Pergamon

Tetrahedron Vol. 51, No. 31, pp. 8423-8436, 1995
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0040-4020/95 $9.50+0.00

0040-4020(95)00458-0

Specific Molecular Recognition by Chiral Cage-type Cyclophanes Having Leucine, Valine, and Alanine Residues

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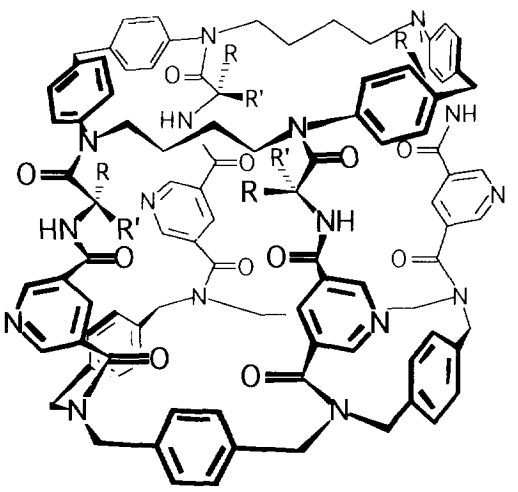
Abstract: Chiral cage-type cyclophanes were constructed with two rigid macrocyclic skeletons and four bridging components bearing chiral leucine, valine, and alanine residues, individually. These host molecules strongly bind anionic and hydrophobic guests, such as 8-anilinonaphthalene-1-sulfonate and 6-*p*-toluidinonaphthalene-2-sulfonate. Thermodynamic parameters were evaluated from temperature-dependent complexation constants determined by fluorescence spectroscopy, and gave negative ΔH and positive ΔS values; especially large values for the cage-type cyclophanes having leucine residues. The positive ΔS values come primarily from effective desolvation of the guest molecules when incorporated into the hydrophobic host cavities, as evidenced by fluorescence parameters. The four bridging segments of the cage-type hosts having chiral amino acid residues seem to undergo chiral twist in the same directions in the light of circular dichroism (CD) spectroscopy. Such helical conformations of the cyclophanes must be caused by chiral nature of the amino acid residues, and the extent of twist in helical conformations is as follows; leucine > valine > alanine. In addition, the twisted direction of bridging segments in the cage-type hosts having L-amino acid residues is opposite to that evaluated for those having D-amino acid residues, so that the former and latter cyclophanes furnish *M*- and *P*-helical cavities, respectively. The chirality-based molecular recognition of the cage-type hosts toward an enantiomeric guest, bilirubin-IXα, was investigated by CD spectroscopy in aqueous media.

INTRODUCTION

Currently, the development of artificial hosts capable of performing functional simulation of naturally occurring receptors has been attracting much attention.[1] Guest-binding sites of the natural hosts are elegantly constructed with various optically active amino acid residues and well shielded from a bulk aqueous phase to become largely hydrophobic. On these grounds, we have developed cage-type cyclophanes bearing L- and D-valine residues [(+)-**2**, (−)-**2**, (+)-**5**, and (−)-**5**],[2] which are constructed with two rigid macrocyclic skeletons, tetraaza[3.3.3.3]paracyclophane and tetraaza[6.1.6.1]paracyclophane, and four chiral bridging components that connect the macrocycles. We have clarified that the hosts having L- and D-valine residues in the respective bridging segments provide helically twisted, globular, and hydrophobic cavities for chiral recognition toward guests, such as (4Z,15Z)-bilirubin IXα (BR) and steroid hormones in aqueous media.[3] In this context, we now prepared cage-type cyclophanes bearing chiral leucine and alanine residues in their bridging segments [(+)-**1**, (−)-**1**, (+)-**3**,[4] (−)-**3**,[4] (+)-**4**, (−)-**4**, (+)-**6**, and (−)-**6**], in order to get further insights into the correlation between a specific molecular recognition ability of the hosts and a hydrophobic and asymmetric property of their internal cavities. We investigated in this work the microenvironmental properties

of internal cavities furnished by the cage-type cyclophanes as demonstrated toward anionic fluorescent guests, 8-anilinonaphthalene-1-sulfonate (ANS) and 6-*p*-toluidinonaphthalene-2-sulfonate (TNS). Furthermore, the enantioselective discrimination by the cage-type hosts toward an enantiomeric guest, BR, was examined in aqueous media.



(+)-1 R = $CH_2CH(CH_3)_2$, R' = H

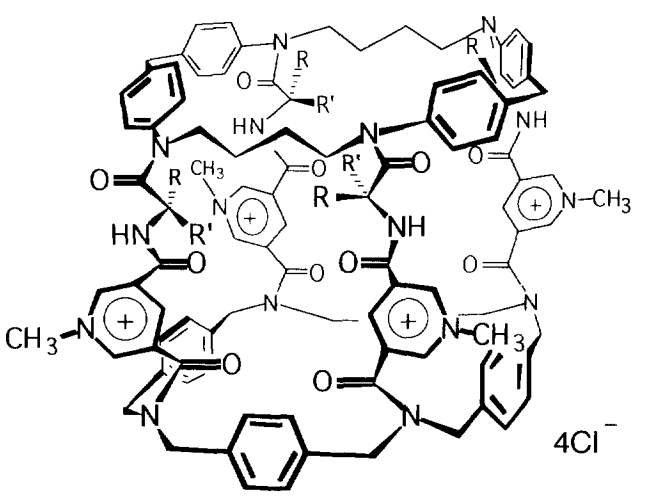
(+)-2 R = $CH(CH_3)_2$, R' = H

(+)-3 R = CH_3, R' = H

(−)-1 R = H, R' = $CH_2CH(CH_3)_2$

(−)-2 R = H, R' = $CH(CH_3)_2$

(−)-3 R = H, R' = CH_3



(+)-4 R = $CH_2CH(CH_3)_2$, R' = H

(+)-5 R = $CH(CH_3)_2$, R' = H

(+)-6 R = CH_3, R' = H

(−)-4 R = H, R' = $CH_2CH(CH_3)_2$

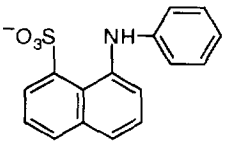
(−)-5 R = H, R' = $CH(CH_3)_2$

(−)-6 R = H, R' = CH_3

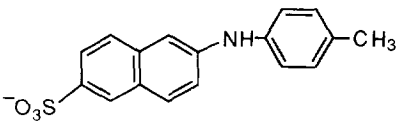
RESULTS AND DISCUSSION

Microenvironmental Properties of Cyclophane Cavities

We adopted anionic fluorescent probes, ANS and TNS, whose emission is extremely sensitive to a microenvironmental polarity of the surrounding medium in both intensity and wavelength,[5] as hydrophobic guest molecules for evaluation of microenvironmental properties of the cyclophane cavities. The guest-binding behavior of cage-type hosts (+)-**1** and (+)-**3** toward the guests was examined by fluorescence spectroscopy in aqueous acetate buffer (0.01 M, pH 4.1, μ 0.10 with KCl) at 20, 30, 40, and 50 °C. Upon addition of the host to the acetate buffer containing each guest (1.0×10^{-6} M), a fluorescence intensity originating from the guest increased along with a concomitant blue shift of the fluorescence maximum, reflecting formation of the corre-



ANS



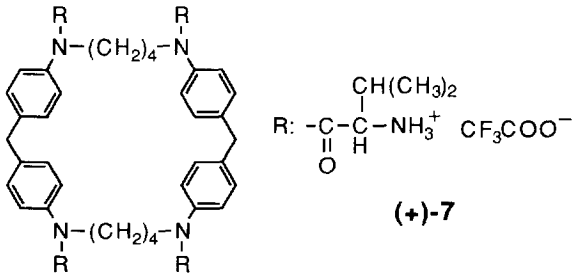
TNS

sponding host–guest complex. The binding constants (K) of (+)-**1** and (+)-**3** toward ANS and TNS were evaluated on the basis of the Benesi-Hildebrand relationship[6] for a 1:1 host–guest interaction in a manner as described previously.[7] The evaluated K values are summarized in Table 1 together with the corresponding values reported for cage-type host (+)-**2**.[8] The K values for the cage-type hosts with the guests are apparently much greater than the corresponding values for the peptide cyclophane (+)-**7**,[2a,2b] which was prepared by introducing four L-valine residues into the rigid tetraaza[6.1.6.1]paracyclophane skeleton; K, $7.5 \times 10^2 - 2.2 \times 10^3$ and $6.4 \times 10^3 - 2.6 \times 10^4$ M^{-1} for complexation with ANS and TNS, respectively.[8] The larger guest-binding affinity of the cage-type hosts is due to an enhanced hydrophobic effect exercised by the three-dimensionally extended internal cavities. Gibbs free energy (ΔG), enthalpy (ΔH), and entropy changes (ΔS)

Table 1. Binding Constants (K / M^{-1}) for Complex Formation of Cyclophanes with Hydrophobic Guests in Aqueous Acetate Buffer (0.01 M, pH 4.1, μ 0.10 with KCl)[a]

Host	Guest	Temperature / K				Ref
		293	303	313	323	
(+)-**1**	ANS	3.4×10^4	3.3×10^4	3.0×10^4	2.7×10^4	
(+)-**2**	ANS	3.3×10^4	2.8×10^4	2.1×10^4	1.8×10^4	8
(+)-**3**	ANS	1.8×10^4	1.5×10^4	1.4×10^4	1.0×10^4	
(+)-**1**	TNS	6.2×10^4	5.0×10^4	4.5×10^4	4.1×10^4	
(+)-**2**	TNS	6.9×10^4	5.8×10^4	4.2×10^4	3.4×10^4	8
(+)-**3**	TNS	7.0×10^4	5.7×10^4	4.8×10^4	3.2×10^4	

a) Concentrations in M: guests, 1.0×10^{-6}; hosts, $5.0 \times 10^{-6} - 3.0 \times 10^{-5}$.



(+)-7

Table 2. Thermodynamic Parameters for Complex Formation of Cyclophanes with Hydrophobic Guests in Aqueous Acetate Buffer (0.01 M, pH 4.1, μ 0.10 with KCl) at 313 K

Host	Guest	ΔG / kJ mol^{-1}	ΔH / kJ mol^{-1}	$T\Delta S$ / kJ mol^{-1}	Ref
(+)-**1**	ANS	−26.8	−6.0	20.8	
(+)-**2**	ANS	−25.9	−17.1	8.8	8
(+)-**3**	ANS	−24.2	−14.3	10.2	
(+)-**1**	TNS	−27.9	−10.2	17.7	
(+)-**2**	TNS	−27.7	−19.5	8.3	8
(+)-**3**	TNS	−28.0	−20.0	7.7	

for formation of the host–guest complexes were evaluated from the temperature-dependent K values as listed in Table 2 together with the corresponding values reported for (+)-**2**.[8] Complexation of the cage-type hosts with the guests gave negative ΔH and positive ΔS values. Moreover, the ΔG values do not significantly differ from each other among the complexation of cage-type cyclophanes (+)-**1**, (+)-**2**, and (+)-**3** with ANS, but the ΔS and ΔH values for formation of the (+)-**1**•ANS complex are surprisingly larger than those for formation of the (+)-**2**•ANS and (+)-**3**•ANS complexes. A similar trend of thermodynamic parameters was also observed for complexation of the cage-type cyclophanes with TNS. These results indicate that the binding affinity of (+)-**1** for the guests is larger than those of cage-type hosts (+)-**2** and (+)-**3** at higher temperatures.

The positive ΔS values must come primarily from effective desolvation of the guest molecules incorporated into the hydrophobic host cavity. In other words, water molecules, surrounding the guest molecule in the uncomplexed state, are removed by complexation with the cage-type host and transferred to a bulk aqueous phase. Such a hydrophobic effect given out by the cage-type hosts was evidenced by fluorescence parameters. The microenvironmental polarity experienced by the incorporated guest molecule was evaluated from the fluorescence maximum in a manner similar to that reported previously.[7] The E_T^N values[9] for ANS and TNS placed in the cage-type hosts are smaller than those for the identical guests incorporated into peptide cyclophane (+)-**7**[8] (Fig.1). Consequently, the three-dimensional cavities provided by the cage-type hosts are significantly apolar and well shielded from the bulk aqueous phase. In addition, the microenvironmental polarity of the guest-binding sites provided by the cage-type cyclophanes was subject to change by hydrophobic nature of the amino acid residues in the bridging segments, and the E_T^N value is in the following sequential order with respect to the cage-type hosts: (+)-**1** < (+)-**2** < (+)-**3**.

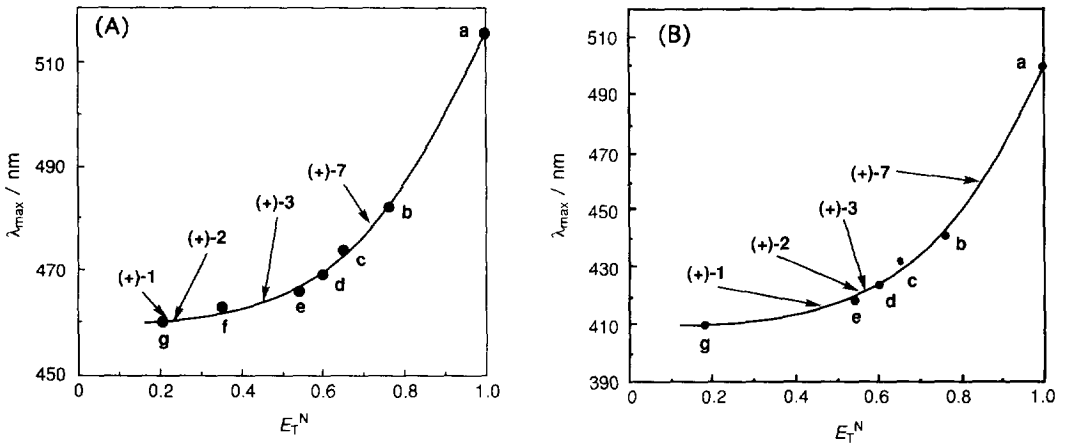


Fig. 1. Solvent effect on fluorescence of ANS (A; 1.0 x 10^{-6} M) and TNS (B; 1.0 x 10^{-6} M) at 30°C: a, aqueous acetate buffer; b, methanol; c, ethanol; d, 1-butanol; e, 2-propanol; f, acetone; g, tetrahydrofuran; fluorescence maxima of the guests incorporated into cyclophanes [(+)-**1**, (+)-**2**, (+)-**3**, and (+)-**7**] are shown by arrows.

Chiral Recognition by Cage-type Cyclophanes

The asymmetric character of the cage-type hosts having leucine and alanine residues was examined by circular dichroism (CD) spectroscopy, and CD spectra for (+)-**1**, (−)-**1**, (+)-**3**, and (−)-**3** are shown in Fig. 2 together with those for (+)-**2** and (−)-**2**. The cage-type hosts having L-amino acid residues showed CD bands with positive sign, while CD bands with inverted sign were observed for the hosts having D-amino acid

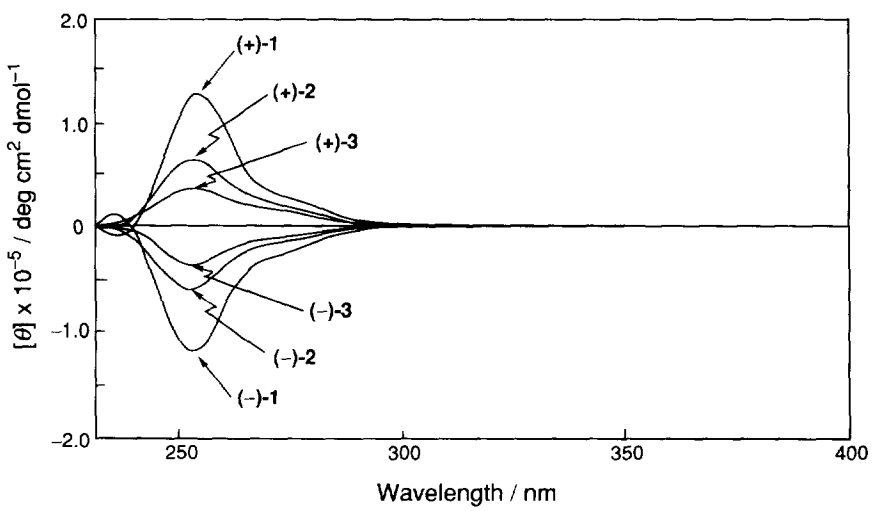


Fig. 2. CD spectra of cage-type cyclophanes [(+)-**1**, (+)-**2**, (+)-**3**, (−)-**1**, (−)-**2**, and (−)-**3**] (5.0 x 10^{-6} M) in methanol–chloroform (1:1 v/v) at 30.0 °C.

residues. A similar asymmetric character was also confirmed for the internal cavities of cationic hosts (+)-**5**, (−)-**5**, (+)-**6**, and (−)-**6** by the identical method in aqueous phosphate buffer (0.01M, pH 7.0, μ 0.1 with KCl):[10] [θ]/deg cm^2 dmol^{-1}, 1.2 x 10^5 (237 nm) for (+)-**5**; −1.3 x 10^5 (236 nm) for (−)-**5**; 3.5 x 10^4 (248nm) for (+)-**6**; −3.4 x 10^4 (245 nm) for (−)-**6**. The results suggest that the four bridging components of each cage-type host, in which all the pyridyl moieties are bound to the chiral amino acid residues, approach close to each other and are twisted in the same direction, in a manner similar to that exercised by cage-type cyclophanes having chiral valine residues, (+)-**2** and (−)-**2**.[2a,3a] Such helical conformations of the cage-type cyclophanes seem to be caused by chiral nature of the amino acid residues, and an extent of twist in the helical conformation is as follows; leucine > valine > alanine. Moreover, the twisted direction of the bridging segments having L-amino acid residues is opposite to that evaluated for those having D-amino acid residues, so that the former hosts furnish an internal cavity of *M*-helicity while the latter ones provide an internal cavity of *P*-helicity, as evidenced by molecular mechanics (BIOGRAF, Dreiding-II)[11] calculations.[2a,3a,3b]

We investigated chirality-based molecular discrimination ability of the cage-type cyclophanes toward an anionic guest, (4Z,15Z)-bilirubin IXα (BR). It is well known that the BR molecule is folded into either of two chiral 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. 3).[12] First, the guest-binding behavior of cage-type hosts (+)-**4**, (−)-**4**, (+)-**5**, (−)-**5**, (+)-**6**, and (−)-**6** 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 host to the carbonate buffer containing BR

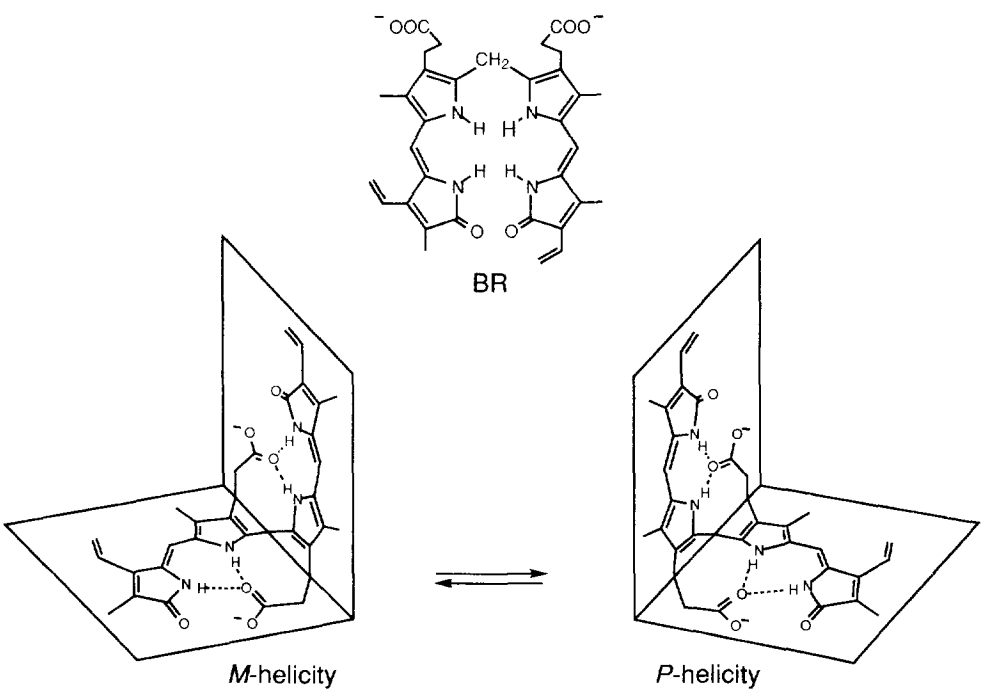


Fig. 3. Three-dimensional representions for two conformational enantiomers of BR stabilized by intramolecular hydrogen bonding, which undergo rapid interconversion in aqueous media.

Table 3. Binding Constants (K/ M^{-1}) for Complex Formation of Cage-type Cyclophanes with BR in Aqueous Carbonate Buffer (0.01 M, pH 10.0, μ 0.10 with KCl) at 28.0 °C

Host	K / M^{-1}	
(+)-4	4.0 x 10^6	
(−)-4	4.9 x 10^6	
(+)-5	1.2 x 10^6	6.4 x 10^5 [a]
(−)-5	1.3 x 10^6	6.9 x 10^5 [a]
(+)-6	5.8 x 10^5	
(−)-6	5.2 x 10^5	

a) Evaluated by CD spectroscopy in aqueous carbonate buffer (0.02 M, pH 10.0, μ 0.10 with KCl) (ref. 3a).

(1.0 x 10^{-5} M), an electronic absorption intensity originating from BR (ε = 47000 at 436 nm) decreased, reflecting formation of the corresponding host–guest complex. The stoichiometry for complex formation was investigated by the molar ratio method. The result revealed that the present hosts underwent complex formation with BR in a 1:1 molar ratio of host to guest. 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,[3b] and are listed in Table 3.

A CD spectrum for a carbonate buffer solution containing **(+)-4** and BR 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; [θ]/deg cm^2 $dmol^{-1}$, −2.1 x 10^4 and 1.9 x 10^4 at 466 and 407 nm, respectively (Fig. 4). The bisignate Cotton effect indicates that the BR molecule selectively assumes a conformational enantiomer of M-helicity upon complexation with **(+)-4** on the basis of an exciton-coupling theory,[13] in a manner similar to that performed by **(+)-5**.[3a] On the other hand, the Cotton effect was inverted in the presence of **(−)-4** in the aqueous carbonate buffer at 28.0 °C; [θ]/deg cm^2 $dmol^{-1}$, 2.6 x 10^4 and −2.4 x 10^4 at 463 and 406 nm, respectively (Fig. 4), indicating that **(−)-4** binds the P-helicity enantiomer of BR in preference to the M-helicity enantiomer. Moreover, the CD band intensities in the presence of cage-type hosts having leucine residues **(+)-4** and **(−)-4** were larger than those in the presence of **(+)-5** ([θ]/deg cm^2 $dmol^{-1}$, −1.7 x 10^4 and 1.3 x 10^4 at 465 and 410 nm, respectively) and **(−)-5** ([θ]/deg cm^2 $dmol^{-1}$, 1.4 x 10^4 and −1.8 x 10^4 at 464 and 416 nm, respectively). A difference in the extent of complexation of BR between **(+)-4** and **(+)-5** is not so large; *e.g.* 48 and 45% fractions of the total BR undergo complexation with **(+)-4** and **(+)-5**, respectively on the basis of the K values under identical conditions. Consequently, the increase in CD band intensity reflects the enhancement of chiral recognition ability performed by cage-type hosts **(+)-4** and **(−)-4** relative to that by **(+)-5** and **(−)-5**. Similar characteristic bisignate Cotton effects were also observed in the presence of cage-type hosts having chiral alanine residues **(+)-6** ([θ]/deg cm^2 $dmol^{-1}$, −1.1 x 10^4 and 5.0 x 10^3 at 465 and 397 nm, respectively) and **(−)-6** ([θ]/deg cm^2 $dmol^{-1}$, 7.3 x 10^3 and −8.4 x 10^3 at 457 and 405 nm, respectively),[14] even though the CD band intensities were much weaker than those observed in the presence of **(+)-5** and **(−)-5**. It seems that the cage-type cyclophanes having valine residues behave as artificial receptors far superior in chiral recognition ability to the cage-type hosts with alanine residues.

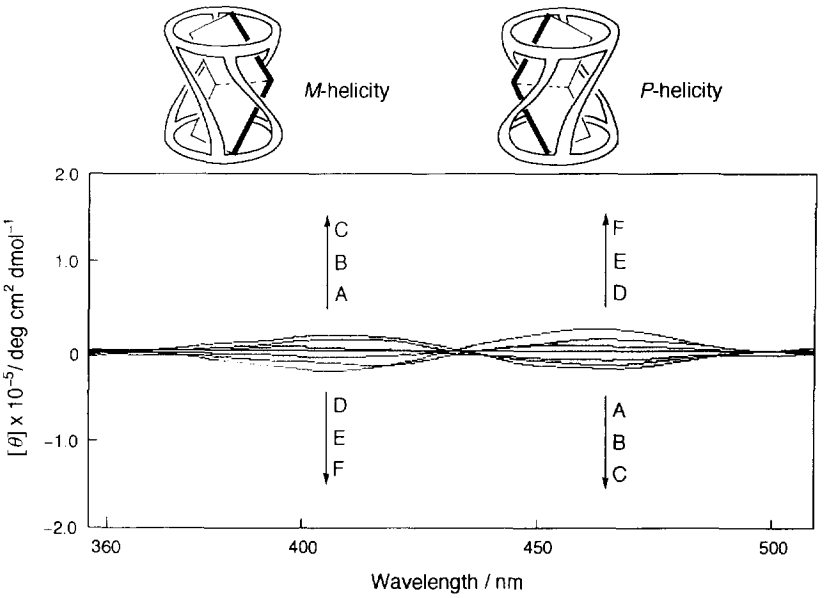


Fig. 4. CD spectra of BR on addition of (+)-**6** (A), (+)-**5** (B), (+)-**4** (C), (−)-**6** (D), (−)-**5** (E), and (−)-**4** (F), respectively, in aqueous carbonate buffer (0.01 M, pH 10.0, μ 0.10 with KCl) at 28.0 °C. Concentrations: BR, 3.0 x 10^{-5} M; hosts, 1.5 x 10^{-5} M

In conclusion, cage-type cyclophanes having chiral leucine, valine, and alanine residues were synthesized on the basis of molecular design that allows to connect two macrocyclic skeletons with four bridging segments. Each of the present cage-type hosts furnishes a hydrophobic internal cavity in aqueous media. The hosts bearing L- and D-amino acids residues in the respective bridging segments afford *M*- and *P*- helical cavities, respectively. The helically twisted cavities enforce on enantiomeric guest, BR to assume specific chiral conformations when they are incorporated, and the chiral recognition ability of the cationic cage-type hosts having leucine residues was most enhanced among the present hosts.

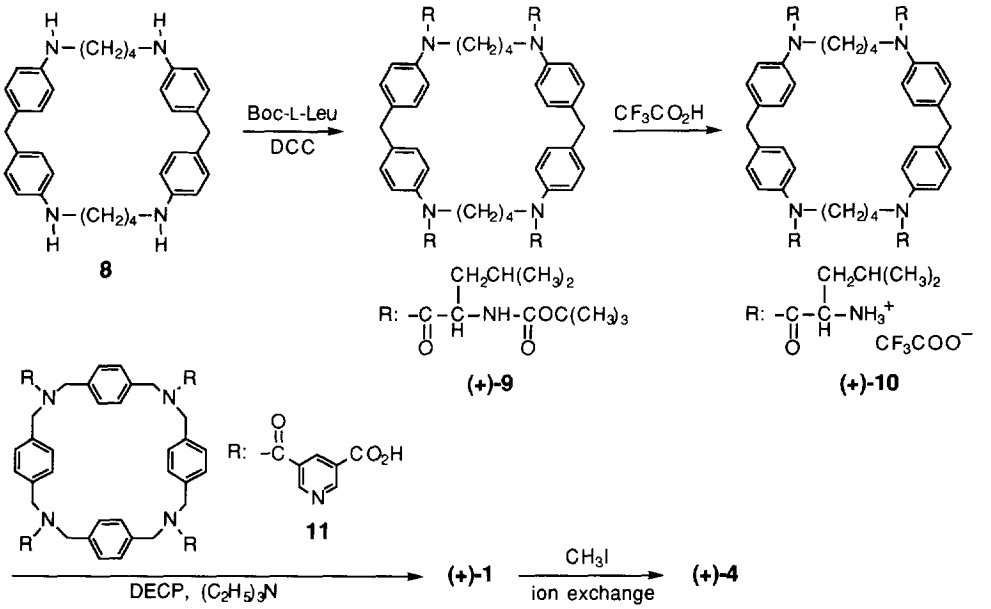
EXPERIMENTAL

General Analyses and Measurements

Melting points were measured with a Yanaco 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 AMX-500 spectrometer installed at the Center of Advanced Instrumental Analysis, Kyushu University. Optical rotations and circular dichroism spectra were measured on a Horiba SEPA polarimeter and a JASCO J-500C spectropolarimeter, respectively.

Materials

The following compounds were obtained from commercial sources as guaranteed reagents and used without further purification: potassium 6-*p*-toluidinonaphthalene-2-sulfonate [K(TNS)] (from Nacalai Tesque, Inc., Kyoto, Japan); sodium 8-anilinonaphthalene-1-sulfonate [Na(ANS)] and (4Z,15Z)-bilirubin IXα (BR) (both from Tokyo Kasei Kogyo Co., Tokyo, Japan). N^{α}-*tert*-Butoxycarbonyl-L-leucine and N^{α}-*tert*-butoxycarbonyl-D-leucine were purchased from Peptide Institute, Inc., Osaka, Japan as guaranteed reagents. 1,6,20,25-Tetraaza[6.1.6.1]paracyclophane (**8**)[15] and *N,N',N'',N'''*-tetrakis(5-carboxynicotinoyl)-2,11,20,29-tetraaza[3.3.3.3]paracyclophane (**11**)[2b] were prepared after methods reported previously. Preparation of cage-type cyclophanes, (+)-**2**, (−)-**2**, (+)-**3**, (−)-**3**, (+)-**5**, and (−)-**5**, and peptide cyclophane (+)-**7** has been described previously.[2a,2b,4] Cyclophanes (+)-**1** and (+)-**4** were synthesized by following the reaction sequence shown in Scheme 1. The use of *tert*-butoxycarbonyl-D-leucine in place of *tert*-butoxycarbonyl-L-leucine afforded the corresponding cyclophanes bearing D-leucine residues, (−)-**1** and (−)-**4**.



Scheme 1

N,N',N'',N'''-Tetrakis(N$^{\alpha}$-tert-butoxycarbonyl-L-leucyl)-1,6,20,25-tetraaza[6.1.6.1]paracyclophane [(+)-9]

Dicyclohexylcarbodiimide (740 mg, 3.6 mmol) was added to a dry dichloromethane (20 mL) solution of *tert*-butoxycarbonyl-L-leucine (800 mg, 3.2 mmol) at 0 °C, and the mixture was allowed to stand at the same temperature while being stirred for 20 min. 1,6,20,25-Tetraaza[6.1.6.1]paracyclophane (**8**; 200 mg, 0.40 mmol) dissolved in dry dichloromethane (20 mL) was added to the mixture, and the resulting mixture was stirred for 4 h at 0 °C and for an additional 48 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 (40 mL). The solution was then allowed to stand overnight at 5 °C, precipitates were removed by filtration, and 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–chloroform (1:1 v/v) as eluant. Evaporation of the product fraction under reduced pressure gave a white solid (380 mg, 71 %): mp 130–131 °C; R_f (Wako Silica Gel 70FM, ethyl acetate) 0.78; IR (KBr disc) 1720 (urethane C=O) and 1660 (amide C=O) cm^{-1}; $[\alpha]_D^{25}$ +134° (c 0.1, CH_3OH); ^1H NMR (500 MHz, $CDCl_3$, 303 K) δ_H 0.37 [d, *J* 6.5 Hz, 12H, $CH(CH_3)_2$ (nonequivalent)], 0.75 [d, *J* 6.5 Hz, 12H, $CH(CH_3)_2$ (nonequivalent)], 1.18 [m, 4H, $COCHCH_2$ (nonequivalent)], 1.38 [m, 4H, $COCHCH_2$ (nonequivalent)], 1.40 [m, 4H, $CH(CH_3)_2$], 1.44 [s, 36H, $C(CH_3)_3$], 1.49 [m, 4H, NCH_2CH_2 (nonequivalent)], 1.55 [m, 4H, NCH_2CH_2 (nonequivalent)], 3.33 [m, 4H, NCH_2CH_2 (nonequivalent)], 3.93 [m, 4H, NCH_2CH_2 (nonequivalent)], 4.01 (s, 4H, $ArCH_2Ar$), 4.22 (m, 4H, COCH), 5.74 (m, 4H, CONH), 7.21 [d, *J* 8.0 Hz, 8H, ArH(ortho)], 7.29 [d, *J* 8.0 Hz, 8H, ArH(meta)]. Anal. Calcd for $C_{78}H_{116}N_8O_{12}$•1/2 H_2O: C, 68.54; H, 8.63; N, 8.20%. Found: C, 68.58; H, 8.51; N, 8.19%.

N,N',N'',N'''-Tetrakis(L-leucyl)-1,6,20,25-tetraaza[6.1.6.1]paracyclophane [(+)-10]

Trifluoroacetic acid (10 mL) was added to a dry dichloromethane (10 mL) solution of (+)-**9** (140 mg, 0.11 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 (130 mg, 86 %): mp 182–183 °C; R_f (Wako Silica Gel 70FM, methanol) 0.63; IR (KBr disc) 1675 (amide C=O) cm^{-1}; $[\alpha]_D^{25}$ +98° (c 0.1, CH_3OH); ^1H NMR (500 MHz, CD_3OD, 303 K) δ_H 0.38 [d, *J* 6.5 Hz, 12H, $CH(CH_3)_2$ (nonequivalent)], 0.74 [d, *J* 6.5 Hz, 12H, $CH(CH_3)_2$ (nonequivalent)], 1.3–1.4 [m, 8H, $CH_2CH(CH_3)_2$], 1.43 [m, 4H, $CH(CH_3)_2$], 1.4–1.6 (m, 8H, NCH_2CH_2), 3.30 [m, 4H, NCH_2CH_2 (nonequivalent)], 3.85 [m, 4H, NCH_2CH_2 (nonequivalent)], 4.00 (m, 4H, COCH), 4.04 (s, 4H, $ArCH_2Ar$), 7.21 [d, *J* 8.5 Hz, 8H, ArH(ortho)], 7.33 [d, *J* 8.5 Hz, 8H, ArH(meta)]. Anal. Calcd for $C_{66}H_{88}F_{12}N_8O_{12}$: C, 56.08; H, 6.28; N, 7.93%. Found: C, 55.95; H, 6.30; N, 7.77%.

Cage-type Cyclophane with L-Leucine Residues [(+)-1]

Individual solutions of (+)-**10** (120 mg, 0.09 mmol) and *N,N',N'',N'''*-tetrakis(5-carboxynicotinoyl)-2,11,20,29-tetraaza[3.3.3.3]paracyclophane (**11**; 93 mg, 0.09 mmol) dissolved in dry *N,N*-dimethylformamide (DMF, 200 mL each) were added dropwise at an identical rate over 3 h to a dry DMF (2800 mL) solution containing diethyl cyanophosphonate (DECP; 65 mg, 0.4 mmol) and triethylamine (66 mg, 0.7 mmol) with vigorous stirring under nitrogen atmosphere at 0 °C. The resulting mixture was stirred for 24 h at the same temperature and for an additional 48 h at room temperature, and then evaporated to dryness under reduced pressure. The residue 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 (50 mg, 31 %): mp 321–323 °C; R_f (Wako Silica Gel 70FM, methanol) 0.58; IR (KBr disc) 1640 (amide C=O) cm^{-1}; $[\alpha]_D^{25}$ +107° (c 0.1, CH_3OH); ^1H NMR (500 MHz, CD_3SOCD_3, 383 K) δ_H 0.4 [m, 12H, $CH(CH_3)_2$ (nonequivalent)], 0.7 [bs, 12H, $CH(CH_3)_2$ (nonequivalent)], 1.4 [m, 4H, $CH(CH_3)_2$], 1.3–1.4 [m, 8H, $CH_2CH(CH_3)_2$], 1.4–1.8 (m, 8H, NCH_2CH_2), 3.3–3.8 (m, 8H, NCH_2CH_2), 4.0 (m, 4H, COCH), 4.0 (s, 4H, $ArCH_2Ar$), 4.5 (m, 16H, $ArCH_2N$), 6.9–7.4

(m, 32H, ArH), 8.2 (m, 4H, Py-H4), 8.8 (m, 4H, Py-H2), 9.1 (m, 4H, Py-H6). Anal. Calcd for $C_{118}H_{124}N_{16}O_{12}$•$7H_2O$: C, 67.99; H, 6.67; N, 10.75%. Found: C, 68.09; H, 6.45; N, 10.53%.

Cationic Cage-type Cyclophane with L-Leucine Residues [(+)-4]

Methyl iodide (0.057 g, 0.4 x 10^{-3} mol) was added to compound (+)-**1** (8.0 mg, 4.1 x 10^{-6} mol) dissolved in dry DMF (10 mL), and the mixture was stirred for 168 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 eluent. 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 as eluant. Evaporation of the solvent under reduced pressure gave a pale yellow solid (8.0 mg, 90 %): mp 253–255 °C (dec.); R_f (Wako Silica Gel 70FM, methanol) 0.62; IR (KBr disc) 1640 (amide C=O) cm^{-1}; NMR (500 MHz, CD_3SOCD_3, 383 K) δ_H 0.4 [m, 12H, $CH(CH_3)_2$ (nonequivalent)], 0.8 [bs, 12H, $CH(CH_3)_2$ (nonequivalent)], 1.4 [m, 4H, $CH(CH_3)_2$], 1.3–1.5 [m, 8H, $CH_2CH(CH_3)_2$], 1.4–1.8 (m, 8H, NCH_2CH_2), 3.2–3.8 (m, 8H, NCH_2CH_2), 4.0 (m, 4H, COCH), 4.0 (s, 4H, $ArCH_2Ar$), 4.5 (m, 12H, NCH_3), 4.5 (m, 16H, $ArCH_2N$), 6.8–7.3 (m, 32H, ArH), 9.2 (m, 4H, Py-H4), 9.2 (m, 4H, Py-H2), and 9.5 (m, 4H, Py-H6). Anal. Calcd for $C_{122}H_{136}N_{16}O_{12}Cl_4$•$4H_2O$: C, 65.64; H,6.50; N, 10.03%. Found: C, 65.53; H, 6.39; N, 10.16%.

N,N',N'',N'''-Tetrakis(N$^{\alpha}$-tert-butoxycarbonyl-D-leucyl)-1,6,20,25-tetraaza[6.1.6.1]paracyclophane [(−)-9]

This compound was prepared by condensation of *tert*-butoxycarbonyl-D-leucine (800 mg, 3.2 mmol) with **8** (200 mg, 0.40 mmol) in a manner similar to that applied to the synthesis of (+)-**9**. 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 (420 mg, 79 %): mp 133–134 °C; R_f (Wako Silica Gel 70FM, ethyl acetate) 0.79; IR (KBr disc) 1720 (urethane C=O) and 1660 (amide C=O) cm^{-1}; $[\alpha]_D^{25}$ −140° (c 0.1, CH_3OH); ^1H NMR (500 MHz, $CDCl_3$, 303 K) δ_H 0.37 [d, *J* 6.5 Hz, 12H, $CH(CH_3)_2$ (nonequivalent)], 0.75 [d, *J* 6.5 Hz, 12H, $CH(CH_3)_2$ (nonequivalent)], 1.18 [m, 4H, $COCHCH_2$ (nonequivalent)], 1.38 [m, 4H, $COCHCH_2$ (nonequivalent)], 1.40 [m, 4H, $CH(CH_3)_2$], 1.44 [s, 36H, $C(CH_3)_3$], 1.49 [m, 4H, NCH_2CH_2 (nonequivalent)], 1.55 [m, 4H, NCH_2CH_2 (nonequivalent)], 3.33 [m, 4H, NCH_2CH_2 (nonequivalent)], 3.93 [m, 4H, NCH_2CH_2 (nonequivalent)], 4.01 (s, 4H, $ArCH_2Ar$), 4.22 (m, 4H, COCH), 5.74 (m, 4H, CONH), 7.21 [d, *J* 8.0 Hz, 8H, ArH(ortho)], 7.29 [d, *J* 8.0 Hz, 8H, ArH(meta)]. Anal. Calcd for $C_{78}H_{116}N_8O_{12}$•1/2 H_2O: C, 68.54; H, 8.63; N, 8.20%. Found: C, 68.50; H, 8.53; N, 8.12%.

N,N',N'',N'''-Tetrakis(D-leucyl)-1,6,20,25-tetraaza[6.1.6.1]paracyclophane [(−)-10]

This compound was prepared by removal of the protecting groups in (−)-**9** (140 mg, 0.11 mmol) with trifluoroacetic acid (10 mL) in a manner similar to that applied to the synthesis of (+)-**10**. 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 (130 mg, 86 %): mp 184–185 °C; R_f (Wako Silica Gel 70FM, methanol) 0.63; IR (KBr disc) 1675 (amide C=O) cm^{-1}; $[\alpha]_D^{25}$ −94° (c 0.1, CH_3OH); ^1H NMR (500 MHz, CD_3OD, 303 K) δ_H 0.38 [d, *J* 6.5 Hz, 12H, $CH(CH_3)_2$

(nonequivalent)], 0.74 [d, *J* 6.5 Hz, 12H, $CH(CH_3)_2$ (nonequivalent)], 1.3–1.4 [m, 8H, $CH_2CH(CH_3)_2$], 1.43 [m, 4H, $CH(CH_3)_2$], 1.4–1.6 (m, 8H, NCH_2CH_2), 3.30 [m, 4H, NCH_2CH_2 (nonequivalent)], 3.85 [m, 4H, NCH_2CH_2 (nonequivalent)], 4.00 (m, 4H, COCH), 4.04 (s, 4H, $ArCH_2Ar$), 7.21 [d, *J* 8.5 Hz, 8H, ArH(ortho)], 7.33 [d, *J* 8.5 Hz, 8H, ArH(meta)]. Anal. Calcd for $C_{66}H_{88}F_{12}N_8O_{12}\cdot H_2O$: C, 55.38; H, 6.34; N, 7.82%. Found: C, 55.22; H, 6.38; N, 7.87%.

Cage-type Cyclophane with D-Leucine Residues [(−)-1]

This compound was prepared by condensation of (−)-**10** (120 mg, 0.09 mmol) with **11** (93 mg, 0.09 mmol) under high dilution conditions at 0 °C in a manner similar to that applied to the synthesis of (+)-**1**. The crude product 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 (60 mg, 37 %): mp 321–323 °C; R_f (Wako Silica Gel 70FM, methanol) 0.58; IR (KBr disc) 1640 (amide C=O) cm^{-1}; $[\alpha]_D^{25}$ −113° (c 0.1, CH_3OH); ^1H NMR (500 MHz, CD_3SOCD_3, 383 K) δ_H 0.4 [m, 12H, $CH(CH_3)_2$ (nonequivalent)], 0.7 [bs, 12H, $CH(CH_3)_2$ (nonequivalent)], 1.4 [m, 4H, $CH(CH_3)_2$], 1.3–1.4 [m, 8H, $CH_2CH(CH_3)_2$], 1.4–1.8 (m, 8H, NCH_2CH_2), 3.3-3.8 (m, 8H, NCH_2CH_2), 4.0 (m, 4H, COCH), 4.0 (s, 4H, $ArCH_2Ar$), 4.5 (m, 16H, $ArCH_2N$), 6.9–7.4 (m, 32H, ArH), 8.2 (m, 4H, Py-H4), 8.8 (m, 4H, Py-H2), 9.1 (m, 4H, Py-H6). Anal. Calcd for $C_{118}H_{124}N_{16}O_{12}\cdot 6H_2O$: C, 68.59; H, 6.63; N, 10.84%. Found: C, 68.43; H, 6.41; N, 11.13%.

Cationic Cage-type Cyclophane with D-Leucine Residues [(−)-4]

This compound was prepared by quaternization of (−)-**1** (8.0 mg, 4.1 x 10^{-6} mol) with methyl iodide (0.057 g, 0.4 x 10^{-3} mol) followed by ion exchange in a manner similar to that applied to the synthesis of (+)-**4**. The crude product was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol as eluant to give a pale yellow solid (8.0 mg, 90%): mp 254–256 °C (dec.); R_f (Wako Silica Gel 70FM, methanol) 0.64; IR (KBr disc) 1640 (amide C=O) cm^{-1}; ^1H NMR (500 MHz, CD_3SOCD_3, 383 K) δ_H 0.4 [m, 12H, $CH(CH_3)_2$ (nonequivalent)], 0.8 [bs, 12H, $CH(CH_3)_2$ (nonequivalent)], 1.4 [m, 4H, $CH(CH_3)_2$], 1.3–1.5 [m, 8H, $CH_2CH(CH_3)_2$], 1.4–1.8 (m, 8H, NCH_2CH_2), 3.2–3.8 (m, 8H, NCH_2CH_2), 4.0 (m, 4H, COCH), 4.0 (s, 4H, $ArCH_2Ar$), 4.5 (m, 12H, NCH_3), 4.5 (m, 16H, $ArCH_2N$), 6.8–7.3 (m, 32H, ArH), 9.2 (m, 4H, Py-H4), 9.2 (m, 4H, Py-H2), and 9.5 (m, 4H, Py-H6). Anal. Calcd for $C_{122}H_{136}N_{16}O_{12}Cl_4\cdot H_2O$: C, 67.27; H,6.39; N, 10.28%. Found: C, 67.17; H, 6.62; N, 10.34%.

Cationic Cage-type Cyclophane with L-Alanine Residues [(+)-6]

This compound was prepared by quaternization of cage-type cyclophane with L-alanine residues (+)-**3** (20 mg, 1.1 x 10^{-5} mol) with methyl iodide (0.140 g, 1.0 x 10^{-3} mol) followed by ion exchange in a manner similar to that applied to the synthesis of (+)-**4**. The crude product was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol as eluant to give a pale yellow solid (16 mg, 73 %): mp 256–257 °C (dec.); R_f (Wako Silica Gel 70FM, methanol) 0.72; IR (KBr disc) 1640 (amide C=O) cm^{-1}; NMR (500 MHz, CD_3SOCD_3, 383 K) δ_H 1.2 (m, 12H, $CHCH_3$), 1.2–1.7 (m, 8H, NCH_2CH_2), 3.3–3.8 (m, 8H, NCH_2CH_2), 4.0 (s, 4H, $ArCH_2Ar$), 4.1 (m, 4H, COCH), 4.5 (m, 12H, NCH_3), 4.5 (m, 16H, $ArCH_2N$), 6.8–7.2 (m, 32H, ArH), 9.2 (m, 4H, Py-H4), 9.3 (m, 4H, Py-H2), and 9.5 (m, 4H, Py-H6).

Anal. Calcd for $C_{110}H_{112}N_{16}O_{12}Cl_4 \cdot H_2O$: C, 65.73; H,5.72; N, 11.14%. Found: C, 65.76; H, 5.89; N, 11.13%.

Cationic Cage-type Cyclophane with D-Alanine Residues [(−)-6]

This compound was prepared by quaternization of cage-type cyclophane with D-alanine residues **(−)-3** (20 mg, 1.1 x 10^{-5} mol) with methyl iodide (0.140 g, 1.0 x 10^{-3} mol) followed by ion exchange in a manner similar to that applied to the synthesis of **(+)-4**. The crude product was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol as eluant to give a pale yellow solid (16 mg, 73 %): mp 256–257 °C (dec.); R_f (Wako Silica Gel 70FM, methanol) 0.72; IR (KBr disc) 1640 (amide C=O) cm^{-1}; NMR (500 MHz, CD_3SOCD_3, 383 K) δ_H 1.2 (m, 12H, $CHCH_3$), 1.2–1.7 (m, 8H, NCH_2CH_2), 3.3–3.8 (m, 8H, NCH_2CH_2), 4.0 (s, 4H, $ArCH_2Ar$), 4.1 (m, 4H, COCH), 4.5 (m, 12H, NCH_3), 4.5 (m, 16H, $ArCH_2N$), 6.8–7.2 (m, 32H, ArH), 9.2 (m, 4H, Py-H4), 9.3 (m, 4H, Py-H2), and 9.5 (m, 4H, Py-H6). Anal. Calcd for $C_{110}H_{112}N_{16}O_{12}Cl_4 \cdot 1.5H_2O$: C, 65.44; H,5.74; N, 11.09%. Found: C, 65.31; H, 5.48; N, 11.02%.

ACKNOWLEDGMENT

The present work was supported by a Special Distinguished Grant for Scientific Research No. 02102006 from the Ministry of Education, Science and Culture of Japan.

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(*Received in Japan* 1 *May* 1995; *accepted* 6 *June* 1995)