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Size- and shape-sensitive molecular discrimination by cage-type cyclophanes in aqueous media

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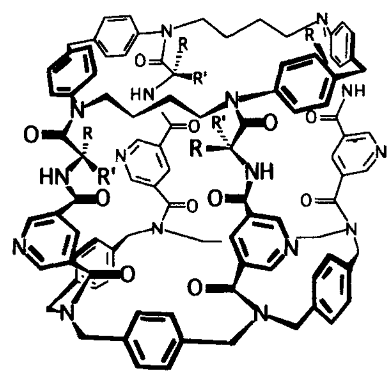
Cage-type cyclophanes, which are constructed with two rigid macrocyclic skeletons, tetraaza[3.3.3.3]paracyclophane and tetraaza[6.1.6.1]paracyclophane, and four chiral bridging components, strongly bind an anionic fluorescent guest, 8-anilinonaphthalene-1-sulfonate, and give out an efficient motional repression effect on the incorporated guest. The cage-type hosts bearing chiral valine residues furnish more apolar internal cavities for the guest molecule than those bearing chiral alanine residues. On the other hand, binding ability and motional repression by peptide cyclophanes, which were prepared by introducing four amino acid residues into a rigid tetraaza[6.1.6.1]paracyclophane skeleton, are less efficient than the cage-type hosts toward the identical guest. Furthermore, microenvironmental polarity of the internal cavities of peptide cyclophanes is much more polar than that of the cage-type hosts. The cage-type hosts perform size- and shape-sensitive molecular discrimination toward nonionic guests of various bulkiness, such as perylene, pyrene, anthracene, and naphthalene, in aqueous media. In addition, binding constants for the cage-type hosts with 4-(1-pyrene)butanoic acid, are greater than those for the identical hosts with pyrene, due to an intermolecular hydrogen-bonding interaction between the butyric acid moiety of the former guest and the bridging segments of the hosts.

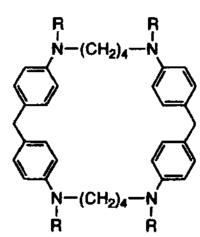


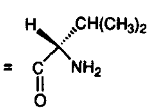
(+)-1 $\begin{cases} R = CH(CH_3)_2 \\ R' = H \end{cases}$

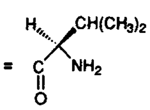
(−)-1 $\begin{cases} R = H \\ R' = CH(CH_3)_2 \end{cases}$

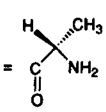
(+)-2 $\begin{cases} R = CH_3 \\ R' = H \end{cases}$

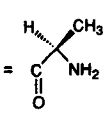
(−)-2 $\begin{cases} R = H \\ R' = CH_3 \end{cases}$



(+)-3 : R = 

(−)-3 : R = 

(+)-4 : R = 

(−)-4 : R = 

In recent years, attention has been focussed on the development of artificial hosts capable of performing functional simulation of naturally occurring supramolecular hosts, such as enzymes and receptors.[1] Guest-binding sites of these natural hosts are elegantly constructed with various optically active amino acid residues and well shielded from bulk aqueous phase to become largely hydrophobic. In addition, these supramolecules generally recognize substrates and other external substances through lock-and-key-type mechanisms. On these grounds, we have developed cage-type cyclophanes which provide three-dimensionally extended internal cavities in aqueous media.[2] Cage-type cyclo-

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phanes (+)-**1** and (−)-**1**, bearing L- and D-valine residues, respectively, 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.[2] We have demonstrated that three-dimensionally extended hydrophobic cavities of the cage-type hosts furnish conformationally forced microenvironments for chiral recognition toward hydrophobic guests, such as (4Z,15Z)-bilirubin IXα and steroid hormones in aqueous media.[3] In this context, we now prepared novel cage-type cyclophanes (+)-**2** and (−)-**2**, bearing L- and D-alanine residues in their bridging segments, respectively, in order to get further insights into the correlation between molecular recognition ability of the hosts and structural and hydrophobic properties of the host cavities. Peptide cyclophanes (+)-**3**,[4] (−)-**3**,[4] (+)-**4**, and (−)-**4**, structural fragments of the cage-type hosts, were also prepared by introducing four L- and D-amino acid residues into a rigid tetraaza[6.1.6.1]paracyclophane skeleton as reference substances for characterization of the specific molecular recognition feature of the cage molecules. We report here on the microenvironmental properties of internal cavities furnished by the cage-type and peptide cyclophanes as demonstrated toward a well-known fluorescent guest, 8-anilinonaphthalene-1-sulfonate (ANS). Furthermore, size- and shape-sensitive molecular discrimination of these hosts were examined in aqueous media by utilizing nonionic fluorescent guests such as perylene, pyrene, anthracene, and naphthalene.

RESULTS AND DISCUSSION

Microenvironmental properties of cyclophane cavities

A fluorescent probe, ANS, whose emission is extremely sensitive to microenvironmental polarity of a surrounding medium in both intensity and wavelength, was adopted as a hydrophobic guest molecule.[5] The guest-binding behavior of the cage-type cyclophanes [(+)-**2** and (−)-**2**] and the peptide cyclophanes [(+)-**4** and (−)-**4**] toward ANS was examined by fluorescence spectroscopy in aqueous acetate buffer [0.01 M, pH 4.1, μ 0.10 (KCl)] at 30.0 °C for evaluation of microenvironmental properties of these host cavities. Upon addition of each host to the acetate buffer containing ANS (1.0×10^{-6} M), a fluorescence intensity originating from ANS increased along with a concomitant blue shift of the fluorescence maximum, reflecting formation of the corresponding host–guest complex. The binding constants (K) of (+)-**2**, (−)-**2**, (+)-**4** and (−)-**4** toward ANS were evaluated on the basis of Benesi-Hildebrand relationship[6] for 1:1 host– guest interactions in a manner as described previously,[7] and are summarized in Table 1 together with the corresponding values for hosts (+)-**1**, (−)-**1**, (+)-**3** and (−)-**3**. The K values for the cage-type hosts with ANS are apparently much greater than the corresponding values for the peptide cyclophanes. Moreover, the guest molecule is strongly incorporated into the cage-type hosts bearing chiral valine residues [(+)-**1** and (−)-**1**] in preference to the cage-type hosts bearing chiral alanine residues [(+)-**2** and (−)-**2**] in aqueous media. The guest-binding affinity is apparently subjected to change by the hydrophobic character of the host cavities and follows the sequence: **1** > **2** > **3** > **4**. The microenvironmental polarity experienced by the incorporated guest molecule was evaluated from the fluorescence maximum in a manner similar to that reported previously[7] (Table 1). The E_T^N values[8] for ANS placed in the cage-type hosts are smaller than those for the identical guest incorporated into the peptide cyclophanes. Consequently, the three-dimensional cavities provided by the cage-type hosts are significantly apolar and well shielded from the bulk aqueous phase. In addition, cage-type host (+)-**1** provides a more apolar microenvironments for ANS than cage-type host (+)-**2** bearing L-alanine residues, because the binding site of the former host involves the hydrophobic L-valine residues. Furthermore, relatively large fluorescence polarization values (P) were obtained for ANS incorporated into the cage-type cyclophanes in comparison with those for the identical guest bound to the peptide cyclophanes (Table 1). This also indicates that a highly desolvated microenvironment is apparently pro-

Table 1 Binding constants (K) for host–guest complexes of cyclophanes with ANS, microenvironmental polarity parameters (E_T^N), and steady-state fluorescence polarization (P) for guest incorporated into cyclophanes in aqueous acetate buffer (0.01 M, pH 4.1, μ 0.10 with KCl) at 30.0°C[a]

Host	K, M^{-1}	E_T^N	(λ_{ex}, nm; λ_{em}, nm)[b]	P	*Ref.*
(+)-**1**	2.8×10^4	0.23	(375, 462)	0.40	4a
(−)-**1**	2.8×10^4	0.23	(375, 462)	0.40	
(+)-**2**	1.5×10^4	0.44	(375, 464)	0.39	
(−)-**2**	1.3×10^4	0.44	(375, 464)	0.39	
(+)-**3**	1.6×10^3	0.73	(375, 479)	0.10	4a
(−)-**3**	1.8×10^3	0.73	(375, 479)	0.10	
(+)-**4**	2.6×10^2	0.75	(375, 482)	0.10	
(−)-**4**	2.6×10^2	0.75	(375, 482)	0.10	

[a]Concentrations: ANS, 1.0×10^{-6} M; (+)−**1**, (−)−**1**, (+)−**2** and (−)−**2**, 1.0×10^{-5} −3.0×10^{-5} M; (+)−**3**, (−)−**3**, (+)−**4** and (−)−**4**, 5.0×10^{-5} −3.0×10^{-4} M. [b] Excitation and emission maxima are given in parentheses, in this sequence.

Table 2 Binding constants (K) for host–guest complexes of cyclophanes with various hydrophobic guests in aqueous acetate buffer (0.01 M, pH 4.0, μ 0.10 with KCl) at 30.0°C[a]

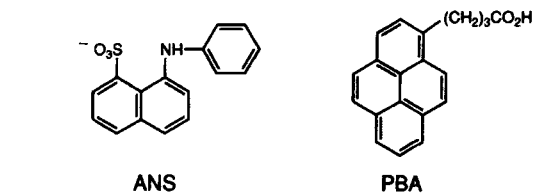
	K, M^{-1}			
Host	*Naphthalene*	*Anthracene*	*Pyrene*	*Perylene*
(−)-**1**	5.8×10^3	2.3×10^4	1.3×10^5	1.0×10^4
(−)-**2**	5.4×10^3	1.3×10^4	9.9×10^4	1.1×10^4
(−)-**3**		1.4×10^4	1.8×10^4	1.1×10^4

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

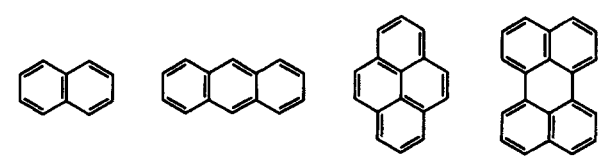
vided by the cage-type hosts so that the tight host–guest interaction, which brings about the marked motional repression of the entrapped guest, becomes effective. Similar microenvironmental properties of the internal cavities furnished by cage-hosts (−)-**1** and (−)-**2** bearing D-amino acid residues were also clarified by the identical methods (Table 1).

Size- and shape-sensitive molecular discrimination

Cage-type cyclophanes (−)-**1** and (−)-**2** have a globular hydrophobic cavity surrounded by two rigid macrocyclic skeletons and four chiral bridging components. In view of investigation of their CPK molecular models, the conformational flexibility of the cage-type hosts seems to be reduced to attain a restricted and rigid geometry of the hydrophobic cavity. On this ground, the cage-type hosts can be expected to perform size- and shape-sensitive molecular discrimination toward hydrophobic guests. The guest-binding behavior of cage-type hosts (−)-**1** and (−)-**2** and non-cage host (−)-**3** toward various fluorescent guests such as perylene, pyrene, anthracene, and naphthalene was examined by fluorescence spectroscopy in aqueous acetate buffer (0.01 M, pH 4.0, μ 0.10 with KCl) at 30.0 °C. A fluorescence intensity originated from each of the guest molecules (1.0×10^{-6} M) was subjected to change upon addition of the hosts, showing simple saturation behavior. The corresponding Benesi-Hildebrand plots based on 1:1 host–guest complexation were found to be linear (r = 0.999–0.990). The binding constants for formation of 1:1 host–guest complexes of the cage-type and peptide cyclophanes with the hydrophobic guests in aqueous media are listed in Table 2. As is obvious from the data in Table 2, pyrene[9] with a molecular volume of about 251 Å^3 fits the cavities of cage-type hosts (−)-**1** and (−)-**2** most tightly among the neutral arenes, even though perylene is more hydrophobic than pyrene. On the other hand, the K values for peptide cyclophane (−)-**3** with the guests are not particularly sensitive to size and shape of the guest molecules and lie in a range of $1 \times 10^4 - 2 \times 10^4$ M^{-1}. Thus, the present cage-type cyclophanes having a three-dimensionally restricted internal cavity were found to discriminate guest molecules ingeniously through the lock-and-key mechanism.



ANS PBA



naphthalene anthracene pyrene perylene

4-(1-Pyrene)butanoic acid (PBA),[10] a pyrene core complemented with a butyric acid moiety as a hydrogen-bonding group, is strongly bound to cage-type host (−)-**2** in the aqueous acetate buffer with a binding constant of 2.0×10^5 M^{-1}. As the butyric acid moiety is removed, the resulting guest (pyrene) shows less binding affinity for the host (Table 2). The result suggests that an intermolecular hydrogen-bonding interaction between the butyric acid moiety of the guest molecule and the bridging segments of (−)-**2** takes advantage over the complex formation. A similar stability enhancement in complex formation of (−)-**1** with PBA was also evidenced by the identical method; K, 6.8×10^5 M^{-1}.

In conclusion, each of the present cage-type hosts furnishes a hydrophobic internal cavity that exhibits size- and shape-sensitive molecular discrimination toward hydrophobic guests through the lock-and-key mechanism. We believe that our concept on molecular design provides a useful guidepost for preparation of artificial receptors that are capable of performing molecular discrimination through the lock-and-key mechanism in aqueous media.

EXPERIMENTAL

General analyses and measurements

Melting points were measured with a 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

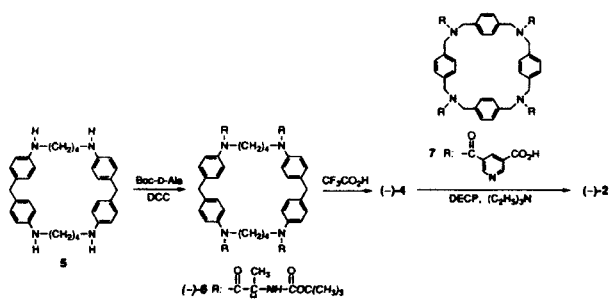
a Bruker AC-250P and a Bruker AMX-500 spectrometer installed at the Center of Advanced Instrumental Analysis, Kyushu University. Optical rotations were measured on a Horiba SEPA polarimeter. A Hitachi M-2500 double-focussing mass spectrometer was used for electrospray ionization (ESI) and a Hitachi M-0301 data acquisition system was used for obtaining ESI-MS data.

Materials

The following compounds were obtained from commercial sources as guaranteed reagents and used without further purification: naphthalene (from Dojin Chemical Laboratories, Kumamoto, Japan), anthracene (from Nacalai Tesque, Inc., Kyoto, Japan), perylene and sodium 8-anilinonaphthalene-1-sulfonate [Na(ANS)] (both from Tokyo Kasei Kogyo Co., Tokyo, Japan), and 4-(1-pyrene)butanoic acid (PBA) (from Molecular Probes, Inc., Oregon, USA). N^{α}-*tert*-Butoxycarbonyl-D-alanine and N^{α}-*tert*-butoxycarbonyl-L-alanine were purchased from Peptide Institute, Inc., Osaka, Japan, as a guaranteed reagent. Pyrene (Nacalai Tesque) was purified by means of liquid chromatography on a column of silica gel (Wakogel C-100) with cyclohexane as eluant; mp 149–151 °C. 1,6,20,25-Tetraaza[6.1.6.1]paracyclophane (**5**)[11] and *N*, *N'*, *N''*, *N'''*-tetrakis(5-carboxynicotinoyl)-2,11,20,29-tetraaza[3.3.3.3]paracyclophane (**7**)[2a] were prepared after methods reported previously. Preparation of cage-type cyclophanes (+)-**1** and (−)-**1** and peptide cyclophanes (+)-**3** and (−)-**3** has been described previously.[4a] Cyclophanes (−)-**2** and (−)-**4** were synthesized by following the reaction sequence shown in Scheme 1. The use of *tert*-butoxycarbonyl-L-alanine in place of *tert*-butoxycarbonyl-D-alanine afforded the corresponding cyclophanes bearing L-alanine residues, (+)-**2** and (+)-**4.**

N, N', N'', N'''-Tetrakis(N^{α}-*tert*-butoxycarbonyl-D-alanyl)-1,6,20,25-triaza[6.1.6.1]paracyclophane [(−)-6]

Dicyclohexylcarbodiimide (3.7 g, 18 mmol) was added to a dry dichloromethane (17 mL) solution of *tert*-butoxycarbonyl-D-alanine (3.0 g, 16 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 (**5**; 1.0 g, 2.0 mmol) dissolved in dry dichloromethane (40 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). After 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 liquid chromatography on a column of silica gel (Wakogel C-300) with ethyl acetate as eluant. Evaporation of the product fraction under reduced pressure gave a white solid (1.6 g, 71 %): mp 151–153 °C; R_f(Wako silica gel 70FM, ethyl acetate) 0.49; IR (KBr disc) 1710 (urethane C=O), 1660 (amide C=O) cm^{-1}; $[\alpha]^{25}_D$ −137° (c = 0.1, CH_3OH); ^1H NMR (500 MHz, $CDCl_3$, 303 K) δ 1.06 (d, *J* = 6.9 Hz, 12H, $CHCH_3$), 1.41 [s, 36H, $C(CH_3)_3$], 1.44 [m, 4H, NCH_2CH_2 (nonequivalent)], 1.53 [m, 4H, NCH_2CH_2 (nonequivalent)], 3.33 [m, 4H, NCH_2CH_2 (nonequivalent)], 3.92 [m, 4H, NCH_2CH_2 (nonequivalent)], 4.00 (s, 4H, $ArCH_2Ar$), 4.20 (m, 4H, COCH), 5.30 (d, *J* = 8.4 Hz, 4H, CONH), 7.10 [d, *J* = 7.9 Hz, 8H, ArH(ortho)], 7.22 [d, *J* = 7.9 Hz, 8H, ArH(meta)]. Anal. Calcd for $C_{66}H_{92}N_8O_{12}$: C, 66.64; H, 7.80; N, 9.42. Found: C, 66.29; H, 7.77; N, 9.33.



Scheme 1

N, N', N'', N'''-Tetrakis(D-alanyl)-1,6,20,25-tetraaza[6.1.6.1]paracyclophane [(−)-4]

Trifluoroacetic acid (10 mL) was added to a dry dichloromethane (50 mL) solution of (−)-**6** (1.0 g, 0.84 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 (630 mg, 60 %): mp 205–209 °C; R_f (Wako silica gel 70FM, methanol) 0.70; IR (KBr disc) 1660 (amide C=O) cm^{-1}; $[\alpha]^{25}_D$ −100° (c = 0.1, CH_3OH); ^1H NMR (500 MHz, CD_3OD, 303 K) δ 1.19 (d, *J* = 6.9 Hz, 12H, $CHCH_3$), 1.43 [m, 4H, NCH_2CH_2 (nonequivalent)], 1.54 [m, 4H, NCH_2CH_2 (nonequivalent)], 3.47 [m, 4H, NCH_2CH_2 (nonequivalent)], 3.82 (m 4H, COCH), 3.86 [m, 4H, NCH_2CH_2 (nonequivalent)], 4.05 (s, 4H, $ArCH_2Ar$), 7.20 [d, *J* = 7.9 Hz, 8H, ArH(ortho)], 7.39 [d, *J* = 7.9 Hz, 8H, ArH(meta)]. Anal. Calcd for $C_{54}H_{64}F_{12}N_8O_{12}$: C, 52.09; H, 5.18; N, 9.00. Found: C, 52.31; H, 5.14; N, 8.88. MS (ESI): *m/z* 395 $(M+2H)^{2+}$, 789 $(M+H)^+$; calcd M for $C_{46}H_{60}N_8O_4$, 788.

Cage-type cyclophane with D-alanine residues [(−)-2]

Individual solutions of (−)-**4** (500 mg, 0.40 mmol) and *N*, *N'*, *N''*, *N'''*-tetrakis(5-carboxynicotinoyl)-2,11,20,29-tetraaza[3.3.3.3]paracyclophane (**7**; 424 mg, 0.40 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 (2600 mL) solution containing diethyl cyanophosphonate (DECP; 299 mg, 1.7 mmol) and triethylamine (300 mg, 3.0 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 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 (110 mg, 15 %): mp 312–313 °C; R_f (Wako silica gel 70FM, methanol) 0.69; IR (KBr disc) 1660 (amide C=O) cm^{-1}; $[\alpha]^{25}_D$ −109° (c = 0.1, CH_3OH); ^1H NMR [500 MHz, $(CD_3)_2SO$, 383 K] δ 1.1 (m, 12H, $CHCH_3$), 1.3–1.5 (m, 8H, NCH_2CH_2), 3.3–3.8 (m, 8H, NCH_2CH_2), 3.9 (m, 4H, COCH), 4.0 (s, 4H, $ArCH_2Ar$), 4.5 (m, 16H, $ArNCH_2$), 6.9–7.4 (m, 32H, ArH), 8.3 (m, 4H, Py-H4), 8.9 (m, 3H, Py-H2), 9.1 (m, 3H, Py-H6). Anal. Calcd for $C_{106}H_{100}N_{16}O_{12}\cdot 6H_2O$: C, 67.06; H, 5.94; N, 11.81. Found: C, 66.95; H, 5.57; N, 11.82.

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

This compound was prepared by condensation of *tert*-butoxycarbonyl-L-alanine (3.0 g, 16 mmol) with **5** (1.0 g, 2.0 mmol) in a manner similar to that applied to the synthesis of (−)-**6**. The crude product was purified by liquid chromatography on a column of silica gel (Wakogel C-300) with ethyl acetate as eluant to give a white solid (2.0 g, 84%): mp 151–153 °C; R_f (Wako silica gel 70FM, ethyl acetate) 0.49; IR (KBr disc) 1710 (urethane C=O), 1660 (amide C=O) cm^{-1}; $[\alpha]^{25}_D$ +134° (c = 0.1, CH_3OH); ^1H NMR (500 MHz, $CDCl_3$, 303 K) δ 1.06 (d, *J* = 6.9 Hz, 12H, $CHCH_3$), 1.41 [s, 36H, $C(CH_3)_3$], 1.44 [m, 4H, NCH_2CH_2 (nonequivalent)], 1.53 [m, 4H, NCH_2CH_2 (nonequivalent)], 3.33 [m, 4H, NCH_2CH_2 (nonequivalent)], 3.92 [m, 4H, NCH_2CH_2 (nonequivalent)], 4.00 (s, 4H, $ArCH_2Ar$), 4.20 (m, 4H, COCH), 5.30 (d, *J* = 8.4 Hz, 4H, CONH), 7.10 [d, *J* = 7.9 Hz, 8H, ArH(ortho)], 7.22 [d, *J* = 7.9 Hz, 8H, ArH(meta)]. Anal. Calcd for $C_{66}H_{92}N_8O_{12}\cdot H_2O$: C, 65.65; H, 7.84; N, 9.28. Found: C, 65.95; H, 7.77; N, 9.29.

***N*,*N'*,*N''*,*N'''*-Tetrakis(L-alanyl)-1,6,20,25-tetraaza-[6.1.6.1]paracyclophane [(+)-4]**

This compound was prepared by removal of the protecting groups of (+)-**6** (1.0 g, 0.88 mmol) with trifluoroacetic acid (10 mL) 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 white solid (630 mg, 60 %): mp 206–210 °C; R_f (Wako silica gel 70FM, methanol) 0.69; IR (KBr disc) 1660 (amide C=O) cm^{-1}; $[\alpha]^{25}_D$ +97° (c = 0.1, CH_3OH); ^1H NMR (500 MHz, CD_3OD, 303 K) δ 1.19 (d, *J* = 6.9 Hz, 12H, $CHCH_3$), 1.45 [m, 4H, NCH_2CH_2 (nonequivalent)], 1.53 [m, 4H, NCH_2CH_2 (nonequivalent)], 3.47 [m, 4H, NCH_2CH_2 (nonequivalent)], 3.84 (m, 4H, COCH), 3.86 [m, 4H, NCH_2CH_2 (nonequivalent)], 4.06 (s, 4H, $ArCH_2Ar$), 7.23 [d, *J* = 7.9 Hz, 8H, ArH(ortho)], 7.38 [d, *J* = 7.9 Hz, 8H, ArH(meta)]. Anal. Calcd for $C_{54}H_{64}F_{12}N_8O_{12}$: C, 52.09; H, 5.18; N, 9.00. Found: C, 52.08; H, 5.11; N, 8.89. MS (ESI): *m/z* 395 $(M+2H)^{2+}$, 789 $(M+H)^+$; calcd M for $C_{46}H_{60}N_8O_4$, 788.

Cage-type cyclophane with L-alanine residues [(+)-2]

This compound was prepared by condensation of (+)-**4** (212 mg, 0.17 mmol) with **7** (180 mg, 0.17 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 (40 mg, 13%): mp 312–315 °C; R_f (Wako silica gel 70FM, methanol) 0.69; IR (KBr disc) 1650 (amide C=O) cm^{-1}; $[\alpha]^{25}_D$ +113° (c = 0.1, CH_3OH); ^1H NMR [500 MHz, $(CD_3)_2SO$, 383 K] δ 1.1 (m, 12H, $CHCH_3$), 1.3–1.5 (m, 8H, NCH_2CH_2), 3.3–3.8 (m, 8H, NCH_2CH_2), 4.0 (s, 4H, $ArCH_2Ar$), 3.9 (m, 4H, COCH), 4.5 (m, 16H, $ArNCH_2$), 6.9–7.4 (m, 32H, ArH), 8.3 (m, 4H, Py-H4), 8.9 (m, 3H, Py-H2), 9.1 (m, 3H, Py-H6). Anal. Calcd for $C_{106}H_{100}N_{16}O_{12}\cdot 6H_2O$: C, 67.06; H, 5.94; N, 11.81. Found: C, 66.88; H, 5.58; N, 11.82.

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