Carbohydrate Clusters

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Carbohydrate Wheels: Cucurbituril-Based Carbohydrate Clusters**

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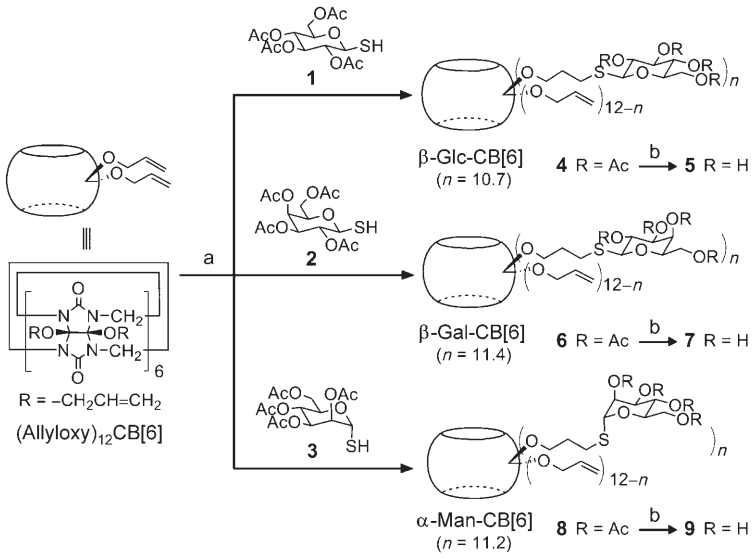
Extracellular carbohydrate–protein interactions are critical in cellular communication processes, such as fertilization, immune response, tumor-cell metastasis, and bacterial or viral infection.[1] Despite its importance in specific recognition processes, the interaction between a single carbohydrate ligand and a protein molecule is usually weak. Therefore, multiple copies of carbohydrates and proteins participate in binding to enhance the affinity and selectivity, which is known as a glycosidic cluster effect.[2] Based on this concept, a number of multivalent carbohydrate clusters with various scaffolds,[3–10] including polymers,[4] dendrimers,[5] calixarenes,[6] cyclodextrins,[7] nanoparticles,[8] and vesicles,[9] have been synthesized to mimic biological systems, and their multivalent binding abilities toward specific lectins or receptors on the cell surface have been investigated.

Cucurbit[*n*]urils (CB[*n*], $n = 5$–10), a family of macrocyclic cavitands comprising *n* glycoluril units, have a hydrophobic cavity accessible through two identical carbonyl-fringed portals, and form stable host–guest complexes with a wide range of guest molecules.[11] Recently, we reported a method for the direct functionalization of CB[*n*], which allowed us to introduce multiple substituents at the "equator" of CB[*n*].[12] In exploring applications of tailor-made CB[*n*] derivatives,[13] we thought that the rigid structure, unique guest-binding ability, and surface that could be tailored would make CB[*n*] a useful multivalent scaffold for carbohydrates. Herein, we present novel CB[6]-based carbohydrate clusters, which have multiple carbohydrate moieties attached to the periphery of a CB[6] core. The CB[6]-based carbohydrate clusters show high selectivity as well as enhanced affinity through multivalent interactions in binding to specific proteins. Moreover, as a result of the CB[6] cavity, they bind molecules to form host–guest complexes, which can be delivered to specific cells that recognize the multivalent carbohydrates.

CB[6]-based glucose, galactose, and mannose clusters (**5**, **7**, and **9**, respectively) were synthesized by photoreaction of $(allyloxy)_{12}$CB[6][12] and acetylthioglycosides **1**, **2**, and **3**,[7a,14] respectively, followed by deacetylation (Scheme 1). The



Scheme 1. Synthesis and yields of CB[6]-based carbohydrate clusters. a) **1**, **2**, or **3** (48 equiv), *hν*, MeOH, 2 days; **4** 76%, **6** 77%, **8** 83%; b) NaOMe, MeOH, 2 h; **5** 85%, **7** 83%, **9** 75%.

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carbohydrate clusters were purified by reversed-phase HPLC and fully characterized by various NMR methods, MALDI-TOF mass spectrometry, and elemental analysis (see the Supporting Information). The MALDI-TOF mass spectra of the carbohydrate clusters revealed species with 9–12 carbohydrate moieties attached to a CB[6] core. Approximately 11 carbohydrates on average are attached to the core, as judged by ^{1}H NMR integration and elemental analysis.[15] The energy-minimized structure of **5** (degree of substitution $n = 12$) is shown in Figure 1. Twelve glucose moieties are attached to the "equator" position of the rigid CB[6] core like a wheel with a diameter and thickness of 2.9 and 1.8 nm, respectively. The size is consistent with the hydrodynamic diameter (2.6 nm) measured by pulsed field gradient NMR techniques (see the Supporting Information).

The binding abilities of the carbohydrate clusters to concanavalin A (ConA), a lectin known to bind selectively α-

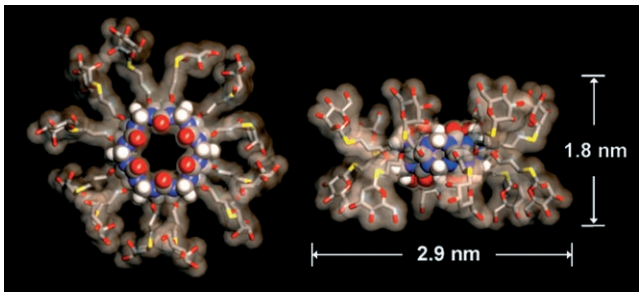

Figure 1. Energy-minimized structure of CB[6]-based glucose cluster **5** ($n = 12$). Hydrogen atoms are omitted for clarity except those in CB[6].

mannosyl or α-glucosyl residues,[16] were investigated by turbidimetric assay.[5a] As shown in Figure 2, immediate aggregation occurred when ConA was added to a solution

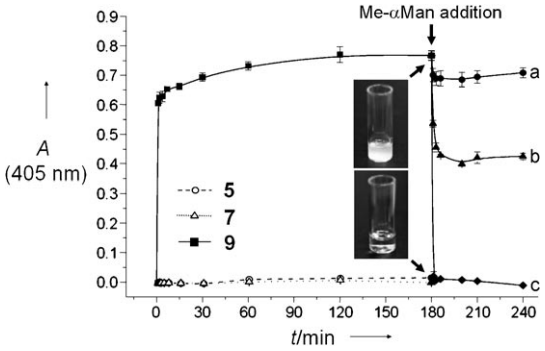

Figure 2. Time courses of the turbidity change for a solution of **5**, **7**, or **9** (7.3 μM) and ConA (20 μM). After 3 h, Me-αMan was added to the solution of ConA and **9**; a, b, and c correspond to the addition of an 11-, 110-, and 1100-fold excess amount of Me-αMan, respectively.

of **9**, whereas no aggregation was observed with **5** or **7** at the same or a higher concentration (see the Supporting Information). These results confirmed the specific binding ability of the carbohydrate clusters to ConA.

To demonstrate the enhanced binding affinity of **9** (in comparison with a monomeric α-mannose) to ConA, an inhibition experiment was performed with monomeric methyl-α-D-mannopyranose (Me-αMan) as an inhibitor for the cross-linking of **9** and ConA. After formation of an aggregate between **9** and ConA, an 11-, 110-, or 1100-fold excess amount of Me-αMan was added to the solution and the change in turbidity was monitored. As shown in Figure 2, a small and moderate decrease in turbidity was observed upon addition of Me-αMan in 11- and 110-fold excess, respectively. Finally, addition of an 1100-fold excess of Me-αMan resulted in complete disruption of the cross-linking interaction between **9** and ConA to give a transparent solution, which qualitatively illustrated a large enhancement in binding affinity of **9** to ConA through a multivalent effect.

The binding stoichiometry between **9** and ConA established by a Job plot was approximately 1:3 (see the Supporting Information).[17,18] Isothermal titration calorimetry (ITC)[19] confirmed that **9** behaves predominantly as a trivalent ligand[20] to the lectin with a binding constant $K = (1.9 \pm 0.2) \times 10^5\ \text{M}^{-1}$,[21] which is 25 times[22] higher than that for Me-αMan to ConA (see Supporting Information). The enhancement in binding affinity is comparable to those (measured by ITC) of other glycoclusters with a similar valency, but smaller than those of glycodendrimers or linear glycopolymers with much higher valency.[3a,19a]

By taking advantage of the cavity provided by the CB[6] scaffold,[11] the CB[6]-based carbohydrate clusters form stable 1:1 host–guest complexes with a wide range of guest molecules including fluorescein isothiocyanate (FITC)–spermine conjugate (**10**)[12] (Figure 3a). To illustrate the potential utility of the CB[6]-based carbohydrate clusters as a drug-delivery vehicle, in vitro targeted delivery experiments were carried out with **10** as a fluorescent probe as well as a model drug, and the HepG2 hepatocellular carcinoma cell with overexpressed galactose receptors as a target cell. Intracellular translocation of the FITC–spermine conjugate complexes of **5**, **7**, and **9** was examined by confocal microscopy. As illustrated in Figure 3b, only **10@7** showed facile internalization into the cell after incubation for 1 h at 37 °C. No significant translocation was observed (Figure 3b, VI) when the experiment was carried out at 4 °C, which suggested that the mechanism of cellular uptake is most likely galactose receptor-mediated endocytosis. Further studies are needed to establish the mechanism of the cellular uptake and the efficiency of CB[6]-based carbohydrate clusters as a drug-delivery vehicle.

In summary, we have synthesized new carbohydrate clusters by using CB[6] as a multivalent scaffold, and

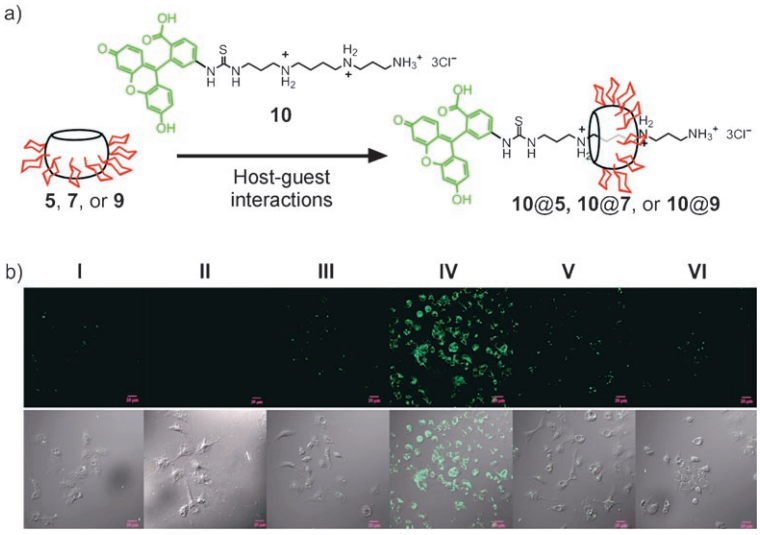

Figure 3. a) Preparation of complexes of **10** and carbohydrate clusters (**5**, **7**, or **9**) through host–guest interactions. b) Confocal microscopy images of HepG2 cells treated with **10** and **10**@CB[6]-based carbohydrate clusters: fluorescence images (top) and fluorescence + differential interference contrast images (bottom). No treatment (I), after incubation with **10** (II), **10@5** (III), **10@7** (IV), and **10@9** (V) at 37 °C, and after incubation with **10@7** at 4 °C (VI). Scale bars 20 μm.

demonstrated their specific and multivalent interactions with a lectin. Furthermore, we demonstrated that the carbohydrate clusters formed a host–guest complex, which was delivered into a specific cell by receptor-mediated endocytosis. As a wide variety of drug–polyamine conjugates can form host–guest complexes with the CB[6]-based carbohydrate clusters, they may be useful in targeted drug delivery and other therapeutic applications. This work can be extended to the use of other members of the CB family as a scaffold and other ligands, to create new multivalent ligands for various applications. Furthermore, polyrotaxanes comprising such CB-based carbohydrate clusters threaded on a suitable polymer may provide even stronger multivalent interactions, and thus be potentially useful in the inhibition of bacterial or viral infection. Further work along these lines is in progress.

Experimental Section

4: **1** (3.5 g, 9.6 mmol) was added to a solution of $(allyloxy)_{12}$CB[6] (0.33 g, 0.20 mmol) in MeOH (60 mL). After degassing with N_2, the mixture was irradiated with UV light for 2 days. The solvent was then removed, and the remaining solid was washed with diethyl ether and dried to give clusters **4** (0.92 mg, 76%). The product was a mixture of partially substituted **4** with an average of about 11 *O*-acetylglucose groups per CB[6] core, as judged by NMR and mass spectral data.

5: NaOMe in MeOH (25%, 400 μL) was added to a stirred solution of **4** (0.90 g, 0.15 mmol) in anhydrous MeOH (50 mL), and the reaction mixture was allowed to stand at room temperature. A precipitate formed during this period of time. After 2 h, the solid was isolated by filtration, redissolved in water, and neutralized with Amberlite IRC-50 (H^+ form) ion-exchange resin. After filtration, the filtrate was freeze-dried and the crude product was purified by reversed-phase HPLC to give cluster **5** (0.51 g, 85%). The isolated product was a mixture of partially substituted **5** with different degrees of substitution. The MALDI-TOF mass spectrum of **5** revealed species with 9–12 glucose units attached to a CB[6] core. The N/S ratio in elemental analysis suggested that the average degree of substitution was 10.7, which was consistent with ^{1}H NMR integration. For further characterization of **4**–**9** and other experimental details, see the Supporting Information.

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[20] ITC is a powerful method to determine the functional valency of a multivalent carbohydrate in binding to lectin,[19a] which may differ from the structural valency. It can be determined from the *n* value that best fits the ITC data. The *n* value corresponds to the number of binding sites per ConA monomer, which is the inverse of the functional valency of a carbohydrate cluster to the lectin. An *n* value of 0.36 (see the Supporting Information) indicates that the binding stoichiometry between **9** and ConA monomer is approximately 1:3, which is consistent with that determined by the turbidimetric Job plot; therefore, **9** behaves predominantly as a trivalent ligand to the lectin.
[21] The observed *K* value for **9** is the average of the three microscopic *K* values at each of its three functional epitopes.[19a]
[22] The binding enhancement corrected for the structural valency (the number of mannose units) of **9** is 2.3.

