Functionalized cucurbiturils and their applications

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Cucurbit[*n*]uril (CB[*n*], *n* = 5–10), a new family of molecular hosts comprising *n* glycoluril units, have gained much attention in the new millennium for their exceptional molecular recognition ability. The CB homologues have brought dynamism to CB chemistry, as witnessed by the heightened interest in the field for the last several years. Compared to the chemistry of cyclodextrins and calixarenes, however, that of CB[*n*] has developed slowly until recently, which may be attributed mainly to their poor solubility in common solvents, and inability to functionalize these molecules. The direct functionalization method of CB[*n*] propelled CB chemistry to a new height as this new method not only solved the solubility problem but also opened up the gateway to the generation of tailor-made CB[*n*] derivatives. The functionalization of CB[*n*] led us to investigate numerous applications including artificial ion channels, vesicles, stationary phases in chromatography, ISEs, polymers, nanomaterials, and many others. This tutorial review describes the recent advances and challenges in the functionalization of CBs along with the applications of functionalized CBs.

1. Introduction

Cucurbit[*n*]uril (CB[*n*]) is a family of macrocyclic compounds comprising *n* glycoluril units, self-assembled from an acid-catalyzed condensation reaction of glycoluril and formaldehyde. Although the synthesis of the parent compound, CB[6] was first reported by Behrend *et al.* in 1905,[1] its chemical nature and structure had been unknown until 1981 when full characterization was reported by Mock and co-workers.[2] The pumpkin-shaped molecule has a hydrophobic cavity and two identical carbonyl-laced portals. Extensive studies on the host–guest behavior of CB[6] were conducted by Mock and others.[3–5] While the hydrophobic interior provides a potential inclusion site for nonpolar molecules, the polar ureido carbonyl groups at the portals allow CB[6] to bind ions and molecules through charge–dipole and hydrogen bonding interactions. The unique structure and recognition properties make CB[6] attractive not only as a synthetic receptor but also as a building block for the construction of supramolecular architectures. A wide variety of supramolecular species such as rotaxanes, catenanes, and molecular machines incorporating CB[6] have been reported.[6] Other applications of CB[6] such as in catalysis[7] have also been demonstrated.

In 2000, we reported the discovery of other members of the CB family, CB[*n*] (*n* = 5–11) and successful isolation of CB[*n*] (*n* = 5, 7 and 8),[8] which has broadened the scope of CB chemistry enormously. Within a few years, their host–guest

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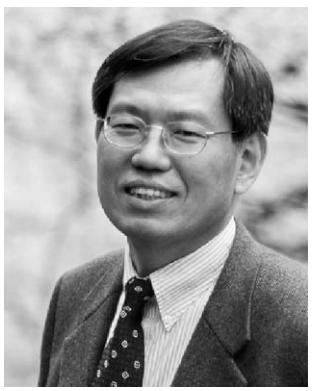


Kimoon Kim

Kimoon Kim studied chemistry at Seoul National University (BS, 1976), Korea Advanced Institute of Science and Technology (KAIST) (MS, 1978) and Stanford University (PhD, 1986). After postdoctoral work at Northwestern University, he joined Pohang University of Science and Technology where he is now a Professor of Chemistry. He is also director of the Center for Smart Supramolecules (CSS) supported by the Creative Research Initiative Program of the Korean Ministry of Science and Technology. His current research focuses on developing novel supramolecular systems toward functional materials and devices.

Narayanan Selvapalam

Narayanan Selvapalam, born in India, received his MS (1990) and PhD (1997) degrees from Mysore University and IIT Delhi, respectively. During 1997–1999, he worked at the R&D center of the Cancer Institute (WIA), Chennai. After working at KAIST as a postdoctoral fellow for a year he joined CSS in 2001. Since 2005, he has been a research assistant professor in the group of Professor Kimoon Kim, and actively involved in the cucurbituril synthesis.

chemistry as well as novel supramolecular assemblies built with CB[*n*] has been extensively studied by us as well as others.[9,10] For example, redox control of guest binding, formation of a macrocycle within a macrocycle, inclusion of two identical or different guest molecules, encapsulation of drugs, promotion of reactions with a large rate acceleration and regio- and stereochemical control, and stabilization of unstable species by encapsulation have been observed. In particular, our discovery of formation of a stable charge-transfer (CT) complex inside CB[8][11] led us and others to build a number of supramolecular assemblies based on this chemistry, including polyrotaxanes, molecular necklaces, dendrimers, vesicles, and molecular machines, and to recognize amino acids and small peptides carrying aromatic residues.[12]

The rich supramolecular chemistry made the host family join other celebrity host families such as crown ethers, cyclodextrins, and calixarenes, but their practical applications were still limited mainly because of their poor solubility in common solvents, and difficulty in introducing functional groups on their surfaces. In particular, unlike other host families, functionalization of these molecules was a daunting task. For example, introduction of substituents at the 'equator' of CBs by condensation of substituted glycoluril with formaldehyde was achieved with only limited success. Reaction of a mixture of substituted glycoluril and unsubstituted glycoluril with formaldehyde produces a complicated mixture of unsubstituted and substituted CBs, separation of desired products from which is practically impossible or laborious. However, our recent discovery of a direct functionalization method of CB[*n*][13] allowed us to synthesize a wide variety of CB derivatives easily and to study many applications. Ion channels, vesicles, polymers, ion selective electrodes incorporating CB[*n*], and CB-immobilized solid surfaces and silica gel have been reported and numerous other applications are being explored. As such, the functionalization has paved the way to applications of CBs, in the same manner as the upsurge in applications of CDs after they were functionalized. This review is the documentation of recent developments in CB chemistry including the synthesis and applications of functionalized CB[*n*] primarily based on the results obtained from our own laboratory.

2. Characteristic features of CB[*n*] and recent developments in CB[*n*] chemistry

Since the chemistry of CB[*n*] has been recently reviewed by us[9] and more comprehensively by Isaacs *et al.*,[10] in this section we will just briefly describe some characteristic features including the host–guest chemistry of the CB family and the latest developments in the chemistry of unfunctionalized CB[*n*].

Young Ho Ko

Young Ho Ko graduated from Konkuk University with a BS degree in 1990, and received his MS and PhD degrees from Pohang University of Science and Technology in NMR spectroscopy under Professor Hee Cheon Lee in 1992 and 1998, respectively. After three years postdoctoral work at the Center for Biofunctional Molecules, he joined the group of Professor Kim in 2001. His current research interests encompass NMR spectroscopy of supramolecular systems and host–guest chemistry.

Kyeng Min Park

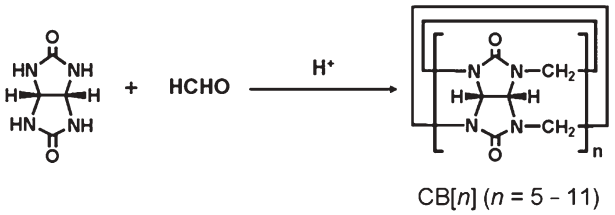
Kyeng Min Park was born in Daejeon, Korea in 1976. He studied industrial chemistry at Hongik University and received a BS degree in 2003. He is currently a PhD student working on biological applications of cucurbituril derivatives at Pohang University of Science and Technology under the supervision of Professor Kimoon Kim.

Dongwoo Kim

Dongwoo Kim received a BS degree in chemistry from Seoul National University in 2000, and MS and PhD degrees in supramolecular chemistry under Professor Kimoon Kim from Pohang University of Science and Technology in 2002 and 2006, respectively. His doctoral thesis focused on the fabrication of supramolecular polymers on surfaces and the direct synthesis of polymer nanocapsules and two-dimensional polymers. He currently is a postdoctoral research fellow at CSS.

Jeeyeon Kim

Jeeyeon Kim was born in Yongin, Korea in 1980. She earned a BS degree in both applied chemistry and bio-engineering from Ajou University in 2004. She is currently a PhD student under Professor Kimoon Kim at Pohang University of Science and Technology. Her research focuses on biological applications of functionalized cucurbiturils.



Scheme 1

2.1. Synthesis, structures, physical properties of CB[*n*]

Reaction of glycoluril with formaldehyde in mineral acids, such as 9 M H_2SO_4 or conc. HCl, at 75–90 °C, yields a mixture of the CB[*n*] family (Scheme 1). The lower reaction temperature compared to that employed in conventional CB[6] synthesis (>110 °C) is the key to the formation of significant amounts of CB homologues in addition to CB[6]. CB[*n*] (*n* = 5, 7 and 8) can be separated in pure form using fractional crystallization and dissolution,[8] or chromatography.[14] From the reaction mixture, Day and co-workers also isolated CB[5]@CB[10], in which CB[5] is trapped in the cavity of CB[10].[15] Recently, Isaacs and co-workers successfully isolated free CB[10] from this complex by replacing the guest CB[5] with melamine diamine followed by removal of the new guest through acylation and excessive washing.[16] We recently discovered that microwave assisted synthesis provides a fast and efficient way of producing a CB[*n*] mixture, which may be useful for the industrial scale production of CBs in the future.[17]

Table 1 compares some structural parameters of the CB homologues. On going from CB[5] to CB[8], the mean diameter of the internal cavity increases progressively from ~4.4 to ~8.8 Å, keeping the height 9.0 Å. Likewise, the portal increases its mean diameter from ~2.4 to ~6.9 Å. Note that both diameters increase by 1.5 Å as we move to the next higher homologue. In terms of the cavity sizes, CB[6], CB[7] and CB[8] are analogous to α-, β- and γ-CD, respectively. No X-ray crystal structure of guest-free CB[10] is available, but those of CB[5]@CB[10][15] and G_2·CB[10] (G = melamine diamine)[16] show an ellipsoidal deformation of the large macrocycle. The diameters of the portal and internal cavity of CB[10] range from ~9.0 to ~11.0 Å and from ~10.7 to ~12.6 Å, respectively, and its cavity volume is almost two times that of CB[8]. It is not clear whether the deformation is intrinsic or due to the encapsulation of guest molecule(s).

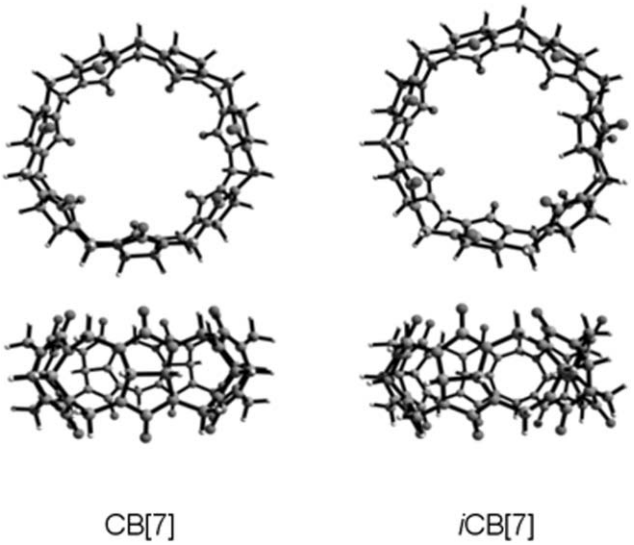
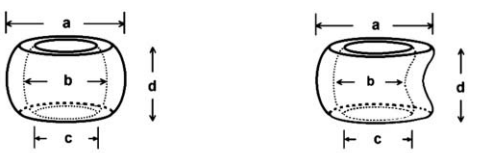


Fig. 1 X-ray crystal structures of CB[7] and *i*CB[7].

In general, solubility of the CB homologues in common solvents is low (<10^{-5} M) except that CB[5] and CB[7] have a moderate solubility in water (2–3 × 10^{-2} M), which is comparable to that of β-CD (1.6 × 10^{-2} M). All the CB homologues are soluble in acidic aqueous solution or aqueous solution containing alkali metal ion. This is why in earlier days guest binding constants of CB[6] were mostly measured in formic acid–water (1 : 1). Most CB[*n*] and their derivatives known thus far are non-toxic, at least in *in vitro* tests.

A new addition to the CB family is inverted cucurbit[*n*]uril (*i*CB[*n*]), which contain an inverted glycoluril unit. We and Isaacs group together isolated *i*CB[6] and *i*CB[7] in 2.0 and 0.4% yields, respectively, from the reaction mixture of glycoluril and formaldehyde.[18] The inverted glycoluril unit with two methine protons pointing inwards makes the cavity less spacious and less symmetrical compared to that of corresponding normal CB[*n*] (Fig. 1), which is manifested in guest binding (see below). *i*CBs are kinetic products as confirmed by product resubmission experiments. For example,

Table 1 Dimensions of CB[*n*] and *i*CB[*n*]



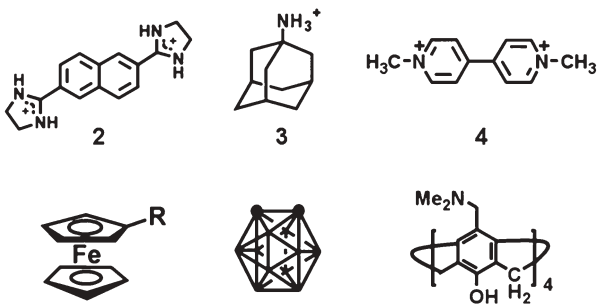
		CB[5]	CB[6]	CB[7]	CB[8]	[a]CB[10]	*i*CB[6]	*i*CB[7]
Outer diameter/Å	a	13.1	14.4	16.0	17.5	18.7–21.0	13.3–14.4	14.9–16.0
Cavity/Å	b	4.4	5.8	7.3	8.8	10.7–12.6	4.2–5.8	5.7–7.3
	c	2.4	3.9	5.4	6.9	9.0–11.0	3.9–5.5	5.4–6.1
Height/Å	d	9.1	9.1	9.1	9.1	9.1	9.1	9.1
Cavity volume/Å^3	—	82	164	279	479	870	—	—

[a] Determined from the X-ray structure of CB[5]@CB[10] complex.[15]

heating *i*CB[6] in conc. HCl transforms it into a mixture of CB[5], CB[6], and CB[7] (24 : 13 : 1).

2.2. Host–guest chemistry

Although CB[*n*] share characteristic features of CB[6], a hydrophobic cavity and polar carbonyl groups surrounding the portals, their varying cavity and portal sizes lead to remarkable molecular recognition properties, different from those of CB[6].[9,10] CB[6] has been known to form stable host–guest complexes with alkyl ammonium ions, particularly α,ω-alkyl diammonium ions of varying chain lengths ($^+NH_3(CH_2)_nNH_3^+$ (**1**), n = 4–7, $K = 10^5$–10^6 M^{-1} measured in formic acid–water). CB[7] forms complexes with larger guest molecules that are not included in CB[6]. For example, CB[7] forms a 1 : 1 complex with 2,6-bis(4,5-dihydro-1*H*-imidazol-2-yl)naphthalene (**2**), protonated adamantanamine (**3**) or methylviologen dication (*N*,*N*′-dimethyl-4,4′-bipyridinium, **4**). Ferrocene derivatives (**5**) and carborane (**6**) get easily encapsulated in the CB[7] cavity in aqueous solution. The cavity of CB[8] is large enough to include two **2** molecules to form a 1 : 2 complex. Also two different guest molecules, such as **4** and 2,6-dihydroxynathphalene can form a hetero-guest pair leading to a stable ternary complex with CB[8]. The cavity is large enough to encapsulate even another macrocycle, such as cyclen, cyclam and their transition metal complexes. The smallest homologue CB[5] strongly binds cations such as NH_4^+ and Pb^{2+}. These ions cannot enter into the cavity, but remain bound to the carbonyl oxygen of the portals.



5a: R = CH_2OH
5b: R = $CH_2N^+(CH_3)_3$
5c: R = COO^-

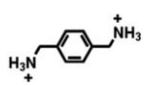
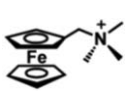
Recently, in collaboration with the Inoue and Kaifer groups, we investigated the binding affinities of CB[7] in water for ferrocene derivatives including charge-neutral (**5a**), positively-charged (**5b**), and negatively-charged (**5c**) ones, by isothermal titration calorimetry (competition method) and compared them with those of β-CDs.[19] All the ferrocene derivatives form inclusion complexes with β-CD with a binding constant of ~2000 M^{-1} regardless of their charge. However, the situation is quite different for CB[7]. First, charge-neutral guest **5a** binds 6 orders of magnitude more tightly to CB[7] than to β-CD. Remarkably, positively-charged guest **5b** exhibits an even higher binding constant (2×10^{12} M^{-1}). This is one of the highest binding affinities ever observed in synthetic receptors along with the CB[7]–adamantanamine (**3**) pair recently discovered by Isaacs *et al.*[20] and quite close to that of the biotin–avidin pair (10^{13}–10^{15} M^{-1}). Equally remarkably, anionic guest **5c** does not bind to CB[7] at all. This remarkably different host–guest behavior of CB[7] and β-CD can be understood from their electrostatic potential (ESP) profiles.[19] While CDs are almost charge-neutral, the portal regions of CBs are negatively charged and the inner cavity surface is also somewhat negatively charged, which explains why CBs binds positively-charged guests very tightly while they do not bind anionic guests.

Isaacs and co-workers also investigated the binding affinities of CB[*n*] (n = 6–8) for various guest molecules in 50 mM NaO_2CCD_3 buffer (pD 4.7) system by a NMR competition method.[20] The binding constants of CB[6] toward several guest molecules are higher than those obtained in formic acid–water (1 : 1) by Mock, while the trends are still maintained. For example, the binding affinity of CB[6] to 1,6-hexanediamine measured by Isaacs *et al.* is 160-fold higher than the reported value (4.5×10^8 *vs.* 2.8×10^6 M^{-1}), and the difference is mainly attributed to the solvent systems chosen for the measurement. In accordance with our discovery, CB[7] tightly binds ferrocene amine **5b** and adamantanamine **3** with K_a values of 10^{11}–10^{12} M^{-1}. CB[8] forms a tight 1 : 1 complex with di-, and trisubstituted adamantanamines as well as monosubstituted adamantaneamines but with lower K_a values compared to those for CB[7].

The large cavity of CB[10] makes the macrocycle form complexes with large guests, including calix[4]arene derivative **7**.[16] The addition of adamantane carboxylic acid (**8**) to CB[10]·**7** lead to formation of termolecular complex CB[10]·**7**·**8**, although **8** alone does not form a complex with **7**. The addition of CB[7] to the ternary complex reverses the process, since CB[7] has the ability to pull back the adamantane derivative into the CB[7] cavity from the ternary complex.

Because of their smaller cavities and more open portals, *i*CB[6] and *i*CB[7] bind most guest molecules less tightly than their CB[*n*] counterparts do (Table 2).[18] Interestingly, *i*CB[6] and *i*CB[7] show a distinct preference for guests with a flatter profile. For example, *i*CB[7] binds aromatic guest **9** more strongly than linear aliphatic guest **1** (n = 6). Also, the K_a value of *i*CB[7] for **9** is higher than that for voluminous guest **5b**, which is in sharp contrast to the behavior of CB[7] which displays much higher affinity for **5b** than **9**. The inverted glycoluril unit modulates their guest binding affinity and rates of dissociation.

Table 2 K_a values of **1**, **9** and **5b** with CB[7] and *i*CB[7]

	Guest	CB[7]	*i*CB[7]
1	$H_3\overset{+}{N}(CH_2)_6\overset{+}{N}H_3$	[b] 9.0×10^7 M^{-1}	[a] 8.8×10^5 M^{-1}
9		[b] 1.8×10^9 M^{-1}	[a] 8.5×10^6 M^{-1}
5b		[a] 4×10^{12} M^{-1}	[a] 2.2×10^6 M^{-1}

[a] In water at 303 K, measured by ITC.[18] [b] In NaOAc buffer at 298 K, measured by NMR.[20]

2.3. Recent developments in applications of unfunctionalized CB[*n*]

The unique structures and remarkable recognition properties of the CB family offer various applications spanning from catalysis to dye removal. Here is a brief summary of some applications of unfunctionalized CB[*n*] reported recently.

CBs have been employed as a reaction chamber to mediate chemical reactions as first demonstrated by Mock *et al*. with a 1,3-dipolar addition reaction inside CB[6].[7] We demonstrated a facile, highly stereoselective [2 + 2] photoreaction of *trans*-diaminostilbene dihydrochloride in the cavity of CB[8] in solution.[21] The ability of the host to stabilize the two guest molecules with a parallel orientation of the olefinic groups in close proximity leads to the high stereoselectivity of the product. Recently, Ramamurthy *et al.* also reported CB[8]-directed photodimerization of *trans*-1,2-bis(4-pyridyl)ethylene and *trans*-*n*-stilbazoles in aqueous solution leading to *syn* dimers in high yield.[22] Macartney *et al.* used CB[7] as a host to mediate a stereoselective [4 + 4] photodimerization of protonated 2-aminopyridine in aqueous solution to yield exclusively the *anti-trans* dimer, which is stabilized by the encapsulation.[23] These studies illustrate that bimolecular reactions between properly designed guest molecules can be facilitated by CB[*n*] in a highly regio- and stereoselective manner.

We examined the inclusion of drugs in CB[7] to explore its potential as a drug carrier. CB[7] forms a stable 1 : 1 inclusion complex with the anticancer drug oxaliplatin in aqueous solution. Following a preliminary study reported in a patent literature, a detailed study of the inclusion complex of oxaliplatin and CB[7] including its reactivity toward guanosine and methionine, and antitumor activity was reported.[24] CB[7] not only increases the stability of the drug but also may reduce unwanted side effects caused by binding of the platinum drug to proteins. Collins and co-workers also reported the encapsulation of a dinuclear platinum complex in CB[7], which does not significantly affect the cytotoxicity of the Pt complex.[25]

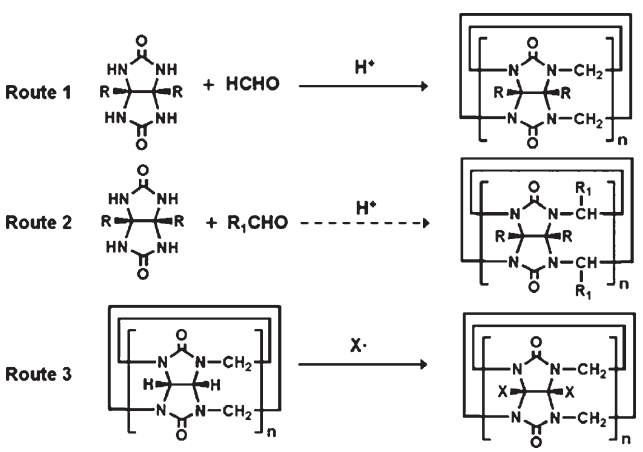
Nau *et al.* investigated the CB[7] complexation of fluorescent dye, rhodamine 6G.[26] The complexation of the dye with CB[7] not only improved its fluorescence intensity but also prevented its aggregation in solution. Furthermore, the complexation reduced the surface adsorption and photobleaching of the dye. These features may enable the applicability of this water-soluble dye to be expanded in the area of biological imaging and screening.

External stimuli dependent host–guest behavior of CB[*n*] has provided operating principles of molecular machines and switches. Mock, and later us, reported pseudorotaxane-based molecular switches incorporating CB[6] that are actuated by a pH change.[6] The discovery of the redox control of the stoichiometry in host–guest complexation of CB[8] prompted us to design and synthesize a [2]pseudorotaxane-based molecular machine that can reversibly form a molecular loop by intramolecular pairing of viologen radical cation units inside CB[8] in response to electrochemical as well as photochemical stimuli.[27] Extending this work, Kaifer *et al*. also reported the self-assembly of redox-switchable dendrimers.[28] The redox-controllable CB[8]-stabilized charge-transfer interaction has also been used in constructing novel molecular machines such as a redox-driven molecular loop-lock. More recently, Kaifer *et al.* reported pH- or redox-driven molecular switches based on pseudorotaxanes incorporating CB[7].[29] We constructed a molecular machine working on a gold surface using a self-assembled monolayer of a pseudorotaxane incorporating CB[6]. Reversible dethreading and rethreading the CB[6] beads in response to a pH change, and ion-gating behavior associated with the threading and dethreading process bead have been observed.[30]

We recently discovered that CB[7] forms a hydrogel.[31] Slow cooling of a warm solution of CB[7] (3–5 wt%) dissolved in a diluted mineral acid such as sulfuric acid to RT leads to gelation of the solution. No other member of the CB family induces gelation of any solvents. The gelation is largely affected by the pH of the solution; the optimum pH for the gel formation ranges between 0 and 2. The gelation is thermoreversible and inhibited by the presence of alkali metal ions. CB[7] hydrogel also shows a guest-induced stimuli-responsive sol–gel transition.

3. Functionalization of cucurbiturils

Although the scope of the CB chemistry was broadened by the discovery of CB homologues, applications of CBs were still limited mainly because of their poor solubility in common solvents, and difficulty in introducing functional groups on their surfaces, which is quite a contrast to other host families including crown ethers, cyclodextrins and calixarenes. In particular, introduction of reactive functional groups at the periphery was a long-standing problem in CB chemistry. In principle, there are three ways to introduce functional groups at the periphery of CB[*n*] (Scheme 2): (1) condensation of glycoluril derivatives carrying substituents at the 'bridging' positions with formaldehyde would give CB[*n*] derivatives bearing the substituents at the "equator" positions; (2) condensation of glycoluril with various aldehydes such as trifluoroacetaldehyde would introduce the corresponding substituents at the methylene bridges of CB[*n*]; (3) direct introduction of substituents at the "equator" position(s) or methylene bridge(s) of the CB[*n*] would produce CB[*n*] derivatives. Since the rediscovery of CB[6],[2] Route 1 has been



Scheme 2

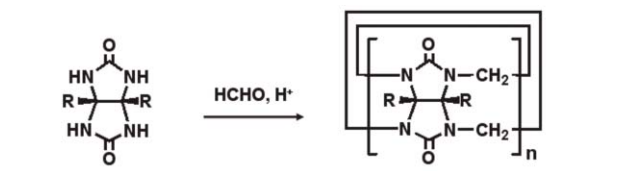
extensively explored but met with a limited success. Despite considerable efforts, Route 2 never gave a trace of success, at least in our laboratory. At the beginning, considering the exceptional chemical stability of CB[*n*], particularly CB[6], the synthesis of CB[*n*] derivatives *via* Route 3 did not seem to have even a remote chance. After many unsuccessful attempts, however, we finally discovered a way to directly introduce a reactive functional group at the equator position(s) of CB[*n*],[13] which now allow us to synthesize a wide variety of CB[*n*] derivatives with tailored properties. In this chapter we will discuss the synthesis of functionalized CBs *via* Routes 1 and 3.

3.1. Synthesis of functionalized CB[*n*] through glycoluril derivatives

3.1.1. Fully substituted CB[*n*]. Glycoluril, a bicyclic product of the reaction of glyoxal with two equiv. of urea, is a fundamental building block for the synthesis of CB[*n*]. Similarly, reaction of RC(=O)C(=O)R (R = Me, Et, Ph, *etc.*) with two equiv. of urea produces glycoluril derivatives carrying substituent R at the 'bridging' positions. During the 1980s and early 1990s, however, attempts to synthesize CB[*n*] derivatives *via* the reaction of glycoluril derivatives with formaldehyde failed with one exception. Decamethylcucurbit[5]uril (Me_{10}CB[5], **11**) can be synthesized from dimethylglycoluril (**10**) under the conventional conditions for CB[6] synthesis, as reported by Stoddart and co-workers in 1992.[32] Recently, we and others found that a small amount of Me_{12}CB[6] (**12**) was also formed from the reaction mixture (Scheme 3).[33]

Introduction of Me groups at the periphery of CB[*n*] does not appreciably improve their solubility in common solvents. However, condensation of cyclohexanoglycoluril (**13**) and formaldehyde produces CB[*n*] derivatives with fused cyclohexyl rings decorating the periphery (CB*[*n*] (*n* = 5 (**14**), 6 (**15**))), which are soluble in water as well as common organic solvents such as DMF.[34] Counterintuitively, they are more soluble in water ($\sim 2 \times 10^{-1}$ M) than in organic solvents ($\sim 3 \times 10^{-2}$ M or less). Their good solubility in common solvents allowed us to study their host–guest chemistry in neutral water and to fabricate membrane electrodes for ion sensing (see below). As illustrated by these examples, however, one serious limitation of this method is that practically no CBs or only smaller homologues (predominantly CB[5] derivatives) are formed when substituents are present on glycoluril.

3.1.2. Partially substituted CB[*n*]. Reaction of a mixture of glycoluril and substituted glycoluril with formaldehyde gives a

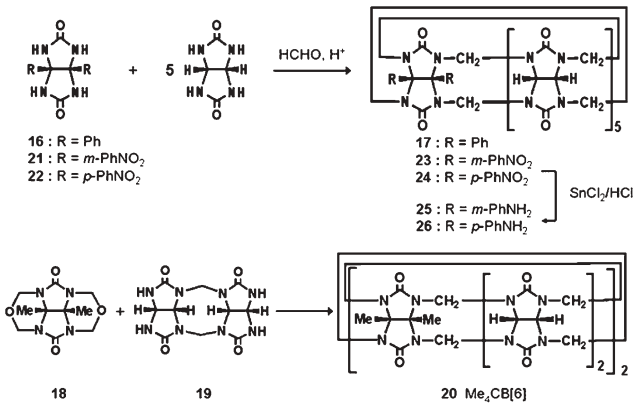


10 : R = Me

13 : R⌒R = $CH_2(CH_2)_2CH_2$

11 : R = Me, n = 5
12 : R = Me, n = 6
14 : R⌒R = $CH_2(CH_2)_2CH_2$, n = 5
15 : R⌒R = $CH_2(CH_2)_2CH_2$, n = 6

Scheme 3



16 : R = Ph
21 : R = *m*-$PhNO_2$
22 : R = *p*-$PhNO_2$

17 : R = Ph
23 : R = *m*-$PhNO_2$
24 : R = *p*-$PhNO_2$
25 : R = *m*-$PhNH_2$
26 : R = *p*-$PhNH_2$

18 **19** **20** Me_4CB[6]

Scheme 4

mixture of partially substituted CB[*n*] along with CB[*n*] (Scheme 4). Nakamura and co-workers reported the synthesis of first partially substituted cucurbituril, diphenylcucurbit[6]uril, (Ph_2CB[6], **17**) by the mixed condensation method. Reaction of a mixture of diphenylglycoluril (**16**) and glycoluril (1 : 5) with formaldehyde produced a mixture of CB[6] and Ph_2CB[6] (**17**) (Scheme 4),[35] from which **17** was separated by gel permeation chromatography. Direct introduction of functional group(s) at the phenyl rings of **17** would produce further functionalized CB[6], which however has not been reported.

Day and co-workers examined the reaction between glycoluril and dimethylglycoluril bis(cyclic ether) (**18**), which is the equivalent of dimethylglycoluril and formaldehyde.[36] The condensation of an equimolar mixture of glycoluril and dimethylglycoluril bis(cyclic ether) rendered a complicated mixture of partially substituted CB[*n*] (*n* = 5–7) including their positional isomers, evidenced by ESI mass spectrometry and NMR. The product mixture typically contains 55–60% of substituted CB[6], and 10–15% of substituted CB[7]. The major product Me_6CB[6], comprising alternating dimethylglycoluril and glycoluril units, was isolated by ion exchange chromatography and successive recrystallization. The condensation of an equimolar mixture of dimethylglycoluril bis(cyclic ether) (**18**) and methylene bridged glycoluril dimer (**19**) produced symmetrical Me_4CB[6] (**20**) in 30% yield.[37]

3.1.3. CB derivatives carrying reactive functional groups. Since CB derivatives carrying reactive functional groups have many potential applications, we decided to synthesize partially substituted CB derivatives having reactive functional groups. The first target molecule that we chose to synthesize was di(aminophenyl)CB[6] (**25**, **26**), which can be obtained by reduction of di(nitrophenyl)CB[6] (**23**, **24**) that in turn can be synthesized by the mixed condensation method using dinitrophenylglycoluril (**21**, **22**) and glycoluril (Scheme 4). To synthesize dinitrophenylglycoluril we first tried nitration of diphenylglycoluril, which however, resulted in a mixture of positional isomers *o*-, *m*-, and *p*-dinitrophenylglycoluril. Thus we moved one step back in our envisaged scheme and synthesized 3,3-dinitrobenzil and 4,4-dinitrobenzil following literature procedures. Condensation of 3,3-dinitrobenzil or 4,4-dinitrobenzil with urea rendered *m*- (**21**), or *p*-dinitrophenylglycoluril (**22**), respectively, in good yield. Reaction of a

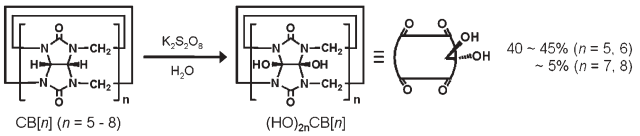
mixture of dinitrophenylglycoluril and glycoluril (1 : 5) with formaldehyde in 9 M H_2SO_4 gave a mixture of CB[6] and dinitrophenylCB[6]. After removal of undesired CB[6] by acid treatment, reduction of crude dinitrophenylCB[6] with $SnCl_2$–HCl or $(NH_4)_2S$ produced diaminophenylCB[6]. Since *m*- (**25**) or *p*-diaminophenylCB[6] (**26**) is fairly soluble in water, the crude product can be further purified by GPC. The preliminary results on the synthesis and applications of diaminophenylCB[6] have been disclosed in a patent literature.[38]

3.1.4 Synthesis of CB analogues and congeners. Isaacs *et al.* synthesized CB[*n*] analogues (*n* = 5–7) containing bis(phthalhydrazide) walls.[39] They were obtained by condensation of bis(phthalhydrazide) and appropriate glycoluril bis(cyclic ether) as nucleophilic and electrophilic building blocks, respectively, which delivers CB[6] and CB[7] analogues in good yield, whereas a CB[5] analogue is formed in low yield. For example, the condensation reaction of bis(phthalhydrazide) with a methylene-bridged glycoluril dimer produced a CB[6] analogue containing two bis(phthalhydrazide) walls linked by two dimeric glycoluril building blocks in a cyclic fashion. A methylene-bridged glycoluril trimer reacts with bis(phthalhydrazide) to yield a CB[7] analogue incorporating a bis(phthalhydrazide). The CB[*n*] analogues are soluble in both organic and aqueous media depending on the substituents attached to the glycoluril bis(cyclic ether) building blocks. The CB[6] analogues maintain a similar binding capacity to that of CB[6]. For example, they form a stable 1 : 1 complex with *p*-xylylenediammonium ion in water, which show slow exchange kinetics on a NMR time scale, similar to CB[6].

They also reported the synthesis of acyclic congeners of CB[*n*].[40] *o*-Xylylene groups were chosen as the linking groups between glycoluril rings, which preorganize them into the (a,a,a,a)-conformation required for an acyclic CB[6] congener. The use of longer terminal and central aromatic rings may result in acyclic CB[*n*] congeners with larger cavity volumes. They measured binding constants of the acyclic CB[6] congener for amine, diol, diacid, guanidinium, and pyridinium species, which indicates that the recognition properties of the acyclic CB[6] congener parallel those of CB[6] with moderate reductions in binding affinity. For example, the acyclic CB[6] congener binds to a wide variety of alkaneammonium and alkanediammonium ions in water ($K_a \leqslant 1.52 \times 10^4\ M^{-1}$), and exhibits length-dependent selectivity. The congener binds hexanediammonium ion 180-fold less tightly than CB[6].

3.2. Direct functionalization of CB[*n*]

For a long time it was thought to be impossible to directly introduce functional groups on the surface of CB[*n*] in part because of their high chemical stability. After numerous unsuccessful attempts, however, we finally developed the first direct functionalization method which leads to hydroxy derivatives of CB[*n*]. Reaction of excess CB[*n*] (*n* = 5–8) with $K_2S_2O_8$ in water produces perhydroxyCB[*n*], $(HO)_{2n}$CB[*n*] (*n* = 5–8) as potassium ion complexes (Scheme 5).[13] While a typical yield of the reaction is 40–45% for CB[5] and CB[6], the higher homologues CB[7] and CB[8] give a low yield (5% or less) presumably due to instability of the perhydroxylated products.



Scheme 5

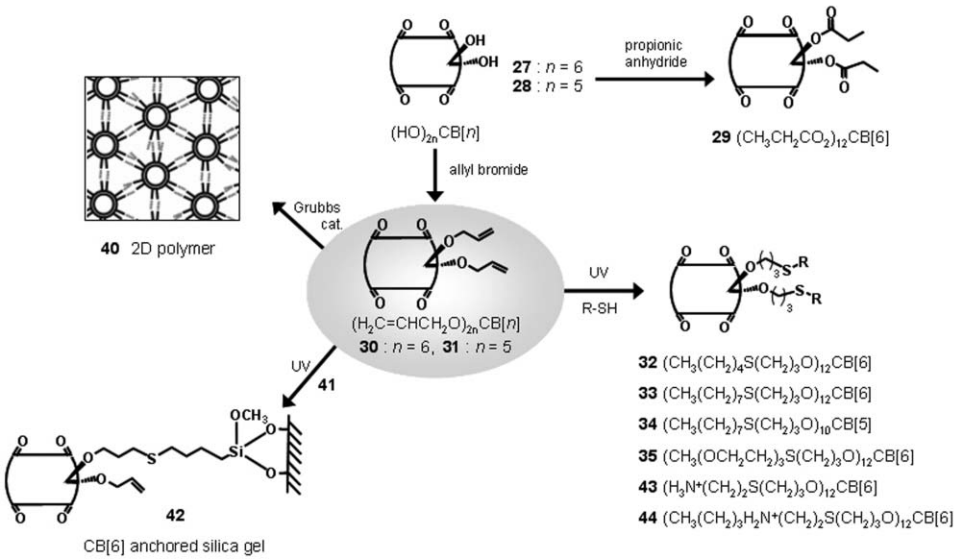
Optimization of the reaction is currently underway. Partial hydroxylation of CB[*n*] is possible but yields a complex mixture of hydroxylated products. The mechanism of the hydroxylation of CB[*n*] has not been fully understood although an OH radical generated by $K_2S_2O_8$ appears to be involved. The X-ray analysis of $(HO)_{12}$CB[6] (**27**) and $(HO)_{10}$CB[5] (**28**) revealed hydroxy groups decorating the periphery of the CB[*n*] frameworks without altering the portal and cavity sizes. Most importantly, the hydroxy derivatives, which are soluble in DMSO and DMF, allow further functionalization *via* conventional organic transformation routes including acylation and alkylation (Scheme 6). For example, **27** can be acylated by treating with propionic anhydride to yield $(CH_3CH_2CO_2)_{12}$CB[6] (**29**). The acylated products have a wide range of solubility depending on the carbon chain lengths attached at the periphery of the CB core. Similarly, alkylation of **27** was demonstrated with allyl bromide to yield perallyloxyCB[6] (**30**), which turned out to be a useful precursor for further transformation because of its good solubility in organic solvents as well as the reactive functional group. A convenient way to utilize the allyl group for further modification is through the photo-addition reaction with suitably designed thiol compounds. For example, **30** undergoes photo-addition reaction with pentanethiol to afford $[CH_3(CH_2)_4S(CH_2)_3O]_{12}$CB[6] (**32**) (Scheme 6). A number of applications of functionalized CBs have been explored and will be covered in the following sections.

4. Applications of functionalized cucurbiturils

4.1. Ion channels

In living cells, ion channels play a crucial role in controlling ion transport across membranes. In 1998, Mackinnon published the first X-ray crystal structure of a potassium ion channel, which revealed the structure of the selectivity filter responsible for discrimination between K^+ and Na^+ ions.[41] The pore size of the selectivity filter, which is aligned with the main chain carbonyl oxygen atoms, is just right for bare K^+ but too large for Na^+, which gives the K^+ ion selectivity. Although many synthetic ion channels based on artificial receptors had been reported,[42] few of them mimic the structural features of the selectivity filter of K^+ channels. The structural resemblance of the carbonyl-fringed portals of CB[*n*] to the selectivity filter of K^+ channels prompted us to study artificial ion channels based on CB[*n*].

Alkylated CBs $[CH_3(CH_2)_7S(CH_2)_3O]_{2n}$CB[*n*](*n* = 6 (**33**); *n* = 5 (**34**)) synthesized from $(allyloxy)_{2n}$CB[*n*] (*n* = 6 (**30**) and 5 (**31**), respectively) and mercaptooctanethiol *via* photo-addition reaction (Scheme 6) were incorporated into a unilamellar vesicle made of EYPC (egg yolk L-α-phosphatidylcholine).[43] Proton transport across the vesicle membrane incorporating **33**



Scheme 6

was studied by fluorometry using a pH sensitive fluorescent dye entrapped inside the vesicle. A sudden pH change of the extravesicular solution caused immediate quenching of the fluorescence. However, addition of neurotransmitter acetylcholine (ACh^+), which is known to form a stable host–guest complex ($K \sim 10^3\ M^{-1}$) with CB[6] derivatives, to the vesicle solution completely inhibited the fluorescence quenching. Taken together these results support that **33** provides a pathway for the proton transport across the membrane, which is blocked by ACh^+, reminiscent of the blocking of the K^+ channels by polyamines.

Alkali metal ion transport across the vesicle membrane was also investigated. The alkali metal ion transport activity of **33** followed the order of $Li^+ > Cs^+ \sim Rb^+ > K^+ > Na^+$, which is exactly opposite to the binding affinity of CB[6] toward alkali metal ions. Similarly, the transport activity of **34** followed the order of $Li^+ > Na^+$, which is also opposite to the binding affinity of **34** toward these metal ions, but virtually no transport was observed for K^+, Rb^+, and Cs^+. This is presumably due to the fact that the carbonyl fringed portal size of **34** (diameter 2.4 Å) is smaller than the diameters of these alkali metal ions (Fig. 2).

The next important question to ask was whether the transport occurs *via* a channel or carrier mechanism. To

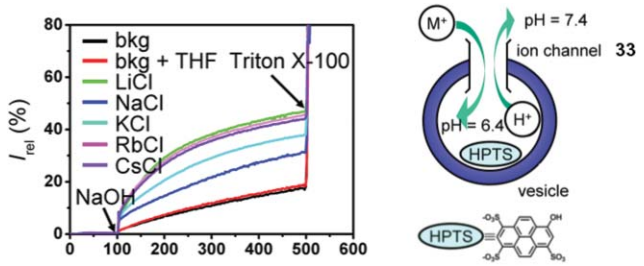


Fig. 2 Changes in the fluorescence intensity ratio (I_{460}/I_{403}) as a function of time associated with alkali metal ion transport across the vesicle membrane incorporating **33** (1 mol%).

answer the question, we performed planar bilayer conductance measurements which revealed a single-channel current of ~ 5 pA for Cs^+ transport across the membrane containing **33**. It corresponds to an ion flux of $\sim 3 \times 10^7$ ions s^{-1}, which is comparable to that of gramicidin. This result is consistent with an ion channel mechanism. However, it is still not well understood how **33** and **34** form an ion channel in membranes. Further studies with higher homologues derivatives are in progress in our laboratory.

4.2. Vesicles

Vesicles are not only important building blocks of all living systems but also potentially useful in many applications such as in development of biomimetic systems, drug/gene delivery systems, and nanostructured materials. In such applications, the incorporation of functional moieties on the surface of vesicles is important, particularly in targeted drug delivery. However, the modification of vesicle surfaces has been mostly achieved by attaching modifier moieties to the vesicle component *via* covalent bonds, which requires laborious, often low-yield, multi-step syntheses. Thus, a noncovalent approach, which would provide a more versatile method for creating vesicles with new properties and functions, has received increasing attention lately. We recently reported a new amphiphilic CB[6] derivative that forms a vesicle, the surface of which can be easily modified through host–guest interactions.[44]

Amphiphilic CB[6] derivative **35** was synthesized by photo-addition reaction of $(allyloxy)_{12}$CB[6] (**30**) and 2-[2-(2-methoxyethoxy)ethoxy]ethanethiol (Scheme 6). Interestingly, **35** forms vesicles of 30–1000 nm diameter in the concentration range of 10^{-3} M to 10^{-4} M. Monodisperse vesicles can be obtained by repeated extrusion through membrane filters with a defined pore size. The high resolution TEM image of the monodisperse sample revealed hollow spheres with a diameter of 170 ± 50 nm and a membrane thickness of 6 ± 1 nm (Fig. 3(a)). A combination of dynamic and static light scattering studies confirms the size and hollow nature of the

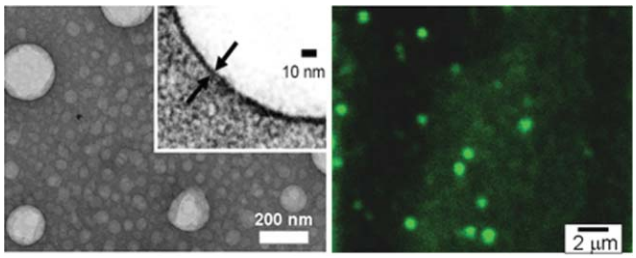


Fig. 3 (a) High resolution TEM image of the vesicles formed by **35** (0.4 mM). The membrane thickness indicated by arrows is 6 ± 1 nm. (b) Confocal microscope image of the vesicles (0.4 mM), the surface of which is decorated with **36**.

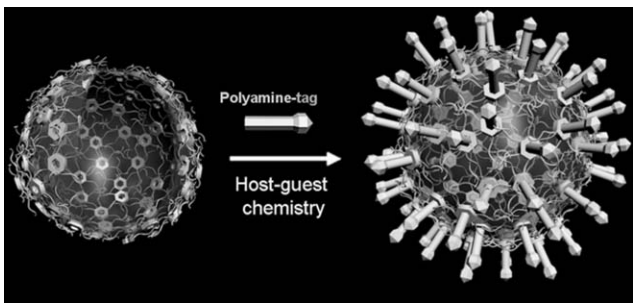
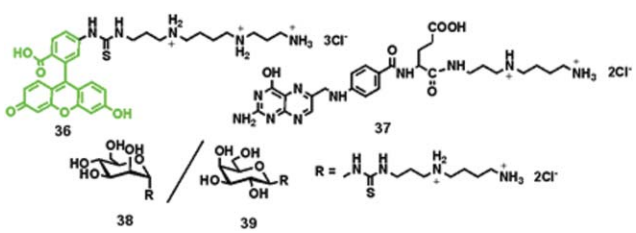


Fig. 4 Illustration of surface modification of vesicle through host–guest chemistry.

vesicles. Further evidence for the formation of vesicles is provided by encapsulation of a fluorescent dye within the interior of the vesicle. It is however not clear how **35**, which does not belong to a common class of vesicle-forming lipids, forms vesicles.

Since the vesicle membrane is made of synthetic receptor CB[6] with an accessible cavity, the vesicle surface can be easily modified using host–guest chemistry. Taking advantage of the exceptionally high affinity of CB[6] toward polyamines, we can decorate the vesicle surface with a specific tag simply by treating the vesicle with a tag-attached polyamine as illustrated in Fig. 4. For example, the surface of vesicle **35** can be decorated with fluorescent tag FITC by treating the vesicle with FITC-spermine conjugate **36** in which spermine serves as a binding motif to the CB[6] unit (Scheme 7). The resulting surface-modified vesicle can be easily visualized by a confocal laser microscope (Fig. 3(b)). Because there are many accessible CB[6] molecules in the vesicle membrane, a large number of and a wide variety of tag moieties can be easily introduced on the vesicle surface using noncovalent interactions, which can interact with specific receptors in a multivalent manner.



Scheme 7

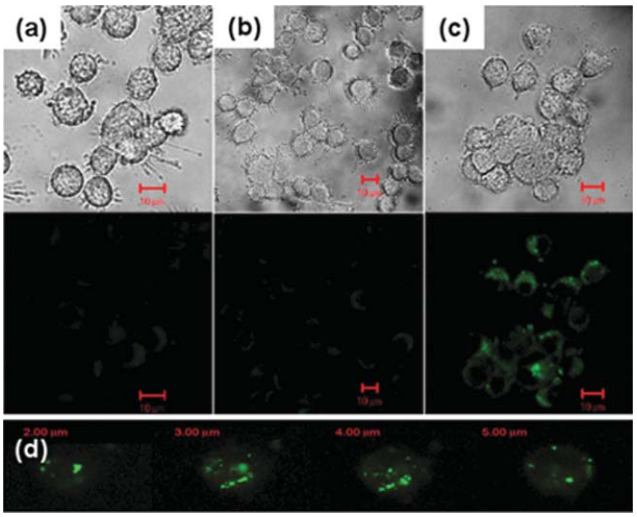


Fig. 5 Confocal laser microscope images of KB cells: (a) untreated, (b) treated with vesicles decorated only with **36**, (c) treated with vesicles decorated with **36** and **37**, and (d) a KB cell in (c) with different focal planes along the view direction.

The multivalent interactions between the surface-modified vesicles and receptors have been demonstrated by agglutination experiments using sugar-decorated vesicles and Concanavalin A (ConA), a lectin with specificity toward α-mannose. α-Mannose-spermidine conjugate **38** was incorporated onto the surface of the vesicle **35** by simply adding **38** to the vesicle solution and shaking it for a few minutes. When the vesicle decorated with **38** was mixed with ConA, aggregation occurred immediately. In contrast, neither free ligand **38** nor the vesicle decorated with β-galactose-spermidine conjugate **39** formed aggregates with ConA. Thus, this observation illustrates the specific and multivalent interactions between the mannose-decorated vesicle and ConA. In fact, the binding constant of the vesicle decorated with **38** to ConA measured by surface plasmon resonance is $\sim 3 \times 10^4$ M^{-1}, which is almost 3 orders of magnitude higher than that of free ligand **38** to ConA (~ 50 M^{-1}).

The potential utility of such surface-decorated vesicles has been demonstrated by receptor-mediated endocytosis of vesicles decorated with folate into human oral cancer KB cells, at the surface of which folate receptors are overexpressed. Their receptor-mediated endocytosis into human oral cancer KB cells was monitored by confocal microscopy As illustrated in the confocal microscope images (Fig. 5), facile endocytosis was evident for vesicle **35** decorated with FITC-spermine conjugate ligand **36** (as a fluorescent probe) and folate-spermine conjugate ligand **37** whereas no significant endocytosis was observed for **35** decorated with only **36**, suggesting that this may provide a potentially viable approach to targeted drug delivery. Besides, the ability for facile surface modification of this vesicle in a noncovalent and modular manner suggests many other practical applications.

4.3. 2D polymers

Two-dimensional (2D) polymers can be defined as a cross-linked network of constituent molecules with nanometre scale

thicknesses and micrometre scale lateral dimensions. Several methods including polymerization of lipid bilayers, self-organization of block copolymers, cross-linking of polymer brush on a patterned surface, and polymerization at the oil–water interface have successfully produced thin polymer sheets with nanometre-scale thickness,[45] Recently, we have developed a new approach to the synthesis of single molecular thick, free-standing 2D polymers, and synthesized a 2D polymer made of a single layer of CB molecules cross-linked in the lateral directions.[46]

Fig. 6(a) depicts our approach to 2D polymers (**40**) made of CB[6] molecules. A self-assembled monolayer (SAM) of 2-aminoethanethiol is first formed on a gold substrate. Immersing the substrate in a solution of $(allyloxy)_{12}$CB[6] (**30**) in chloroform produces an adlayer of **30** through hydrogen bonding and charge–dipole interactions between the carbonyl portal of the CB derivative and the terminal ammonium groups on the SAM. In contrast, no adlayer is formed on a SAM terminated by nonpolar moieties under the same condition, which clearly indicates that the surface recognition *via* the carbonyl portal of **30** plays an important role in the formation of an adlayer.

Since **30** is a rigid disk-shaped molecule with twelve polymerizable allyl groups at the periphery, the adlayer of **30** is expected to be easily in-plane, cross-linked *via* olefin cross metathesis. When the adlayer is immersed in a solution of the second generation Grubbs catalyst in chloroform, a 2D polymer (**40**) with a fish-net polymer network is generated on the SAM. The thickness of the polymerized adlayer plus the base SAM measured by ellipsometry is ~2 nm, which indicates that the 2D polymer (**40**) is a single molecule thick. The free-standing 2D polymer detached from the substrate by treating with 1 N NaOH solution is flexible enough to be wrinkled as shown in a SEM image (Fig. 6(b)). In addition, 2D polymers with various shapes and sizes in the lateral dimensions can be prepared by the microcontact printing technique.

The molecular cavities incorporated into the 2D polymer (**40**) allow us to decorate its surface noncovalently in a similar manner to the one described above. For example, FITC-decorated 2D polymer can be visualized by a confocal microscope, which may enable it to be used to directly observe

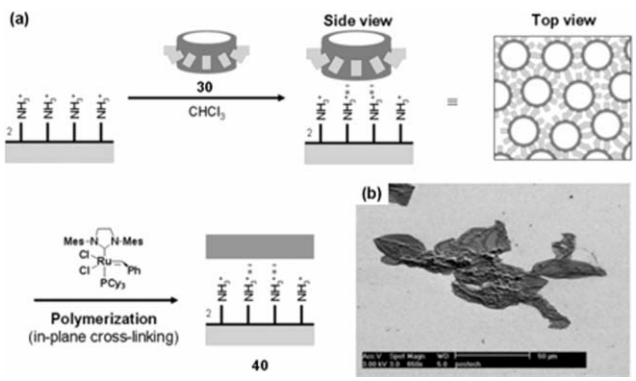


Fig. 6 (a) Synthetic approach to 2D polymers with an in-plane cross-linked network and (b) SEM image of a 2D polymer detached from 2-aminoethanethiol SAM.

their folding–unfolding transitions in solution for the first time. We are currently investigating the dynamic behavior of the 2D polymer including the theoretically predicted folding–unfolding transitions in solution, and exploring its use as a scaffold for organization of interesting nanomaterials by taking advantage of the unique host–guest chemistry of CB[6].

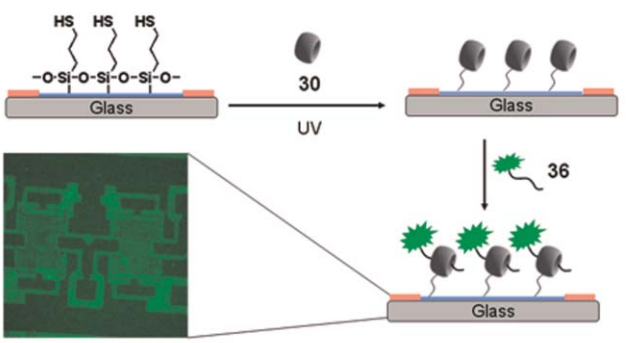
4.4. Immobilization of CB[*n*] on solid surfaces

Immobilization of receptors on a solid surface is important for many applications including developing a sensor. Anchoring a functionalized CB[6] on a glass surface has been demonstrated.[13] Immersing a glass substrate whose surface has been modified to carry thiol terminal groups, in a solution of **30** followed by irradiating UV light produces a CB[6] modified glass (Scheme 8). The CB[6] modified glass recognizes small molecules and ions such as alkyl ammonium ions which are known to bind to CB[6]. For example, a fluorescence microscopic image of the CB[6] modified glass plate taken after brief immersion of the plate in an aqueous solution containing FITC-spermine conjugate **36** shows a pattern made by a microcontact printing technique. This gives clear evidence of complex formation between the fluorescent guest and the CB[6] unit anchored on the surface. Such a CB[6] modified surface may be useful in designing sensors and biochips.

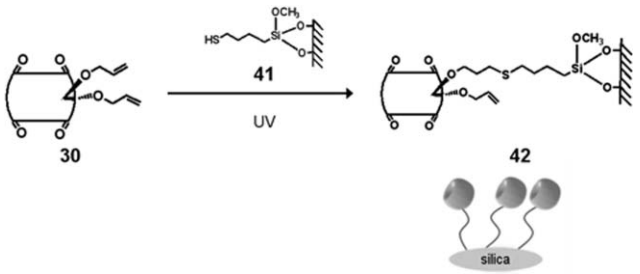
4.5. CB[*n*]-anchored silica gel for chromatography

Because of the unique recognition properties, as well as chemical stability, of CBs, CB[*n*]-immobilized silica gel or polymer beads would be useful in separation science, but they were not ventured until recently as no method to introduce reactive functional groups to CB[*n*] was available. The discovery of the direct functionalization method of CB[*n*] prompted us to explore their applications in separation science. Our approach and preliminary results on the preparation of CB[*n*]-based stationary phase materials and their uses in chromatography have been described in patent literature.[47]

While we were expanding this work, Liu *et al.* independently reported the preparation of a perhydroxyCB[6] bonded silica gel and its use as a stationary phase in HPLC to separate alkaloids.[48] They utilized perhydroxyCB[6] (**27**) as a starting material for the immobilization of CB[6] on silica gel. The reaction of **27** with (3-isocyanatopropyl)triethoxysilane followed by *in situ* reaction with silica gel rendered the CB[6]



Scheme 8



Scheme 9

immobilized silica gel. The separation efficiency of the CB[6] anchored silica gel column was examined with a mixture of alkaloids. Other parameters affecting the separation such as pH, solvent polarity and ionic strength were also investigated for the column.

Our concern with the direct use of **27** for anchoring purposes is, however, the contamination of K_2SO_4 in **27**. It is known that **27** synthesized by oxidation of CB[6] using potassium persulfate usually contains a large amount of K_2SO_4 originating from the decomposition of potassium persulfate. Thus, we prepared CB[6] anchored silica gel using perallyloxyCB[6] (**30**) as a starting material instead of **27**.[49] Irradiation of UV light on a mixture of **30** and mercaptopropyl-functionalized silica gel **41** followed by removal of unreacted **30** by washing afforded perallyloxyCB[6]-anchored silica gel **42** (Scheme 9). The amount of CB[6] anchored on silica surface was quantified using FITC-spermine conjugate (**36**) as a fluorescent probe, which binds tightly to CB[6]. The amount of accessible CB[6] measured by this method (52 μmol g^{-1}), was considerably smaller than that estimated from elemental analysis (86 μmol g^{-1}), indicating that not all the CB[6] units attached on the silica surface are accessible by the guest.

Anchoring of other members of the CB family on silica gel and polymer beads is being studied in our laboratory. Such materials may be useful as a stationary phase in chromatography and also find useful applications in other areas including extraction of metal ions and dye removal.

4.6. Ion selective electrodes

PVC membrane ion-selective electrodes (ISEs) provide one of the most popular and versatile sensing devices to detect electroinactive ionic species. A wide variety of synthetic receptor molecules have been incorporated into ISEs as sensing elements. Despite their remarkable recognition properties, the use of CB[*n*] in ISE was hampered by their poor solubility in organic solvents, and difficulty in introducing functional groups on their surfaces until our synthesis of soluble CB[*n*] derivatives. Utilizing organic soluble CB[*n*] derivatives we have successfully fabricated membrane ISEs and demonstrated their uses in detecting biologically and environmentally important ions.[34]

Developing effective detection methods of neurotransmitter acetylcholine (ACh) is important because of its critical role in nervous systems in many organisms including human. However, detection of ACh, particularly in the presence of interfering ions, poses a significant analytical challenge because it lacks a chromophore or electroactive center. Based on our observation that CB*[6] (**15**) forms a stable 1 : 1 host–guest complex with acetylcholine in neutral water, we developed an ISE for detecting ACh. The good solubility of CB*[6] (**15**) in organic solvents allows fabrication of a membrane electrode which detects ACh with high selectivity over choline and other interfering ions such as Na^+, K^+, and NH_4^+ (Fig. 7).[34] Similarly, a membrane electrode incorporating CB*[5] (**14**) functions as an ISE for Pb^{2+}, potentially useful for monitoring the toxic element in the environment. This work can be extended to other soluble CB[*n*] derivatives to prepare ISEs that may find useful applications.

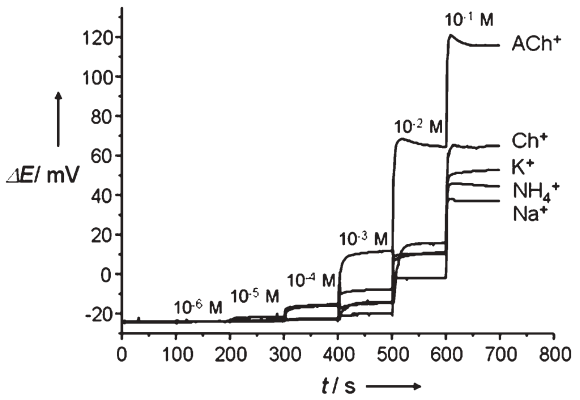
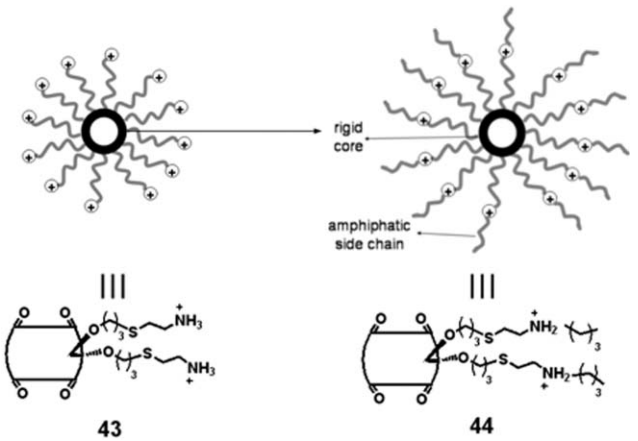


Fig. 7 Responses of an ISE based on CB*[6] (**15**) to acetylcholine (ACh^+) and other interfering ions including choline (Ch^+) (pH 7.2 with 10 mM Tris buffer).

4.7. New scaffold for antibiotics

Since pathogenic bacteria have been acquiring resistance to conventional antibiotics, there is a surge of interest in developing new antibiotics based on novel chemical scaffolds. Recent studies suggested that both amphiphilicity and multivalency should be considered simultaneously in designing good antibiotics. We and Jon's group thought that perallyloxyCB[6] (**30**) may serve as a new scaffold for antibiotics as it carries twelve identical functional groups surrounding a rigid macrocyclic core, enabling modification with amphiphatic side chains. Indeed, we recently discovered a new CB[6]-based antibacterial agent that kills a broad spectrum of bacteria from Gram-negative to positive with comparable efficacy to that of natural antibacterial peptides (Scheme 10).[50]

Primary and secondary amine-modified CB[6] derivatives (**43** and **44**, respectively) were synthesized as shown in Scheme 6 and their *in vitro* antibacterial activities were assessed against various Gram-negative and positive strains. **44** showed significantly higher antibacterial activity than **43** with several μM ranges of minimum inhibition concentration (MIC) as well as minimum bactericidal concentration (MBC). To examine the antibacterial mechanism of the CB[6] derivatives, calcein dye leakage experiments were carried out using small unilamellar vesicles as a model bacterial membrane, which showed that **44** disrupts the model bacterial membrane whereas **43** does not. Another experiment demonstrated that **44** is able to kill bacteria with high selectivity over mammalian cells. Furthermore, the CB[6]-based antibiotic is stable toward enzymatic degradation. Since the hydrophobicity and overall



Scheme 10

charge density can be easily tuned by introduction of other derivatives, highly efficient antibacterial agents for clinical applications may be developed based on this approach.

5. Conclusions and perspectives

Though the parent molecule CB[6] is a century old, the chemistry of the CB family is beginning to blossom. The recent developments, including the synthesis of CB homologues and derivatives, have brought dynamism to CB chemistry, as witnessed by the heightened interest in the field for the last several years. In particular, the direct functionalization of CB[*n*] and subsequent synthesis of tailor-made CB derivatives have removed one of the major obstacles of CB chemistry and henceforth they are now available for exhaustive synthetic manipulations to pursue many useful applications as we have demonstrated in this review article. The functionalization led us to investigate numerous applications including artificial ion channels, vesicles, stationary phases in chromatography, ISEs, polymers, nanomaterials, and many others. Indeed, CB homologues and functionalized CB derivatives hold great promise for many practical applications including waste water treatment, drug/gene delivery, immunization, catalysis, sensors, biochips, separation of biologically important molecules, odor removal, slow release of fragrance, and reduction of toxicity of anticancer drugs.

Although CB chemistry has grown up enormously since the beginning of the new millennium, there are still many challenges as well as opportunities. For example, the recent discovery of *i*CB[*n*] provided a fresh insight into the CB formation reaction, but its mechanism is still not fully understood. Unlike CDs, CBs are achiral molecules and the synthesis of chiral CBs with enantioselective properties will find many uses including chiral columns for chromatography. Although the discovery of the direct functionalization method has paved the way to applications of CBs, developing more efficient functionalization methods, particularly for higher homologues such as CB[7] and CB[8], is important in expanding their applications. From the practical application point of view, the field of CB-based functional materials and devices is only in its infancy and prospects are enormous. For example, CB-based molecular switches/machines and sensors may be developed leading to new nanotechnology. The unique ability of CB[*n*] in forming exceptionally stable host–guest complexes with binding constants comparable to that of biotin–avidin suggests that the synthetic host–guest systems may be used in the same way as the natural system is currently used in many biochemical applications. With such great promise, the future of the CB chemistry is brighter than ever.

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