would be readily decomplexed from CB[7] upon base treatment, while the *meta*-carborane would act as a control.

The electronic non-uniformity of carboranes is widely recognized, resulting in different electronic influences on bound substituents depending on the cage vertex.[43] The B9 positions of *ortho*- and *meta*-carborane are the most electron-rich positions in the corresponding carboranes. Given the similar inductive electronic effects of B-bound substituents on carboranes compared to bulky alkyl groups, we targeted N-substitution at the B(9) vertices of the carboranes to most closely mimic the electronic environment of adamantylamine. Despite a variety of methods to functionalize *ortho*- and *meta*-carborane,[44–48] the synthetic methodology developed for the amination of 9-Br-*meta*-carborane[49] is incompatible with 9-Br-*ortho*-carborane (**1**) due to deboronation by the basic reaction conditions. Alternative routes to nitrogen-substituted *ortho*-carboranes were similarly unsuccessful for the generation of free amine at the B9-position.[50]

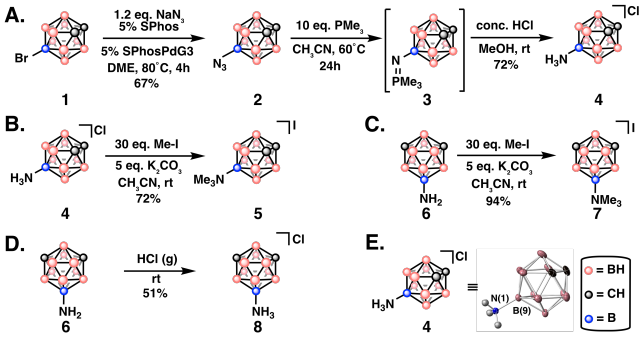
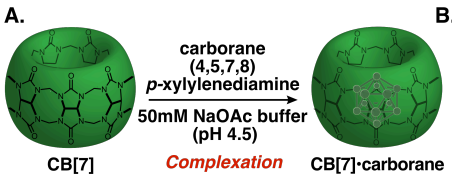


Figure 2. (**A**) Synthetic route to 9-amino-*ortho*-carborane (**4**) through 9-azido-*ortho*-carborane (**2**). (**B, C**) Treatment of **4** and **6** with Me-I affords trimethylated derivatives **5,7**. **(D**) Formation of salt **8**. **(E**) Single crystal X-ray diffraction of **4** (chloride counterion and cage-based hydrogens removed for clarity). SPhos and SPhosPdG3 are the Pd(II) catalysts and precatalysts used for the transformation, respectively.

Thus, a new synthetic route (Figure 2A) to furnish the B9 aminated *ortho*-carborane target was necessary. Treatment of 9-Br-*ortho*-carborane (**1**) with NaN_3 under Pd-catalyzed cross-coupling conditions afforded the desired 9-N_3-*ortho*-carborane (**2**)[49] in 67% yield.[51] This is a rare example of Pd-catalyzed cross-coupling of an azide anion. Subsequent Staudinger reduction using trimethylphosphine (PMe_3) and hydrolysis with concentrated HCl gave the desired hydrochloride salt **4**, confirmed by crystallography (Figure 2E), in 72% overall yield. To probe

the effect of a permanent positive charge on the guest, the trimethylammonium derivatives of **4** and **6** (**5** and **7**, respectively) were prepared in good yield through treatment with MeI (Figure 2B,C). We prepared the analogous hydrochloride salt 9-NH_3-*meta*-carborane (**8**) by treating the corresponding amine with gaseous HCl (Figure 2D) to aid in binding affinity investigations.



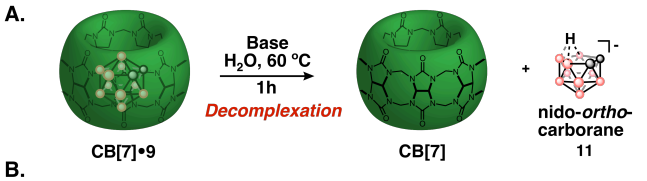
B.

#	K_a (M^{-1})
4	(3±2) x 10^{10}
5	(1.1±0.2) x 10^{10}
8	(2±2) x 10^{10}
7	(1.8±0.8) x 10^{9}

Figure 3. (**A**) Association of carborane with CB[7]. (**B**) Binding affinities of water-soluble carborane derivatives (**4**,**5**,**7**,**8**) (1 eq.) determined by ^{1}H NMR spectroscopy competition experiments with *p*-xylylenediamine (2 eq.) in 50mM NaOAc buffer.[18]

The aqueous solubility of the amino and ammonium functionalized carboranes (**4, 5, 7, 8**) allowed binding affinities to be determined by ^{1}H-NMR spectroscopy in acetate buffer. Competition experiments against *p*-xylylenediamine were performed.[18] Interestingly, three of the four 9-aminocarboranes (**4, 5, 8**) displayed similar binding affinities of $K_a \approx 10^{10}$ M^{-1} in pH 4.5 NaOAc buffer (Figure 3). The exception was 9-trimethylammonium-*meta*-carborane (**7**) where methylation decreases the K_a by an order of magnitude. We note that the binding affinities are not quite as high as those observed with adamantylamine, despite the structural analogy between carborane and adamantane.[52] This could be could be due to the greater inherent net dipole of carboranes that alters the positioning in the CB[7] hydrophobic pocket. Nevertheless, at $K_a = 10^{10}$ M^{-1} we have established carboranes as a new class of high affinity guests for CB[7].

Next, we investigated the ability for carborane guests to be decomplexed from CB[7] on demand. For initial studies we employed *ortho*-and *meta*-carborane, which we predicted to have orthogonal decomplexation properties (Figure 4A). We characterized the **CB[7]•carborane** complexes in trifluoroacetic acid (TFA), due to limited water solubility of unfunctionalized carborane.[62] We found that a stable complex between CB[7] and *ortho*- and *meta*-carborane (35–55 mM, 1.2 eq) readily formed upon sonication and could be purified by washing with organic solvent. Carborane is clearly seen in the aqueous solution of host-guest complex via ^{11}B-NMR spectroscopy when both *ortho*- (**9**) and *meta*-carborane (**10**) are introduced (Figure S7).



B.

Entry	Carborane	Base	1h	2h	8h	24h
1	**9**	$HNMe_2$ (1M THF)	9%	17%	45%	86%
2	**9**	$HNMe_2$ (40% in H_2O)	<5%	<5%	<5%	<5%
3	**9**	Pyridine	<5%	<5%	<5%	<5%
4	**9**	**Piperidine**	**>95%**	**>95%**	**>95%**	**>95%**
5	**4**	Piperidine	8%	19%	>95%	>95%
6	**10**	Piperidine	<5%	<5%	<5%	<5%
7	**8**	Piperidine	<5%	<5%	<5%	<5%

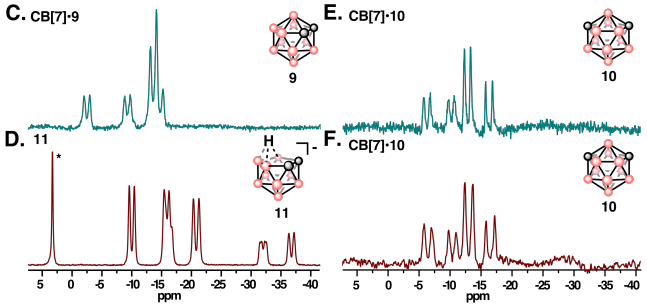


Figure 4: (**A**) Decomplexation of the **CB[7]•9** complex through deboronation of *ortho*-carborane (**9**) with base to yield to *nido-ortho*-carborane (**11**) and free **CB[7]**. (**B**) Table of conditions screened to evaluated the decomplexation of **CB[7]•carborane** complexes. Generation of **11** was calculated by relative integration of baseline corrected ^{11}B NMR spectra. **CB[7]•carborane** complex and base (5 equiv.) were combined in H_2O, stirred at 60 °C, and monitored by ^{11}B NMR spectroscopy. (**E, F**) ^{11}B NMR spectra taken before (**C**) and after (**D**) 1h of subjecting **CB[7]•9** complex (B,C) or **CB[7]•10** complex (E,F) to 20% piperidine/H_2O ($^v/_v$) at 60 °C. * denotes borate side-product known to form during **9** deboronation.

We screened a panel of bases that are known to deboronate *ortho*-carborane in solution to efficiently yield *nido-ortho*-carborane (Figure 4B). For decomplexation of *ortho*-carborane from CB[7], we found that aqueous solubility was the most important factor determining the rate of deboronation as exemplified by piperidine's superiority compared to dimethylamine ($HNMe_2$) (Figure 3B, entries 1–2, 9). The volatility of dissolved $HNMe_2$ likely also contributes to its inferior deboronating ability under the reaction conditions (Figure 4B, entries 1–2).

After establishing 50% aqueous piperidine at 60 °C as optimal decomplexation conditions for **CB[7]•9**, we applied similar conditions to the *meta*-carborane complex (**CB[7]•10**). Distinct differences are immediately apparent with the *ortho*-carborane undergoing deboronation to yield *nido-ortho*-carborane, **11**, while no transformation was apparent with the *meta*-carborane (Figure 4C-F, Figure 4B, entry **6**). These results are consistent with the diminished electrophilicity of the boron vertices

adjacent to carbon vertices in **10** as compared to **9** and demonstrate the potential for orthogonal chemical behavior of sterically identical guest molecules encapsulated by CB[7]. Importantly, the 9-aminocarboranes **4** and **8** (Figure 4B, entries 5, 7) follow the same deboronation trends as the unfunctionalized compounds.

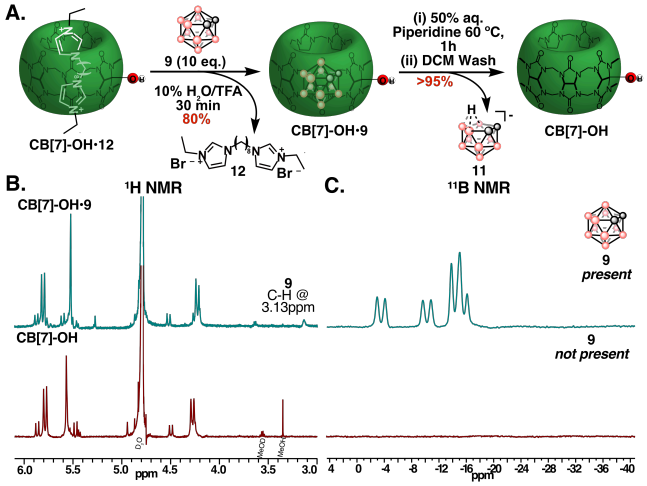


Figure 5: (A) Straightforward isolation of guest-free **CB[7]-OH** by guest exchange with **9** and subsequent decomplexation with piperidine. (**B,C**) Displacement of **12** by *ortho*-carborane (**9**) and formation of **CB[7]-OH•9** (top, blue) and decomplexation and removal of (**11**) to form a guest-free **CB[7]-OH** cavity (bottom, red) was observed by ^{1}H-NMR (B) and ^{11}B-NMR spectroscopy (C).

To showcase the utility of the "on-demand" decomplexation offered by the *ortho*-carborane guests we used **9** to isolate **CB[7]-OH** (Figure 5A). **CB[7]-OH** is an important intermediate for the creation of CB[7] conjugates, materials, and devices. CB[7] can be readily monohydroxylated by treatment of CB[7]•**12** with persulfate salts; however we found efficient removal of **12** from the **CB[7]-OH** cavity to be difficult.[29] Gratifyingly, **12** could be displaced with **9** in as little as 30 min in a H_2O/TFA mixture. Upon evaporation of the TFA, treatment with piperidine for 1 hour followed by a dichloromethane wash provided guest free **CB[7]-OH**. The progress of complexation of **9**, deboronation, and removal of **11** by washing can be easily monitored by ^{1}H NMR and ^{11}B NMR spectroscopy (Figure 5B,C). In our hands, this is the fastest and highest yielding procedure to synthesize and isolate guest-free **CB[7]-OH** to-date. The decomplexation method was also used to prepare guest-free **CB[7]-N$_3$**, another important functionalized **CB[7]**.[53,54]

To demonstrate the ability to recycle CB[7]-constructs via decomplexation of *ortho*-carborane guests, we conjugated **CB[7]-N$_3$**[53,55] to bicyclononyne-functionalized Wang resin (Wang-BCN, Figure 6A, Scheme S2) using copper-free click chemistry and confirmed successful immobilization using fluorescein adamantylamine conjugate **13**.

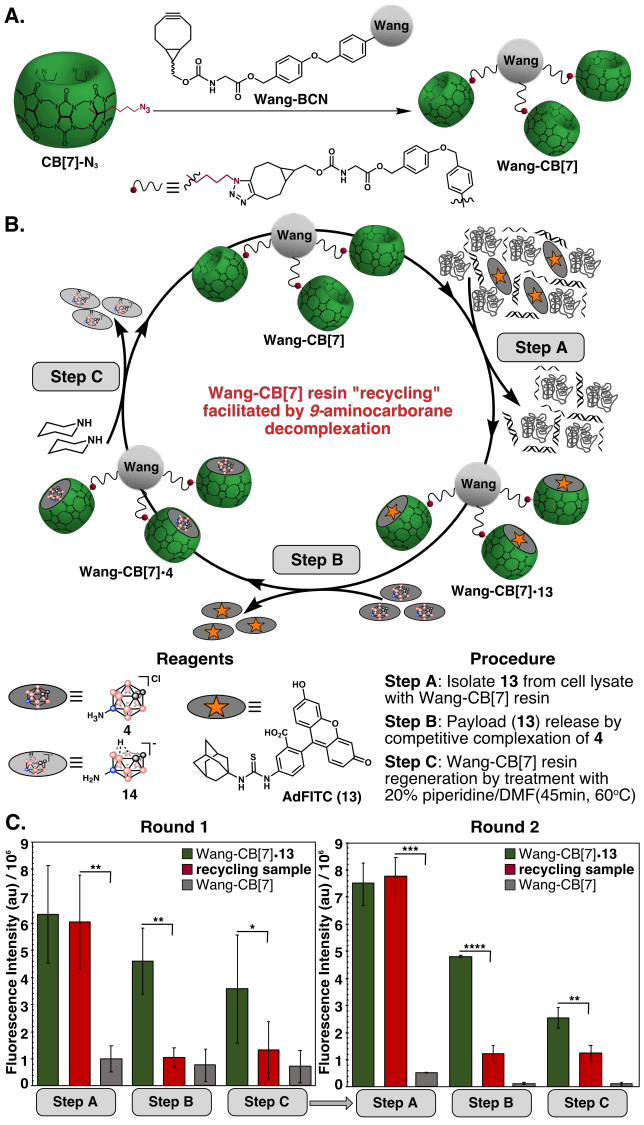


Figure 6: (**A**) Scheme depicting attachment of CB[7]-N_3 to bicyclononyne (BCN)-functionalized Wang resin to produce Wang-CB[7]. (**B**) Wang-CB[7] was used to isolate payload, **13**, from Jurkat lysate in 50% DMF/H_2O (Step A), and was regenerated by complexation of **4** (Step B) followed by decomplexation (Step C). (**C**) Fluorescence of Wang-CB[7] throughout the recycling sequence (red) compared to fluorescent Wang-CB[7]•**13**–samples not incubated with **4** in Step B–(green) and non-fluorescent Wang-CB[7]–samples not incubated with **13** or **4** in Steps A and B–(gray). Error bars represent standard deviation of 3 replicate samples. *P≤0.05, **P≤0.01, ***P≤0.001, ****P≤0.0001.

The resulting **Wang-CB[7]** resin was added to Jurkat lysate (2 mg/mL) containing **13** to selectively isolate the fluorescent guest from a complex mixture (Figure 6B, Step A). After washing away cell lysate, displacement with **4** rapidly releases **13** (Figure 6B, Step B). Finally, treatment with piperidine deboronates **4** to produce **14** and regenerate **Wang-CB[7]** (Figure 6B, Step C). The success of each step was monitored through the fluorescence of **Wang-CB[7]** (red) and compared to fluorescent

Wang-CB[7]•13, where displacement by **4** was omitted (green) and non-fluorescent **Wang-CB[7]**, where addition of **13** and **4** were omitted (gray) controls (Figure 6C). The cycle was repeated twice, at which point signal became too low due to significant loss of resin in the washing steps. Recycling of the resin represents a novel method of reusing precious CB[7]-constructs that can be applied for payload isolation in many biological and materials application settings.

In this report, we present a new cucurbit[7]uril guest scaffold based on icosahedral boron clusters (carboranes), which are high affinity ($K_a \approx 10^{10}\ M^{-1}$) binders for CB[7] that can be removed on demand through deboronation chemistry. We designed 9-aminocarborane guests to mimic the size and charge of adamantylamine and took advantage of the differential reactivity of carborane isomers to prepare guests that were (*ortho*) and were not (*meta*) readily deboronated. To access 9-amino-*ortho*-carborane, we report a rare example of Pd-catalyzed cross coupling with azide. We utilized this scaffold to efficiently prepare guest-free CB[7]-OH and showcase the opportunity to "recycle" CB[7]-constructs that can be continuously employed in biological assays and materials applications. We envision this work will overcome limitations of traditional biotin-(strept)avidin systems and enable CB[7] sensors and technologies. This work further highlights how unique stimuli-responsive features of boron clusters can aid the development of new complex hybrid chemical systems.[56–64]

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