# Molecular Recognition by Novel Macrotetracyclic Cyclophanes Having Dipeptide Segments

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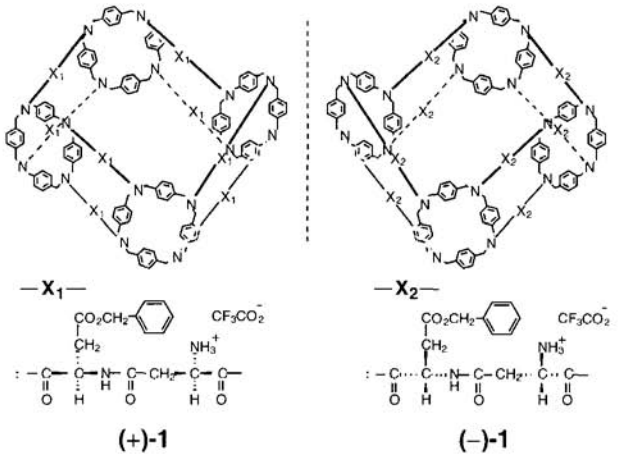
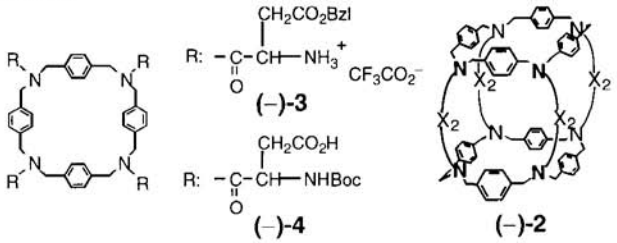
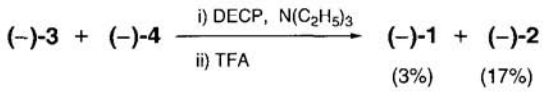
Novel macrotetracyclic cyclophanes, which are constructed with four cyclophane units having eight dipeptide moieties, have been prepared and characterized by various spectroscopic methods. The guest-binding behavior of the host toward water-soluble porphyrines was examined by electronic spectroscopy.

Cage-type cyclophanes are artificial host molecules, which provide a three-dimensionally extended hydrophobic cavity for inclusion of guest molecules in aqueous media.[1] We have previously prepared various cage-type cyclophanes as chiral hosts bearing chiral binding sites furnished by optically active amino acid moieties such as leucine, valine, and alanine.[2] In addition, we have also succeeded further modification by introducing dipeptide either L-Asp-L-Asp or D-Asp-D-Asp residues.[3] In this context, we have prepared novel macrotetracyclic cyclophanes having much larger size of intramolecular cavity than those of cage-type cyclophanes prepared by us, and examined their molecular recognition behavior.




The macrotetracyclic cyclophane (−)-**1** was synthesized by the reaction sequences as shown in Scheme 1. Both peptide cyclophanes having β-benzyl-L-aspartyl moieties (−)-**3** and *tert*-butyloxycarbonyl-L-aspartyl moieties (−)-**4** were prepared by the method reported previously.[3,4] The mixtures of cage-type cyclophane (−)-**2** and macrotetracyclic cyclophane (−)-**1** was obtained by intermolecular condensation of (−)-**3** with (−)-**4** in the presence of diethyl cyanophosphonate (DECP) and triethylamine under high dilution conditions in dry *N*,*N*-dimethylformamide at 0 °C. Both hosts were purified by gel-filtration chromatography on a column of Sephadex LH-20 with methanol–chloroform (1:1 v/v) as an eluent, followed by a column of Toyopearl HW-40F with the same eluent. Water-soluble macrotetracyclic cyclophane (−)-**1** was obtained by removal of the α-amino-protecting groups with trifluoroacetic acid (TFA). The product was purified by gel-filtration chromatography on a column of Sephadex LH-20 with methanol as an eluent; mp >250 °C (dec.). $^1$H NMR [500 MHz, $(CD_3)_2$-SO, 373 K] δ=2.5–3.2 (m, 32H, $CH_2CO_2$, $CH_2CONH$), 3.9–4.8 (m, 64H, $ArCH_2N$), 5.1 (m, 16H, $ArCH_2O$), 4.7 and 5.3 (m, 16H, $CHCH_2CO_2$ and $CHNH_3$), 6.6–7.0 (m, 64H, $ArHCH_2N$), 7.2–7.4 (m, 40H, $ArHCH_2O$). Found: C, 60.63; H, 5.29; N, 8.89%. Calcd for $C_{264}H_{264}F_{24}N_{32}O_{56}$: C, 60.55; H, 5.08; N, 8.56%. MALDI-TOF-MS m/z 4326.0 ($M - 7CF_3CO_2H - CF_3CO_2^-)^+$ ; calcd M for $C_{264}H_{264}F_{24}N_{32}O_{56}$, 5237.1. Compound (+)-**1** was also prepared by the same method.




**Scheme 1.**

Asymmetric characters of the present hosts have been examined by means of circular dichroism (CD) spectroscopy. Macrotetracyclic hosts, (−)-**1** and (+)-**1**, show CD bands opposite to each other in aqueous acetate buffer (0.01 mol dm$^{-3}$, pH 3.5) at 30 °C, reflecting the asymmetric character of their internal cavities: [θ] −3.6 x 10$^5$ and −3.3 x 10$^5$ deg cm$^2$ dmol$^{-1}$ for (−)-**1** at the CD peaks of 212 and 222 nm, respectively; +3.6 x 10$^5$ and +3.4 x 10$^5$ deg cm$^2$ dmol$^{-1}$ for (+)-**1** at the CD peaks of 214 and 222 nm, respectively.

The preliminary guest-binding behavior of the macrotetracyclic host (−)-**1** and the cage-type cyclophanes (−)-**2** toward water-soluble porphyrin derivatives was examined by means of electronic spectroscopy in aqueous acetate buffer (0.01 mol dm$^{-3}$, pH 3.5) at 303 K.[5] 5, 10, 15, 20-Tetraphenyl-21H, 23H-porphine tetrasulfonic acid ($H_2$TPPS) was proved to be exist in the monomeric form without formation of intermolecular aggregates at the concentration below 5.0 x 10$^{-5}$ mol dm$^{-3}$.[6] Under the conditions, the porphyrin was protonated to be $H_4^{2+}TPPS^{4-}$ (5.0 x 10$^{-6}$ mol dm$^{-3}$), which showed Soret- and Q-bands at 433 and 644 nm, respectively. Upon addition of (−)-**1** to a solution of $H_2$TPPS, the absorbance based on di-protonated $H_4^{2+}TPPS^{4-}$ was decreased and a new species, which has Soret band at 420 nm and Q-bands at 518 and 555 nm, appeared by the formation of the host-guest complexes, which proceed with concomitant deprotonation from $H_4^{2+}TPPS^{4-}$ to $H_2TPPS^{4-}$ as shown in Figure 1. Besides the change of absorbance, the Soret- and Q- bands were red-shifted and clearly were different from those of free $H_2TPPS^{4-}$, which shows Soret

band at 412 nm and Q-bands at 515 and 551 nm in aqueous acetate buffer (0.01 mol $dm^{-3}$, pH 3.5) at 303 K.[6] This result suggests that the guest molecule exists in an apolar microenvironment by the formation of host-guest complex.

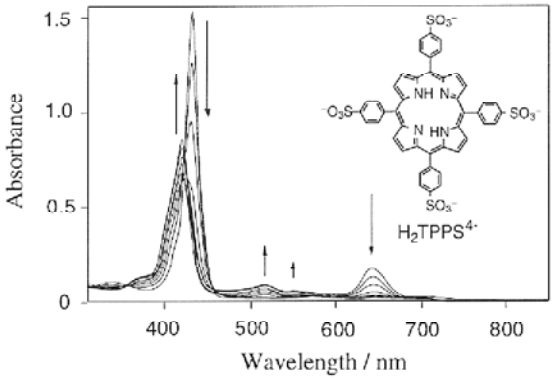



**Figure 1.** Electronic spectral change for the formation of host-guest complexes upon addition of (−)-**1** to a solution of $H_2TPPS$ ($5.0 \times 10^{-6}$ mol $dm^{-3}$) in aqueous acetate buffer (0.01 mol $dm^{-3}$, pH 3.5) at 303 K. Concentration of (−)-**1**; 0.5, 1.0, 2.0, 2.5, 3.0, 5.0, 7.0 x $10^{-6}$, 1.0, 2.5, 5.0 x $10^{-5}$ mol $dm^{-3}$.

Stoichiometry for the host-guest complexes was investigated by the molar ratio method as shown in Figure 2. Figure 2 indicates that it appeared to form 1:2 host-guest complex between host (−)-**1** and $H_2TPPS$, while cage-type cyclophane (−)-**2** forms 1:1 host-guest complex toward the identical guest. Binding constants ($K$) of (−)-**1** toward $H_2TPPS$ was evaluated on the basis of the mathematical treatment previously described by Conners.[7] As for a neutral porphyrin, 5, 10, 15, 20-tetrakis(4-hydroxyphenyl)-21H, 23H-porphine ($H_2TPP(OH)$), the same spectral change was observed upon the addition of (−)-**1**, while a cationic porphyrin, 5, 10, 15, 20-tetrakis(1-methylpyridinium-4-yl)-21H, 23H-porphine ($H_2TMPyP$) showed no spectral change. The evaluated $K$ values are summarized in Table 1. The guest-binding ability of the macrotetracyclic host (−)-**1** toward porphyrin derivatives was subjected to change by electrostatic nature of the guests. Anionic

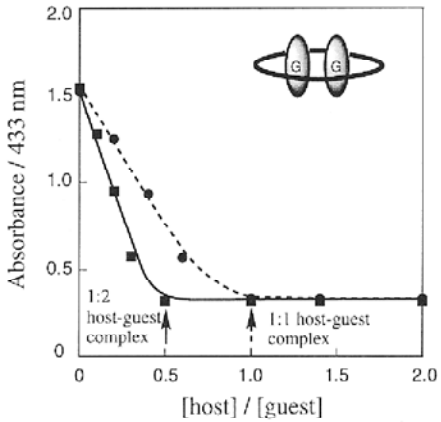



**Figure 2.** Stoichiometry for the formation of complexes with the hosts and $H_2TPPS$ ($5.0 \times 10^{-6}$ mol $dm^{-3}$): Macrotetracyclic cyclophane (−)-**1**, solid line; cage-type cyclophane (−)-**2**, broken line.

porphyrin is more favorable to be incorporated into octa-cationic macrotetracyclic host (−)-**1** than the others. These results indicate that electrostatic interactions as well as hydrophobic ones serve an important role for inclusion of $H_2TPPS$ .

**Table 1.** Binding constants ($K$ / $dm^3$ $mol^{-1}$) for the formation of the complex of macrotetracyclic host (−)-**1** and cage-type hosts (−)-**2** with porphyrin derivatives in aqueous acetate buffer (0.01 mol $dm^{-3}$, pH 3.5) at 303 K

| Guest | $K$ / $dm^3$ $mol^{-1}$ | |
|---|---|---|
| | (−)-**1** | (−)-**2** [b] |
| $H_2TPPS$ | $1.0 \times 10^6$ ($K_1$)<br>$9.7 \times 10^4$ ($K_2$) | $1.3 \times 10^5$ |
| $H_2TPP(OH)$ | $5.4 \times 10^4$ ($K_1$)<br>$2.2 \times 10^4$ ($K_2$) | $9.5 \times 10^3$ |
| $H_2TMPyP$ | – [a] | – [a] |

[a] No spectral change was observed. [b] Binding constants were estimated on the basis of Benesi-Hildebrand relationship.

In conclusion, the novel macrotetracyclic cyclophanes bearing dipeptide residues are capable of binding toward large guest molecules, such as porphyrin derivatives, and expected to be utilized as a receptor model.

## References and Notes

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