Preparation and Characterization of Novel Cage-type Cyclophanes Having Three or Four Bridging Dipeptide Segments

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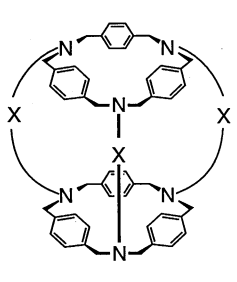
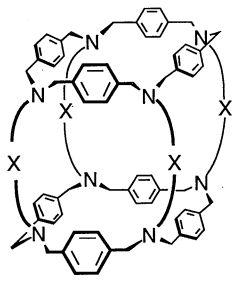
Novel cage-type cyclophanes constructed with two rigid macrocyclic skeletons, triaza[3.3.3]paracyclophanes or tetraaza-[3.3.3.3]paracyclophanes, and three or four dipeptide moieties, α-L-aspartyl-L-aspartyl residues, were prepared. The guest-binding behavior of the cage-type hosts toward fluorescent guests, such as 8-anilinonaphthalene-1-sulfonate and 6-*p*-toluidinonaphthalene-2-sulfonate, was examined in comparison with that demonstrated by non-cage hosts toward the identical guests.

Cage-type cyclophanes are artificial host molecules, each providing a three-dimensionally extended hydrophobic cavity for inclusion of guest molecules in aqueous media.[1] We have previously prepared various cage-type cyclophanes bearing chiral binding sites furnished by optically active amino acid residues such as leucine, valine, and alanine as chiral hosts.[2] The binding constants of the hosts for inclusion of fluorescent guests, such as 8-anilinonaphthalene-1-sulfonate (ANS) and 6-*p*-toluidinonaphthalene-2-sulfonate (TNS) were in the range of $1.5 \times 10^4 - 5.8 \times 10^4$ dm^3 mol^{-1} in aqueous acetate buffer at 303 K.[2] In order to get further insights into the correlation between a molecular recognition ability of the hosts and a three-dimensional extent of the guest-binding site, we now designed novel cage-type cyclophanes having three or four bridging dipeptide segments, **1** and **2**.



1



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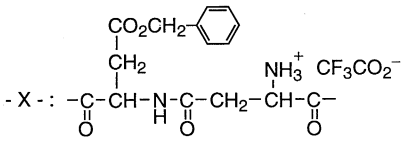
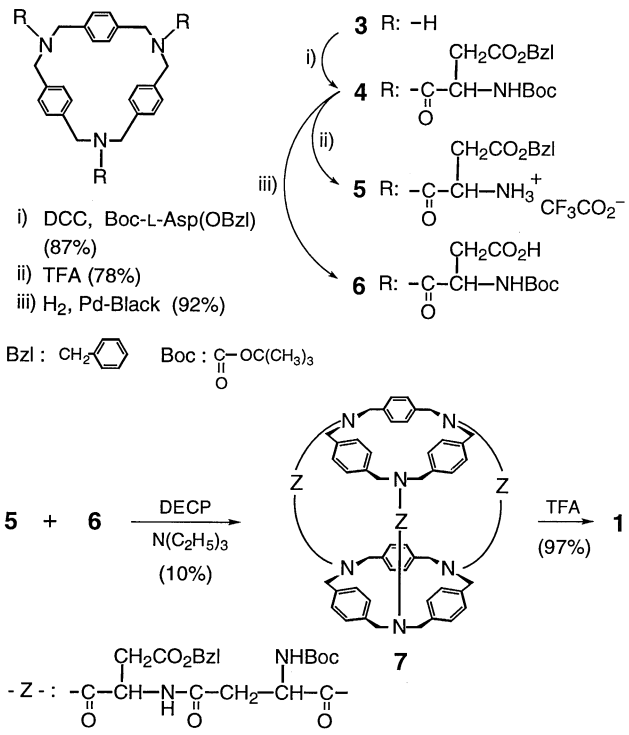


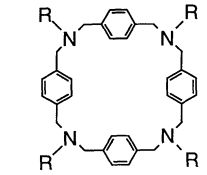
Each of the present host molecules was constructed with two rigid macrocyclic skeletons, triaza[3.3.3]paracyclophanes or tetraaza[3.3.3.3]paracyclophanes, and three or four dipeptide moieties that connect two macrocycles. The size and hydrophobic property of the internal cavity provided by host **1** must be different from those by host **2** for molecular recognition. Moreover, the bridging segments composed of α-L-aspartyl-L-aspartyl residues may confer chirality-based multi-point interaction capability on the resulting hosts.

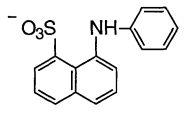
The cage-type cyclophane having three bridging segments (**1**) was synthesized by following the reaction sequences shown in Scheme 1. A peptide cyclophane having *tert*-butyloxycarbonyl-β-benzyl-L-aspartyl moieties (**4**) was prepared by condensation of 2,11,20-triaza[3.3.3]paracyclophane (**3**)[3] with β-benzyl N^{α}-(*tert*-butyloxycarbonyl)-L-aspartate [Boc-L-Asp-(OBzl)] in the presence of *N,N*-dicyclohexylcarbodiimide (DCC). A peptide cyclophane having β-benzyl-L-aspartyl moieties (**5**) was prepared by removal of the α-amino-protecting groups of **4** with trifluoroacetic acid (TFA). The β-carboxy-protecting groups of **4** were also selectively removed by hydrogenolysis with palladium black to afford a peptide cyclophane having *tert*-butyloxycarbonyl-L-aspartyl moieties (**6**). Cage-type cyclophane **7** was synthesized by condensation of **5** with **6** in the presence of diethyl cyanophosphonate (DECP) and triethylamine under high dilution conditions in dry *N,N*-dimethylformamide at 0 °C. All the novel products mentioned above were purified by gel-filtration chromatography on a column of Sephadex LH-20 with methanol or methanol–chloroform (1:1 v/v) as eluent, and identified by ^{1}H NMR and IR spectroscopy as well as by elemental analyses. Water-soluble cage-type cyclophane **1** was prepared by removal of the α-amino-protecting groups of **7** with TFA. The product was purified by gel-filtration chromatography on a column of Sephadex LH-20 with methanol–chloroform (1:1 v/v) as eluent; mp 276–279 °C (dec.). ^{1}H NMR [500 MHz, CD_3OD, 303 K] δ=2.5–3.0 (m, 12H, CH_2CO_2, CH_2CONH),



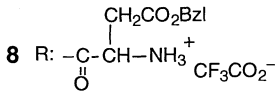
Scheme 1.

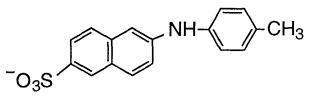
4.35 (m, 3H, C*H*NHCO), 4.5–4.8 (m, 24H, ArC*H*$_2$N), 5.05 (m, 6H, OCH$_2$Ar), 5.23 (m, 3H, C*H*NH$_3$), 6.8–7.0 (m, 24H, NCH$_2$Ar*H*), 7.2–7.4 (m, 15H, OCH$_2$Ar*H*). Found: C, 60.26; H, 5.35; N, 8.72%. Calcd for $C_{99}H_{99}F_9N_{12}O_{21}$: C, 60.55; H, 5.08; N, 8.56%. ESI-MS m/z 1621 (M – $2CF_3CO_2H$ – $CF_3CO_2^-)^+$ and 811 (M – CF_3CO_2H – $2CF_3CO_2^-)^{2+}$; calcd M for $C_{99}H_{99}F_9N_{12}O_{21}$, 1694. The use of 2,11,20,29-tetraaza[3.3.3.3]paracyclophane in place of **3** afforded a corresponding peptide cyclophane having four β-benzyl-L-aspartyl moieties (**8**) and a cage-type cyclophane having four bridging segments (**2**) after the method applied to the preparation of **1**.





ANS

8 R: 



TNS

The preliminary guest-binding behavior of cage-type hosts **1** and **2** and peptide cyclophanes **5** and **8**, as reference substances, toward ANS and TNS was examined by means of fluorescence spectroscopy in aqueous acetate buffer (0.01 mol dm^{-3}, pH 4.0, μ 0.1 with KCl) at 303 K. Stoichiometries for the complexes formed with the hosts and the guests were investigated by the Job's continuous variation method.[4] Binding constants (*K*) of these hosts toward ANS and TNS were evaluated on the basis of the Benesi-Hildebrand relationship for a 1:1 host-guest interaction in a manner as described previously.[5] The evaluated *K* values are summarized in Table 1. The guest-binding ability of the hosts toward ANS and TNS was subject to change by size- and hydrophobic nature of the internal cavity of each host: **2** > **1** > **8** > **5**. The inclusion interaction of host **2** with ANS was investigated by ^{1}H NMR spectroscopy in D_2O–$(CD_3)_2SO$ (80 : 20 v/v) at 303 K. Upon addition of **2** to a solution of ANS, all ^{1}H NMR signals due to the guest were subjected to substantial upfield shifts. The evaluated complexation-induced shifts (CIS) were relatively small: 0.02 – 0.07 ppm for H-4, H-5, H-6, H-7, H-2', H-3', and H-4'. Therefore, the ANS molecule was incorporated into the three-dimensional cavity provided intramoleculary by the two macrocylic skeletons and four bridging components, in a manner similar to that reported for another cage-type host toward the identical guest.[6] A similar molecular arrangement in a complex formed with host **1** and ANS was also confirmed by the identical method.

Table 1. Binding Constants (K / dm^3 mol^{-1}) for complex formation of cage-type hosts **1** and **2** and peptide cyclophanes **5** and **8** with ANS and TNS in aqueous acetate buffer (0.01 mol dm^{-3}, pH 4.0, μ 0.10 with KCl) at 303 K

	K / dm^3 mol^{-1}	
Host	ANS	TNS
1	4.1 x 10^3	1.2 x 10^4
2	2.0 x 10^4	4.9 x 10^4
5	1.1 x 10^3	1.6 x 10^3
8	3.4 x 10^3	4.4 x 10^3

Cage-type cyclophane **1** showed circular dichroism (CD) bands in aqueous acetate buffer (0.01 mol dm^{-3}, pH 4.0, μ 0.1 with KCl) at 283 K, reflecting asymmetric character of its internal cavity; [Θ], –7.8 x 10^4 and +3.8 x 10^4 deg cm^2 dmol^{-1} at 215 and 235 nm (respective CD peak wavelengths). On the other hand, cyclophane **2** showed a negative CD band at 212 nm with [Θ] = –2.6 x 10^5 deg cm^2 dmol^{-1} under identical conditions. Asymmetric properties of the internal cavities provided by both hosts for guest recognition were confirmed to be different from each other in aqueous media. The chirality-based molecular discrimination behavior of the present hosts is now under investigation in our laboratory.

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Reference and Notes

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