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Synthesis of Cyclophane Dimer Using Cyclophane-tethered Fmoc-amino Acid Derivatives as a Multivalent Host

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Water-soluble cyclophane dimer was synthesized by Fmoc-chemistry of cyclophane-tethered Fmoc-lysine derivative, as a multivalent host. The host showed enhanced guest binding affinity relative to that by monocyclic cyclophane, as confirmed by fluorescence spectroscopy as well as surface plasmon resonance measurements.

Cyclophanes are macrocyclic compounds that form inclusion complexes with various organic compounds. Cyclophanes play a broad and prominent role in host–guest chemistry, biomimetic chemistry, and supramolecular chemistry.[1] Much effort has been devoted to develop sophisticated cyclophanes by introducing various functional groups such as polar moieties for water-solubility,[2] saccharide ligands for lectin-targeting,[3] and fluorophores for guest-sensing[4] on the macrocyclic skeleton. We have recently developed cyclophane oligomers on the basis of molecular design to connect several macrocyclic skeletons directly as a multivalent host.[5] On the other hand, peptide synthesis using Fmoc-chemistry has been utilized to connect natural and non-natural amino acids as building blocks.[6] In the course of our ongoing research on cyclophane oligomers, we became interested in the utilization of cyclophane-tethered Fmoc-amino acid derivative as a building block. In this regard, we have adopted a simple strategy by conjugating the cyclophane derivatives with Fmoc-lysine residue through amide linkage.[7] We report here the synthesis of water-soluble cyclophane bearing a dipeptide moiety **1** and analogous cyclophane dimer **2** by synthesis using Fmoc-chemistry of the cyclophane-tethered Fmoc-lysine derivative **3** (Figure 1). Furthermore, cyclophane dimer **2** was demonstrated as a host for effective incorporation of hydrophobic guests in aqueous media by means of fluorescence spectroscopy as well as surface plasmon resonance (SPR) measurements.

Water-soluble cyclophanes **1** and **2** were synthesized by following the reaction sequence shown in Scheme 1. Cyclophane-tethered Fmoc-lysine derivative **3** was prepared by a reaction of Fmoc-lysine with succinimidyl ester derivative of cyclophane **4**.[8] Then, we applied compound **3** to synthesis a dipeptide **5**, which was prepared by condensation of **3** with L-leucine amide in the presence of benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (PyBOP). Moreover, a precursor **6** of cyclophane dimer **2** was synthesized by condensation of **3** with monoamino derivative of the dipeptide, which was easily prepared from **5** by removal of the protecting group with piperidine, in the presence of benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP). Cationic cyclophane dimer **2** was obtained from **6** by reaction with piperidine and trifluoroacetic acid (TFA) in this sequence to remove Fmoc and Boc protecting groups, respectively. Cyclophane **1** was also derived from **5** in a manner similar to that applied to the synthesis of **2**. New compounds were characterized by means of spectroscopy (^{13}C NMR and ESI-MS) and elemental analysis (see the Supporting Information).[8]

A possible computer-generated CPK model for cyclophane **2** suggested that hydrophobic cavities were reasonably separated from branched hydrophilic groups that are required for giving water solubility to cyclophanes (see the Supporting Information).[8,9] In addition, the secondary structural features on the peptide backbone of

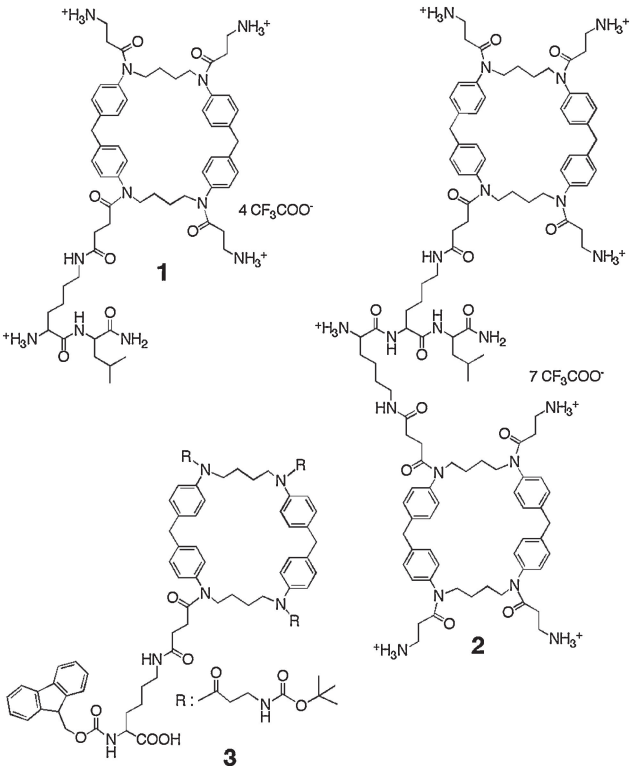
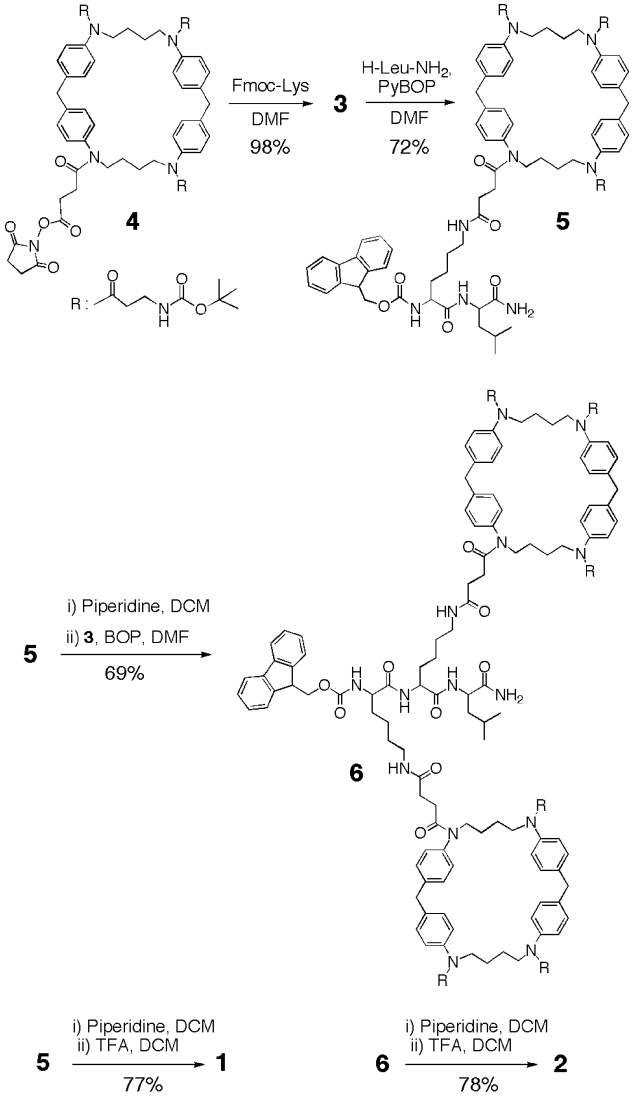


Figure 1. Water-soluble cyclophanes **1** and **2** and cyclophane-tethered Fmoc–lysine derivative **3**.

2 were hardly observed by circular dichroism measurements. Cyclophane **2** had good H_2O-solubility of $>0.17\,g\,mL^{-1}$. These results suggest that cyclophane **2** having hydrophobic cavities will act as water-soluble hosts.

The guest-binding behavior of cyclophane dimer **2** toward fluorescent guests such as 6-*p*-toluidinonaphthalene-2-sulfonate (TNS) and 6-anilinonaphthalene-2-sulfonate (2,6-ANS), whose emission is extremely sensitive to change in microenvironmental polarity experienced by the molecules,[10] was examined by fluorescence spectroscopy in aqueous HEPES {2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid} buffer (0.01 M, pH 7.4, 0.15 M with NaCl) at 298 K. The fluorescence intensity originating from each guest increased along with a concomitant blue shift of the fluorescence maximum upon addition of a large excess amount of **2** as shown in Figure 2. Binding constants for the formation of inclusion complexes of **2** with TNS and 2,6-ANS in a 1:1 molar ratio[11] (K) were evaluated on the basis of the Benesi–Hildebrand relationship ($K = 7.8 \times 10^4$ and $7.2 \times 10^4\,M^{-1}$, respectively). On the other hand, K values for monocyclic cyclophane **1** upon complexation with TNS and 2,6-ANS were relatively small: $K = 5.1 \times 10^3$ and $5.7 \times 10^3\,M^{-1}$, respectively (see the Supporting Information).[8] Therefore, the K values of **2** with TNS and 2,6-ANS were enhanced about 15- and 13-fold, respectively, relative to that of **1**, reflecting multivalent effects in macrocycles.



Scheme 1. Preparation of water-soluble cyclophanes **1** and **2**.

In addition, guest-binding affinities of cyclophanes **1** and **2** with immobilized (1-pyrenylmethyl)amine (PMA) on a surface were also investigated by SPR measurements in a manner similar to that reported previously.[12] When a solution of **2** in HEPES buffer was applied into the surfaces of immobilized PMA (4600 RU), the association between **2** and immobilized PMA was observed to increase in the signal in the form of response (RU) as shown in Figure 3. Then, by changing the HEPES running buffer, the dissociation was initiated (Figure 3). The K value of **2** with immobilized PMA was evaluated to be $1.4 \times 10^6\,M^{-1}$ on the basis of kinetic analysis by using curve fitting methods applied to the SPR sensorgram, which was about 14-fold larger than that of **1** for the identical guest ($K = 9.7 \times 10^4\,M^{-1}$). The enhancement in guest binding seems to be achieved by multiplying cyclophane cavities of **2**, which was comparable to those examined by fluorescence experiments.

In conclusion, cyclophane dimer **2** was successfully synthesized by using cyclophane-tethered Fmoc-lysine derivative **3** as a building block in a fairly good yield. The guest-binding affinities of **2** were about 14-fold larger than those of a corresponding monocyclic cyclophane **1** reflecting the multivalency effects in macrocycles. Development of longer peptides bearing cyclophane moieties is quite promising by using this strategy.

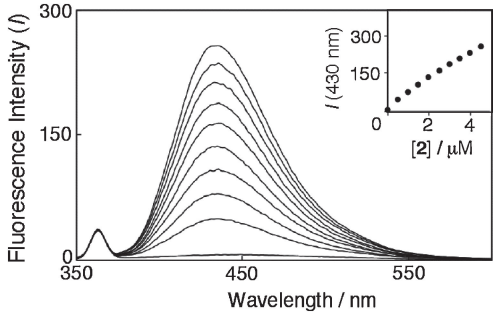


Figure 2. Fluorescence spectral changes for an aqueous solution of TNS (0.25 μM) upon addition of **2** in HEPES at 298 K, [**2**] = 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, and 4.5 μM (from bottom to top). Ex. 326 nm. Inset: the corresponding titration curve.

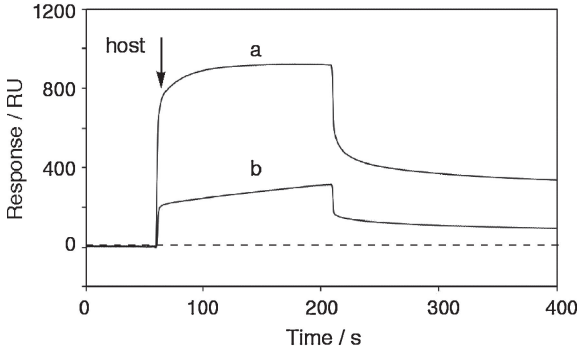


Figure 3. Oberlay sensorgrams of **2** (a, 50 μM) and **1** (b, 50 μM) with immobilized pyrene surface. Flow rate: 20 μL min^{-1}, in HEPES buffer. Arrow represents an injection of each of the hosts.

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