Synthesis of Water-soluble Tris(cyclophane) Hosts and Surface Plasmon Resonance Study on Guest-binding Interaction with Immobilized Guests

Osamu Hayashida*[1,3] and Atsushi Kitaura[2]
[1]*Institute for Materials Chemistry and Engineering, Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812-8581*
[2]*Department of Chemistry and Biochemistry, Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812-8581*
[3]*PRESTO, JST, 4-1-8 Honcho Kawaguchi 332-0012*

(Received May 11, 2006; CL-060561; E-mail: ohaya@ms.ifoc.kyushu-u.ac.jp)

Tris(cyclophane) derivatives were prepared as water-soluble hosts by connecting three macrocyclic skeletons. The present hosts showed enhanced guest-binding affinity relative to that by simple cyclophane, as confirmed by surface plasmon resonance measurements.

Naturally occurring multivalent clusters of receptors are known to exhibit extremely strong binding capability toward substrates, even though individually these substrates bind only weakly to each other.[1] Bio-inspired molecular hosts such as cyclodextrin oligomers,[2] water-soluble cyclodextrin polymers,[3] and dendritic cyclophanes[4] have been widely used to mimic the functions of multivalent receptors. We have previously synthesized polytopic macrocycles such as cage-type cyclophanes[5a] and cyclophane-based multi(cyclophane)[5b] in order to enhance guest-binding ability. In this context, we now report the preparation of linear-type tris(cyclophane) having eight polar side-chains **3a** and **3b** and the guest-affinity of the resulting hosts to hydrophobic guests by fluorescence spectroscopy and surface plasmon resonance (SPR) in comparison with that of water-soluble cyclophane **1a** (Chart 1).[5c]

Tris(cyclophane) having eight polar side chains with terminal galactose residues **3a** was prepared by following the reaction sequence given in Scheme 1. A cyclophane bearing two boc-β-alanine moieties **4** was synthesized in a 23% yield by condensation of 1,6,20,25-tetraaza[6.1.6.1]paracyclophane[6] with boc-β-alanine (2.2 equiv.) in the presence of dicyclohexylcarbodiimide (DCC). A cyclophane bearing three boc-β-alanine moieties **5** was prepared in a similar manner using boc-β-alanine (3.2 equiv) A cyclophane having two carboxy groups **6** was derived

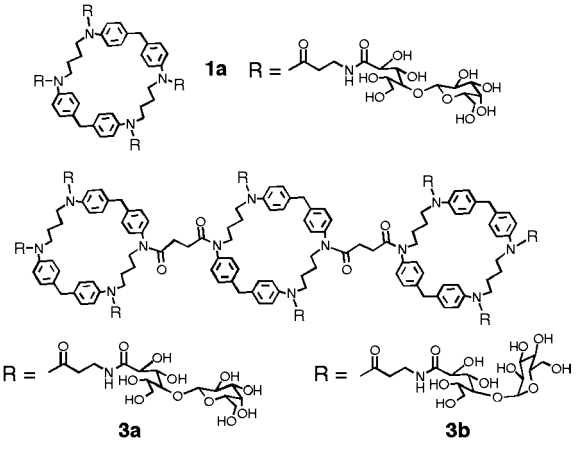
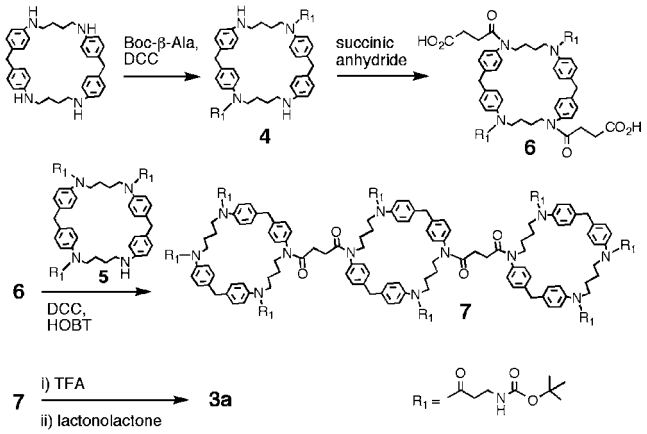


Chart 1.



Scheme 1. Preparation of tris(cyclophane) having eight polar side chains with terminal galactose residues.

from **4** by a reaction of succinic anhydride and was isolated as dicarboxylic acid. Precursor **7** was prepared by condensation of **6** with **5** in the presence of DCC and 1-hydroxybenzotriazole (HOBT). Tris(cyclophane) derivative **3a** was prepared from **7** by treatment with TFA and subsequent aminolysis with lactonolactone. The use of maltonolactone in place of lactonolactone afforded the corresponding tris(cyclophane) having eight polar side-chains with terminal glucose residues **3b**. All the new compounds were characterized by means of spectroscopy (^{1}H and ^{13}C NMR), MS, and elemental analysis.[7] Both cyclophanes **3a** and **3b** had good solubility of >1 g mL^{-1} in aqueous 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) buffer (0.01 M, pH 7.4, with 0.15 M NaCl).

First, fluorescence spectroscopy at 293 K was used to examine the guest-binding abilities of **3a** and **3b** as host toward well-known fluorescent guests such as 6-*p*-toluidinonaphthalene-2-sulfonate (TNS) and pyrene, in a manner similar to that reported previously.[8] The fluorescence intensity originating from TNS (1 μM) increased with a concomitant blue shift of the fluorescence maximum upon addition of a large excess of **3a**, as shown in Figure 1. The microenvironmental polarity experienced by the entrapped guest molecule was evaluated on the basis of the correlation between λ_{max} and the solvent polarity parameter (E_T^N)[9] as described previously.[8] The E_T^N value for TNS placed in **3a** was estimated to 0.62 (425 nm), which was equivalent to the value for 1-propanol.[10] Similar complexation behavior was confirmed for **3b** with TNS. The binding constants (K) for 1:1 complex in the presence of a large excess of the hosts were evaluated on the basis of the Benesi–Hildebrand relationship: K, 3.9×10^4 and 3.6×10^4 M^{-1} for the complexes of **3a** and **3b**, respectively. The guest-binding affinity of **3a** was enhanced 24-fold relative to

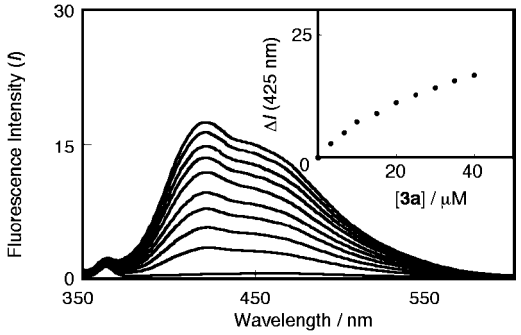


Figure 1. Fluorescence spectral changes for aqueous solution of TNS (1 μM) upon addition of **3a** in H_2O at 293 K: [**3a**] = 0, 3.3, 6.6, 10, 15, 20, 25, 30, 35, and 40 μM (from bottom to top). Inset: the corresponding titration curve, Ex, 326 nm.

that by the corresponding monocyclic cyclophane **1a** (K, $1.6 \times 10^3\ M^{-1}$). Since **3a** has 3 unit-binding sites, the guest-binding enhancement of **3a** per binding site is eightfold that reflects cooperative and multivalency effects in macrocycles. A similar enhancement in the guest-binding of hosts toward pyrene was also confirmed by identical methods: K, 2.3×10^5 and $9.4 \times 10^3\ M^{-1}$, for the complexes of **3a** and **1a**, respectively.

We examined the binding interactions of **3a** and **1a** to an immobilized pyrene as a guest on a sensor chip by SPR measurements. First, immobilization of 1-aminomethyl pyrene (Py–N) to the carboxylated dextran sensor chip surface (CM5), which was set in Biacore X (Pharmacia Biotech), was performed by utilizing the EDC-NHS coupling protocol (Figure 2A).[11] The amount of immobilization of guest molecules was given as a resonance signal of 8,580 RU (resonance units). Second, when a solution of **3a** in HEPES buffer was applied into surfaces of immobilized pyrene, the association shown in Figure 2B(a) was observed. Then, by changing the HEPES buffer to wash away the noncovalently bound **3a**, the dissociation shown in Figure 2B(a) was initiated. The binding constant (K) of **3a** with immobilized pyrene was determined to be $6.7 \times 10^5\ M^{-1}$ on the

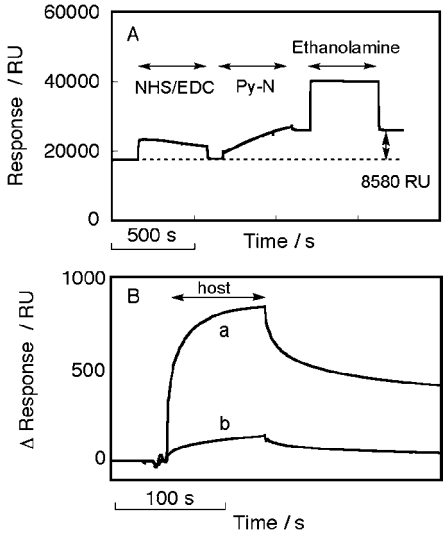


Figure 2. (A) Immobilization of Py–N onto CM5 sensor chip. (B) Oberlay sensorgrams of **3a** (a, 40 μM) and **1a** (b 40 μM) with immobilized pyrene surface, Flow rate: 20 μL min^{-1} in HEPES buffer.

basis of kinetic analysis using 1:1 Langmuir fitting in a manner similar to that reported previously.[12] In addition, the cluster effect achieved by multiplying cyclophane cavities seems to be reflected in the binding ability of **3a**, because the binding affinity of monocyclic cyclophane **1a** (K, $2.3 \times 10^4\ M^{-1}$) toward the immobilized pyrene was much weaker than that of **3a** (Figure 2B(b)). A similar enhancement by ca. 30-fold[13] in guest-binding was also confirmed for **3b** by identical methods.

In summary, novel tris(cyclophane) hosts showed enhanced guest-binding affinity relative to that by simple cyclophane, which was confirmed by fluorescence spectroscopy. Moreover, the enhancement for the guest-binding affinity was retained by the tris(cyclophane) hosts toward immobilized pyrene derivatives on a sensor chip. Thus, well-qualified host molecules, such as tris(cyclophane) hosts that meet various requirements of strong guest-binding and microenvironmental properties, are expected to be utilized as artificial receptors in order to simulate biological functions.

References and Notes

1 Y. C. Lee, R. T. Lee, *Acc. Chem. Res.* **1995**, *28*, 321.
2 S. Aime, M. Botta, F. Fedeli, E. Gianolio, E. Terreno, P. Anelli, *Chem. Eur. J.* **2001**, *7*, 5262.
3 G. Crini, *Prog. Polym. Sci.* **2005**, *30*, 38.
4 F. Diederich, B. Felber, *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4779.
5 a) Y. Murakami, O. Hayashida, Y. Nagai, *J. Am. Chem. Soc.* **1994**, *116*, 2611. b) O. Hayashida, Y. Takaoka, I. Hamachi, *Tetrahedron Lett.* **2005**, *46*, 6589. c) O. Hayashida, I. Hamachi, *J. Org. Chem.* **2004**, *69*, 3509.
6 K. Odashima, A. Itai, A. Iitaka, K. Koga, *J. Am. Chem. Soc.* **1980**, *102*, 2504.
7 Compound **4**; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41 (s, 18H), 1.5 (m, 8H), 2.1 (m, 4H), 3.1 (m, 4H), 3.3 (m, 4H), 3.6 (m, 4H), 3.83 (s, 4H), 5.31 (m, 2H), 6.4 (d, 4H), 6.9 (m, 8H), and 7.2 (m, 4H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 25.9, 26.1, 26.8, 27.0, 28.9, 35.2, 36.8, 40.9, 43.8, 44.3, 49.3, 79.4, 113.2, 128.5, 129.9, 130.5, 139.9, 142.8, 147.0, 156.3, and 171.9. HRMS (FAB) calcd for $C_{50}H_{66}N_6O_6$: 846.5044. Found: 846.5087. Compound **6**; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.3 (m, 26H), 1.9–2.2 (m, 8H), 2.5 (m, 4H), 3.2 (m, 4H), 3.6 (m, 8H), 3.91 (s, 4H), 5.33 (m, 2H), 6.9 (d, 8H), and 7.2 (m, 8H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 25.1, 28.9, 29.7, 30.0, 35.2, 36.8, 41.4, 48.9, 49.6, 79.5, 128.7, 130.6, 140.6, 141.1, 156.4, 171.9, 172.1, and 176.4. Anal. Found: C, 64.09; H, 7.04; N, 7.81%. Calcd for $C_{58}H_{74}N_6O_{12}\cdot 2H_2O$: C, 64.31; H, 7.26; N, 7.76%. HRMS (FAB) calcd for $C_{58}H_{75}N_6O_{12}$: 1047.5443. Found: 1047.5446. Compound **7**; ^{13}C NMR (150 MHz, $CDCl_3$) δ 25.2, 28.5, 33.2, 35.2, 36.7, 41.5, 49.2, 49.3, 79.4, 128.2, 129.9, 130.5, 140.7, 141.3, 156.3, 171.2, and 174.0. Anal. Found: C, 67.54; H, 7.53; N, 9.39%. Calcd for $C_{174}H_{228}N_{20}O_{28}\cdot 2H_2O$: C, 67.77; H, 7.58; N, 9.08%. MS (MALDI–TOF) m/z, 3070 $[M + Na]^+$. Compound **3a**; ^{13}C NMR (150 MHz, D_2O) δ 23.5, 29.4, 34.0, 35.8, 41.0, 47.0, 48.0, 61.5, 62.4, 69.0, 69.1, 70.8, 71.4, 71.6, 71.8, 72.0, 72.2, 73.2, 75.8, 81.5, 82.0, 103.9, 128.4, 130.5, 139.3, 141.4, 173.0, and 174.0. Anal. Found: C, 52.50; H, 6.91; N, 5.12%. Calcd for $C_{230}H_{324}N_{20}O_{100}\cdot 16H_2O$: C, 52.54; H, 6.83; N, 5.33%. MS (MALDI–TOF) m/z, 4970 $[M + H]^+$. Compound **3b**; ^{13}C NMR (150 MHz, D_2O) δ 25.0, 29.3, 32.6, 35.8, 41.0, 47.0, 52.5, 60.7, 62.5, 69.7, 72.0, 72.7, 72.8, 73.3, 82.4, 100.8, 100.9, 128.6, 130.5, 139.3, 141.6, 173.0, and 174.2. Anal. Found: C, 52.84; H, 6.44; N, 5.16%. Calcd for $C_{230}H_{324}N_{20}O_{100}\cdot 14H_2O$: C, 52.91; H, 6.80; N, 5.37%. MS (MALDI–TOF) m/z, 4970 $[M + H]^+$.
8 O. Hayashida, K. Ono, Y. Murakami, *Tetrahedron* **1995**, *51*, 8423.
9 C. Reichardt, in *Solvents and Solvent Effects in Organic Chemistry*, VCH Verlagsgesellschaft, Weinheim, **1988**, Chap. 7.
10 The E_T^N value for TNS placed in **1a** was estimated to 0.68.
11 The carboxyl groups on the sensor surfaces were activated with an injection of a solution containing 0.4 M *N*-ethyl-*N*′-(3-diethylaminopropyl)carbodiimide (EDC) and 0.1 M *N*-hydroxysuccinimide (NHS). Specific surfaces were obtained by injecting a solution of Py-N (0.4 mM). The immobilization procedure was completed by a 7 min injection of 1 M ethanolamine hydrochloride to block remaining ester groups.
12 O. Hayashida, K. Mizuki, K. Akagi, A. Matsuo, T. Kanamori, T. Nakai, S. Sando, Y. Aoyama, *J. Am. Chem. Soc.* **2003**, *125*, 594.
13 The guest-binding enhancement of **3a** per binding site is ca. 10-fold.

Published on the web (Advance View) June 17, 2006; doi:10.1246/cl.2006.808