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Saccharide-Coated $M_{12}L_{24}$ Molecular Spheres That Form Aggregates by Multi-interaction with Proteins

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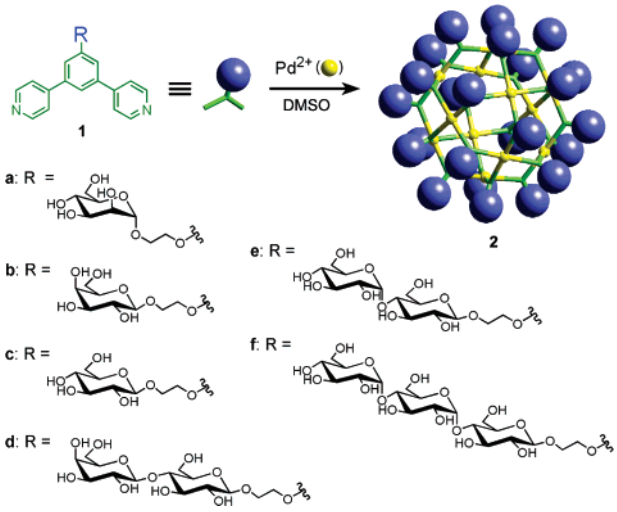
Saccharide−protein interactions play essential roles in vivo, particularly in cellular adhesion and infection.[1] Such biomolecular interactions take place via the recognition of not a monosaccharide but a saccharide cluster by the binding site of protein surfaces.[2] Many efforts have been made for synthesizing saccharide clusters to study how saccharides work on cellular recognition and to add their functions to synthetic molecules. Previously, saccharide clusters have been prepared by the covalent or noncovalent immobilization of saccharides on artificial scaffolds, such as polymers,[3] dendrimers,[2b,4] cyclodextrins,[5,6] calixarenes,[6,7] gold nanoparticles,[8] vesicles,[9] and so on.[10] However, the facile library preparation of structurally well-defined saccharide clusters has been still unexplored. Here, we report a self-assembly approach to the precise clusterization of saccharides at the periphery of ca. 4-nm-sized $M_{12}L_{24}$ molecular sphere (where M and L denote metal ions and bridging ligands, respectively). We designed mono-, di-, or trisaccharide-substituted bridging ligands **1a**−**f**. On complexation with Pd(II) ions, the bridging ligands are assembled into an $M_{12}L_{24}$ spherical core (Scheme 1).[11] Since each ligand carries one (oligo)-saccharide unit, the periphery of the self-assembled sphere is covered precisely with 24 (oligo)saccharides. We show the saccharide-dependent interaction of a series of spherical complexes with lectins to form colloidal aggregates.

α-Mannopyranoside derivative **1a**, synthesized in three steps from 2-bromoethyl 2,3,4,6-tetra-*O*-acetyl-*β*-D-mannopyranoside, was treated with $Pd(NO_3)_2$ in DMSO-d_6 for 12 h at 70 °C. The quantitative formation of spherical complex **2a** was indicated by ^{1}H NMR (Figure 1). Only one set of signals for the ligand was observed, in good agreement with the highly symmetric structure of **2a**. A large downfield shift of the signals of pyridine protons (e.g., $\Delta\delta_{PyH\alpha} = 0.77$ ppm, $\Delta\delta_{PyH\beta} = 0.48$ ppm) is ascribed to the metal−pyridine coordination. The signals of aromatic ring protons involved in the spherical core broadened because of the slow dynamic motion of the rigid large core.

Diffusion-ordered NMR spectroscopy (DOSY) confirmed the selective formation of a single species. All proton signals showed the same diffusion coefficient at $(3.7 \pm 0.3) \times 10^{-11}$ m^2 s^{-1}, consistent with the estimated diameter of 5.2 nm (Supporting Information).[12] A large spherical structure was also ascertained by direct AFM observation (Supporting Information). Cold-spray ionization mass spectrometry (CSI−MS) confirmed an $M_{12}L_{24}$ composition with the molecular weight of 15762 Da after exchange of counterions from nitrate to triflate ions (Figure 2). A series of $[\mathbf{2a}-(OTf^-)_n]^{n+}$ ($n = 8-14$) peaks was observed. For example, a peak at m/z 1283.5 was assigned to $[\mathbf{2a}-(OTf^-)_{11}]^{11+}$ (calculated as 1283.8).

Using the self-assembly method, we easily established the library of saccharide-appended spherical complexes. In addition to monosaccharide-appended ligands **1b** and **1c**, di- and trisaccharide derivatives **1d**−**f** were easily prepared, all of which were successfully

Scheme 1. Self-Assembly of $M_{12}L_{24}$ Complexes with 24 Saccharide Moieties at the Periphery



assembled into the corresponding $M_{12}L_{24}$ complexes **2b**−**f**. The estimated diameters of the mono-, di-, and trisaccharide-appended spheres are 5.2, 6.1, and 7.0 nm, respectively (Figure 3). All of the six complexes have 24 saccharide units equivalently aligned at the

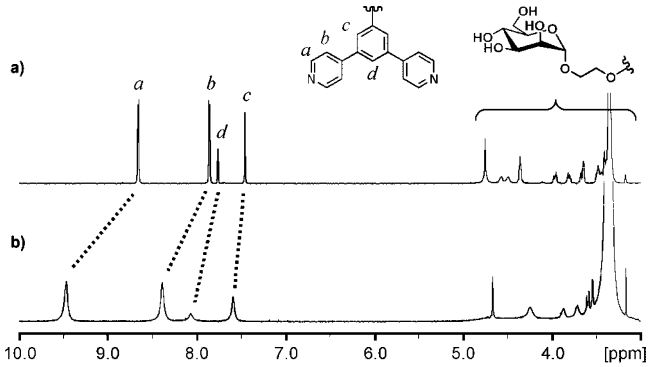


Figure 1. ^{1}H NMR spectra of (a) ligand **1a** and (b) complex **2a**.

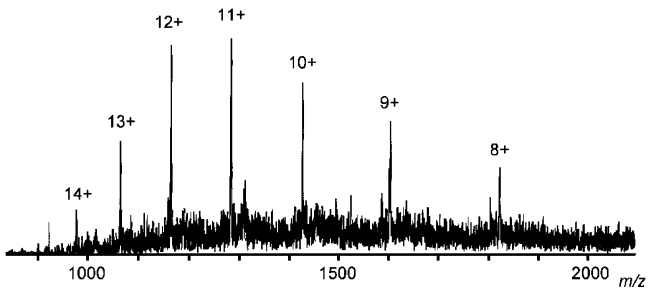


Figure 2. CSI−MS spectrum of **2a** (CH_3CN:DMSO = 20:1, OTf^- salt).

10.1021/ja0693082 CCC: $37.00

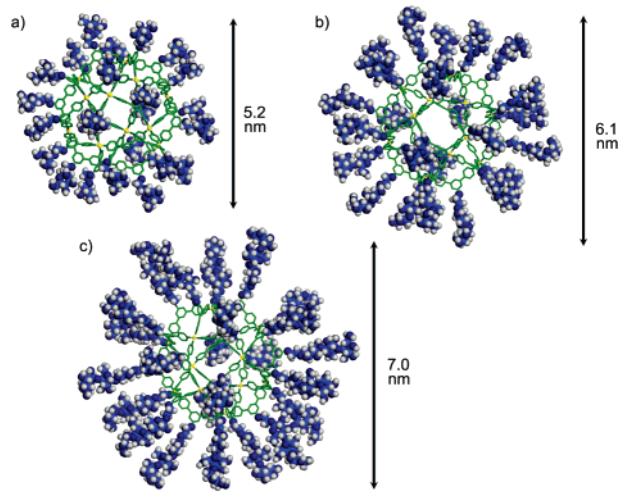


Figure 3. Optimized molecular models and estimated diameters of (a) **2a**, (b) **2d**, and (c) **2f**.

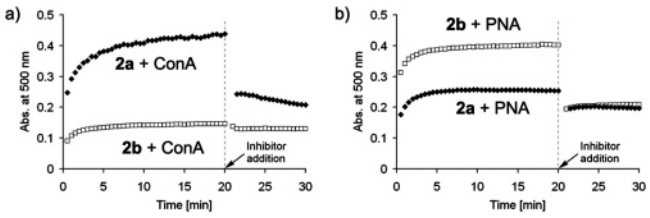


Figure 4. Turbidity changes, monitored by the absorbance at 500 nm, on addition of **2a** and **2b** to (a) ConA or (b) PNA. For clarity, results with **2c**−**f** are given in Supporting Information. A large excess of (a) α-methyl mannopyranoside or (b) D-galactose was added as an inhibitor at 20 min.

periphery of the sphere. The structures of **2b**−**f** were reliably characterized by NMR and CSI−MS; with the latter, even the molecular weight of 21455 Da (for **2f**) was unambiguously determined (Supporting Information).

Saccharide clusters on the spheres **2a**−**f** are expected to show specific interactions with proteins. Thus, we studied the interaction of **2a**−**f** with concanavalin A (ConA), which is a well-studied lectin from *Canavalia ensiformis*.[13] ConA is known to selectively recognize α-mannopyranoside and α-glucopyranoside at its four binding sites. Therefore, **2a** appending 24 α-mannopyranoside units at its periphery is expected to cross-link ConA to form an aggregate. When the DMSO solution of **2a** was added to the solution of ConA in HEPES buffer, the solution became opaque, indicating the formation of the colloidal aggregate of **2a** and ConA (Figure 4a). To this solution was added an excess amount of α-methyl mannopyranoside as an inhibitor against **2a**. Then the opaque solution turned back almost clear, suggesting the disaggregation of the **2a**−ConA aggregate. The aggregation and disaggregation were monitored by measuring the turbidity of the solution.[4b,c,14] The clusterization of the α-mannopyranoside units at the periphery of **2a** is essential because the monomeric component **1a** did not cause the aggregate formation; the transparency of the solution did not change at all on addition of **1a** to the ConA solution.

The aggregate formation was also observed on the addition of **2e** and **2f** bearing α-glucopyranoside. In contrast, aggregates were not obtained in the case of **2b**, **2c**, and **2d** bearing α-galactopyranoside or β-glucopyranoside (Figure 4a). Aggregation of ConA with the spherical complexes depended on the kind of terminal saccharide unit, indicating that ConA recognizes the terminal saccharide unit of **2a**−**f**.

When peanut agglutinin (PNA), a galactose-binding lectin from *Arachis hypogaea*,[15] was used, **2b** and **2d** bearing β-galactopyranoside formed aggregates as indicated by the turbidity measurement. However, the other spherical complexes (**2a**, **2c**, **2e**, and **2f**) did not (Figure 4b).

In conclusion, we established the facile preparation of the library of spherical complexes having 24 saccharide units at the periphery by metal-directed self-assembly. Clustered saccharides on the spherical platform are efficiently bound to ConA or PNA recognizing the conformation of the saccharides. We expect that the combination of the protein recognition at the periphery and molecular encapsulation at the interior of the spheres will lead to many biomedical applications.

Supporting Information Available: Preparation and physical properties of **1a**−**f** and **2a**−**f**, and detailed methods of turbidity experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Taylor, M. E.; Drickamer, K. *Introduction to Glycobiology*; Oxford University Press: New York, 2003. (b) Lis, H.; Sharon, N. *Chem. Rev.* **1998**, *98*, 637−674.
(2) (a) Mammen, M.; Choi, S.-K.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2754−2794. (b) Lundquist, J. J.; Toone, E. J. *Chem. Rev.* **2002**, *102*, 555−578.
(3) (a) Spaltenstein, A.; Whitesides, G. M. *J. Am. Chem. Soc.* **1991**, *113*, 686−687. (b) Mann, D. A.; Kanai, M.; Maly, D. J.; Kiessling, L. L. *J. Am. Chem. Soc.* **1998**, *120*, 10575−10582. (c) Hasegawa, T.; Kondoh, S.; Matsuura, K.; Koyabashi, K. *Macromolecules* **1999**, *32*, 6595−6603.
(4) (a) Lindhorst, T. K.; Kieburg, C. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1953−1956. (b) Zanini, D.; Roy, R. *J. Am. Chem. Soc.* **1997**, *119*, 2088−2095. (c) Aoi, K.; Itoh, K.; Okada, M. *Macromolecules* **1995**, *28*, 5391−5393.
(5) Mellet, C. O.; Defaye, J.; Fernandez, J. M. G. *Chem.—Eur. J.* **2002**, *8*, 1982−1990.
(6) A review of saccharide-appended cyclodextrins and calixarenes: Fulton, D. A.; Stoddart, J. F. *Bioconjugate Chem.* **2001**, *12*, 655−672.
(7) (a) Marra, A.; Scherrmann, M.-C.; Dondoni, A.; Casnati, A.; Minari, P.; Ungaro, R. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2479−2481. (b) Fujimoto, T.; Shimizu, C.; Hayashida, O.; Aoyama, Y. *J. Am. Chem. Soc.* **1997**, *119*, 6676−6677. (c) Aoyama, Y. *Chem.—Eur. J.* **2004**, *10*, 588−593.
(8) (a) Otsuka, H.; Akiyama, Y.; Nagasaki, Y.; Kataoka, K. *J. Am. Chem. Soc.* **2001**, *123*, 8226−8230. (b) de la Fuente, J. M.; Barrientos, A. G.; Rojas, T. C.; Rojo, J.; Canada, J.; Fernandez, A.; Penades, S. *Angew. Chem., Int. Ed.* **2001**, *40*, 2258−2261.
(9) (a) Kingery-Wood, J. E.; Williams, K. W.; Sigal, G. B.; Whitesides, G. M. *J. Am. Chem. Soc.* **1992**, *114*, 7303−7305. (b) Lee, H.-K.; Park, K. M.; Jeon, Y. J.; Kim, D.; Oh, D. H.; Kim, H. S.; Park, C. K.; Kim, K. *J. Am. Chem. Soc.* **2005**, *127*, 5006−5007.
(10) (a) Complex: Sakai, S.; Sasaki, T. *J. Am. Chem. Soc.* **1994**, *116*, 1587−1588. (b) Micelle: Yasugi, K.; Nakamura, T.; Nagasaki, Y.; Kato, M.; Kataoka, K. *Macromolecules* **1999**, *32*, 8024−8032.
(11) (a) Tominaga, M.; Suzuki, K.; Kawano, M.; Kusukawa, T.; Ozeki, T.; Sakamoto, S.; Yamaguchi, K.; Fujita, M. *Angew. Chem., Int. Ed.* **2004**, *43*, 5621−5625. (b) Tominaga, M.; Suzuki, K.; Murase, T.; Fujita, M. *J. Am. Chem. Soc.* **2005**, *127*, 11950−11951. (c) Sato, S.; Iida, J.; Suzuki, K.; Kawano, M.; Ozeki, T.; Fujita, M. *Science* **2006**, *313*, 1273−1276. (d) Murase, T.; Sato, S.; Fujita, M. *Angew. Chem., Int. Ed.* **2007**, *46*, 1083−1085.
(12) Estimated diameter from the Stokes−Einstein equation (standardization with X-ray structure of a relevant complex)[11a] is 4.0 ± 0.4 nm.
(13) Naismith, J. H.; Emmerich, C.; Habash, J.; Harrop, S. J.; Helliwell, J. R.; Hunter, W. N.; Raftery, J.; Kalb, A. J.; Yariv, J. *Acta Crystallogr.* **1994**, *D50*, 847−858.
(14) Fujimoto, T.; Shimizu, C.; Hayashida, O.; Aoyama, Y. *J. Am. Chem. Soc.* **1998**, *120*, 601−602.
(15) Banerjee, R.; Das, K.; Ravishankar, R.; Suguna, K.; Surolia, A.; Vijayan, M. *J. Mol. Biol.* **1996**, *259*, 281−296.

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