doi:10.1246/cl.2010.1321
Published on the web November 13, 2010

Guest Binding, Cellular Uptake, and Molecular Delivery of Water-soluble Cyclophanes Having a Pyrene Moiety

Osamu Hayashida,* Chika Eguchi, Keiichiro Kimura, Yu Oyama, Tomomi Nakashima, and Kosei Shioji
Department of Chemistry, Faculty of Science, Fukuoka University, 8-19-1 Nanakuma, Fukuoka 814-0180

(Received October 12, 2010; CL-100871; E-mail: hayashida@fukuoka-u.ac.jp)

Cationic water-soluble cyclophane bearing a pyrene **1** was synthesized as a host for inclusion of anionic guest molecules. Cationic host **1** was found to be incorporated into human hepatoblastome HepG2 cells effectively. Moreover, 8-anilinonaphthalene-1-sulfonate (ANS) having an inherent poor cell permeability was taken up into the cells in the presence of **1** through formation of host–guest complexes.

The development of artificial carriers exhibiting cellular uptake capability is of great importance for applications such as drug delivery systems[1] and live-cell imaging.[2] Organic chemists have made numerous attempts to develop artificial carriers based on polymers,[3] dendrimers,[4] and other molecular assemblies[5] that can permeate and invade cells. The cellular uptake of the artificial carriers is claimed to depend principally on the size, hydrophobicity, and hydrophilicity of the molecules.[6] On these grounds, we have focused on water-soluble cyclophanes having a well-defined molecular structure and an inherent potential to act as a host for inclusion of guest molecules. In order to develop functionalized cyclophanes[7] as a carrier, we have now designed new cationic and anionic fluorescent cyclophanes **1** and **2**, respectively, which are composed of a tetraaza[6.1.6.1]paracyclophane skeleton, three polar side chains, and a pyrene moiety (Figure 1). We report here the synthesis of water-soluble cyclophanes having a pyrene moiety, their guest-binding abilities, and cellular uptake with emphasis on the molecular delivery.

Cationic cyclophane bearing pyrene group **1** was prepared by following the reaction sequence given in Scheme 1. Anionic cyclophane **2** was derived from **1** by a reaction with succinic anhydride (Scheme 1). All the novel products were purified by gel-filtration chromatography and identified by ^{13}C NMR and IR spectroscopy as well as by elemental analyses (see the Supporting Information).[8] Even though compounds **1** and **2** contain a hydrophobic cavity and a pyrene group, both compounds are soluble in aqueous neutral media at biological pH owing to three polar side chains.

Pyrene-appended water-soluble cyclophanes **1** and **2** showed characteristic fluorescence spectra originating from pyrene moieties in aqueous media (Figure 2). The guest-binding behavior of **1** and **2** toward a well-known fluorescent guest, 8-anilinonaphthalene-1-sulfonate (ANS),[9] was examined by fluorescence spectroscopy in aqueous HEPES {2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid} buffer (0.01 M, pH 7.4, 0.15 M with NaCl). Upon addition of ANS to an aqueous HEPES buffer containing **1**, a fluorescence intensity originating from pyrene moiety of **1** at 379 and 396 nm decreased along with a concomitant increase of the fluorescence intensity of entrapped ANS molecules at around 510 nm, as shown in Figure 2a, reflecting the formation of host–guest complexes. Such fluorescence quenching of **1** at 379 and 396 nm seems to be caused by the interactions between the pyrene group of **1** and entrapped ANS molecules. A similar trend of guest-binding behavior was also observed for complexation of **2** with ANS (Figure 2b). Binding constants (K) for the 1:1 host–guest complexes were calculated on the basis of a Benesi–Hildebrand relationship on the basis of spectroscopic data obtained at various concentrations of the guests in a manner as described previously;[10] 7.2×10^4 and $2.1 \times 10^4\,M^{-1}$ for the complexes of **1** and **2**, respectively.

First, we investigated the cell (human hepatoblastome HepG2 cells) uptake of water-soluble cyclophanes with a pyrene moiety **1** and **2** using microscopic observation with a fluorescent microscope. The cells were exposed to either cationic host **1** or anionic host **2** for 60 min and incubated for 6 h at 37 °C followed by washing. Fluorescence images were recorded at the excitation

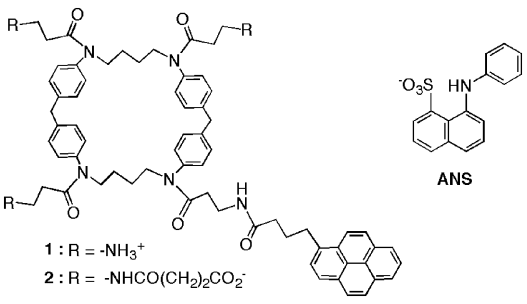
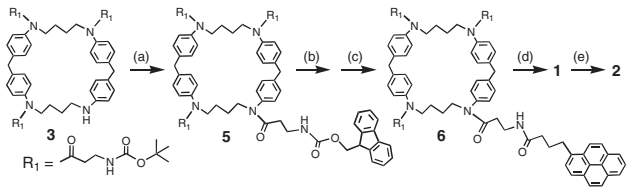


Figure 1. Water-soluble cyclophanes having a pyrene moiety **1** and **2** and ANS.



Scheme 1. Preparation of **1** and **2**. Reagents and conditions: (a) Fmoc-β-Ala, DCC, DCM; (b) piperidine, DCM; (c) 1-pyrenebutylic acid, DCC, DCM; (d) 30% TFA; (e) succinic anhydride, DCM.

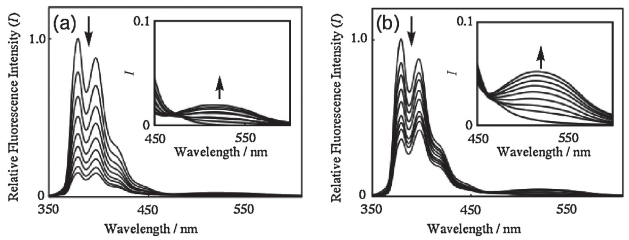


Figure 2. Fluorescence spectral changes for aqueous solution of **1** (a) and **2** (b) upon addition of ANS in HEPES buffer (0.01 M, pH 7.4, 0.15 M with NaCl) at 298 K. [**1**] = [**2**] = 1.0 μM. [ANS] = 0, 5.0, 10, 15, 20, 25, 30, 35, and 40 μM (from top to bottom at 379 and 396 nm). Ex. 322 nm.

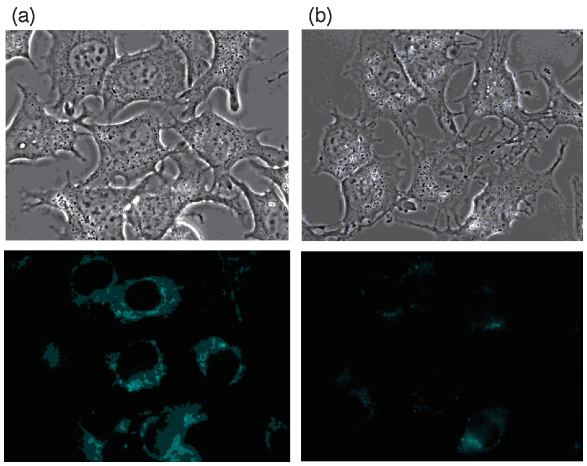


Figure 3. Microphotographs (top) and their fluorescence images (bottom) of HepG2 cells after a 60 min incubation with an aqueous solution (pH 7.3 with PBS) of (a) **1** (5 nM) and (b) **2** (5 nM) at 37 °C, followed by washing twice the PBS buffer. The cells were then incubated for 6 h and analyzed by fluorescence microscopy.

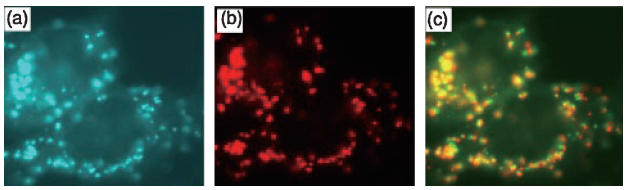


Figure 4. Cellular distribution of **1** (blue, a) and LysoTracker Red (red, b) in HepG2 cells after incubation in the presence of **1** (5 nM) for 1 h and then, LysoTracker RED (50 nM) for 10 min at 37 °C. The image shown in (c) is the overlay of the green (a) and red (b) image.

wavelength of pyrene and the emission wavelength for the blue fluorescence. As shown in Figure 3, the microscopic images revealed that cationic host **1** was taken up efficiently by the cells, while anionic host **2** was taken up poorly. These results suggested that the poor cellular uptake of anionic host **2** seems to be caused by the electrostatic repulsion between anionic host and negative charges at the cell membrane surfaces. In the case of cationic host **1**, the resulting host was distributed in the cytoplasm rather than in the nucleus of the cell. The localization of the host upon cell uptake was explored in more detail using double staining experiments[11] with LysoTracker Red[12] which was commonly used as a lysosomal marker (Figure 4). Red-labeled lysosomes manifest themselves upon excitation, as shown in Figure 4b. Computer overlap of the two micrographs, i.e., red LysoTracker Red and green pyrene host **1**, affords merged yellow-to-orange spots (Figure 4c). These results indicated that cationic host **1** was incorporated in the lysosomes and hence may have been taken into the cells via endocytosis.

We further investigate cellular uptake of ANS molecules in the absence and presence of **1** using microscopic observation. As a control experiment in the absence of **1**, HepG2 cells were exposed to ANS alone for 60 min and incubated for 6 h at 37 °C followed by washing. The fluorescence intensity originating from internalized ANS was hardly detectable (Figure 5a). On the other hand, the amount of ANS internalized into the cells was enhanced in the presence of **1** compared to the case in the absence of **1** (Figure 5b). Therefore, ANS molecules were delivered into the cells to some extent through formation of the host–guest complexes.[13]

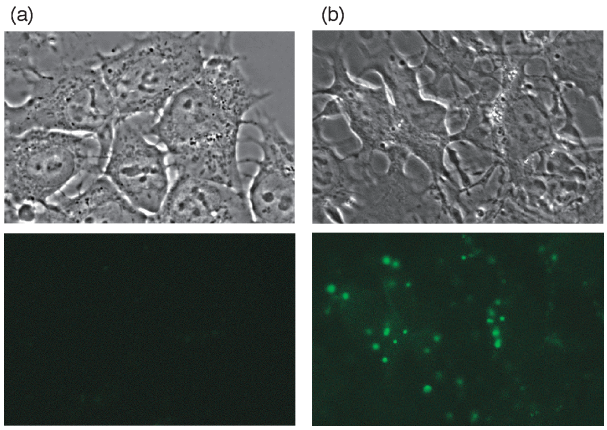


Figure 5. Microphotographs (top) and their fluorescence images (bottom) of HepG2 cells after a 60 min incubation with an aqueous solution (pH 7.3 with PBS) of (a) ANS (5 nM) and (b) ANS (5 nM) + **1** (5 nM) at 37 °C, followed by washing twice the PBS buffer. The cells were then incubated for 6 h and analyzed by fluorescence microscopy.

In conclusion, cationic cyclophane **1** was incorporated into HepG2 cells effectively as confirmed by fluorescent microscopic observation. Moreover, ANS having poor cell permeability was taken up into the cells in the presence of **1** through formation of the host–guest complexes. We believe that our concept of molecular design provides a useful guidepost for preparation of carrier models that are capable of performing as drug delivery systems.

This work was supported in part by MEXT (No. 21550136), JST Research Seeds Quest Program (No. 090540). We are grateful to Prof. H. Nakagawa for helpful discussions on microscope experiments.

References and Notes

1 Q. He, Y. Cui, J. B. Li, *Chem. Soc. Rev.* **2009**, *38*, 2292.
2 O. Thoumine, H. Ewers, M. Heine, L. Groc, R. Frischknecht, G. Giannone, C. Poujol, P. Legros, B. Lounis, L. Cognet, D. Choquet, *Chem. Rev.* **2008**, *108*, 1565.
3 J. K. Oh, D. J. Siegwart, H. Lee, G. Sherwood, L. Peteanu, J. O. Hollinger, K. Kataoka, K. Matyjaszewski, *J. Am. Chem. Soc.* **2007**, *129*, 5939.
4 W. D. Jang, K. Kataoka, *J. Drug Delivery Sci. Technol.* **2005**, *15*, 19.
5 S. Mourtas, C. A. Aggelopoulos, P. Klepetsanis, C. D. Tsakiroglou, S. G. Antimisiaris, *Langmuir* **2009**, *25*, 8480.
6 a) T. Nakai, T. Kanamori, S. Sando, Y. Aoyama, *J. Am. Chem. Soc.* **2003**, *125*, 8465. b) K. Matsui, S. Sando, T. Sera, Y. Aoyama, Y. Sasaki, T. Komatsu, T. Terashima, J. Kikuchi, *J. Am. Chem. Soc.* **2006**, *128*, 3114.
7 a) O. Hayashida, I. Hamachi, *J. Org. Chem.* **2004**, *69*, 3509. b) O. Hayashida, N. Ogawa, M. Uchiyama, *J. Am. Chem. Soc.* **2007**, *129*, 13698. c) O. Hayashida, M. Uchiyama, *Org. Biomol. Chem.* **2008**, *6*, 3166.
8 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.
9 J. Slavik, *Biochim. Biophys. Acta* **1982**, *694*, 1.
10 O. Hayashida, D. Sato, *J. Org. Chem.* **2008**, *73*, 3205.
11 J. Rinehart, K. Kahle, P. Heros, N. Vazquez, P. Meade, F. H. Wilson, S. C. Hebert, I. Gimenez, G. Gamba, R. Lifton, *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 16777.
12 E. C. Freundt, M. Czapiga, M. J. Lenardo, *Cell Res.* **2007**, *17*, 956.
13 The Hamachi group has previously reported the similar concept. See: T. Kohira, K. Honda, A. Ojida, I. Hamachi, *ChemBioChem* **2008**, *9*, 698.

