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Specific Molecular Recognition by Cage-type Cyclophanes Having a Helically Twisted and Cylindrical Internal Cavity

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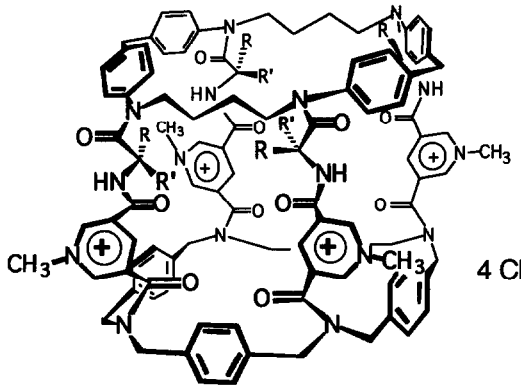
Abstract: Cage-type cyclophanes, which are constructed with two rigid 2,11,20-triaza[3.3.3]paracyclophane skeletons and three chiral bridging components, were prepared. Temperature-dependent ^{1}H NMR measurements in $(CD_3)_2SO$ indicate that the molecular framework of the cage-type cyclophane having a cylindrical internal cavity is more rigid than those of the corresponding non-cage hosts. The guest-binding behavior of the cage-type hosts toward various guests was examined by electronic absorption spectroscopy and electrospray ionization (ESI) mass spectrometry. The present hosts were found to bind anionic guests, such as 1-hydroxy-2,4-dinitronaphthalene-7-sulfonate, 2-hydroxy-1-(phenylazo)naphthalene-6,8-disulfonate, 2,7-bis[(4-methyl-2-sulfophenyl)azo]-1,8-dihydroxynaphthalene-3,6-disulfonate, 8-anilinonaphthalene-1-sulfonate, 6-*p*-toluidinonaphthalene-2-sulfonate, naphthalene-1-sulfonate, and 3,5-bis(methoxycarbonyl)benzene-1-sulfonate, to form host–guest complexes. The computer-aided molecular modeling study reveals that the three pyridinium moieties bound to the chiral L- and D-valine residues in the bridging segments undergo chiral twist in the same directions. However, the twisted direction in the host bearing L-valine residues is opposite to that evaluated for the host bearing D-valine residues so that the former and latter cage-type cyclophanes furnish *M* and *P*-helical cavities, respectively, as reflected in their circular dichroism (CD) spectra. The chirality-based molecular recognition of the cage-type hosts toward enantiomeric guests such as bilirubin-IXα and pamoic acid in aqueous media was investigated by CD spectroscopy.

INTRODUCTION

The development of artificial hosts capable of performing chiral recognition toward guest molecules in aqueous media is of great importance for creating supramolecules which exercise functional simulation of cell-surface receptors.[1] The specific molecular recognition ability of artificial receptors in aqueous media is highly dependent on the hydrophobic character of their internal cavities, because non-covalent host–guest interactions become more effective in well-desolvated and hydrophobic microenvironments. On this ground, we have recently prepared and characterized water-soluble cage-type cyclophanes (+)-**1** and (–)-**1** constructed with two rigid macrocyclic skeletons, tetraaza[6.1.6.1]paracyclophane and tetraaza[3.3.3.3]paracyclophane, and four bridging components that connect the macrocycles.[2] The hosts bearing L- and D-valine residues in the respective bridging segments provide helically twisted and globular hydrophobic cavities for chiral recognition toward guest molecules.[3] In order to explore the possibility of specific molecular recognition by artificial receptors, it is required to develop various cage-type cyclophanes having an internal cavity different from those provided by previous cage-type hosts. We now designed novel cage-type hosts constructed with two

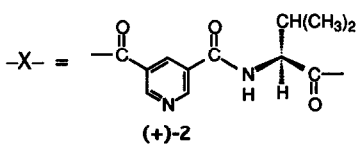
triaza[3.3.3]paracyclophanes[4] as rigid macrocyclic skeletons and three chiral bridging components [(+)-**2**, (−)-**2**, (+)-**3**, and (−)-**3**];[5] all the bridging components are connected to the macrocyclic skeletons in the same direction. The present hosts can be expected to provide helically twisted and cylindrical internal cavities and to perform chirality-based molecular discrimination toward guest molecules in aqueous media. We now report the preparation, structural and asymmetric characteristics, and guest-binding behavior of these novel cage-type cyclophanes with emphasis on their chiral recognition toward guest molecules.



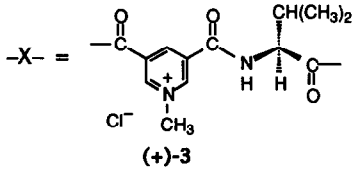
4 Cl⁻

(+)-**1** { R = CH(CH$_3$)$_2$, R' = H

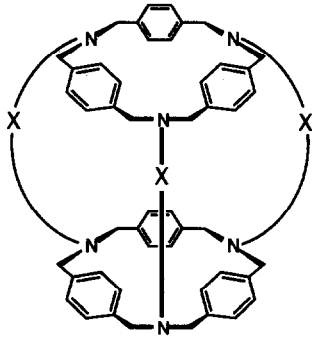
(−)-**1** { R = H, R' = CH(CH$_3$)$_2$

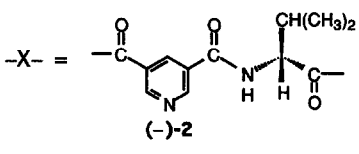


(+)-**2**

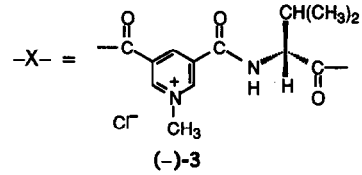


(+)-**3**





(−)-**2**



(−)-**3**

RESULTS AND DISCUSSION

Structural and Asymmetric Properties Characteristic of Cage-type Cyclophanes

Host (+)-**2** provides a cylindrical hydrophobic cavity surrounded by two rigid macrocyclic skeletons, triaza[3.3.3]paracyclophanes, and three bridging components in reference to its CPK molecular model. The upper and lower holes provided by the macrocyclic skeletons are relatively small; the maximum inner diameter of the holes is *ca.* 4 Å. However, each of the side holes constructed with the two bridging segments and two *p*-xylylene moieties are relatively large, so that a guest molecule as large as a naphthalene derivative is capable of passing through it.

Temperature-dependent ^1H NMR spectroscopy was applied on (+)-**2**, **7**, and (+)-**6** to differentiate molecular rigidity between the cage-type and non-cage cyclophanes. In $(CD_3)_2SO$ at 293 K, both phenyl and methylene proton signals for the non-cage hosts appear as complicated split patterns [Fig. 1 (A) and (B)].

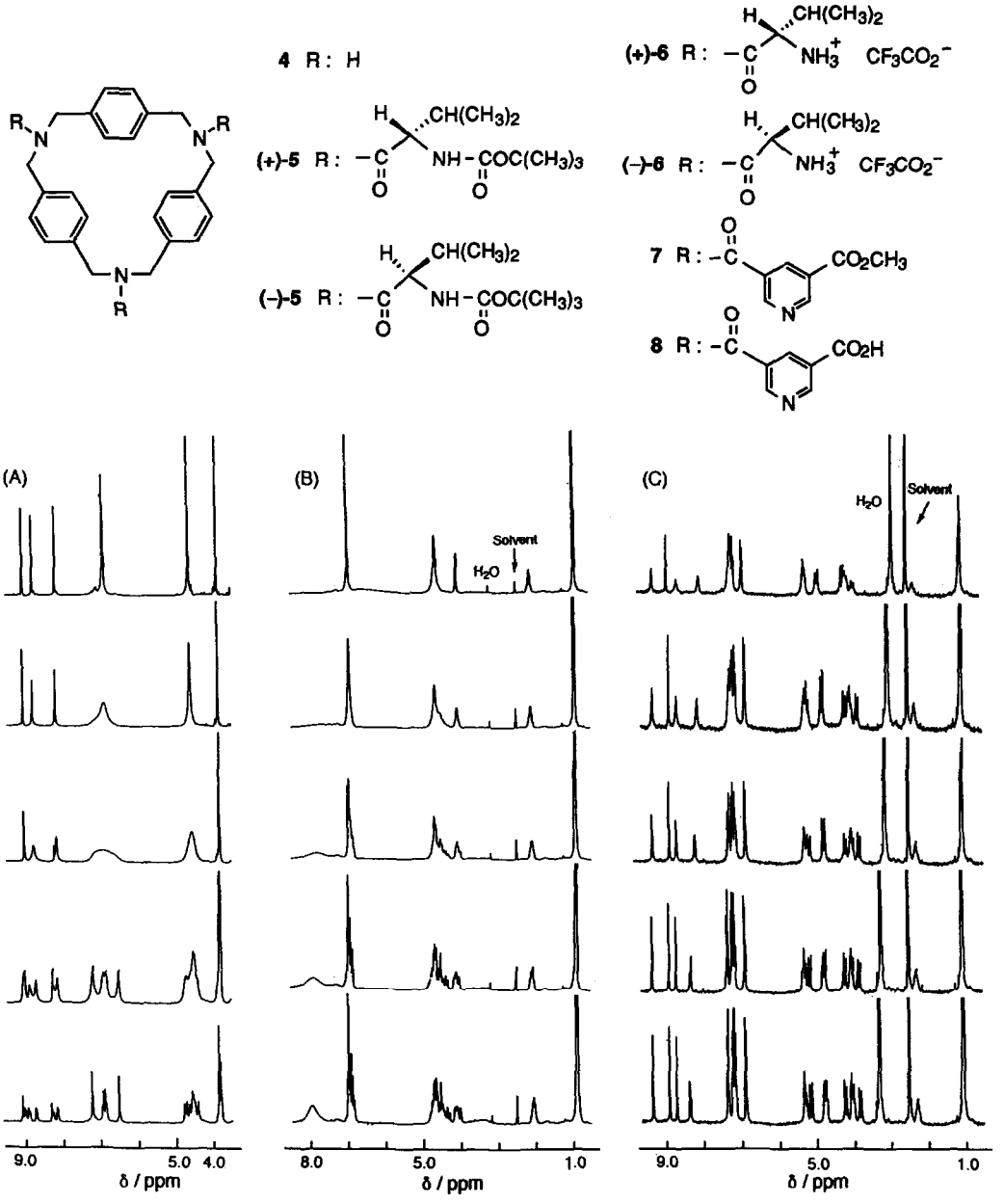


Fig. 1. ^1H NMR spectra of **7** (A), (+)-**6** (B) and (+)-**2** (C) at 293, 313, 333, 353, and 383 K (from bottom to top) in $(CD_3)_2SO$.

The result indicates that the introduction of pyridyl moieties and valine residues into the macrocyclic skeleton through amide linkages suppresses the conformational freedom because of intramolecular steric hindrance and results in formation of conformational isomers.[6] When the measurement temperature was raised in $(CD_3)_2SO$, these proton signals attributable to **7** and (+)-**6** were gradually changed and finally converted into singlet signals. The apparent coalescence temperatures (T_c) evaluated from these signals are *ca.* 333 and 353 K for **7** and (+)-**6**, respectively. As for cage-type cyclophane (+)-**2**, complicated split patterns were also observed for both phenyl and methylene protons in $(CD_3)_2SO$ at 293K, indicating the presence of conformational isomers [Fig. 1 (C)]. However, these signals do not undergo drastic changes as observed for **7** and (+)-**6** even at higher temperatures. The results indicate that the conformational flexibility of the macrocyclic framework of (+)-**2** is remarkably diminished relative to that of the non-cage hosts. The asymmetric character of the present hosts was examined by means of circular dichroism (CD) spectroscopy and computer-aided molecular modeling study. Cage-type hosts (+)-**3** and (−)-**3** showed CD bands in aqueous phosphate buffer (0.01 M, pH 7.0, μ 0.10 with KCl) at 30°C: $[\theta]$/deg cm^2 dmol^{-1}, +3.9 x 10^4 (233 nm) and +8.4 x 10^3 (286 nm) for (+)-**3**; −4.0 x 10^4 (233 nm) and −9.2 x 10^3 (285 nm) for (−)-**3** (Fig. 2). This result suggests that the three bridging components of (+)-**3** and (−)-**3**, each having a pyridinium moiety and a chiral valine residue, approach close to each other and are twisted in the same direction, in a manner similar to those exercised by cage-type cyclophanes having four bridging segments such as (+)-**1**, and (−)-**1**.[3a] Such helical conformations of (+)-**3** and (−)-**3** seem to be caused by chiral nature of the valine residues in the bridging segments in the light of minimum energy conformations for these hosts in the gas phase, as evaluated on the basis of molecular mechanics calculations (BIOGRAF, Dreiding-II). The optimized conformation for (+)-**3** indicates that the upper macrocyclic ring is rotated clockwise around the molecular axis, which goes through the center of the two macrocyclic skeletons, by *ca.* 45° with respect to the lower one, so that (+)-**3** furnishes an *M*-helical internal cavity (Fig. 3). Moreover, the twisted direction of bridging components in (−)-**3** is opposite to that evaluated

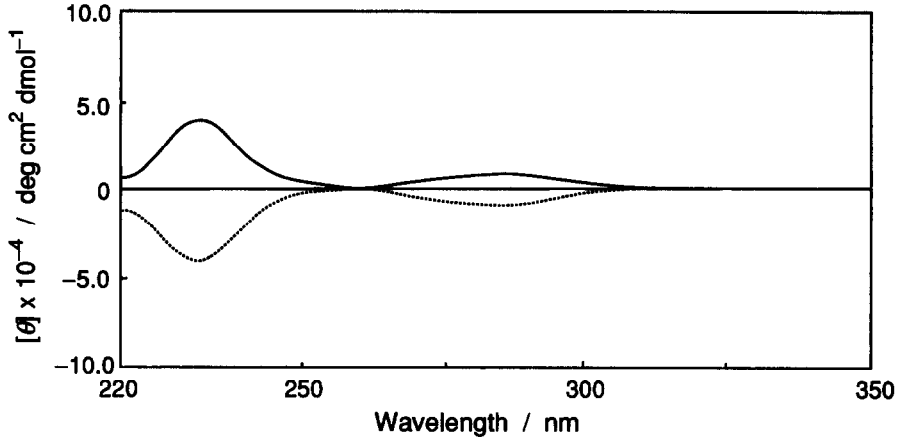


Fig. 2. Circular dichroism spectra for (+)-**3** (3.0 x 10^{-5} M) and (−)-**3** (3.0 x 10^{-5} M) indicated by solid and dotted lines, respectively, in aqueous phosphate buffer (0.01 M, pH 7.0, μ 0.10 with KCl) at 30.0 °C.

for (+)-3, so that (–)-3 provides a *P*-helical internal cavity for chiral recognition toward guest molecules. A similar asymmetric character was confirmed for the internal cavities of hosts (+)-2 and (–)-2 by the identical method.

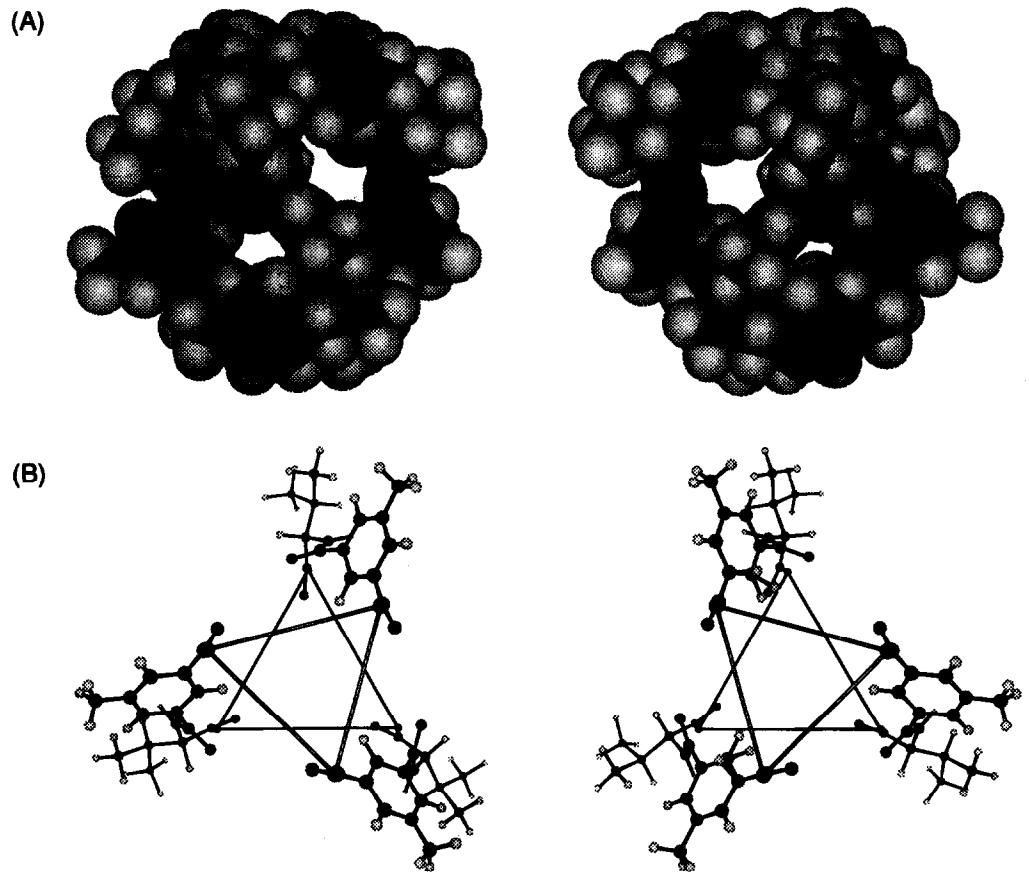
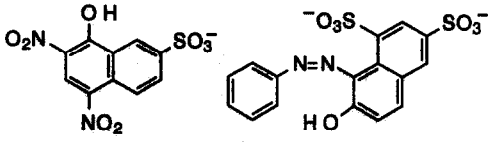


Fig. 3. Optimized lowest energy conformations of (+)-3 (left) and (–)-3 (right); (A) CPK models, (B) schematic representations of helically twisted conformations. Each triangle represents a triaza[3.3.3]paracyclophane skeleton.

Molecular Recognition by Cage-type Cyclophanes

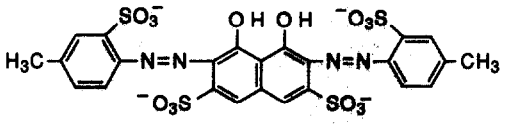
The guest-binding behavior of cage-type host (+)-3 toward various hydrophobic molecules was examined by electronic absorption spectroscopy in aqueous phosphate buffer (0.01 M, pH 7.0, μ 0.10 with KCl) at 30.0 °C. The following dyes were adopted as guest molecules: 1-hydroxy-2,4-dinitronaphthalene-7-sulfonate (NYS), 2-hydroxy-1-(phenylazo)naphthalene-6,8-disulfonate (OG), 2,7-bis[(4-methyl-2-sulfophenyl)azo]-1,8-dihydroxynaphthalene-3,6-disulfonate (DSA), and 2-[4-(dimethylamino)styryl]-1-ethylquinolinium (QR). An absorption intensity originated from each of the guest molecules, except for QR, decreased along with a concomitant red shift of its absorption maximum upon addition of host (+)-3, reflecting

the formation of host–guest complexes. Binding constants for the formation of complexes of the hosts with various guest molecules in a 1:1 molar ratio (K) were evaluated on the basis of the Benesi-Hildebrand relationship[7] in a manner as described previously[8]; 1.2 x 10^4, 1.0 x 10^4, and 3.0 x 10^4 M^{-1} for complexation of NYS, OG, and DSA, respectively. The binding affinity of (+)-**3** for these guests is nearly 1 order of magnitude weaker than that of a cage-type cyclophane having four bridging segments, (+)-**1**; 3.7 x 10^5, 1.2 x 10^5, and 3.4 x 10^5 M^{-1} for complexation of NYS, OG, and DSA, respectively.[3a] The structural differences

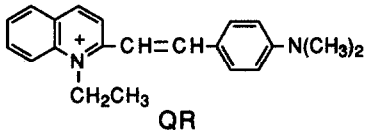


NYS

OG



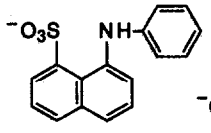
DSA



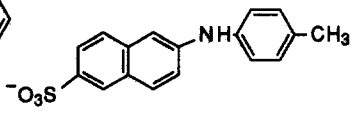
QR

between the former and latter hosts, as regards size, shape, and hydrophobic character of their internal cavities, seem to be reflected in the binding affinity for anionic guests. On the other hand, host (+)-**3** shows no capacity of binding a cationic guest, QR. Consequently, cationic cage-type host (+)-**3** recognizes hydrophobic guests in aqueous media through hydrophobic and electrostatic interactions in a manner as observed with other water-soluble cyclophanes.[3a,8]

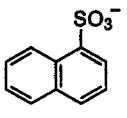
We have recently shown that electrospray ionization (ESI) mass spectrometry[9] is a useful method for direct detection of host–guest complexes.[10] The guest-binding behavior of (+)-**3** toward 8-anilinonaphthalene-1-sulfonate (ANS), 6-*p*-toluidinonaphthalene-2-sulfonate (TNS), naphthalene-1-sulfonate (NS), 3,5-bis(methoxycarbonyl)benzene-1-sulfonate (SIP), and QR was examined by ESI mass spectrometry. The mass spectrum of a host–guest complex of (+)-**3** with ANS is shown in Fig. 4 (A). An ESI peak originating from the complex was clearly observed: $m/z(M_{complex} - 2Cl)^{2+}$ 874; calcd $M_{complex}$[11] for $C_{103}H_{105}Cl_2N_{13}O_{12}S_1$, 1819. Similar mass spectra were also observed for other complexes of host (+)-**3** involving anionic guests TNS, NS, and SIP: $m/z(M_{complex} - 2Cl)^{2+}$ 881, 829, and 862 for TNS, NS, and SIP complexes, respectively; calcd $M_{complex}$ for the TNS, NS, and SIP complexes of (+)-**3** are 1833 ($C_{104}H_{107}Cl_2N_{13}O_{12}S_1$), 1728 ($C_{97}H_{100}Cl_2N_{12}O_{12}S_1$), and 1794 ($C_{97}H_{102}Cl_2N_{12}O_{16}S_1$), respectively. However, any ESI peak attributable to the (+)-**3** complex of QR was not detected.



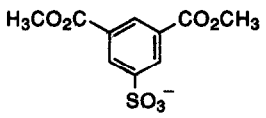
ANS



TNS



NS



SIP

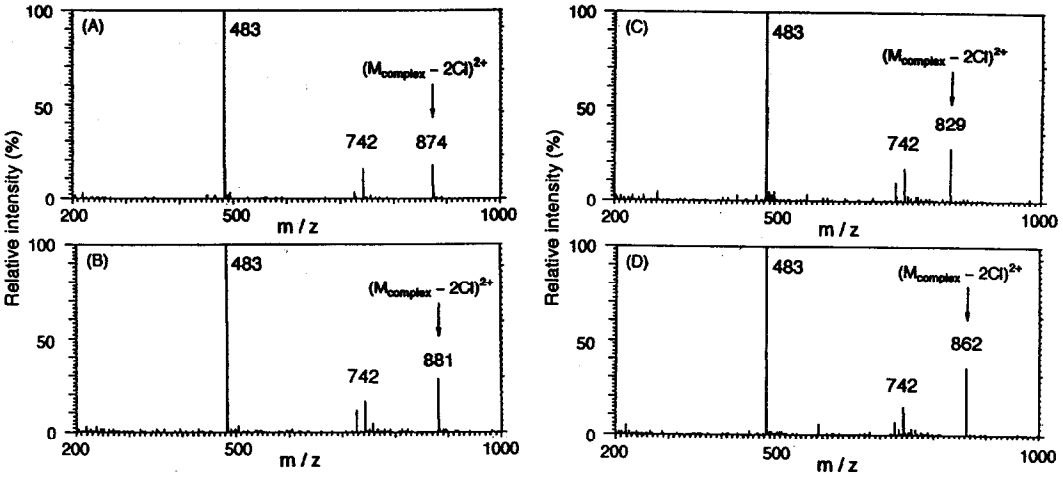
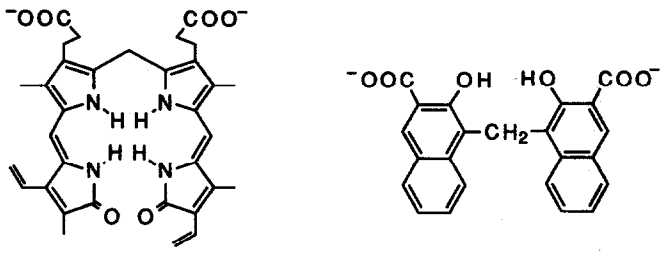


Fig. 4. ESI mass spectra for complexes of (+)-**3** involving anionic guests ANS (A), TNS (B), NS (C), and SIP (D).

Chiral Recognition by Cage-type Cyclophanes

Cage-type hosts (+)-**3** and (–)-**3** furnish chiral internal cavities as mentioned above. On this ground, we investigated chirality-based molecular discrimination ability of the cage-type cyclophanes toward anionic guests such as (4Z,15Z)-bilirubin IXα (BR) and pamoic acid (PA). The former guest, the cytotoxic yellow-orange tetrapyrrole pigment of jaundice, consists of two dipyrrinone units conjoined by a methylene group. X-Ray crystallography and NMR spectroscopy applied on BR indicate that the molecule is folded into either of two ridge-tile-shaped enantiomers stabilized by intramolecular hydrogen-bonding interactions between the propionic acid group of one dipyrinone moiety and the pyrrole and lactam residues of the other dipyrrinone (Fig. 5).[12]



BR PA

First, the guest-binding behavior of (+)-**3** and (–)-**3** toward BR was examined by electronic absorption spectroscopy in aqueous carbonate buffer (0.01 M, pH 10.0, μ 0.10 with KCl) at 28.0 °C. Upon addition of the cage-type hosts to the carbonate buffer containing BR, an electronic absorption intensity originating from BR (ε = 47000 at 436 nm) decreased, reflecting formation of the corresponding host–guest complex. The stoichiometry for the complexes formed with the hosts and the guest was investigated by the Job's continuous variation method.[13] The result revealed that the present hosts underwent complex formation with

BR in a 1:1 molar ratio of host to guest (Fig. 6). Binding constants (K) for formation of the 1:1 host–guest complexes were calculated on the basis of spectroscopic data obtained at various concentrations of the hosts in a manner as described previously;[10] 3.7 x 10^4 and 3.8 x 10^4 M^{-1} for the complexes of (+)-3 and (–)-3, respectively, at 28.0 °C. A CD spectrum for a carbonate buffer solution containing equimolar amounts of (+)-3

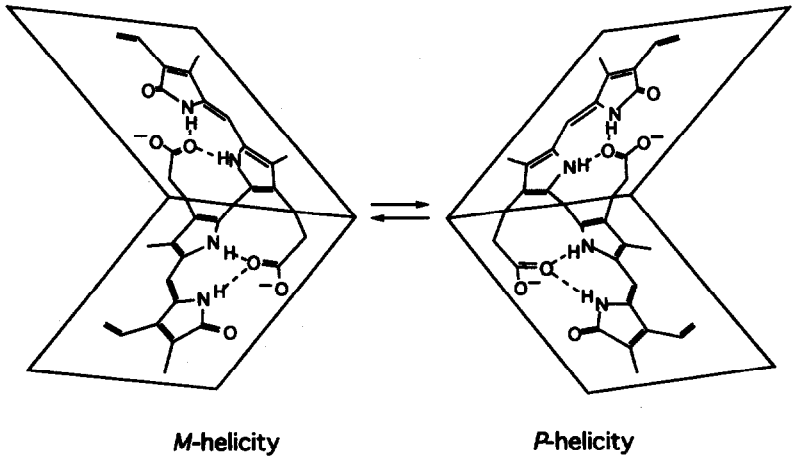


Fig. 5. Three-dimensional representations for two conformational enantiomers of BR stabilized by intramolecular hydrogen bonding, which undergo rapid interconversion in aqueous media.

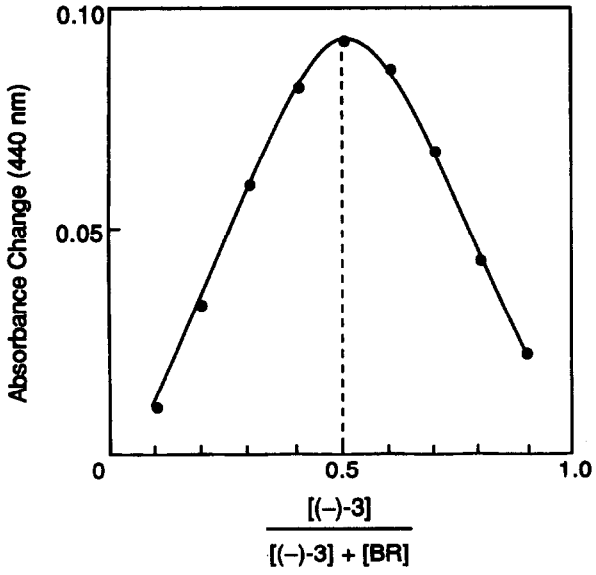


Fig. 6. Job's continuous variation plot for a combination of (–)-3 and BR in aqueous carbonate buffer (0.02 M, pH 10.0, μ 0.1 with KCl) at 28.0 °C; [(–)-3] + [BR] = 5.0 x 10^{-5} M.

and BR (3.0×10^{-5} M each) showed negatively and positively signed CD bands, which are due to an exciton coupling between the two proximal dipyrrinone chromophores within the incorporated guest molecule, in a longer wavelength range at 28.0 °C; $[\theta]$/deg cm^2 dmol^{-1}, -1.7×10^4 and 1.4×10^4 at 460 and 409 nm, respectively (Fig. 7). The characteristic bisignate Cotton effect indicates that the BR molecule selectively assumes a conformational enantiomer of *M*-helicity upon complexation with (+)-**3** on the basis of an exciton-coupling theory,[14] in a manner similar to that performed by (+)-**1**[15] and cyclodextrins.[16] On the other hand, the Cotton effect was inverted in the presence of (−)-**3** in aqueous carbonate buffer at 28.0 °C; $[\theta]$/deg cm^2 dmol^{-1}, 2.3×10^4 and -1.4×10^4 at 462 and 408 nm, respectively (Fig. 7), indicating that (−)-**3** binds the *P*-helicity enantiomer of BR in preference to the *M*-helicity enantiomer.

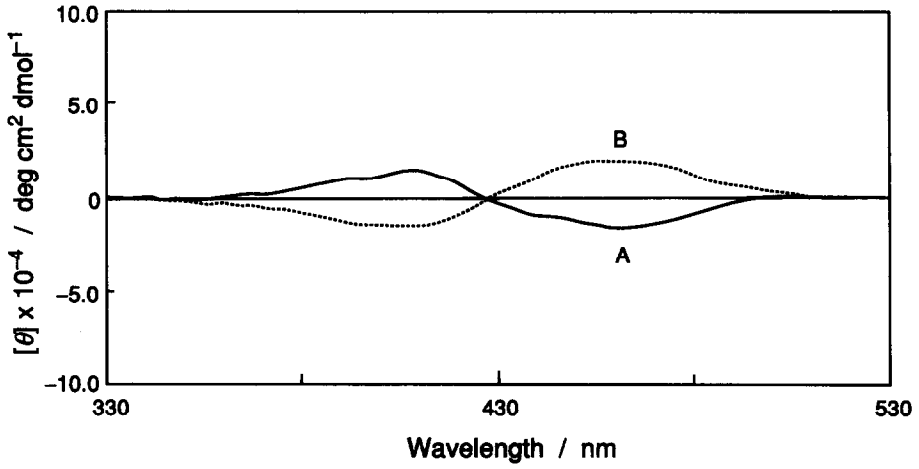


Fig. 7. Circular dichroism spectra of BR (3.0×10^{-5} M) in aqueous carbonate buffer (0.01 M, pH 10.0, μ 0.1 with KCl) at 28.0 °C upon addition of cage-type cyclophanes: A, (+)-**3** (3.0×10^{-5} M); B, (−)-**3** (3.0×10^{-5} M).

Both hosts (+)-**3** and (−)-**3** also bind PA with identical binding constants of 1.9×10^3 M^{-1} in the aqueous carbonate buffer at 28.0 °C as evaluated by florescence spectroscopy. It must be noted that enantioselective binding behavior of the cage-type hosts toward PA was markedly different from that of the identical hosts toward BR. Upon addition of (+)-**3** to the carbonate buffer containing PA, bisignate CD bands due to two exciton transitions within the incorporated guest molecule were observed ($[\theta]$/deg cm^2 dmol^{-1}, 1.1×10^3 and -8.9×10^2 at 377 and 329 nm, respectively), indicating that the PA molecule bound to (+)-**3** selectively assumes a *P*-helicity conformation (Fig. 8). Moreover, PA bound to (−)-**3** exhibited similar bisignate Cotton bands with inverted CD signs ($[\theta]$/deg cm^2 dmol^{-1}, -9.7×10^2 and 1.3×10^3 at 376 and 329 nm, respectively), verifying that the incorporated PA molecule is present as the *M*-helicity conformer in the chiral internal cavity of (−)-**3** (Fig. 8). Such enantioselective discrimination capabilities of the hosts toward BR and PA, opposite to each other, reflect a difference in mutual stereochemical interactions between the host cavities and these guests when they are incorporated into the host cavities. Although a CPK molecular model study suggests that such a difference in incorporation behavior between BR and PA is reasonable, direct evidences for the conformational

differences among these host–guest complexes were not obtained due to lack of appropriate media for ^{1}H NMR measurements.

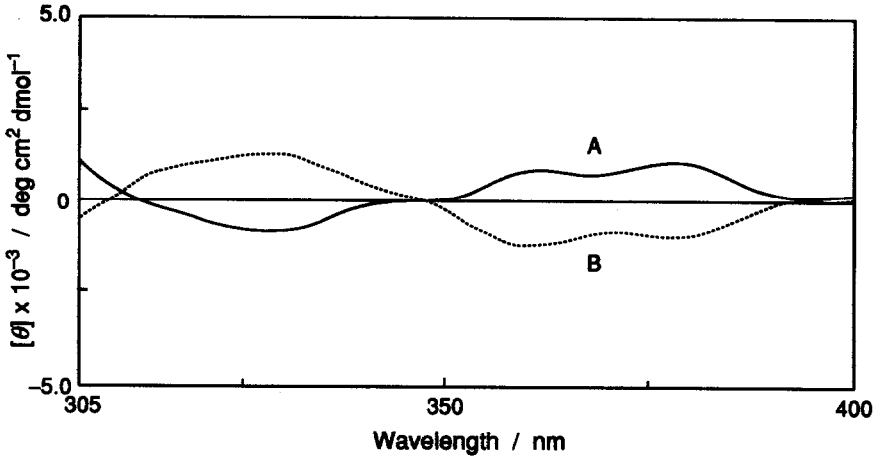


Fig. 8. Circular dichroism spectra of PA (3.0 x 10^{-5} M) in aqueous carbonate buffer (0.01 M, pH 10.0, μ 0.1 with KCl) at 28.0 °C upon addition of cage-type cyclophanes: A, (+)-**3** (3.0 x 10^{-5} M); B, (−)-**3** (3.0 x 10^{-5} M).

In conclusion, cage-type cyclophanes (+)-**2**, (−)-**2**, (+)-**3**, and (−)-**3** were synthesized on the basis of molecular design that allows to connect two macrocyclic skeletons with three bridging segments. The hosts bearing L- and D-valine residues in the respective bridging segments afford *M*- and *P*-helical cavities, respectively. The helically twisted cavities enforce on enantiomeric guests, such as BR and PA, to assume specific chiral conformations when they are incorporated. We believe that the present study provides a useful guidepost for preparation of multifunctional receptor models that are capable of performing effective molecular discrimination.

EXPERIMENTAL

General Analyses and Measurements

Melting points were measured with Yanako MP-500D apparatus (hot-plate type). Elemental analyses were performed at the Microanalysis Center of Kyushu University. IR spectra were recorded on a JASCO IR-810 spectrophotometer, while ^{1}H NMR spectra were taken on a Bruker AC-250P and a Bruker AMX-500 spectrometer installed at the Center of Advanced Instrumental Analysis, Kyushu University. Optical rotations were measured on a Horiba SEPA polarimeter. A Hitachi M-2500 double-focussing mass spectrometer was used for electrospray ionization (ESI)[16] and a Hitachi M-0301 data acquisition system was used for obtaining ESI-MS data. Circular dichroism and electronic absorption spectra were recorded on a JASCO J-500C spectropolarimeter and a Hitachi 220A spectrophotometer, respectively. Molecular mechanics [BIOGRAF,

Dreiding-II][17] calculations were carried out on an IRIS-4D/220GTX workstation (Silicon Graphics, Mountain View, California, U.S.A.).

Materials

The following compounds were obtained from commercial sources as guaranteed reagents and used without further purification: sodium 1-hydroxy-2,4-dinitronaphthalene-7-sulfonate [Na(NYS)] and disodium 2-hydroxy-1-(phenylazo)naphthalene-6,8-disulfonate [Na_2(OG)] (both from Wako Pure Chemical, Industries, Osaka, Japan); disodium 2,7-bis[(4-methyl-2-sulfophenyl)azo]-1,8-dihydroxynaphthalene-3,6-disulfonate [Na_2(DSA)] (from Dojin Chemical Laboratories, Kumamoto, Japan); 2-[4-(dimethylamino)styryl]-1-ethylquinolinium iodide [(QR)I] and potassium 6-*p*-toluidinonaphthalene-2-sulfonate [K(TNS)] (both from Nacalai Tesque, Inc., Kyoto, Japan); sodium naphthalene-1-sulfonate [Na(NS)], sodium 3,5-bis(methoxycarbonyl)benzene-1-sulfonate [Na(SIP)], sodium 8-anilinonaphthalene-1-sulfonate [Na(ANS)], (4Z,15Z)-bilirubin IXα (BR), and 4,4'-methylenebis(3-hydroxy-2-naphthalenecarboxylic acid) (pamoic acid; PA) (all from Tokyo Kasei Kogyo Co., Tokyo, Japan). N^{α}-*tert*-Butoxycarbonyl-L-valine and N^{α}-*tert*-butoxycarbonyl-D-valine were purchased from Peptide Institute, Inc., Osaka, Japan, as guaranteed reagents. 2,11,20-Triaza[3.3.3]paracyclophane (**4**) was prepared after a method reported previously.[5]

N,N',N"-Tris(N^{α}-tert-butoxycarbonyl-L-valyl)-2,11,20-triaza[3.3.3]paracyclophane [(+)-5]

Dicyclohexylcarbodiimide (755 mg, 3.8 mmol) was added to a dry dichloromethane (20 mL) solution of *tert*-butoxycarbonyl-L-valine (685 mg, 3.2 mmol) and 1-hydroxybenzotriazole (610 mg, 4.5 mmol) at 0 °C, and the mixture was allowed to stand at the same temperature while being stirred for 30 min. 2,11,20-Triaza[3.3.3]paracyclophane (**4**; 159 mg, 0.45 mmol) dissolved in dry dichloromethane (20 mL) was added to the mixture, and the resulting mixture was stirred for 5 h at 0 °C and for an additional 24 h at room temperature. An insoluble material (*N,N'*-dicyclohexylurea) was removed by filtration, the filtrate was evaporated to dryness under reduced pressure, and the residue was dissolved in ethyl acetate (50 mL). The solution was then allowed to stand overnight at 5 °C, precipitates were removed by filtration, and the filtrate was evaporated under reduced pressure. The crude product was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol–chloroform (1:1 v/v) as eluant. Evaporation of the product fraction under reduced pressure gave a white solid (384 mg, 91%): mp 128–130 °C; R_f [Wako Silica Gel 70FM, methanol–chloroform (1:1 v/v)] 0.87; IR/cm^{-1} (KBr disc) 1720 (urethane C=O) and 1640 (amide C=O); $[\alpha]^{25}_{D}$ −23° (c = 0.1, CH_3OH); ^{1}H NMR [250 MHz, $(CD_3)_2SO$, 373 K] δ_H 0.79 [d, *J* 6.7 Hz, 9H, $CH(CH_3)_2$ (nonequivalent)], 0.86 [d, *J* 6.7 Hz, 9H, $CH(CH_3)_2$ (nonequivalent)], 1.38 [s, 27H, $C(CH_3)_3$], 2.00 [m, 3H, $CH(CH_3)_2$], 4.27 (dd, *J* 9.0 and 7.5 Hz, 3H, COCH), 4.59 (m, 12H, $ArCH_2$), 6.19 (d, 3H, *J* 9.0 Hz, NHCO), 6.89 (s, 12H, ArH). Anal. Calcd for $C_{54}H_{78}N_6O_9$•1/2 H_2O: C, 67.26; H, 8.26; N, 8.72%. Found: C, 67.14; H, 8.28; N, 8.70%.

N,N',N"-Tris(L-valyl)-2,11,20-triaza[3.3.3]paracyclophane [(+)-6]

Trifluoroacetic acid (10 mL) was added to a dry dichloromethane (10 mL) solution of (+)-**5** (150 mg, 0.16 mmol), and the mixture was stirred for 2 h at room temperature. After the solvent was evaporated off under reduced pressure, the crude product was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol as eluant. The product fraction was evaporated to dryness under reduced pressure to give a white solid (151 mg, 97%): mp 177–179 °C; R_f (Wako Silica Gel 70FM, ethanol) 0.85;

IR/cm^{-1} (KBr disc) 1670 (amide C=O); $[\alpha]^{25}_{D}$ +55° (c = 0.1, CH_3OH); ^{1}H NMR [250 MHz, $(CD_3)_2SO$, 383 K] δ_H 0.93 [d, *J* 6.8 Hz, 9H, $CH(CH_3)_2$ (nonequivalent)], 0.96 [d, *J* 6.8 Hz, 9H, $CH(CH_3)_2$ (nonequivalent)], 2.12 [m, 3H, $CH(CH_3)_2$], 4.05 (d, *J* 5.2 Hz, 3H, COCH), 4.61 (m 12H, $ArCH_2$), 6.93 (12H, s, ArH). Anal. Calcd for $C_{45}H_{57}F_9N_6O_9 \cdot 3/2H_2O$: C, 52.78; H, 5.91; N, 8.21%. Found: C, 52.92; H, 5.88; N, 8.22%.

N,N',N"-Tris[5-(methoxycarbonyl)nicotinoyl]-2,11,20-triaza[3.3.3]paracyclophane (7)

A mixture of 5-(methoxycarbonyl)nicotinic acid (1.0 g, 5.0 mmol) and thionyl chloride (30 mL, 150 mmol) was refluxed for 1 h. The reaction mixture was cooled to room temperature and stirred for 24 h at the same temperature. The excess thionyl chloride was removed under reduced pressure, and dry dichloromethane (20 mL) was added to the residue with stirring. The solvent was evaporated off under reduced pressure to give 5-(methoxycarbonyl)nicotinoyl chloride quantitatively. A dry dichloromethane (20 mL) solution of 2,11,20-triaza[3.3.3]paracyclophane (4) (216 mg, 0.6 mmol) was added dropwise in 30 min to a dry dichloromethane (20 mL) solution of the acid chloride (1.0 g, 5.0 mmol) with stirring at room temperature. The resulting mixture was stirred for 40 h at the same temperature and evaporated to dryness under reduced pressure. The mixture was stirred at room temperature for 1 h after 150 mL of methanol–chloroform (1:1 v/v) was added to the residue, and insoluble materials were removed by filtration. The crude product, obtained by evaporation of the solvent under reduced pressure, was purified by gel filtration chromatography on columns of Sephadex LH-20 and Toyopearl HW-40F, in this sequence, with methanol–chloroform (1:1 v/v) as eluant. The product fraction was evaporated to dryness under reduced pressure to give a white solid (207 mg, 41%): mp 105–107 °C; R_f (Wako Silica Gel 70FM, methanol) 0.66; IR/cm^{-1} (KBr disc) 1730 (ester C=O) and 1640 (amide C=O); ^{1}H NMR [250 MHz, $(CD_3)_2SO$, 383 K] δ_H 3.87 (s, 9H, $COOCH_3$), 4.60 (m, 12H, $ArCH_2$), 6.90 (m, 12H, ArH), 8.18 (m, 3H, Py-H4), 8.77 (d, *J* 2.1 Hz, 3H, Py-H2), 9.02 (d, *J* 2.1 Hz, 4H, Py-H6). Anal. Calcd for $C_{48}H_{42}N_6O_9 \cdot 3/2H_2O$: C, 65.97; H, 5.19; N, 9.61%. Found: C, 65.73; H, 4.96; N, 9.44%.

N,N',N"-Tris(5-carboxynicotinoyl)-2,11,20-triaza[3.3.3]paracyclophane (8)

A mixture of **7** (242 mg, 0.29 mmol), aqueous sodium hydroxide (0.9 M; 10mL), and methanol (120 mL) was refluxed for 28 h and cooled to room temperature. Methanol was evaporated off under reduced pressure, and water (40 mL) was added to the concentrate. The pH of the solution was adjusted to 3 by adding aqueous hydrochloric acid (1 M) after removal of a small amount of insoluble materials by filtration, and the solution was allowed to stand overnight at 5 °C. Precipitates were recovered, washed with methanol, and dried under reduced pressure at room temperature to give a white solid (204 mg, 89%): mp 205–207 °C (dec.); R_f (Wako Silica Gel 70FM, methanol) 0.89; IR/cm^{-1} (KBr disc) 1720 (carboxyl C=O) and 1630 (amide C=O); ^{1}H NMR [250 MHz, $(CD_3)_2SO$, 373 K] δ_H 4.60 (m, 12H, $ArCH_2$), 6.89 (m, 12H, ArH), 8.17 (m, 3H, Py-H4), 8.74 (d, *J* 2.0 Hz, 3H, Py-H2), 9.02 (d, *J* 2.0 Hz, 3H, Py-H6), 13.60 (bs, 3H, COOH). Anal. Calcd for $C_{45}H_{36}N_6O_9 \cdot H_2O$: C, 65.69; H, 4.65; N, 10.21%. Found: C, 65.93; H, 4.67; N, 10.02%.

Cage-type Cyclophane with L-Valine Residues [(+)-2]

Individual solutions of **8** (119 mg, 0.15 mmol) and (+)-**6** (148 mg, 0.15 mmol) dissolved in dry *N,N*-dimethylformamide (DMF, 200 mL each) were added dropwise at an identical rate over 10 h to a dry DMF (2200 mL) solution containing diethyl cyanophosphonate (DECP; 100 mg, 0.6 mmol) and triethylamine (100

mg, 1.0 mmol) with vigorous stirring under nitrogen atmosphere at 0 °C. The resulting mixture was stirred for 36 h at the same temperature and for an additional 6 h at room temperature, and then evaporated to dryness under reduced pressure. The residue was purified by liquid chromatography on a column of silica gel (Wakogel C-300) with methanol–chloroform (1:1 v/v) as eluant, followed by gel filtration chromatography on columns of Sephadex LH-20 and Toyopearl HW-40F, in this sequence, with methanol–chloroform (1:1 v/v) as eluant. The product fraction was evaporated to dryness under reduced pressure to give a white solid (51 mg, 25%): mp 292–294 °C (dec.); R_f [Wako Silica Gel 70FM, methanol–chloroform (1:1 v/v)] 0.42; IR/cm^{-1} (KBr disc) 1640 (amide C=O); $[\alpha]^{25}_D$ +86° (c = 0.1, $CHCl_3$); ^{1}H NMR [250 MHz, $(CD_3)_2SO$, 303 K] δ_H 1.0 [m, 18H, $CH(CH_3)_2$], 2.3 [m, 3H, $CH(CH_3)_2$], 3.8–5.2 [m, 24H, $ArCH_2$], 5.3 (m, 3H, COCH), 6.9–7.3 (m, 24H, ArH), 8.3 (d, 3H, *J* 6.5 Hz, CONH), 8.7 (m, 3H, Py-H4), 8.9 (m, 3H, Py-H2), 9.3 (m, 3H, Py-H6). Anal. Calcd for $C_{84}H_{84}N_{12}O_9 \cdot H_2O$: C, 70.87; H, 6.09; N, 11.81%. Found: C, 70.79; H, 5.99; N, 11.48%. ESI-MS *m/z* 703 $(M+2H)^{2+}$,1405 $(M+H)^+$; calcd M for $C_{84}H_{84}N_{12}O_9$, 1404.

Cationic Cage-type Cyclophane with L-Valine Residues [(+)-3]

Methyl iodide (0.50 g, 3.52 mmol) was added to compound **(+)-2** (30 mg, 2.1 x 10^{-5} mol) dissolved in dry DMF (5mL), and the mixture was stirred for 170 h at room temperature. After the mixture was evaporated to dryness under reduced pressure, the resulting iodide salt was converted into the chloride salt by ion-exchange chromatography on a column of Amberlite IRA-401 with methanol as eluant. The solvent was evaporated off under reduced pressure, and the crude product was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol–chloroform (1:1 v/v) as eluant. Evaporation of the solvent under reduced pressure gave a pale yellow solid (31 mg, 94%): mp 270–272 °C (dec.); IR/cm^{-1} (KBr disc) 1640 (amide C=O); $[\alpha]^{25}_D$ +49° (c = 0.1, CH_3OH); ^{1}H NMR [500 MHz, $(CD_3)_2SO$, 303 K] δ_H 1.07 [d, *J* 6.5 Hz, 9H, $CH(CH_3)_2$ (nonequivalent)], 1.10 [d, *J* 6.5 Hz, 9H, $CH(CH_3)_2$ (nonequivalent)], 2.4 [m, 3H, $CH(CH_3)_2$], 3.8–5.2 (m 24H, $ArCH_2$), 4.43 (s, 9H, NCH_3), 5.4 (m, 3H, COCH), 6.8–7.2 (m, 24H, ArH), 8.68 (d, *J* 7.5 Hz, 3H, CONH), 9.13 (m, 3H, Py-H4), 9.50 (m, 3H, Py-H2), 9.76 (m, 3H, Py-H6). Anal. Calcd for $C_{87}H_{93}N_{12}O_9Cl_3 \cdot 2H_2O$: C, 65.59; H,6.14; N, 10.55%. Found: C, 65.51; H, 6.04; N, 10.31%. ESI-MS *m/z* 483 $(M-3Cl)^{3+}$, 742 $(M-2Cl)^{2+}$; calcd M for $C_{87}H_{93}N_{12}O_9Cl_3$, 1556.

N,N',N''-Tris(N$^{\alpha}$-tert-butoxycarbonyl-D-valyl)-2,11,20-triaza[3.3.3]paracyclophane [(−)-5]

This compound was prepared by condensation of *tert*-butoxycarbonyl-D-valine (754 mg, 3.47 mmol) with **4** (176 mg, 0.50 mmol) in a manner similar to that applied to the synthesis of **(+)-5**. The crude product was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol–chloroform (1:1 v/v) as eluant to give a white solid (392 mg, 83%): mp 128–130 °C; R_f [Wako Silica Gel 70FM, methanol–chloroform (1:1 v/v)] 0.87; IR/cm^{-1} (KBr disc) 1720 (urethane C=O) and 1640 (amide C=O); $[\alpha]^{25}_D$ +26° (c = 0.1, CH_3OH); ^{1}H NMR [250 MHz, $(CD_3)_2SO$, 373 K] δ_H 0.78 [d, *J* 6.7 Hz, 9H, $CH(CH_3)_2$ (nonequivalent)], 0.85 [d, *J* 6.7 Hz, 9H, $CH(CH_3)_2$ (nonequivalent)], 1.38 [s, 27H, $C(CH_3)_3$], 2.00 [m, 3H, $CH(CH_3)_2$], 4.25 (dd, *J* 9.0 and 7.5 Hz, 3H, COCH), 4.59 (m, 12H, $ArCH_2$), 6.20 (d, *J* 9.0 Hz, 3H, NHCO), 6.89 (s, 12H, ArH). Anal. Calcd for $C_{54}H_{78}N_6O_9 \cdot 1/2\ H_2O$: C, 67.26; H, 8.26; N, 8.72%. Found: C, 67.20; H, 8.23; N, 8.75%.

N,N',N"-Tris(D-valyl)-2,11,20-triaza[3.3.3]paracyclophane [(−)-6]

This compound was prepared by removal of the protecting groups of (−)-**5** (355 mg, 0.35 mmol) with trifluoroacetic acid (10 mL) in a manner similar to that applied to the synthesis of (+)-**6**. The crude product was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol as eluant to give a white solid (344 mg, 98 %): mp 177–179 °C; R_f (Wako Silica Gel 70FM, ethanol) 0.85; IR/cm^{-1} (KBr disc) 1670 (amide C=O); $[\alpha]^{25}_D$ −51° (c = 0.1, CH_3OH); ^1H NMR [250 MHz, $(CD_3)_2SO$, 383 K] δ_H 0.93 [d, *J* 6.8 Hz, 9H, $CH(CH_3)_2$ (nonequivalent)], 0.96 [d, *J* 6.8 Hz, 9H, $CH(CH_3)_2$ (nonequivalent)], 2.12 [m, 3H, $CH(CH_3)_2$], 4.04 (d, *J* 5.2 Hz, 3H, COCH), 4.61 (m 12H, $ArCH_2$), 6.93 (12H, s, ArH). Anal. Calcd for $C_{45}H_{57}F_9N_6O_9$: C, 54.21; H, 5.76; N, 8.43%. Found: C, 54.03; H, 5.76; N, 8.33%.

Cage-type Cyclophane with D-Valine Residues [(−)-2]

This compound was prepared by condensation of (−)-**6** (162 mg, 0.16 mmol) with **8** (130 mg, 0.16 mmol) under high dilution conditions at 0 °C in a manner similar to that applied to the synthesis of (+)-**2**. The crude product was purified by liquid chromatography on a column of silica gel (Wakogel C-300) with methanol–chloroform (1:1 v/v) as eluant, followed by gel filtration chromatography on columns of Sephadex LH-20 and Toyopearl HW-40F, in this sequence, with methanol–chloroform (1:1 v/v) as eluant to give a white solid (60 mg, 26%): mp 292–294 °C (dec.); R_f [Wako Silica Gel 70FM, methanol-chloroform (1:1 v/v)] 0.42; IR/cm^{-1} (KBr disc) 1640 (amide C=O); $[\alpha]^{25}_D$ −82° (c = 0.1, $CHCl_3$); ^1H NMR [500 MHz, $(CD_3)_2SO$, 303 K] δ_H 1.0 [m, 18H, $CH(CH_3)_2$], 2.3 [m, 3H, $CH(CH_3)_2$], 3.8–5.2 [m, 24H, $ArCH_2$], 5.3 (m, 3H, COCH), 6.9–7.3 (m, 24H, ArH), 8.3 (d, *J* 6.5 Hz, 3H, CONH), 8.7 (m, 3H, Py-H4), 8.9 (m, 3H, Py-H2), 9.3 (m, 3H, Py-H6). Anal. Calcd for $C_{84}H_{84}N_{12}O_9 \cdot 2H_2O$: C, 69.98; H, 6.15; N, 11.66%. Found: C, 70.09; H, 6.05; N, 11.36%. ESI-MS *m/z* 703 $(M+2H)^{2+}$, 1405 $(M+H)^+$; calcd M for $C_{84}H_{84}N_{12}O_9$, 1404.

Cationic Cage-type Cyclophane with D-Valine Residues [(−)-3]

This compound was prepared by quaternization of (−)-**2** (30 mg, 2.1 x 10^{-5} mol) with methyl iodide (0.50 g, 3.52 mmol) followed by ion exchange in a manner similar to that applied to the synthesis of (+)-**3**. The crude product was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol–chloroform (1:1 v/v) as eluant to give a pale yellow solid (32 mg, 95%): mp 270–272 °C (dec.); IR/cm^{-1} (KBr disc) 1640 (amide C=O); $[\alpha]^{25}_D$ −56° (c = 0.1, CH_3OH); ^1H NMR [500 MHz, $(CD_3)_2SO$, 303 K] δ_H 1.07 [d, *J* 6.5 Hz, 9H, $CH(CH_3)_2$ (nonequivalent)], 1.10 [d, *J* 6.5 Hz, 9H, $CH(CH_3)_2$ (nonequivalent)], 2.4 [m, 3H, $CH(CH_3)_2$], 3.8–5.2 (m 24H, $ArCH_2$), 4.43 (s, 9H, NCH_3), 5.4 (m, 3H, COCH), 6.8–7.2 (m, 24H, ArH), 8.7 (d, *J* 7.5 Hz, 3H, CONH), 9.13 (m, 3H, Py-H4), 9.50 (m, 3H, Py-H2), 9.78 (m, 3H, Py-H6). Anal. Calcd for $C_{87}H_{93}N_{12}O_9Cl_3 \cdot 1/2H_2O$: C, 66.72; H,6.05; N, 10.73%. Found: C, 66.61; H, 6.02; N, 10.65%. ESI-MS *m/z* 483 $(M-3Cl)^{3+}$, 742 $(M-2Cl)^{2+}$; calcd M for $C_{87}H_{93}N_{12}O_9Cl_3$, 1556.

ESI-MS Measurements for Host-guest Complexes

Mass spectra of host–guest complexes were measured by flow injection analysis without use of columns. A solution of (+)-**3** (15 μg, 0.01 μmol) and ANS (6 μg, 0.01 μmol) in water (10μL) was injected with a loop injector. Water–methanol (1:1 v/v) was used for the samples as a mobile phase at a flow rate of 20–50 ml min^{-1}. Small droplets, including cluster ions produced by nebulizer (99.999% N_2) at atmospheric pressure, were introduced into the ion source housing and the mass analyzing region through the intermediate region,

which was located between the first electrode with an aperture and the second electrode with an aperture. A drift voltage was applied to the intermediate region to dissociate cluster ions into cationized molecules. The temperature of the steel block in the nebulizer and the drift voltage in the intermediate region were set at 200 °C and *ca.* 100 V, respectively, because maximum signal intensities for the cationized host–guest complexes were obtained under these conditions. The intermediate and mass analyzing regions were evacuated with a diffusion pump to about 0.2 and 10^{-5} Torr, respectively. Intense signals for other host–guest complexes were also observed under the identical conditions.

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REFERENCES AND NOTES

1. (a) Murakami, Y.; Kikuchi, J.; Hayashida, O. *Top. Curr. Chem.* in press. (b) Lehn, J. -M, *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1304.
2. (a) Murakami, Y.; Ohno, T.; Hayashida, O.; Hisaeda, Y. *Chem. Lett.* **1991**, 1595. (b) Murakami, Y.; Hayashida, O.; Ito, T. *Chem. Lett.* **1992**, 497. (c) Murakami, Y.; Hayashida, O. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 1140.
3. (a) Murakami, Y.; Hayashida, O.; Ito, T.; Hisaeda, Y. *Pure Appl. Chem.* **1993**, *65*, 551. (b) Murakami, Y.; Hayashida, O.; Ono, K. *Pure Appl. Chem.* **1993**, *65*, 2319. (c) Murakami, Y.; Hayashida, O.; Nagai, Y. *Recl. Trav. Chem. Pays-Bas*, **1994**, *113*, 209.
4. Fujita, T; Lehn, J. -M. *Tetrahedron Lett.* **1988**, *29*, 1709.
5. Preliminary communication of this work: Murakami, Y.; Hayashida, O.; Matsuura, S. *Recl. Trav. Chem. Pays-Bas*, **1993**, *112*, 421.
6. For phenyl and methylene proton signals of non-substituted macrocycle **4**, singlet signals were observed at the same temperature.
7. Benesi, H. A.; Hildebrand, J. H. *J. Am. Chem. Soc.* **1949**, *71*, 2703.
8. Murakami, Y.; Kikuchi, J.; Ohno, T.; Hayashida, O.; Kojima, M. *J. Am. Chem. Soc.* **1990**, *112*, 7672.
9. (a) Yamashita, M.; Fenn, J. B. *J. Phys. Chem.*, **1984**, *88*, 4451. (b) Whitehouse, M.; Dreyer, R. N.; M. Yamashita, M.; Fenn, J. B. *Anal. Chem.*, **1985**, *57*, 675.
10. Murakami, Y.; Hayashida, O.; Nagai, Y. *Tetrahedron Lett.* **1993**, *34*, 7935.
11. $M_{complex}$ stands for M of (1:1) host-guest complexes bearing no charge.
12. (a) LeBas, G.; Allegret, A.; Mauguen, Y.; DeRango, C.; Bailly, M. *Acta Crystallogr., Sect. B* **1980**, *B36*, 3007. (b) Trull, F. R.; Ma, J. -S.; Landen, G. L.; Lightner, D. A. *Isr. J. Chem.* **1983**, *23*, 211.
13. Likussar, W.; Boltz, D. F. *Anal. Chem.*, **1973**, *43*, 1265.
14. Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy-Exciton Coupling in Organic Stereochemistry*; University Science Books: Mill Valley, CA, 1983.
15. Murakami, Y.; Hayashida, O.; Nagai, Y. *J. Am. Chem. Soc.* **1994**, *116*, 2611.

16. (a)Lightner, D. A.; Gawronski, J. K.; Gawronska, K. *J. Am. Chem. Soc.* **1985**, *107*, 2456. (b)Kano, K.; Yoshiyasu, K.; Hashimoto, S. *J. Chem. Soc., Chem. Commun.* **1988**, 801.
17. Mayo, L.; Olafson, B. D.; Goddard III, W. A. *J. Phys. Chem.* **1990**, *94*, 8897.

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