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Preparation and Host–Guest Interactions of Novel Cage-type Cyclophanes Bearing Chiral Binding Sites Provided by Dipeptide Residues

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Abstract: Novel cage-type cyclophanes, which are constructed with two rigid macrocyclic skeletons, tetraaza[3.3.3.3]paracyclophanes, and four bridging segments composed of either β-L-aspartyl-L-aspartyl or β-D-aspartyl-D-aspartyl residues were prepared [(–)-**1** and (+)-**1**, respectively]. Structural and asymmetric properties of these cage-type cyclophanes were characterized by ^{1}H NMR and circular dichroism (CD) spectroscopy. The guest-binding behavior of the cage-type hosts toward fluorescent guests, such as 8-anilinonaphthalene-1-sulfonate and 6-*p*-toluidinonaphthalene-2-sulfonate, was examined in comparison with those demonstrated by the corresponding non-cage hosts in aqueous media. The microenvironmental polarity around the fluorescent guests and their fluorescence polarization values in the presence of the cage-type hosts revealed that these guest molecules were incorporated into apolar host cavities, and their rotational motion was largely restricted. Furthermore, chiral host–guest interactions between the hosts and a hydrophobic guest, pamoic acid (PA), were examined by CD spectroscopy. PA was subjected to stereochemical changes to assume *P*- and *M*-helix configurations in the presence of (–)-**1** and (+)-**1**, respectively.

INTRODUCTION

Much attention has been focused on artificial receptors capable of performing enantioselective or diastereoselective molecular recognition to mimic specific molecular functions performed by naturally occurring receptors in aqueous media.[1] In such cases, amino acid residues of a host molecule and/or a guest molecule play a significant role as binding sites reflecting multipoint interactions which are generally observed with many natural receptors or enzymes toward substrates.[2] The molecular design of peptides and amino acid derivatives as synthetic receptors may lead to the development of biosensors, drug carriers, and catalysts. In addition, the recent progress of combinatorial chemistry has made peptides to be more favorable target compounds than ever before.[3] Even though successive researches on synthetic receptors have been reported, there still remains some problems on how to formulate chemical structures that are readily synthesized and furnish a large, three-dimensionally extended cavity with well-organized binding sites. We have previously prepared various cage-type cyclophanes having optical active amino acid residues such as leucine, valine, and alanine in their bridging segments, and examined their chiral recognition behavior toward hydrophobic guests in aqueous media.[4]

As the next stage, we became interested in introducing dipeptide moieties into bridging segments of the cage-type cyclophanes, which are constructed with two rigid macrocyclic skeletons, tetraaza[3.3.3.3]-paracyclophanes, and four bridging segments, and those cyclophanes having β-L-aspartyl-L-aspartyl residues and β-D-aspartyl-D-aspartyl residues, (–)-**1** and (+)-**1**, respectively, as the bridging segments were prepared.[5]

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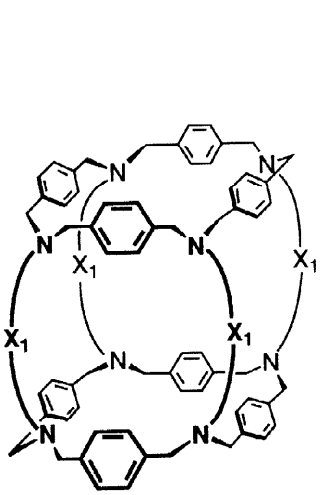
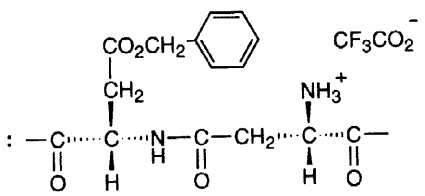
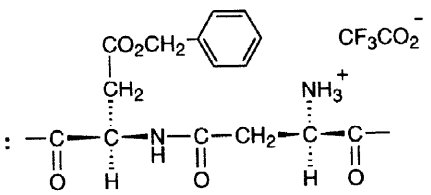
We report here the preparation, structural and asymmetric character, and stereospecific guest recognition behavior of such cage-type hosts toward fluorescent guests, such as 8-anilinonaphthalene-1-sulfonate (ANS) and 6-*p*-toluidinonaphthalene-2-sulfonate (TNS) in comparison with that demonstrated by the corresponding non-cage hosts in aqueous media. Furthermore, chirality based molecular recognition was also investigated with emphasis on the chiral host–guest interactions between the cage-type hosts and 4, 4'-methylenebis(3-hydroxy-2-naphthalene carboxylic acid) [pamoic acid (PA)].



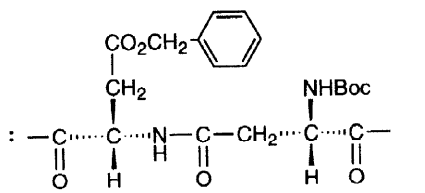
—X_1—



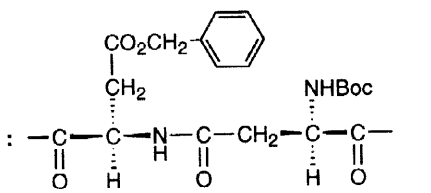
(–)-1



(+)-1



(–)-2



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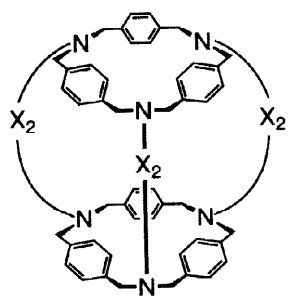

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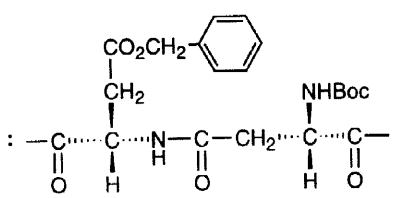
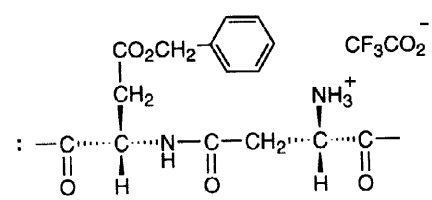
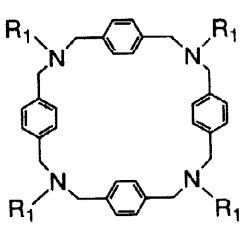
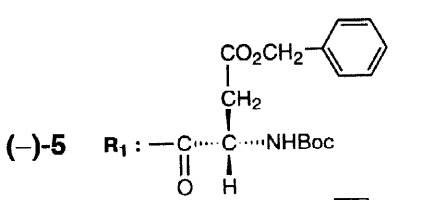


(–)-3

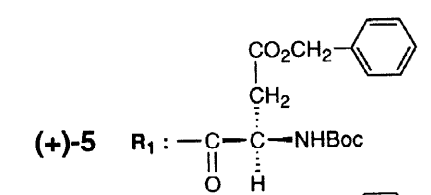


(–)-4

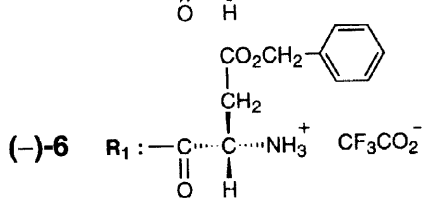




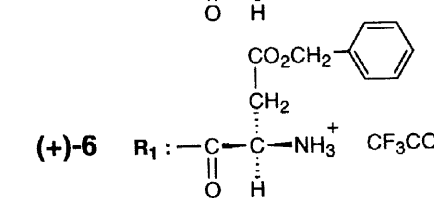
(–)-5



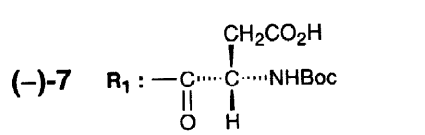
(+)-5



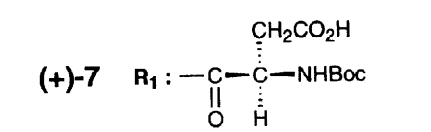
(–)-6



(+)-6



(–)-7



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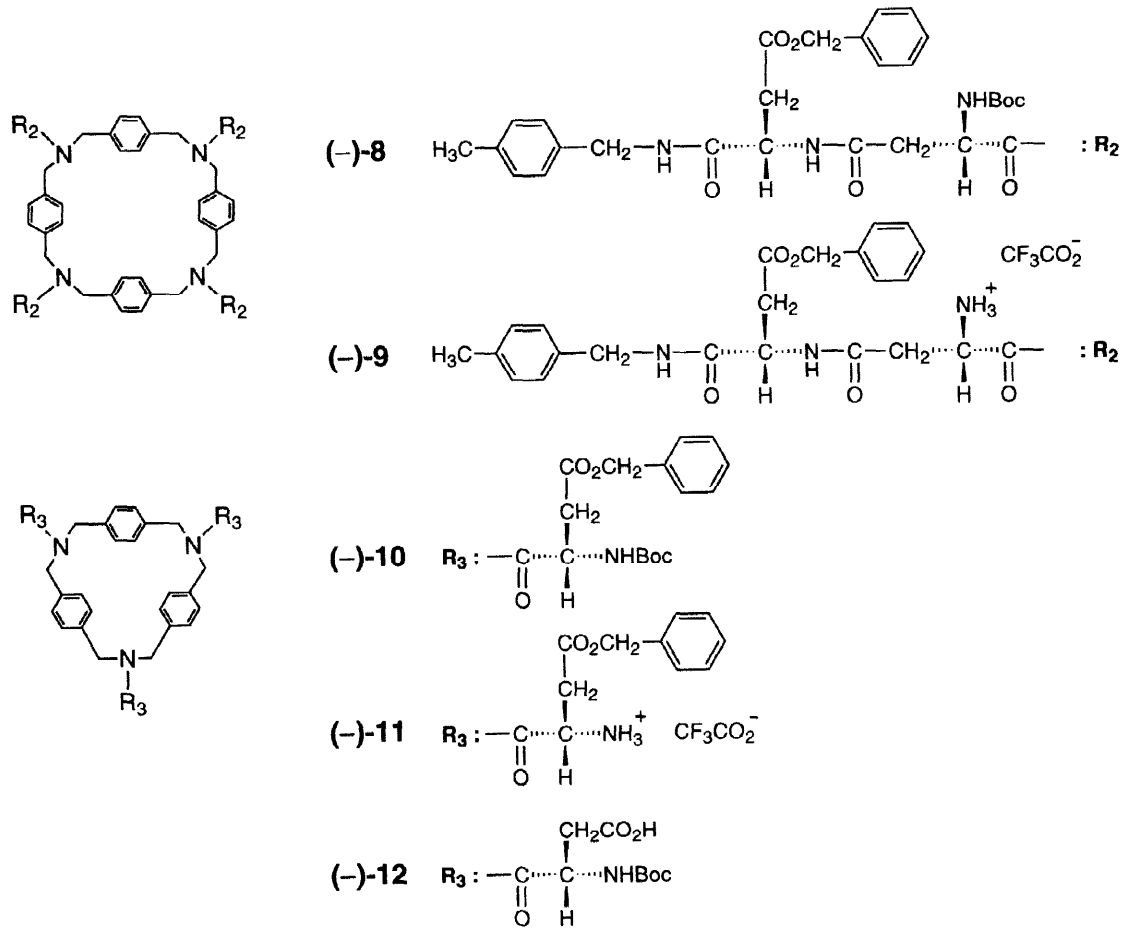
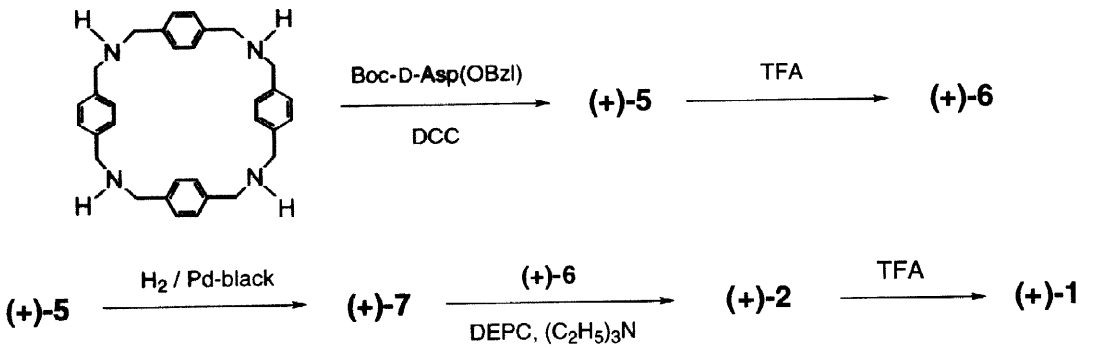


RESULTS AND DISCUSSION

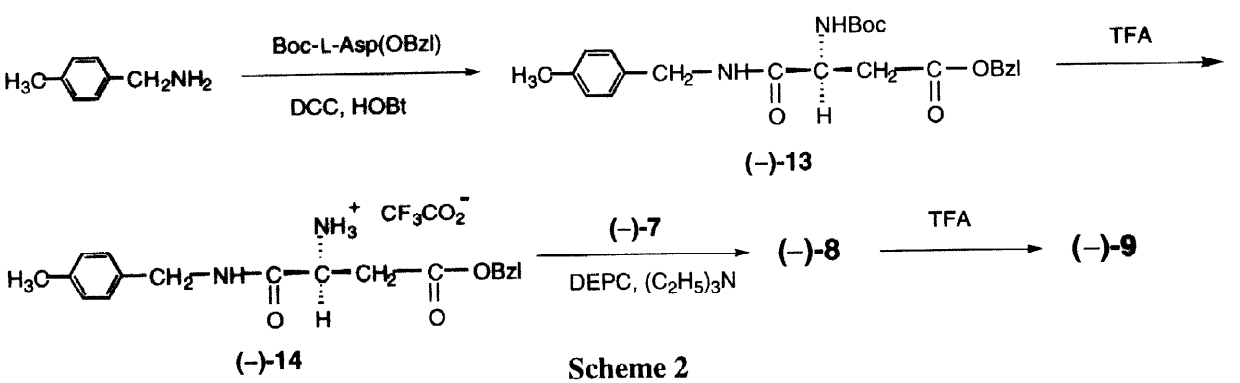
Preparation of Various Cyclophanes Having Dipeptide Segments

In order to introduce dipeptide units into bridging segments of the cage-type molecules, we adopted two major synthetic steps. The first one is the formation of amide bonds between secondary amines of the macrocycle and carboxylic acids of appropriate amino acid residues. Secondly, the resulting cyclophanes are linked by intermolecular condensation of amino groups with carboxyls at four points. Four-bridged cage-type cyclophane, **(+)-1**, was synthesized by a preparative sequence shown in Scheme 1. A non-cage cyclophane, **(+)-5**, having *tert*-butyloxycarbonyl-β-benzyl-D-aspartyl residues was prepared by condensation of 2,11,20,29-tetraaza[3.3.3.3]paracyclophane[6] with β-benzyl N^{α}-(*tert*-butyloxycarbonyl)-D-aspartic acid [Boc-D-Asp-(OBzl)] in the presence of *N,N*-dicyclohexylcarbodiimide (DCC). A non-cage cyclophane, **(+)-6**, having β-benzyl-D-aspartyl residues was prepared from **(+)-5** by removal of the α-amino-protecting Boc-groups with trifluoroacetic acid (TFA). The β-carboxy-protecting OBzl-groups of **(+)-5** were also selectively removed by hydrogenolysis with palladium black to afford a protection-free non-cage cyclophane, **(+)-7**. Cage-type cyclophane **(+)-2** was obtained by condensation of **(+)-6** with **(+)-7** in the presence of diethyl cyanophosphonate (DECP) and triethylamine under high dilution conditions in dry *N,N*-dimethylformamide at 0 °C. Water-soluble cage-type cyclophane **(+)-1** was prepared from **(+)-2** by removal of the α-amino-protecting Boc-groups with TFA. The same synthetic strategy was applied to the preparation of **(–)-1** from **(–)-6** and **(–)-7**.[4] A non cage-type cyclophane, **(–)-9**, was synthesized as a reference compound, where one

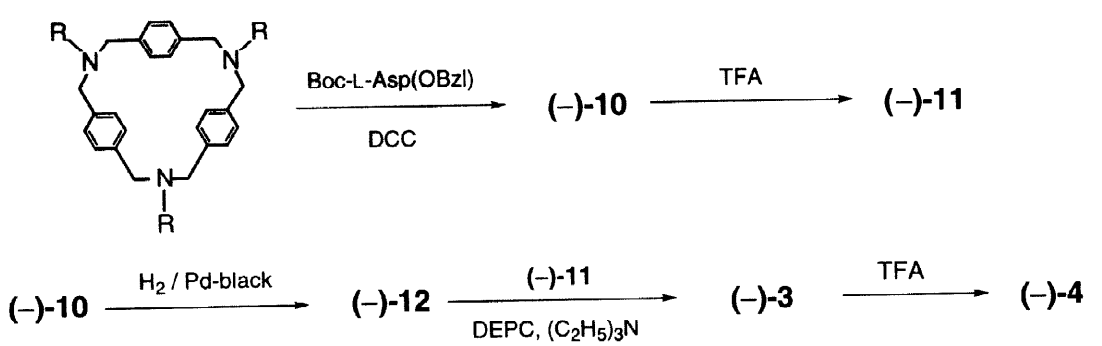
of the macrocycles was removed from the cage-type cyclophane, as shown in Scheme 2. In addition, a cage-type cyclophane, (−)-**4**, which is constructed with two triaza[3.3.3]paracyclophanes[7] and three dipeptide bridging segments composed of β-L-aspartyl-L-aspartyl residues, was prepared as shown in Scheme 3. Both dimension and hydrophobic property of the internal cavity of (−)-**4** is obviously different from those of (−)-**1**. All the novel products were identified by ^{1}H NMR, ESI-MS, and IR measurements as well as by elemental analyses.



Scheme 1



Scheme 2



Scheme 3

Structural Properties of Cage-type Cyclophanes Having Dipeptide Segments

The structural feature of cage-type cyclophane (–)-**1** was characterized by ^{1}H NMR in comparison with the corresponding data for reference compounds. Methylene and phenyl proton signals originated from the macrocyclic skeleton appeared as complicated splitting patterns (at 303K, CD_3OD). As for non-cage cyclophanes, minor difference in chemical shift were detected for methylenes between (–)-**6** and (–)-**9**, and the signals for both species remained in a narrow region (Figs. 1b, 1c; 4.2 – 5.1ppm). On the other hand, methylene proton signals for cage-type cyclophane (–)-**1** were observed over an extremely wide range (Fig. 1a; 2.3 – 5.7ppm) relative to those for the non-cage cyclophanes. The result indicates that the introduction of β-L-aspatyl-L-aspartic residues into the macrocyclic skeleton through amide linkages causes conformational restriction due to intramolecular steric hindrance around the amide bonds. The cage-type cyclophane is rigid enough to restrict rotational and twisting motions of the three dimensional framework, while the non-cage cyclophanes more or less retain rotational freedom. A 2D NMR study with the COSY technique was carried out (at 303K, CD_3OD) to define a homonuclear shift correlation on methylene protons (Fig. 2). Each cross peak represents the existence of geminally coupled protons, suggesting formation of conformational isomers in the NMR time scale. The temperature-dependent ^{1}H NMR spectroscopy furnished structural information on the molecular rigidity of cage-type cyclophane (–)-**1**. In $(CD_3)_2SO$ at 293K, both phenyl and methylene proton signals for non-cage hosts (–)-**6** and (–)-**9** showed complicated splitting patterns (Figs. 3a and 3b, respectively). These complicated proton signals gradually turned to sharper ones as the temperature increased from 293 to 373 K, and a coalescence temperature (*Tc*) was estimated to be ca.353 K for both (–)-**6** and (–)-**9**. As for (–)-**1**, however, these proton signals still appeared in a wide range and are rather complicated even at 373 K (Fig. 3c). These results also indicate that the macrocyclic framework of cage-type cyclophane (–)-**1** is less flexible than the corresponding non-cage cyclophanes (–)-**6** and (–)-**9**. Two couples of doublet signals are expected to appear, since all methylene protons H_a–H_d are nonequivalent as can be seen by Fig. 4a. Similarly, the cis-trans isomerization shown in Fig. 4b gives us another two couples of doublet signals, while protons shown in Figs. 4c and 4d are exactly equivalent to those of the corresponding enantiomers shown in Figs. 4a and 4b, respectively.[8] This cis-trans conversion is expected to be observable on the other side of the macrocycle, thus the total number of coupling pairs being consistent with the COSY spectral data.

Molecular Recognition toward Fluorescent Guests

The binding ability of the cage-type hosts and the non-cage hosts toward fluorescent guests, such as 8-anilinonaphthalene-1-sulfonate (ANS) and 6-*p*-toluidinonaphthalene-2-sulfonate (TNS), was evaluated by fluorescence spectoroscopy in an aqueous acetate buffer (0.01 mol dm^{-3}, pH 4.0, μ 0.10 with KCl) at 30°C. Upon addition of the hosts to the acetate buffer containing each individual guest molecule, a fluorescence intensity originating from the guest molecule increased along with a concomitant blue shift of the fluorescence maximum, reflecting formation of the corresponding host–guest complexes. Binding constants (K) for these hosts were evaluated on the basis of Benesi-Hildebrand relationship[9] for a 1:1 host–guest interaction in a manner as described previously,[4] and are listed in Table 1. The K values for the hosts toward ANS and TNS are subject to change in accordance with size and hydrophobic nature of the internal cavity: (–)-**1** > (–)-**4** > (–)-**6** > (–)-**11**. The microenvironment around the guest molecules in the presence of hosts are apolar even in an aqueous medium in the light of E_T^N values[10] which were determined from fluorescence maxima of the guests.

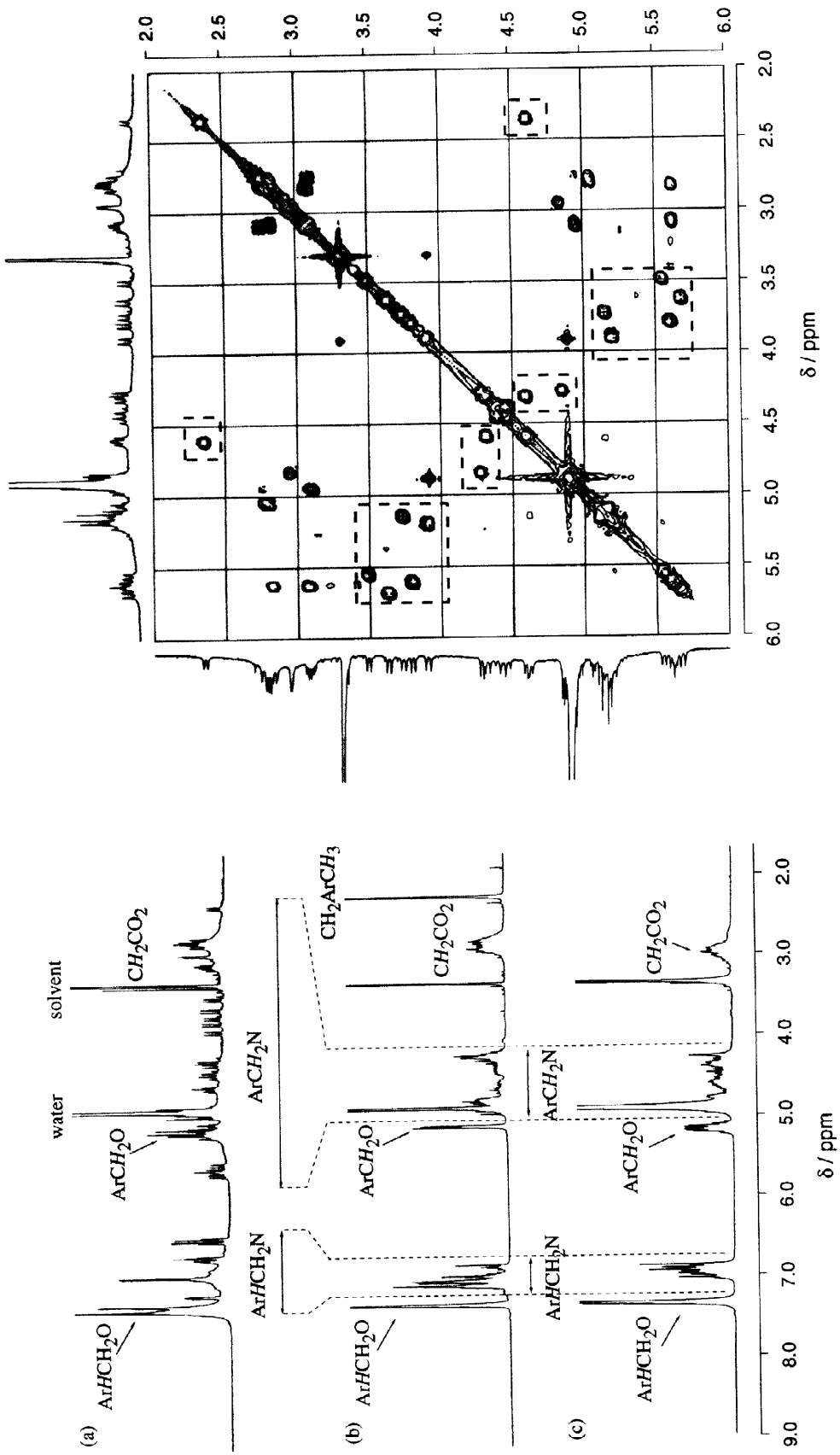


Fig. 1. ^{1}H NMR spectra for various cyclophanes; (a) four bridged cage-type cyclophane (–)-**1**, (b) non cage-cyclophane with dipeptide moiety (–)-**9**, and (c) non-cage cyclophane (–)-**6** in CD_3OD at 303K.

Fig. 2. COSY spectrum of cage-type cyclophane (–)-**1** in CD_3OD at 303K; geminally coupled protons appeared inside the broken square circles.

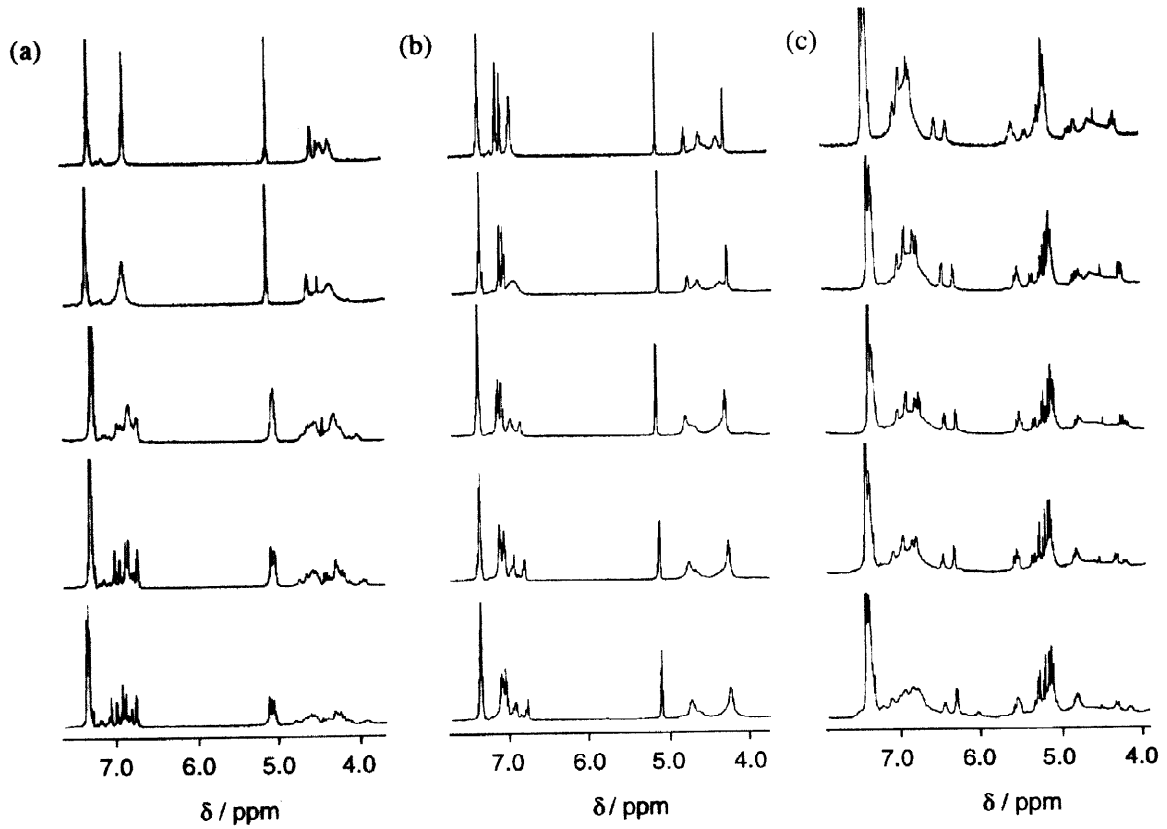


Fig. 3. Temperature dependent ^{1}H NMR spectra of (a) non-cage cyclophane (–)-**6**, (b) non-cage cyclophane (–)-**9** and (c) cage-type cyclophane (–)-**1** at 293, 313, 333, 353, and 373 K (from bottom to top) in $(CD_3)_2SO$.

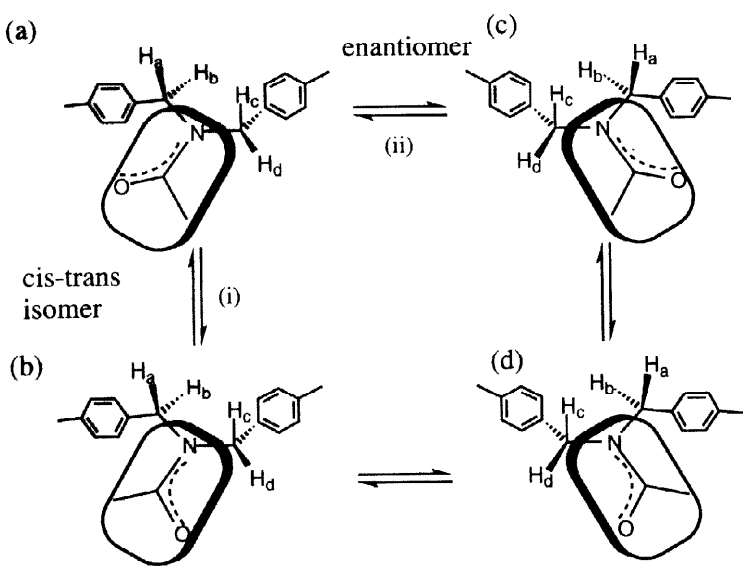


Fig. 4. Four possible stereochemical isomers of the cage-type cyclophane as caused by (i) cis-trans isomerization and (ii) enantiomeric inversion with respect to an amide bond placed on the macrocycle.

In addition, large fluorescence polarization values (P), which reflect high microscopic viscosity in the hydrophobic cavities of the hosts where the guest molecule is incorporated, were obtained in the presence of the hosts, especially in the presence of the cage-type cyclophane. Thus, these guests are effectively incorporated into the host cavities, and the rotational motion of the guests is largely restricted. The stoichiometry for the host–guest complexes was confirmed by the Job's continuous variation method[11] and consistent with 1:1 host–guest complexation (Fig. 5).

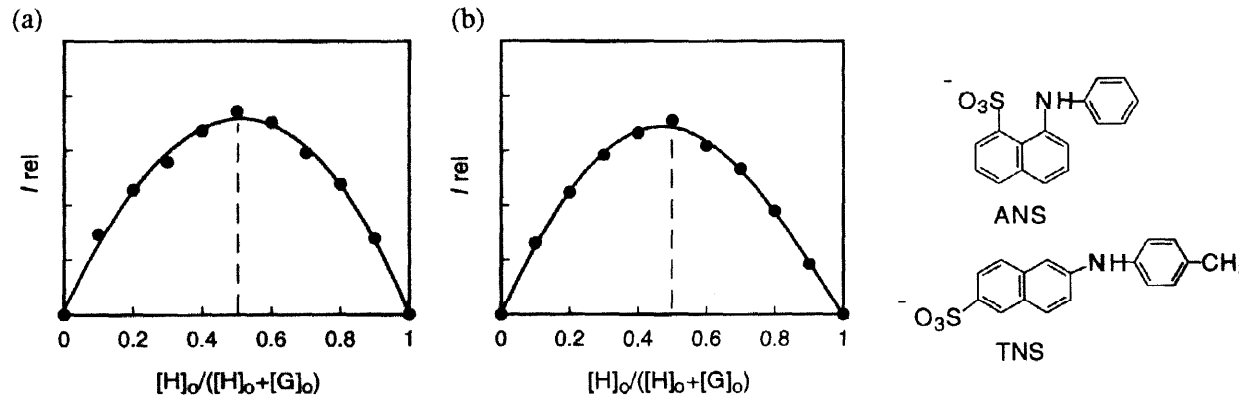


Fig. 5. Job's plots for complex formation of cage-type host (–)-**1** with guests [(a) ANS, (b) TNS] in aqueous acetate buffer (0.01 mol dm^{-3}, pH 4.0, μ 0.10 with KCl) at 30 °C with attention to fluorescence intensity (I); total concentration of (–)-**1** and the guests, $[H]_0+[G]_0$, being maintained at 1.0 x 10^{-5} mol dm^{-3}.

Table 1. Binding constants (K / dm^3 mol^{-1}), microenvironmental polarity parameters (E_T^N) and steady-state fluorescence polarization values (P) for guests incorporated into cage-type hosts (–)-**4** and (–)-**1** and non-cage hosts (–)-**11** and (–)-**6** in aqueous acetate buffer (0.01 mol dm^{-3}, pH 4.0, μ 0.10 with KCl) at 30°C.

Host [a]	Guest	K / dm^3 mol^{-1}	E_T^N	P
(–)-**11**	ANS	1.1 x 10^3	0.73	0.22
	TNS	1.6 x 10^3	0.69	0.19
(–)-**6**	ANS	3.4 x 10^3	0.48	0.26
	TNS	4.4 x 10^3	0.68	0.20
(–)-**4**	ANS	4.1 x 10^3	0.42	0.32
	TNS	1.2 x 10^4	0.66	0.24
(–)-**1**	ANS	2.0 x 10^4	0.36	0.33
	TNS	4.9 x 10^4	0.63	0.25

a) Because of limited solubility of (–)-**9**, a binding constant (K) for (–)-**9** was evaluated in an aqueous medium containing 30% methanol at 30°C. Under these conditions, K values for (–)-**1** toward ANS and TNS were evaluated to be as low as 4.1 x 10^3 and 9.5 x 10^3 dm^3 mol^{-1}, while those for (–)-**9** were 1.5 x 10^3 and 2.0 x 10^3 dm^3 mol^{-1}, respectively.

Chiral Host-Guest Interaction between Cage-Type Cyclophane and Pamoic Acid

The asymmetric character of the present hosts was examined by circular dichroism (CD) spectroscopy. Chiral cage-type cyclophanes (–)-**1** and (+)-**1** show CD bands, patterns of which are opposite to each other in aqueous acetate buffer (0.01 mol dm^{-3}, pH 5.5, μ 0.1 with KCl) at 30°C, reflecting the asymmetric character of their internal cavities; [θ] -2.0×10^5 and -1.3×10^5 deg cm^2 $dmol^{-1}$ for (–)-**1** at their respective CD peak wavelengths of 209 and 225 nm, respectively; $+1.8 \times 10^5$ and $+1.4 \times 10^5$ deg cm^2 $dmol^{-1}$ for (+)-**1** at their respective CD peak wavelengths of 207 and 225 nm, respectively (Fig. 6). On the other hand, non-cage cyclophanes (–)-**6** and (+)-**6** show relatively weak CD bands as compared to those of the cage-type hosts; [θ] $+2.8 \times 10^4$ and -8.1×10^4 deg cm^2 $dmol^{-1}$ for (–)-**6** at their respective CD peak wavelengths of 203 and 227 nm, respectively; -3.0×10^4 and $+8.0 \times 10^4$ deg cm^2 $dmol^{-1}$ for (+)-**6** at their respective CD peak wavelengths of 203 and 227 nm, respectively. These results also indicate that conformational flexibility of the cage framework of (–)-**1** and (+)-**1** is largely reduced relative to those of the non-cage hosts in agreement with the results of structural analysis obtained by ^1H NMR spectroscopy.

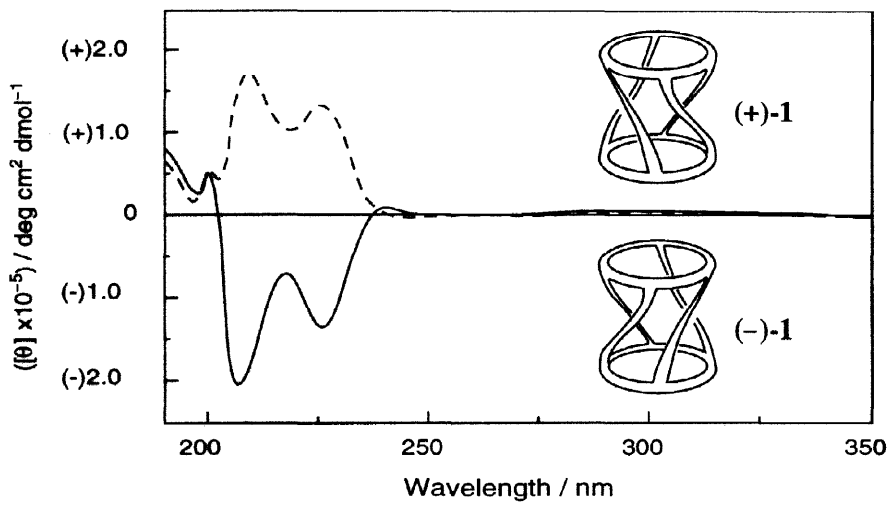


Fig. 6. CD spectra of (–)-**1** (1.0×10^{-5} mol dm^{-3}) and (+)-**1** (1.0×10^{-5} mol dm^{-3}) (solid and dashed lines, respectively) in aqueous acetate buffer (0.01 mol dm^{-3}, pH 5.5, μ 0.1 with KCl) at 30 °C

The guest-binding behavior of cage-type hosts (–)-**1** and (+)-**1** toward anionic guest PA was also examined by fluorescence spectroscopy in aqueous acetate buffer (0.01 mol dm^{-3}, pH5.5, μ 0.1 with KCl) at 30 °C. Upon addition of the individual hosts to the acetate buffer containing PA (1.0×10^6 mol dm^{-3}), a fluorescence intensity originating from the guest increased along with a concomitant blue shift of the fluorescence maximum, reflecting formation of the corresponding host–guest complex.[12] The stoichiometry for the complex formed with the host and PA was investigated by the Job's continuous variation method (Fig. 7).[11] The result reveals that the present hosts undergo complex formation with the guest in a 1:1 molar ratio of host to guest. Binding constants (K) for (–)-**1** and (+)-**1** toward PA were evaluated on the basis of Benesi-Hildebrand relationship for 1:1 host-guest interaction in a manner as described previously.[13] The K values for (–)-**1** and (+)-**1** with PA are 7.2×10^5 and 7.1×10^5 dm^3 mol^{-1}, respectively. On the other hand, both non-cage cyclophanes (–)-**6** and (+)-**6** showed no capacity of binding PA.

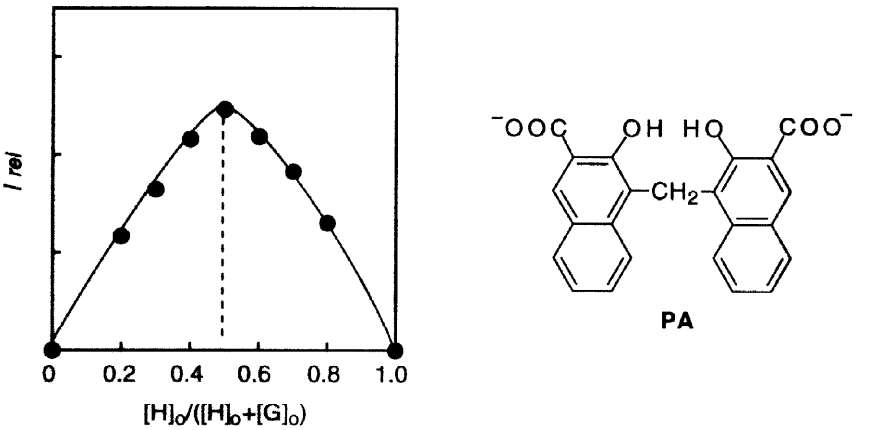


Fig. 7. Job's plot for complex formation of (–)-**1** with PA in aqueous acetate buffer (0.01 mol dm^{-3}, pH 5.5, μ 0.10 with KCl) at 30°C; total concentration of (–)-**1** and PA, 1.0 x 10^{-5} mol dm^{-3}.

The formation of such inclusion complexes of the cage-type hosts was also detected by CD spectroscopy. Upon addition of PA to an aqueous acetate buffer (0.01 mol dm^{-3}, pH 5.5, μ 0.10 with KCl) containing (–)-**1**, bisignate CD bands due to double exciton transitions originated from the incorporated guest appeared in a longer wavelength range ([θ], −1.9 x 10^4 and +1.9 x 10^4 deg cm^2 $dmol^{-1}$ at 237 and 261 nm, respectively) (Fig. 8; solid line). On the other hand, bisignate CD bands with inverted signs were observed for PA in the presence of (+)-**1**; [θ], +1.5 x 10^4 and −1.0 x 10^4 deg cm^2 $dmol^{-1}$ at 236 and 259 nm, respectively (Fig. 8; broken line). As a consequence, the CD phenomena were induced by the incorporated guest molecule through its stereochemical interaction with the chiral host cavity; the bisignate Cotton bands with inverted CD signs verifying that the incorporated PA molecule exists as a *P*- or a *M*-helical conformer[14] in the presence of (–)-**1** and (+)-**1**, respectively.

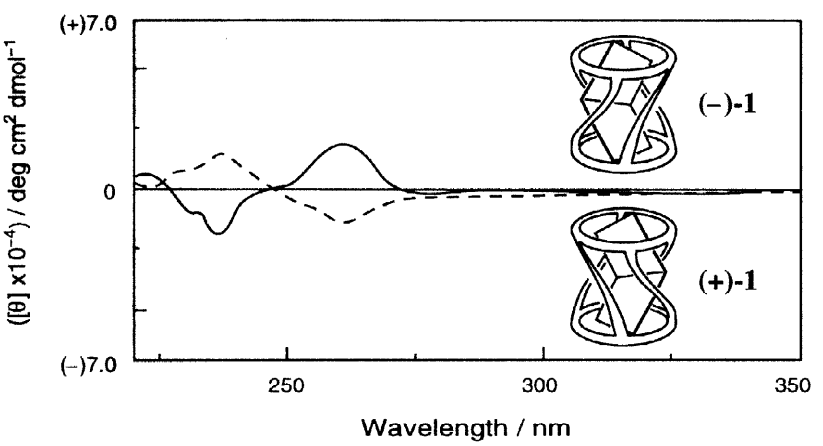


Fig. 8. CD spectra of PA (2.0 x 10^{-5} mol dm^{-3}) in the presence of (–)-**1** (1.0 x 10^{-5} mol dm^{-3}) and (+)-**1** (1.0 x 10^{-5} mol dm^{-3}) (solid and dashed lines, respectively) in aqueous acetate buffer (0.01 mol dm^{-3}, pH5.5, μ 0.1 with KCl) at 30 °C

The inclusion behavior of cage-type cyclophane (–)-**1** toward PA was also investigated by means of ^1H NMR spectroscopy in aqueous media at 303K. Because of the limited solubility of (–)-**1**, the measurements were carried out in D_2O/DMSO-d_6 (1:1 v/v). ^1H NMR signals of PA were subject to change in chemical shift

upon addition of the host molecule. Binding constants (K) decreased under these conditions in comparison with those in aqueous acetate buffer; K, 5.0 x 10^2 dm^3 mol^{-1}. Aromatic protons H_4 (8.31 ppm), H_5 (7.76 ppm), and H_6 (7.27 ppm) shifted to a higher magnetic field in the presence of (–)-**1**. In contrast, the doublet signal due to H_8 (8.07 ppm) shifted to a lower magnetic field upon complexation with (–)-**1**. The evaluated CIS values for PA are shown in Fig.9(a). These CIS values prove that the PA molecule is incorporated into the host cavity, and the two naphthalene groups of the guest are presumably placed individually in opposite macrocyclic skeletons as shown in Fig.9(b). The present molecular arrangement was also supported by the observed 1:1 stoichiometry for the complexes and the bisignate CD band originated from PA in the presence of (–)-**1**.

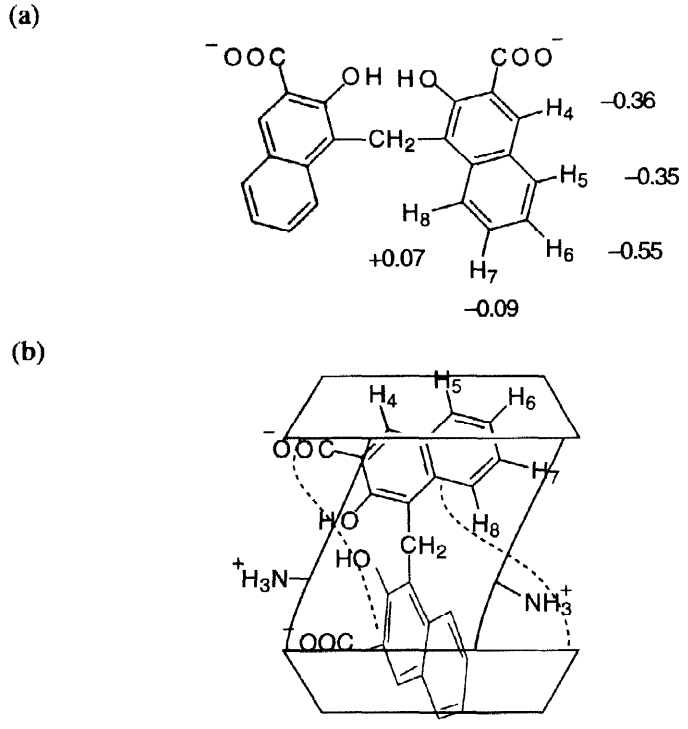


Fig. 9. Complexation of cage-type cyclophane (–)-**1** with PA: (a) CIS values (ppm) for PA upon complexation with (–)-**1** in D_2O/DMSO-d_6 (1:1 v/v) at 303K; (b) schematic illustration for the molecular arrangement within the complex.

In conclusion, we have prepared novel cage-type cyclophanes having dipeptide bridging segments. The structural property of cage-type cyclophane (–)-**1** was characterized by means of ^1H NMR spectroscopy. These hosts are capable of furnishing three-dimensionally extended hydrophobic cavities and effectively incorporate fluorescent guest, such as ANS and TNS, in aqueous media. Microenvironmental polarity parameters (E_T^N) around the guest molecules and their fluorescence polarization values (P) in the presence of cage-type hosts reveal that these guests molecules are placed in an apolar microenvironment and their rotational motion is largely restricted. Furthermore, the helically twisted cavities of (–)-**1** and (+)-**1** tend to furnish conformation-enforced microenvironments for chiral recognition of the enantiomeric guest, PA.

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. CD spectra were recorded on a JASCO J-720w spectropolarimeter. Purity of compounds was confirmed by HPLC (TOSOH Corporation); column TSK gel Silica-60, 4.6 x 25 (mmID x cm) and TSK gel Silica-60, 21.5 x 30 (mmID x cm). A Hitachi M-2500 double-focusing mass spectrometer was used for electrospray ionization (ESI) and a Hitachi M-0301 data acquisition system was used for processing ESI-MS data.

Materials

N,N',N'',N'''-Tetrakis[3-benzyloxycarbonyl-2-[(*tert*-butoxycarbonyl)amino]propanoyl]-2,11,20,29-tetraaza[3.3.3.3]paracyclophane, **(–)-5**, and *N,N',N'',N'''*-tetrakis[2-(*tert*-butoxycarbonyl)amino]-3-carboxypropanoyl]-2,11,20,29-tetraaza[3.3.3.3]paracyclophane, **(–)-7**, were prepared after the method reported previously.[13]

N,N',N'',N'''-Tetrakis[3-benzyloxycarbonyl-2-[(*tert*-butoxycarbonyl)amino]propanoyl]-2,11,20,29-tetraaza[3.3.3.3]paracyclophane, (+)-5

Dicyclohexylcarbodiimide (2.9g, 14mmol) was added to a dry dichloromethane (35mL) solution of N^{α}-(*tert*-butoxycarbonyl)-D-aspartic acid β-benzyl ester (4.5g, 14mmol) at 0°C, and the mixture was allowed to stand at the same temperature while being stirred for 30 min. 2,11,20,29-Tetraaza[3.3.3.3]paracyclophane (1.0g, 2.1mmol) dissolved in dry dichloromethane (60mL) was added to the mixture, and the resulting mixture was stirred for 4 h at 0°C and for an additional 24 h at room temperature. After an insoluble material (*N,N'*-dicyclohexylurea) was removed by filtration, 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 eluent. Evaporation of the product fraction gave a white solid (3.4g, 93%): mp 92–93 °C; R_f (Wako Silica Gel 70FM, ethyl acetate) 0.89; IR (KBr) 1740 (ester C=O), 1700 (urethane C=O), 1650 (amide C=O) cm^{-1}; $[\alpha]^{25}_D$ +79° (c=0.1, chloroform); ^{1}H NMR [500 MHz, $(CD_3)_2SO$, 373 K] δ=1.34 [s, 36H, $C(CH_3)_3$], 2.64–2.68 [dd, J_{vic}=6.3 Hz and J_{gem}=16.0 Hz, 4H, CH_2CO_2 (nonequivalent)], 2.91–2.96 [dd, J_{vic}=7.2 Hz and J_{gem}=16.0 Hz, 4H, CH_2CO_2 (nonequivalent)], 4.39 (m, 16H, NCH_2Ar), 4.96 (m, 4H, NHC*H*CO), 5.10 (s, 8H, CO_2CH_2), 6.79 (m, 4H, N*H*CHCO), 6.86 (m, 16H, NCH_2Ar*H*), 7.30 (m, 20H, CO_2CH_2Ar*H*). Anal. Calcd for $C_{96}H_{112}N_8O_{20}$•3/2H_2O: C, 66.84; H, 6.72; N, 6.50%. Found: C, 66.89; H, 6.54; N, 6.43%.

N,N',N'',N'''-Tetrakis[[2-amino-3-(benzyloxycarbonyl)]propanoyl]-2,11,20,29-tetraaza-[3.3.3.3]paracyclophane, (+)-6

Trifluoroacetic acid (10 mL) was added to a dry dichloromethane(30 mL) solution of (+)-**5** (1.5 g, 0.88 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 eluent. The product fraction was evaporated to dryness under reduced pressure to give a white solid (1.2 g, 74 %): mp 124–126 °C; R_f (Wako Silica Gel 70FM, chloroform) 0.25; IR (KBr) 1740 (ester C=O), 1680 (amide C=O) cm^{-1}; $[\alpha]^{25}_D$ +32° (c=0.1, methanol); ^1H NMR [500 MHz, $(CD_3)_2SO$, 373 K] δ=2.86–2.91 [dd, J_{vic}=7.0 Hz and J_{gem}=17.0 Hz, 4H, CH_2CO_2 (nonequivalent)], 2.97–3.01 [dd, J_{vic}=5.0 Hz and J_{gem}=17.0 Hz, 4H, CH_2CO_2 (nonequivalent)], 4.25–4.60 (m, 16H, NCH_2Ar), 4.67 (m, 4H, CHCO), 5.14 (s, 8H, CO_2CH_2), 6.91 (m, 16H, NCH_2ArH), 7.34 (m, 20H, CO_2CH_2ArH). Anal. Calcd for $C_{84}H_{84}N_8O_{20}F_{12}$•3/2$H_2O$: C, 56.68; H, 4.92; N, 6.29%. Found: C, 56.76; H, 4.81; N, 6.21%. ESI-MS m/z 1297 (M – $2CF_3CO_2H$ – $2CF3CO_2^-)^+$, 649 (M – CF_3CO_2H – $3CF_3CO_2^-)^{2+}$; calcd M for $C_{84}H_{84}N_8O_{20}F_{12}$, 1754.

N,N',N'',N'''-Tetrakis[2-[(*tert*-butoxycarbonyl)amino]-3-carboxypropanoyl]-2,11,20,29-tetraaza[3.3.3.3]paracyclophane, (+)-7

Palladium-black (Pd: 98–99%, 1.0g) was added to a tetrahydrofuran (50 mL) solution of (+)-**5** (1.2 g, 0.71 mmol), and hydrogen gas was introduced into the solution with stirring for 20 h at room temperature. After the catalyst was removed by filtration, 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 eluent. The product fraction was evaporated to dryness under reduced pressure to give a white solid (730 mg, 77 %): mp 269–272 °C (dec.); R_f (Wako Silica Gel 70FM, methanol) 0.51; IR (KBr) 1710 (carboxylic acid C=O), 1710 (urethane C=O), 1640 (amide C=O) cm^{-1}; $[\alpha]^{25}_D$ +115° (c=0.1, methanol); ^1H NMR [500 MHz, $(CD_3)_2SO$, 373 K] δ=1.35 [s, 36H, $C(CH_3)_3$], 2.48–2.52 [dd, J_{vic}=6.5 Hz and J_{gem}=16.5 Hz, 4H, CH_2CO_2 (nonequivalent)], 2.78–2.83 [dd, J_{vic}=7.5 Hz and J_{gem}=16.5 Hz, 4H, CH_2CO_2 (nonequivalent)], 4.42 (m, 16H, NCH_2Ar), 4.91 (m, 4H, CHCO), 6.76 (m, 4H, N*H*CHCO), 6.90 (m, 16H, NCH_2ArH). Anal. Calcd for $C_{68}H_{88}N_8O_{20}$: C, 61.07; H, 6.63; N, 8.38%. Found: C, 60.87; H, 6.58; N, 8.16%.

Cage-type Cyclophane Having Boc-D-Asp-D-Asp(OBzl), (+)-2

Individual solutions of (+)-**6** (500 mg, 0.29 mmol) and (+)-**7** (380 mg, 0.29 mmol) dissolved in dry *N,N*-dimethylformamide (DMF, 100 mL each) were added dropwise at an identical rate over 3 h to a dry DMF (2.0 L) solution containing diethyl cyanophosphonate (DECP; 490 mg, 2.9 mmol) and triethylamine (440 mg, 4.3 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 24 h at room temperature, and then evaporated to dryness under reduced pressure. The residue was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol–chloroform (1:1 v/v) as eluent. The product fraction was evaporated to dryness under reduced pressure to give a white solid (170 mg, 24 %): mp 279–283 °C (dec.); IR (KBr) 1650 (amide C=O), 1720 (urethane C=O), 1740 (ester C=O) cm^{-1}; ^1H NMR [500MHz, $(CD_3)_2SO$, 373 K] δ = 1.3–1.4 [m, 36H, $C(CH_3)_3$], 2.5–3.1 [m, 16H, $CHCH_2CO_2$ and $CHCH_2CON$], 3.3–5.5 [m, 32H, $ArCH_2N$], 5.1–5.2 [m, 8H, $ArCH_2O$], 5.2 and 5.6 [m, 8H, $CHCH_2CO_2$ and $CHNHCO_2$], 6.3–6.9 [m, 32H, $ArHCH_2N$], 7.2–7.4 [m, 20H, $ArHCH_2O$]. Purity of the product was confirmed by HPLC [TSK gel Silica 60, 21.5 x 30 (mmID x cm), methanol–chloroform 7:3 (v/v), 6 mL/min, retension time 10.7 min (single component)]. Anal. Calcd for $C_{144}H_{160}N_{16}O_{28}$•4$H_2O$: C, 65.64; H, 6.43; N, 8.51%. Found: C, 65.53; H, 6.14; N, 8.38%.

Cage-type Cyclophane Having L-Asp-L-Asp(OBzl), (+)-1

Trifluoroacetic acid (3.0 mL) was added to a dry dichloromethane (20 mL) solution of (+)-**2** (70 mg, 2.7 x 10^{-5} mol), and the mixture was stirred for 2 h at room temperature. After the solvent was evaporated under reduced pressure, the crude product was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol as eluent. The product fraction was evaporated to dryness under reduced pressure to give a white solid (56 mg, 78 %): mp 236–240 °C (dec.); IR (KBr) 1650 (amide C=O), 1740 (ester C=O) cm^{-1}; $[\alpha]^{25}_D$ +49° (c=0.1, methanol); ^1H NMR [500MHz, $(CD_3)_2SO$, 373 K] δ = 2.5–3.2 [m, 16H, $CHCH_2CO_2$ and $CHCH_2CON$], 3.3–5.6 [m, 32H, $ArCH_2N$], 5.2 [m, 8H, $ArCH_2O$], 4.8 and 5.6 [m, 8H, $CHCH_2CO_2$ and $CHNH_3$], 6.3–6.9 [m, 32H, $ArHCH_2N$], 7.0–7.4 [m, 20H, $ArHCH_2O$]. Anal. Calcd for $C_{132}H_{132}N_{16}O_{28}F_{12}$: C, 60.54; H, 5.08; N, 8.56%. Found: C, 60.87; H, 5.35; N, 8.52%. ESI-MS *m/z* 1082 (M – $2CF_3CO_2H$ – $2CF_3CO_2^-)^{2+}$, 721 (M – CF_3CO_2H – $3CF_3CO_2^-)^{3+}$; calcd M for $C_{132}H_{132}N_{16}O_{28}F_{12}$, 2619.

***N,N',N'',N'''*-Tetrakis[[2-amino-3-(benzyloxycarbonyl)]propanoyl]-2,11,20,29-tetraaza-[3.3.3.3]paracyclophane, (–)-6**

Trifluoroacetic acid (10 mL) was added to a dry dichloromethane(30 mL) solution of (–)-**5** (1.0 g, 0.59 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 eluent. The product fraction was evaporated to dryness under reduced pressure to give a white solid (680 mg, 66 %): mp 123-125 °C; R_f (Wako Silica Gel 70FM, methanol) 0.83; IR (KBr disc) 1730 (ester C=O), 1660 (amide C=O) cm^{-1}; $[\alpha]^{25}_D$ –32° (c=0.1, methanol); ^1H NMR [500 MHz, $(CD_3)_2SO$, 373 K] δ=2.86–2.91 [dd, J_{vic}=7.0 Hz and J_{gem}=17.0 Hz, 4H, CH_2CO_2 (nonequivalent)], 2.97–3.01 [dd, J_{vic}=5.0 Hz and J_{gem}=17.0 Hz, 4H, CH_2CO_2 (nonequivalent)], 4.25–4.60 (m, 16H, NCH_2Ar), 4.67 (m, 4H, CHCO), 5.14 (s, 8H, CO_2CH_2), 6.91 (m, 16H, NCH_2ArH), 7.34 (m, 20H, CO_2CH_2ArH). Anal. Calcd for $C_{84}H_{84}N_8O_{20}F_{12}$•3/2$H_2O$: C, 56.68; H, 4.92; N, 6.29%. Found: C, 56.59; H, 4.73; N, 6.10%. ESI-MS m/z 1297 (M – $2CF_3CO_2H$ – $2CF_3CO_2^-)^+$, 649 (M – CF_3CO_2H – $3CF_3CO_2^-)^{2+}$; calcd M for $C_{84}H_{84}N_8O_{20}F_{12}$, 1754.

Cage-type Cyclophane Having Boc-L-Asp-L-Asp(OBzl) Residues, (–)-2

Individual solutions of (–)-**6** (500 mg, 0.29 mmol) and (–)-**7** (380 mg, 0.29 mmol) dissolved in dry *N,N*-dimethylformamide (DMF, 100 mL each) were added dropwise at an identical rate over 3 h to a dry DMF (2.0 L) solution containing diethyl cyanophosphonate (DECP; 490 mg, 2.9 mmol) and triethylamine (440 mg, 4.3 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 24 h at room temperature, and then evaporated to dryness under reduced pressure. The residue was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol–chloroform (1:1 v/v) as eluent. The product fraction was evaporated to dryness under reduced pressure to give a white solid (120 mg, 20 %): mp 281–284 °C (dec.); IR (KBr) 1650 (amide C=O), 1720 (urethane C=O), 1740 (ester C=O) cm^{-1}; ^1H NMR [500MHz, $(CD_3)_2SO$, 373 K] δ = 1.3–1.4 [m, 36H, $C(CH_3)_3$], 2.5–3.1 [m, 16H, $CHCH_2CO_2$ and $CHCH_2CON$], 3.3–5.5 [m, 32H, $ArCH_2N$], 5.1–5.2 [m, 8H, $ArCH_2O$], 5.2 and 5.6 [m, 8H, $CHCH_2CO_2$ and $CHNHCO_2$], 6.3–6.9 [m, 32H, $ArHCH_2N$], 7.2–7.4 [m, 20H, $ArHCH_2O$]. Purity of the product was confirmed by HPLC analysis [TSK gel Silica 60, 21.5 x 30

(mmID x cm), methanol–chloroform 7:3 (v/v), 6 mL/min, retension time 10.7 min (single component)]. Anal. Calcd for $C_{144}H_{160}N_{16}O_{28}$•$4H_2O$: C, 65.64; H, 6.43; N, 8.51%. Found: C, 65.44; H, 6.28; N, 8.55%.

Cage-type Cyclophane Having L-Asp-L-Asp(OBzl) Residues, (–)-1

Trifluoroacetic acid (2.0 mL) was added to a dry dichloromethane (20 mL) solution of **(–)-2** (30 mg, 1.4 x 10^{-5} mol), and the mixture was stirred for 2 h at room temperature. After the solvent was evaporated under reduced pressure, the crude product was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol as eluent. The product fraction was evaporated to dryness under reduced pressure to give a white solid (30 mg, 84 %): mp 237–241 °C (dec.); IR (KBr) 1650 (amide C=O), 1740 (ester C=O) cm^{-1}; $[\alpha]^{25}_D$ –49° (c=0.1, methanol); ^1H NMR [500MHz, $(CD_3)_2SO$, 373 K] δ = 2.5–3.2 [m, 16H, $CHCH_2CO_2$ and $CHCH_2CON$], 3.3–5.6 [m, 32H, $ArCH_2N$], 5.2 [m, 8H, $ArCH_2O$], 4.8 and 5.6 [m, 8H, $CHCH_2CO_2$ and $CHNH_3$], 6.3–6.9 [m, 32H, $ArHCH_2N$], 7.0–7.4 [m, 20H, $ArHCH_2O$]. Anal. Calcd for $C_{132}H_{132}N_{16}O_{28}F_{12}$: C, 60.54; H, 5.08; N, 8.56%. Found: C, 60.57; H, 5.41; N, 8.79%. ESI-MS *m/z* 1082 ($M - 2CF_3CO_2H - 2CF_3CO_2^-)^{2+}$, 721 ($M - CF_3CO_2H - 3CF_3CO_2^-)^{3+}$; calcd M for $C_{132}H_{132}N_{16}O_{28}F_{12}$, 2619.

***N,N',N''*-Tris[[3-(benzyloxycarbonyl)-2-[(*tert*-butoxycarbonyl)amino]propanoyl]-2,11,20-triaza[3.3.3]paracyclophane, (–)-10**

Dicyclohexylcarbodiimide (530 mg, 2.6 mmol) was added to a dry dichloromethane (50 mL) solution of N^{α}-(*tert*-butoxycarbonyl)-L-aspartic acid β-benzyl ester (700 mg, 2.2 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 (110 mg, 0.31 mmol) dissolved in dry dichloromethane (30mL) was added to the mixture, and the resulting mixture was stirred for 4 h at 0°C and for an additional 24 h at room temperature. An insoluble material (*N,N'*-dicyclohexylurea) was 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 eluent. Evaporation of the product fraction under reduced pressure gave a white solid (340 mg, 87%): mp 86–88 °C; R_f [Wako Silica Gel 70FM, chloroform–ethyl acetate (1:1 v/v)] 0.69; IR (KBr disc) 1740 (ester C=O), 1720 (urethane C=O), 1650 (amide C=O) cm^{-1}; ^1H NMR [500 MHz, $(CD_3)_2SO$, 373 K] δ=1.34 [s, 27H, $C(CH_3)_3$], 2.58 [dd, J_{vic}=6.5 Hz and J_{gem}=16.5 Hz, 3H, CH_2CO_2 (nonequivalent)], 2.79 [dd, J_{vic}=6.5 Hz and J_{gem}=16.5 Hz, 3H, CH_2CO_2 (nonequivalent)], 4.53 (m, 12H, NCH_2Ar), 5.83 (m, 3H, $NHCHCO$), 5.04 (s, 6H, CO_2CH_2), 6.63 (m, 3H, $NHCHCO$), 6.83 (m, 12H, NCH_2ArH), 7.30 (m, 15H, CO_2CH_2ArH). Anal. Calcd for $C_{72}H_{84}N_6O_{15}$•$3/2H_2O$: C, 66.50; H, 6.74; N, 6.46%. Found: C, 66.58; H, 6.75; N, 6.32%.

***N,N',N''*-Tris[[2-amino-3-(benzyloxycarbonyl)]propanoyl]-2,11,20-triaza[3.3.3]paracyclophane, (–)-11**

Trifluoroacetic acid (3.0 mL) was added to a dry dichloromethane(30 mL) solution of **(–)-10** (340 mg, 0.27 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 eluent. The product fraction was evaporated to dryness under reduced pressure to give a white solid (300 mg, 85 %): mp 120–122 °C; R_f [Wako Silica Gel 70FM, methanol–

:chloroform (1:1 v/v)] 0.80; IR (KBr) 1730 (ester C=O), 1650 (amide C=O) cm^{-1}; $[\alpha]^{25}_D$ +7.0° (c=0.1, methanol); ^1H NMR [500 MHz, $(CD_3)_2SO$, 373 K] δ=2.75 [dd, J_{vic}=7.0 Hz and J_{gem}=16.5 Hz, 3H, CH_2CO_2 (nonequivalent)], 2.99 [dd, J_{vic}=5.0 Hz and J_{gem}=16.5 Hz, 3H, CH_2CO_2 (nonequivalent)], 4.37 (m, 3H, CHCO), 4.57 (m, 12H, NCH_2Ar), 5.11 (s, 6H, CO_2CH_2), 6.88 (m, 12H, NCH_2ArH), 7.34 (m, 15H, CO_2CH_2ArH). Anal. Calcd for $C_{63}H_{63}N_6O_{15}F_9$: C, 57.53; H, 4.83; N, 6.39%. Found: C, 57.48; H, 4.88; N, 6.41%. ESI-MS m/z 973 $(M - 2CF_3CO_2H - CF_3CO_2^-)^+$, 487 $(M - CF_3CO_2H - 2CF_3CO_2^-)^{2+}$; calcd M for $C_{63}H_{63}N_6O_{15}F_9$, 1315.

N,N',N''-Tris[2-(*tert*-butoxycarbonyl)amino]-3-carboxypropanoyl]-2,11,20-triaza[3.3.3]-paracyclophane, (−)-12

Palladium-black (Pd: 98–99%, 300 mg) was added to a tetrahydrofuran (40 mL) solution of (−)-**10** (180 mg, 0.14 mmol), and hydrogen gas was introduced into the mixture with stirring for 19 h at room temperature. The catalyst was removed by filtration, and the solvent was evaporated under reduced pressure. The crude product was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol as eluent. The product fraction was evaporated to dryness under reduced pressure to give a white solid (130 mg, 92 %): mp 154–156 °C; Rf [Wako Silica Gel 70FM, methanol–chloroform (1:1 v/v)] 0.78; IR (KBr disc) 1710 (urethane C=O), 1640 (amide C=O) cm^{-1}; $[\alpha]^{25}_D$ −54° (c=0.1, methanol); ^1H NMR [500 MHz, $(CD_3)_2SO$, 373 K] δ=1.35 [s, 27H, $C(CH_3)_3$], 2.45 [dd, J_{vic}=6.5 Hz and J_{gem}=16.0 Hz, 3H, CH_2CO_2H (nonequivalent)], 2.69 [dd, J_{vic}=7.0 Hz and J_{gem}=16.0 Hz, 3H, CH_2CO_2H (nonequivalent)], 4.55 (m, 12H, NCH_2Ar), 4.79 (m, 3H, NHC*H*CO), 6.57 (m, 3H, $NHCO_2$), 6.87 (m, 12H, NCH_2ArH). Anal. Calcd for $C_{51}H_{66}N_6O_{15}\cdot H_2O$: C, 59.99; H, 6.71; N, 8.23%. Found: C, 60.07; H, 6.74; N, 8.25%.

Cage-type Cyclophane Having Boc-L-Asp-L-Asp(OBzl) Residues, (−)-3

Individual solutions of (−)-**11** (170 mg, 0.13 mmol) and (−)-**12** (130 mg, 0.13 mmol) dissolved in dry *N,N*-dimethylformamide (DMF, 100 mL each) were added dropwise at an identical rate over 3 h to a dry DMF (700 mL) solution containing diethyl cyanophosphonate (DECP; 220 mg, 1.3 mmol) and triethylamine (260 mg, 2.6 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 2 h at room temperature, and then evaporated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel (Wakogel C-300) with methanol–chloroform (1:1 v/v) as eluent, followed by gel filtration chromatography on a column of Sephadex LH-20 with methanol–chloroform (1:1 v/v) as eluent. The product fraction was evaporated to dryness under reduced pressure to give a white solid (24 mg, 10 %): mp 185–187 °C (dec.); R_f [Wako Silica Gel 70FM, methanol–chloroform (1:10 v/v)] 0.67; IR (KBr) 1730 (ester C=O), 1720 (urethane C=O), 1650 (amide C=O) cm^{-1}; ^1H NMR [500 MHz, $(CD_3)_2SO$, 373 K] δ=1.4 [m, 27H, $C(CH_3)_3$], 2.4–2.8 (m, 12H, CH_2CO_2 and CH_2CONH), 4.5 (m, 24H, NCH_2Ar), 4.9 (m, 6H, $CHCH_2CO_2$ and $CHNHCO_2$), 5.1 (s, 6H, CO_2CH_2Ar), 5.3 (m, 3H, $NHCOCH_2$), 6.6 (m, 3H, $NHCO_2$), 6.9 (m, 24H, NCH_2ArH), 7.3 (m, 15H, CO_2CH_2ArH). Purity of the product was confirmed by HPLC [TSK gel Silica 60, 21.5 x 30 (mmID x cm), methanol-chloroform 7:3 (v/v), 6 mL/min, retension time 12.4 min (single component)]. Anal. Calcd for $C_{108}H_{120}N_{12}O_{21}\cdot 3H_2O$: C, 65.64; H, 6.43; N, 8.51%. Found: C, 65.86; H, 6.31; N, 8.60%.

Cage-type Cyclophane Having L-Asp-L-Asp(OBzl) Residues, (–)-4

Trifluoroacetic acid (2.0 mL) was added to a dry dichloromethane (20 mL) solution of **(–)-3** (15 mg, 7.8 x 10^{-6} mol), and the mixture was stirred for 2 h at room temperature. After the solvent was evaporated under reduced pressure, the crude product was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol as eluent. The product fraction was evaporated to dryness under reduced pressure to give a white solid (13 mg, 85 %): mp 276–279 °C (dec.).; ^{1}H NMR [500 MHz, CD_3OD, 303 K] δ=2.3–3.0 [m, 12H, $CH_2CO_2CH_2Ph$, CH_2CONH], 4.4 [m, 3H, $CHNHCO$], 4.6–4.8 [m, 24H, $ArCH_2N$], 5.0 [d, J_{gem}=12.5 Hz, 3H, OCH_2Ar (non-equivalent)], 5.1 [d, J_{gem}=12.5 Hz, 3H, OCH_2Ar (nonequivalent)], 5.2 [m, 3H, $CHNH_3$], 6.9–7.0 [m, 24H, $ArHCH_2N$], 7.3–7.4 [m, 15H, $ArHCH_2O$]. Anal. Calcd for $C_{99}H_{99}N_{12}O_{21}F_9$: C, 60.55; H, 5.08; N, 8.56%. Found: C, 60.26; H, 5.35; N, 8.72%. ESI-MS m/z 1621 $(M - 2CF_3CO_2H - CF_3CO_2^-)^+$, 811 $(M - CF_3CO_2H - 2CF_3CO_2^-)^{2+}$, 541 $(M - 3CF_3CO_2^-)^{3+}$; calcd M for $C_{99}H_{99}N_{12}O_{21}F_9$, 1694.

3-(Benzyloxycarbonyl)-2-[(*tert*-butoxycarbonyl)amino]-*N*-(4-methylbenzyl)propanamide, (–)-13

Dicyclohexylcarbodiimide (960 mg, 4.6 mmol) was added to a dry dichloromethane (30 mL) solution of N^{α}-(*tert*-butoxycarbonyl)-L-aspartic acid β-benzyl ester (1.0 g, 3.1 mmol) and 1-hydroxybenzotriazole (620mg, 4.0 mmol) at 0°C, and the mixture was allowed to stand at the same temperature while being stirred for 30 min. After 4-methylbenzylamine (750 mg, 4.6 mmol) dissolved in dry dichloromethane (30mL) was added to the mixture, the resulting mixture was stirred for 4 h at 0°C and for additional 24 h at room temperature. An insoluble material was removed by filtration, and the filtrate was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel (Wakogel C-300) with ethyl acetate as eluent. Evaporation of the product fraction under reduced pressure gave a white solid (1.3 g, 95%): mp 98–100 °C; R_f [Wako Silica Gel 70FM, ethyl acetate] 0.76; IR (KBr) 1740 (ester C=O), 1680 (urethane C=O), 1660 (amide C=O) cm^{-1}; ^{1}H NMR [500 MHz, $(CD_3)_2SO$, 303 K] δ=1.37 [s, 9H, $C(CH_3)_3$], 2.45 (s, 3H, $ArCH_3$), 2.59–2.64 [dd, J_{vic}=8.5 Hz and J_{gem}=16.0 Hz, 1H, CH_2CO_2 (nonequivalent)], 2.75–2.80 [dd, J_{vic}=5.5 Hz and J_{gem}=16.0 Hz, 1H, CH_2CO_2 (nonequivalent)], 4.20 (m, 2H, NCH_2Ar), 4.35 (m, 1H, $NHCHCO$), 5.07 (s, 2H, CO_2CH_2), 7.07 and 7.10 (d, *J*=8.0 Hz, 4H, CH_2ArHCH_3), 7.14 (d, *J*=8.0 Hz, 1H, $NHCHCO$), 7.30–7.36 (m, 5H, CO_2CH_2ArH), 8.28 (m, 1H, CH_2NHCO). Anal. Calcd for $C_{24}H_{30}N_2O_5$: C, 67.59; H, 7.09; N, 6.57%. Found: C, 67.54; H, 7.06; N, 6.55%.

2-Amino-3-(benzyloxycarbonyl)-*N*-(4-methylbenzyl)propanamide, (–)-14

Trifluoroacetic acid (3.0 mL) was added to a dry dichloromethane (20 mL) solution of **(–)-13** (540 mg, 1.2 mmol), and the mixture was stirred for 2 h at room temperature. After the solvent was evaporated under reduced pressure, the crude product was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol as eluent. The product fraction was evaporated to dryness under reduced pressure to give a colorless oily product (460 mg, 86 %): R_f [Wako Silica Gel 70FM, ethyl acetate] 0.39; IR (KBr) 1730 (ester C=O), 1660 (amide C=O) cm^{-1}; ^{1}H NMR [500 MHz, $(CD_3)_2SO$, 303 K] δ=2.26 (s, 3H, $ArCH_3$), 2.89–2.93 [dd, J_{vic}=8.0 Hz and J_{gem}=17.5 Hz, 1H, CH_2CO_2 (nonequivalent)], 2.96–3.00 [dd, J_{vic}=5.0 Hz and J_{gem}=17.5 Hz, 1H, CH_2CO_2 (nonequivalent)], 4.14 (m, 1H, CHCO), 4.26 (d, *J*=5.5 Hz, 2H, NCH_2Ar), 5.13 (s, 2H, CO_2CH_2), 7.12 and 7.14 (d,*J*=8.5 Hz 4H, CH_2ArHCH_3), 7.33–7.40 (m, 5H, CO_2CH_2ArH),

8.25 (bs, 3H, CHNH_3), 8.83 (t, J=5.5 Hz, 1H, CH_2NHCO). Anal. Calcd for $C_{21}H_{23}N_2O_5F_3 \cdot 1/2H_2O$: C, 56.12; H, 5.28; N, 6.23%. Found: C, 55.79; H, 5.23; N, 6.21%. ESI-MS m/z 327 $(M - CF_3CO_2^-)^+$; calcd M for $C_{21}H_{23}N_2O_5F_3$, 440.

N,N',N'',N'''-Tetrakis[[3-[N-[2-benzyloxycarbonyl-1-N-(4-methylbenzylcarbamoyl)]ethylcarbamoyl]-2-[(*tert*-butoxycarbonyl)amino]propanoyl]-2,11,20,29-tetraaza[3.3.3.3]paracyclophane, (–)-8

A sample of (–)-**11** (440 mg, 1.0 mmol) was added to a dry N,N-dimethylformamide (DMF, 40 mL) solution containing diethyl cyanophosphonate (DECP; 330 mg, 2.0 mmol), triethylamine (200 mg, 2.0 mmol) and (–)-**7** (300 mg, 0.22 mmol) with vigorous stirring under nitrogen atmosphere at 0 °C. The resulting mixture was stirred for 4 h at the same temperature and for an additional 24 h at room temperature, and then evaporated to dryness under reduced pressure. The crude product was purified by gel filteration chromatography on a column of Sephadex LH-20 and then on a column of Toyopearl HW-40F both with methanol–chloroform (1:1 v/v) as eluent. The product fraction was evaporated to dryness under reduced pressure to give a white solid (470 mg, 82 %): mp 122–124 °C; R_f [Wako Silica Gel 70FM, methanol–chloroform (1:5 v/v)] 0.83; IR (KBr) 1720 (ester C=O), 1680 (urethane C=O), 1650 (amide C=O) cm^{-1}; ^1H NMR [500 MHz, $(CD_3)_2SO$, 373 K] δ=1.34 [s, 36H, $C(CH_3)_3$], 2.22 (s, 12H, $ArCH_3$), 2.43–2.49 and 2.66–2.74 (m, 16H, $CHCH_2CO_2$ and $CHCH_2CON$), 4.15–4.25 (m, 8H, CH_2ArCH_3), 4.36–4.52 (m, 16H, NCH_2Ar), 4.73 and 4.94 (m, 8H, $CHCH_2CO_2$ and CHNHCO), 5.09 (s, 8H, CO_2CH_2), 6.68 and 7.95 (m, 8H, $CHNHCO_2$ and CONHCH), 6.85 (m, 16H, NCH_2ArH), 7.02 and 7.09 (d, J=8.0 Hz, 16H, CH_2ArHCH_3), 7.18 (m, 20H, CO_2CH_2ArH), 7.79 (m, 4H, $CONHCH_2$). Anal. Calcd for $C_{116}H_{144}N_{16}O_{28}$: C, 63.03; H, 6.57; N, 10.13%. Found: C, 62.85; H, 6.60; N, 10.09%.

N,N',N'',N'''-Tetrakis[3-[N-[2-benzyloxycarbonyl-1-N-(4-methylbenzylcarbamoyl)]ethylcarbamoyl]-2-aminopropanoyl]-2,11,20,29-tetraaza[3.3.3.3]paracyclophane, (–)-9

Trifluoroacetic acid (4.0 mL) was added to a dry dichloromethane (30 mL) solution of (–)-**8** (230 mg, 9.0 x 10^{-5} mol), and the mixture was stirred for 2 h at room temperature. After the solvent was evaporated under reduced pressure, the crude product was purified by gel filtration chromatography on a column of Sephadex LH-20 with methanol as eluent. The product fraction was evaporated to dryness under reduced pressure to give a white solid (220 mg, 93 %): mp 148–151 °C; R_f [Wako Silica Gel 70FM, methanol-chloroform (1:5 v/v)] 0.63; IR (KBr) 1730 (ester C=O), 1660 (amide C=O) cm^{-1}; ^1H NMR [500 MHz, $(CD_3)_2SO$, 373 K] δ=2.24 (s, 12H, $ArCH_3$), 2.63–2.90 (m, 16H, $CHCH_2CO_2$ and $CHCH_2CON$), 4.22 (m, 8H, CH_2ArCH_3), 4.31 (m, 16H, NCH_2Ar), 4.57 and 4.72 (m, 8H, $CHCH_2CO_2$ and CHNHCO), 5.10 (s, 8H, CO_2CH_2), 6.93 (m, 16H, NCH_2ArH), 7.04 and 7.09 (d, J=8.0 Hz 16H, CH_2ArHCH_3), 7.28–7.36 (m, 20H, CO_2CH_2ArH), 7.95 (m, 4H, $CONHCH_2$), 8.20 (m, 4H, CONHCH). Anal. Calcd for $C_{132}H_{140}N_{16}O_{28}F_{12} \cdot H_2O$: C, 59.95; H, 5.41; N, 8.47%. Found: C, 59.92; H, 5.44; N, 8.55%. ESI-MS m/z 2172 $(M - 3CF_3CO_2H - CF_3CO_2^-)^+$, 1086 $(M - 2CF_3CO_2H - 2CF_3CO_2^-)^{2+}$, 724 $(M - CF_3CO_2H - 3CF_3CO_2^-)^{3+}$; calcd M for $C_{132}H_{140}N_{16}O_{28}F_{12}$, 2627.

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