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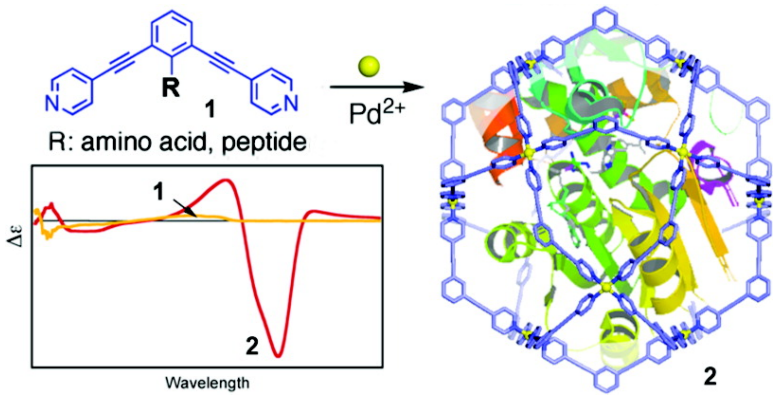

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Endohedral Peptide Lining of a Self-Assembled Molecular Sphere To Generate Chirality-Confined Hollows

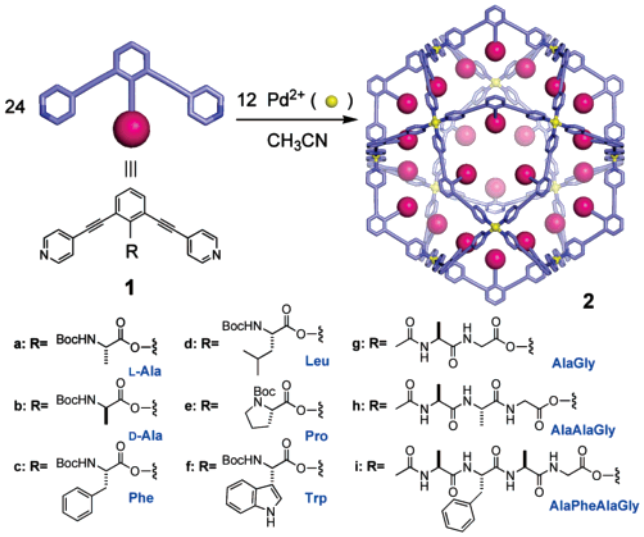
Kosuke Suzuki, Masaki Kawano, Sota Sato, and Makoto Fujita*

Department of Applied Chemistry, School of Engineering, The University of Tokyo and CREST, Japan Science and Technology Agency (JST), 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8656, Japan

Received May 21, 2007; E-mail: mfujita@appchem.t.u-tokyo.ac.jp

Enzyme pockets are chiral cavities surrounded by amino acid residues, where highly elaborated molecular recognition and chemical transformations take place.[1] Although there are many reports on the de novo design and control of peptide 3D-structures,[2,3] the construction of chiral pockets fully surrounded by amino acid residues has been seldom achieved. In the present study, peptide fragments are tethered to the interior of an $M_{12}L_{24}$ nanosized spherical complex[4,5] to generate peptide-lined chiral cavities. The shell of the spherical complex self-assembles from 12 Pd(II) ions and 24 bent bridging ligands with dangling peptide fragments (Scheme 1). In this simple way, we succeeded in decorating the interior surface of the 4 nm shell with 24−96 amino acid residues. As the ligand synthesis is modular, we expect that the combinatorial replacement of peptide residues enables the artificial evolution of enzymelike pockets.

Scheme 1



We designed bent ligands **1a**−**i** with amino acids or peptides attached at the benzene core. The ligands were synthesized in one step by the condensation reactions of 2,6-bis(4-pyridylethynyl)-phenol and *N*-protected amino acids or peptides using *N*,*N*′-dicyclohexylcarbodiimide as a condensation agent. When a mixture of L-alanine anchored ligand **1a** (5.0 μmol) and $Pd(CF_3SO_3)_2$ (2.5 μmol) in CD_3CN (1 mL) was stirred for 4 h at 50 °C, the quantitative formation of a single and highly symmetric product, **2a**, was indicated by ^{1}H NMR spectroscopic analysis. The large downfield shift of the product signals as compared to ligand **1a**, particularly for pyridyl α-hydrogen atoms ($\Delta\delta$ = 0.33 ppm), is attributed to the metal−pyridine coordination. Cold-spray ionization mass spectrometry (CSI-MS)[6] clearly confirmed the $M_{12}L_{24}$ stoichiometry from a series of prominent peaks of $[\mathbf{2a}-(OTf^-)_n]^{n+}$ (n = 5−13). For example, intense peaks at m/z 1860.6, 1637.2, and 1458.7 were assigned to $[\mathbf{2a}-(OTf^-)_8]^{8+}$, $[\mathbf{2a}-(OTf^-)_9]^{9+}$, and $[\mathbf{2a}-(OTf^-)_{10}]^{10+}$, respectively (Figure 1).

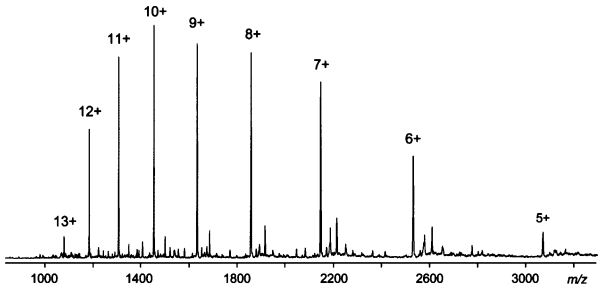


Figure 1. CSI-MS spectrum of complex **2a** (CH_3CN, OTf^- salt).

The structure of complex **2a** was unambiguously determined by single-crystal X-ray diffraction.[7] Single crystals suitable for X-ray crystallographic analysis were obtained by the slow vapor diffusion of 1,4-dioxane into an acetonitrile solution of **2a**. Despite the severe disorder of solvent molecules and some of the triflate counterions, synchrotron X-ray irradiation with high flux and low divergence provided high quality data, from which the structure of $M_{12}L_{24}$ spherical complex with a diameter of 4.6 nm was revealed (Figure 2). The locations of the L-alanine moieties on the interior surface were precisely determined. The positions of the *t*-Boc protection groups, however, could not be clearly observed because of disorder.

The accumulation of 24 asymmetrical amino acid moieties generates a chiral environment within the spherical shell. The intensity of the circular dichromism (CD) spectra of ligands **1a**

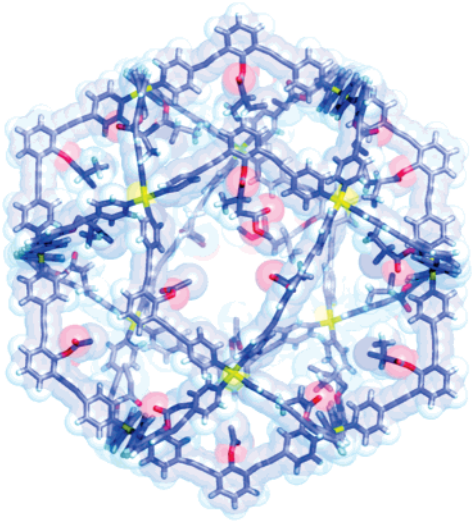


Figure 2. Crystal structure of complex **2a**. Counterions and solvent molecules are omitted for clarity (Pd, yellow; C, blue; N, purple; O, red; H, gray).

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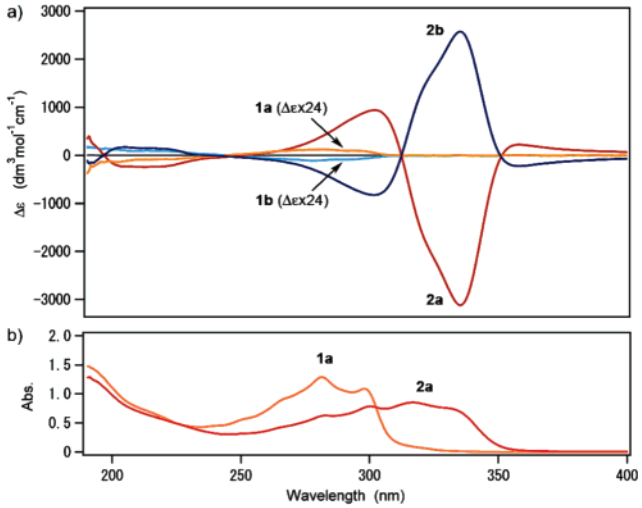

Figure 3. (a) CD spectra and (b) UV spectra of ligand **1a** (L-Ala, 24 μM), **1b** (D-Ala, 24 μM) and complex **2a**,**b** (1 μM) in CH_3CN at 25 °C with a 1 cm cell. The CD spectra of **1a**,**b** were multiplied by a factor of 24 for comparison. $\Delta\epsilon$ = molar circular dichroism.

(L-Ala) and **1b** (D-Ala) is very weak (Figure 3). After complexation, however, sphere **2a** shows distinct Cotton effects in the absorptive region of the complex framework. At equimolar concentration of alanine moieties of **1a** and **2a**, the intensity of the Cotton effects of **2a** was about thirty times higher than that of **1a**. Mirror image Cotton effects were exhibited for **2a** and **2b**, reflecting the absolute configurations of L- and D-alanine moieties. We believe that the Cotton effects stem from the chiral conformation of the ligand frameworks.[8] Namely, the chirality of 24-amino acids is transferred to the backbone of the spherical complex, resulting in twisting of the coordinated ligands in solution.

In addition to the alanine-lined spherical complexes, we obtained spheres endo-functionalized with various types of amino acids or peptides. Ligands **1c**−**i** were assembled into spherical complexes **2c**−**i**, whose structures were characterized by NMR and CSI-MS. These complexes contain 24- (**2c**−**f**), 48- (**2g**), 72- (**2h**), and 96- (**2i**) amino acid residues, respectively (Figure S1). When the 5-residue peptide (Ala-Val-Phe-Ala-Gly) was tethered to the ligand, the spherical complex was no longer formed as a single product, probably because of the limitation of the cavity volume. These results reveal that the spherical shell (4.6 nm in diameter) can contain up to ca. 100 amino acid residues, corresponding to small proteins in size and the number of amino acid residues.

The interior of the spheres can be combinatorially functionalized with a variety of amino acid residues. For the simplest demonstration, we examined the self-assembly of the spherical complex from two different ligands. When the mixture of ligand **1a** and **1c** (10:1) was treated with $Pd(CF_3SO_3)_2$, CSI-MS analysis indicated the formation of several isomers of $M_{12}L_{24}$ spherical complexes containing Ala and Phe residues in 24/0 to 20/4 ratios (Figure 4). In a similar way, the Ala-lined sphere can incorporate a few L-proline residues of **1e** that potentially show organocatalysis for some organic transformations.[9] Since the sphere formation is a thermodynamic process, the spherical shell assembling from two or more ligands constitutes a dynamic combinatorial library[10] of amino acid residues at the interior.

In summary, we have constructed 4.6 nm-sized spherical complexes containing up to 96 amino acid residues. The number and the sequence of amino acid residues are modified and controlled. These peptide-lined complexes generate chiral hollow cavities, similar to enzyme pockets, which could be utilized as

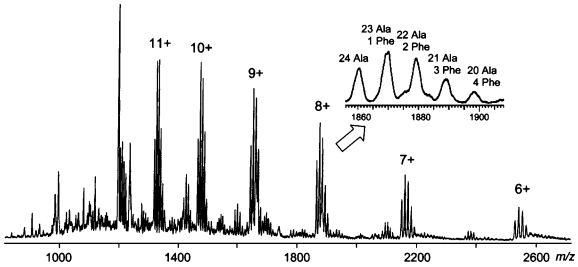

Figure 4. CSI-MS spectrum of $M_{12}L_{24}$ spherical complex self-assembled from the mixture of ligand **1a** (Ala) and **1c** (Phe). The ratio of **1a**/**1c** was 10:1 (CH_3CN, OTf^- salt).

binding pockets for asymmetric molecular recognition and reactions. We also demonstrated the dynamic combinatorial libraries of spherical cavities by combining two or more different ligands bearing amino acids. Although the ratio of amino acids within the complexes is statistical, we expect that the ratio and position of amino acid residues can be controlled by template molecules and external environment and that the interior confined in the sphere can be ultimately evolved toward artificial enzyme pockets.

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Supporting Information Available: Preparation and physical properties of **1a**−**i** and **2a**−**i**, and crystallographic data of **2a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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