

## Solution-to-Surface Molecular-Delivery System Using a Macrocyclic Sugar Cluster. Sugar-Directed Adsorption of Guests in Water on Polar Solid Surfaces

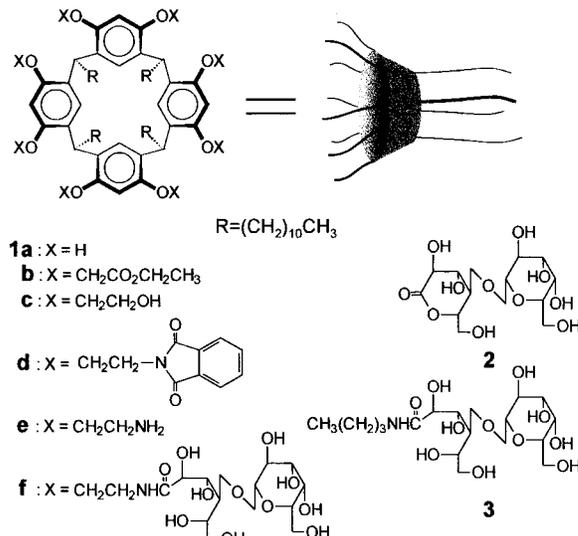
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Cell-surface oligosaccharides occur as clusters. They play essential roles in various cell-recognition processes,<sup>1</sup> where saccharide–receptor interactions are often claimed to be multivalent.<sup>2</sup> The so-called cluster effect<sup>2</sup> has led many workers to investigate the receptor-binding properties of a variety of multiantennal synthetic saccharide derivatives (polymers and oligomers,<sup>3</sup> dendrimers,<sup>4</sup> surfactant aggregates,<sup>5</sup> and metal complexes<sup>6</sup>). The present work is concerned with the interaction of a well-defined macrocyclic sugar cluster with a nonbiological polar solid surface as a simplified multivalent receptor site. We report here that the clustering sugar moieties *in water* can be irreversibly adsorbed or assembled on the silica surface. Coupled with the guest-binding ability of the macrocycle as a host, this leads to a novel sugar-directed solution-to-surface molecular delivery system.<sup>7</sup>

An octagalactose derivative of calix[4]resorcinarene (**1f**; R = (CH<sub>2</sub>)<sub>10</sub>CH<sub>3</sub>)<sup>8</sup> was obtained by the reaction of lactonolactone (**2**) with octamine **1e**, which was prepared in four steps from the parent macrocycle **1a**.<sup>9</sup> Compound **1f** is highly soluble in water (>0.1 M). Nevertheless, it is readily adsorbed on the surface of quartz plate (silica glass) dipped in an aqueous solution of the former.<sup>10</sup> Electronic absorption spectroscopy for the recovered plates<sup>10</sup> indicates the following. (1) The



adsorption is practically irreversible; **1f** adsorbed can not be rinsed out by repeated washing with water, although it is desorbed when washed with an aqueous solution of amine such as ethanolamine and pyridine. (2) There is a maximal or saturation amount of **1f** adsorbed, whose absorbance corresponds to a packing density of 0.28 molecules/nm<sup>2</sup> or an occupation area of 3.5 nm<sup>2</sup>/molecule.<sup>11</sup> (3) The saturation binding is achieved almost instantaneously (~10 s) when [1f]<sub>aq</sub> ≥ 1 mM. The occupation area shown above is very similar to the cross-sectional area of the sugar-cluster part of compound **1f** ( $\phi \cong 2$  nm at the most folded conformation; Figure 1). These results suggest that cluster **1f** forms a closely-packed monolayer on the quartz surface, as schematically shown in step a of Scheme 1.<sup>12</sup> In marked contrast to **1f**, a nonsugar analog (**1a**, R = (CH<sub>2</sub>)<sub>2</sub>SO<sub>3</sub>Na) in water shows no sign of adsorption on quartz. Adsorption on silica gel also occurs readily. Thus, compound **1f** in water (0.1 mM, 10 mL) can be removed completely from the aqueous phase upon addition of a sufficiently excess amount (3 g) of chromatographic silica gel (Kaseigel 60–75). This is not the case for simple sugar derivatives such as galactose and phenyl  $\beta$ -galactopyranoside.

The lack of affinity to quartz of galactose (1 M) and lactonolactone–butylamine adduct **3** (1 M) as monovalent references and  $\gamma$ -cyclodextrin (0.1 M) as a 1,4-linked cyclic glucose octamer is evidenced by the competition experiments. Even in a large excess as indicated above, they neither inhibit the adsorption of cluster **1f** (0.1–1 mM) on the quartz surface nor promote desorption of **1f** adsorbed thereon. Thus, the octamer/monomer selectivity factor must be **1f/3**  $\gg$  10<sup>4</sup>. This reflects a remarkable efficiency of the multivalent interaction (step a in Scheme 1) between clustering sugar side chains of **1f** in water and the multivalent silica surface composed of silanol and silyl ether functionalities. There is little doubt that the interaction involves multiple **1f**–silica hydrogen-bonding, since it is disrupted by an amine base with decreasing efficiencies CH<sub>3</sub>NH<sub>2</sub> (pK<sub>a</sub> = 10.7) > 4-(CH<sub>3</sub>)<sub>2</sub>NC<sub>5</sub>H<sub>4</sub>N (9.7) > HO(CH<sub>2</sub>)<sub>2</sub>NH<sub>2</sub> (9.5) > NH<sub>3</sub> (9.2) > C<sub>3</sub>H<sub>5</sub>N (5.2). Use of hydrogen-bonding in the formation of membranous aggregates in water has been noted.<sup>13</sup>

(1) The accurate amounts of host **1f** adsorbed and guest ANS coadsorbed on the quartz surface were determined after desorbing them into a 30% aqueous ethanolamine solution.

(2) Ichimura et al. have shown that derivatives **1a** (X = H, CH<sub>2</sub>CO<sub>2</sub>H, or CH<sub>2</sub>CH<sub>2</sub>OH) in apolar organic media can be adsorbed on a quartz plate to form closely-packed monolayers. For the adsorption on colloidal silica, see: Ueda, M.; Fukushima, N.; Kudo, K.; Ichimura, K. *J. Mater. Chem.* **1997**, *7*, 641–645. For the formation of monolayers of sulfide derivatives of macrocycle **1a** on the gold surface, see: Thoden van Velzen, E. N.; Engbersen, J. F. J.; de Lange, P. J.; Mahy, J. W. G.; Reinhoudt, D. N. J. *Am. Chem. Soc.* **1995**, *117*, 6853–6862.

(1) *Carbohydrate-Protein Interactions*; Clarke, A. E., Wilson, I. A., Eds.; Springer-Verlag: Heidelberg, Germany, 1988.

(2) (a) Connolly, D. T.; Townsend, R. R.; Kawaguchi, K.; Bell, W. R.; Lee, Y. C. *J. Biol. Chem.* **1982**, *257*, 939–945. (b) Lee, R. T.; Lee, Y. C. *Biochem. Biophys. Res. Commun.* **1988**, *155*, 1444–1451.

(3) (a) Spaltenstein, A.; Whitesides, G. M. *J. Am. Chem. Soc.* **1991**, *113*, 686–687. (b) Glick, G. D.; Knowles, J. R. *Ibid.* **1991**, *113*, 4701–4703. (c) Salesan, S.; Duus, J. O.; Domaille, P.; Kelm, S.; Paulson, J. C. *Ibid.* **1991**, *113*, 5865–5866. (d) Callstrom, M. R. *Ibid.* **1992**, *114*, 378–380. (e) Roy, R.; Andersson, F. O.; Harms, G.; Kelm, S.; Schauer, R. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 1478–1481. (f) Mortell, K. H.; Gingras, M.; Kiessling, L. L. *J. Am. Chem. Soc.* **1994**, *116*, 12053–12054.

(4) Aoi, K.; Itoh, K.; Okada, M. *Macromolecules* **1995**, *28*, 5391–5393.

(5) Kingery-Wood, J. E.; Williams, K. W.; Sigal, G. B.; Whitesides, G. M. *J. Am. Chem. Soc.* **1992**, *114*, 7303–7305.

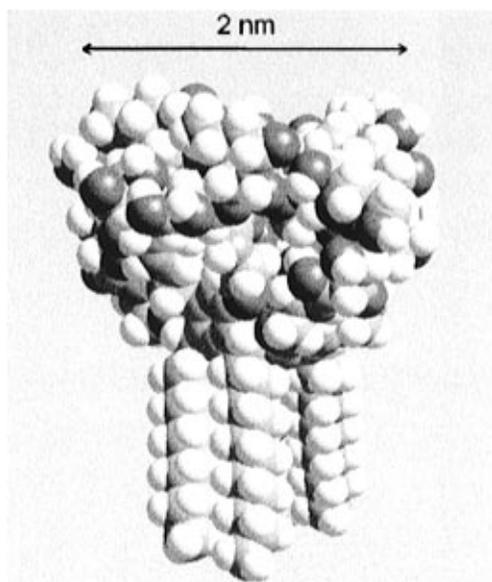
(6) Sakai, S.; Sasaki, T. *J. Am. Chem. Soc.* **1994**, *116*, 1587–1588.

(7) For an example of the saccharide-directed molecular delivery systems, see for example: Gordon, S.; Rabinowitz, S. *Adv. Drug. Delivery Rev.* **1989**, *4*, 27–47.

(8) For a calix[4]arene derivative having four side chains with a terminal sugar moiety, see: Marra, A.; Scherrmann, M.-C.; Dondoni, A.; Casnati, A.; Minari, P.; Ungaro, R. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2479–2481.

(9) **1a**  $\rightarrow$  **1b** (BrCH<sub>2</sub>CO<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub> + K<sub>2</sub>CO<sub>3</sub> in acetone at 80 °C for 3 h; 97%), **1b**  $\rightarrow$  **1c** (LiAlH<sub>4</sub> in THF at 70 °C for 3 h; 90%), **1c**  $\rightarrow$  **1d** (diethyl azodicarboxylate + P(C<sub>6</sub>H<sub>5</sub>)<sub>3</sub> + phthalimide in THF at 70 °C for 30 h; 68%), **1d**  $\rightarrow$  **1e** (N<sub>2</sub>H<sub>4</sub> in ethanol at 60 °C for 4 h; 53%), **1e**  $\rightarrow$  **1f** (**2** in methanol at 70 °C for 4 h; 79%). Compound **1f** was fully characterized by means of spectroscopy (IR, <sup>1</sup>H and <sup>13</sup>C NMR, and TOF-MS) and elemental analysis. Dynamic light scattering indicates it is monomeric at 0.1 mM but forms aggregates ( $\phi \cong 20$  nm) at 1 mM in water.

(10) Experimental procedure: 10 quartz plates (12 × 50 × 1 mm) were dipped for 10 min in an aqueous solution of compound **1f** ([1f]<sub>aq</sub> ≥ 0.1 mM) at 25 °C, washed three times with 5 mL of pure water, and dried under nitrogen. The combined plates show an absorption band for adsorbed **1f** at  $\lambda_{\text{max}} = 285$  nm ( $\lambda_{\text{max}} = 283$  nm for **1f** in water). The maximal absorbance of A = 0.0035 per **1f**-coated plate, i.e., A = 0.00175 for each side of a plate is practically independent of [1f]<sub>aq</sub> (0.1–10 mM).



**Figure 1.** CPK model for compound **1f**. Dark, light dark, and white colors represent oxygen, carbon, and hydrogen atoms, respectively.

Another characteristic aspect of compound **1f** is that of a host.<sup>14</sup> For example, it forms a stable 1:1 (from Job plot) complex with 8-anilinnaphthalene-1-sulfonate (ANS) in water (step b in Scheme 1). The large binding constant ( $K = 2.2 \times 10^5 \text{ M}^{-1}$  at 25 °C,<sup>15</sup> from fluorimetric titration at 479 nm ( $[\text{ANS}] = 1.0 \times 10^{-6} \text{ M}$  and  $[\mathbf{1f}] \leq 2.2 \times 10^{-5} \text{ M}$ ) and the spectroscopic evidence<sup>16</sup> strongly suggest that guest ANS is included, as for host **1a**,<sup>14</sup> in the polyaromatic cavity of the host flexibly capped<sup>17</sup> by the sugar side chains, as schematically shown by the structure for **1f**·guest in Scheme 1.

ANS alone in water is not adsorbed on a quartz plate (step c in Scheme 1) but is readily coadsorbed with host **1f** (step d). Spectroscopic analysis<sup>18</sup> of the plates dipped in an aqueous solution of host and guest ( $[\mathbf{1f}] = 1 \text{ mM}$  and  $[\text{ANS}] \geq 1 \text{ mM}$ ) shows that (1) the host/guest molar ratio on the plate is  $\sim 1:1$  (step d)<sup>11</sup> and (2) adsorbed guest ANS is gradually rinsed out upon repeated washing with water (step e), while (3) adsorbed host **1f** is not desorbed unless washed with an aqueous amine solution as above.<sup>11</sup> These results indicate that host **1f** not only as such but also as the complex **1f**·ANS is irreversibly adsorbed on the quartz surface (step d),<sup>19</sup> where the included guest molecule is not completely encapsulated but exhibits at least

(13) Kimizuka, N.; Kawasaki, T.; Kunitake, T. *J. Am. Chem. Soc.* **1993**, *115*, 4387–4388.

(14) The guest-binding properties of lipophilic and hydrophilic derivatives of parent macrocycle **1a** ( $\text{R} = (\text{CH}_2)_{10}\text{CH}_3$  or  $(\text{CH}_2)_2\text{SO}_3\text{Na}$ ) have been extensively studied. For examples, see: (a) Aoyama, Y.; Tanaka, Y.; Sugahara, S. *J. Am. Chem. Soc.* **1989**, *111*, 5397–5404. (b) Kikuchi, Y.; Kato, Y.; Tanaka, Y.; Toi, H.; Aoyama, Y. *Ibid.* **1991**, *113*, 1349–1354. (c) Kobayashi, K.; Asakawa, Y.; Kato, Y.; Aoyama, Y. *J. Am. Chem. Soc.* **1992**, *114*, 10307–10313.

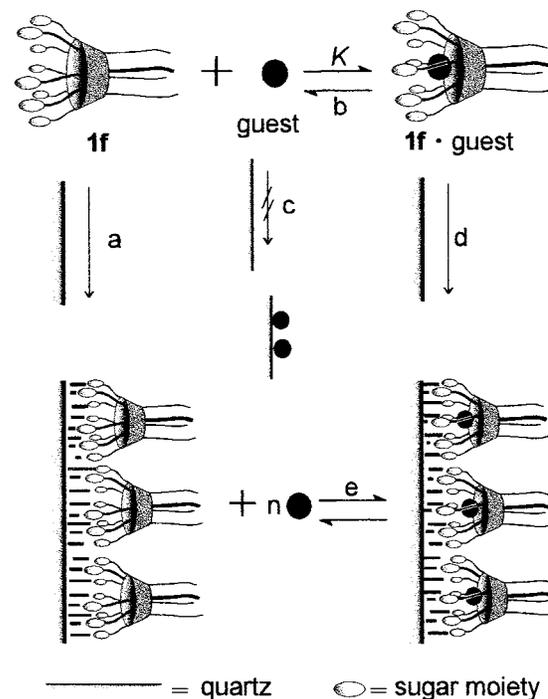
(15) The  $K$  values for ANS range from  $\sim 10^2 \text{ M}^{-1}$  for  $\beta$ -cyclodextrin up to  $\sim 10^6 \text{ M}^{-1}$  for octopus cyclophanes having eight alkyl chains (Murakami, Y.; Kikuchi, J.; Ohno, T.; Hayashida, O.; Kojima, M. *J. Am. Chem. Soc.* **1990**, *112*, 7672–7681).

(16) NMR spectra indicate significant complexation-induced upfield shifts for the protons of bound ANS. A remarkable enhancement in the fluorescence intensities ( $I$ ) of ANS upon binding ( $I = 103$  (arbitrary unit) at 479 nm for complex **1f**·ANS and  $I \approx 0.3$  at 520 nm for free ANS) indicates that bound ANS is effectively insulated from bulk water.

(17) Emert, J.; Breslow, R. *J. Am. Chem. Soc.* **1975**, *97*, 670–672.

(18) Each plate was washed successively with a minimal amount (0.5 mL) of water. The absorbance at 285 nm (for **1f**) and 352 nm (for ANS) was monitored for the plate after each washing.

### Scheme 1



some degree of reversibility (step e). In fact, complexation on the surface takes place when a **1f**-coated quartz plate is dipped in an aqueous solution of the guest (step e).

Silica gel experiments are consistent. Addition of the silica gel (Kaseigel, 3 g) to an aqueous solution of ANS (0.05 mM, 10 mL) results in preferential adsorption of water on the silica ( $\sim 1.3 \text{ mL/g}$ , as independently confirmed); the aqueous solution of ANS is concentrated by  $\sim 50\%$  as a consequence. In the presence of host **1f** (0.1 mM), the fluorescent guest is completely transferred from the aqueous phase to the silica surface.

The significance of the present study is 3-fold. First, it demonstrates a remarkable sugar-cluster effect. Water effectively interferes with polar host–guest interactions. Sugar-binding via hydrogen-bonding has so far been effective only in apolar organic media.<sup>14a,20</sup> The present work thus provides a new strategy for sugar-binding *in water*.<sup>14c,21</sup> Second, the dual roles of compound **1f** as a host as well as an adsorbate leads to a novel solution-to-surface molecular transport or delivery system. Most carrier-mediated transport systems studied so far are concerned with membrane transport between two fluid phases (gas or liquid). Third, the present system thus suggests a potential utility in sugar-directed delivery or targeting of particular guest molecules as drugs or probes to the specific saccharide-receiving biological surfaces.

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(19) Methyl orange (MO) can also be coadsorbed with a 1:1 (**1f** to MO) stoichiometry;  $K = 6.4 \times 10^5 \text{ M}^{-1}$  for complex **1f**·MO in water.

(20) For examples, see: (a) Huang, C.-Y.; Cabell, L. A.; Anslyn, E. V. *J. Am. Chem. Soc.* **1994**, *116*, 2778–2792. (b) Das, G.; Hamilton, A. D. *Ibid.* **1994**, *116*, 11139–11140.

(21) (a) Coterón, J. M.; Vicent, C.; Bosso, C.; Penadés, S. *J. Am. Chem. Soc.* **1993**, *115*, 10066–10076. (b) Shiomi, Y.; Saisho, M.; Harada, T.; Shinkai, S. *Tetrahedron* **1992**, *48*, 8239–8252.