Pure & Appl. Chem., Vol. 65, No. 3, pp. 551–556, 1993.
Printed in Great Britain.

Molecular recognition by novel cage-type azaparacyclophanes bearing chiral binding sites in aqueous media

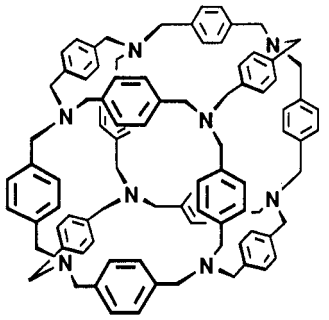
Yukito Murakami, Osamu Hayashida, Toshihiro Ito and Yoshio Hisaeda

Department of Chemical Science and Technology, Faculty of Engineering, Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812, Japan

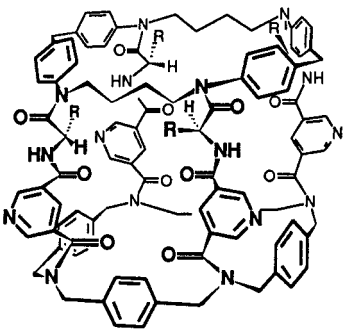
Abstract – Novel cage-type cyclophanes which are constructed with two rigid macrocyclic skeletons, tetraaza[6.1.6.1]paracyclophane and tetraaza[3.3.3.3]-paracyclophane, and four chiral bridging components were prepared. An asymmetric character of the internal cavity was clarified by means of circular dichroism (CD) spectroscopy and a computer-aided molecular modeling study based on molecular mechanics and dynamics (BIOGRAF, Dreiding-I and Dreiding-II) conformational search. In aqueous media, the present hosts strongly bound anionic and nonionic hydrophobic guests to form inclusion complexes in 1:1 stoichiometry and the CD phenomena were induced in an incorporated achiral guest molecule through its stereochemical interaction with the chiral host cavity. In addition, the present hosts exhibited discriminative recognition toward steroid hormones in D_2O/CD_3OD (3:1 v/v) as effected by hydrophobic and π–π interactions. The chirality-based discrimination of estrogens was attributed to their different modes of hydrogen bonding with the hosts.

INTRODUCTION

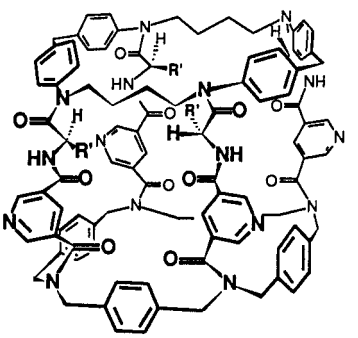
Currently, there is growing interest in molecular recognition by cyclophanes in order to mimic specific functions of naturally occurring supramolecular hosts, such as enzymes and receptors (ref. 1). The overall guest-binding ability of a host molecule in aqueous media is highly dependent on the hydrophobic character of the host cavity, and enhanced as the hydrophobicity increases since noncovalent host–guest interactions become more effective in well-desolvated and hydrophobic microenvironments. Although moderate guest recognition has been exercised by various cyclophanes composed of a single macrocyclic skeleton, more specific molecular recognition can be achieved by modified cyclophane hosts capable of providing a three-dimensionally extended internal cavity. On these grounds, we have recently developed Kyuphane (**1**) composed of six faces, each being constructed with a 2,11,20,29-tetraaza[3.3.3.3]-paracyclophane ring, as a cage-type host (ref. 2). Host **1** is soluble in acidic aqueous media below pH 4, and exhibits the following unique functions with regard to molecular recognition: (1) **1** demonstrates a pH-dependent guest-binding ability due to change in the specific microenvironmental polarity of its three dimensional cavity upon variable protonation of the nitrogen atoms. (2) **1** shows size- and shape-sensitive molecular discrimination originating from the rigid geometry of the hydrophobic cavity and the specific protonation geometry. (3) The proton NMR signals of guest molecules, such as naphthalene-2,6-disulfonate, 8-anilinonaphthalene-1-sulfonate and 6-*p*-toluidinonaphtalene-2-sulfonate, completely disappear upon complexation with **1**. Meanwhile, binding sites of naturally occurring enzymes and receptors are constructed with various optically active amino acid residues so that these supramolecules show outstanding chiral recognition toward substrates and other external substances. In this context, the next strategy is to get further insights into the chirality-based molecular recognition behavior of cage-type cyclophanes toward various guests in aqueous media. From such a viewpoint, we prepared novel cage-type peptide cyclophanes bearing chiral binding sites provided by L- and D-valine residues [(+)-**2** and (–)-**2**, respectively] (ref. 3). Both (+)-**2** and (–)-**2** are soluble in acidic aqueous media and behave as polycationic hosts. In order to obtain water-soluble hosts in aqueous media over a wide pH range, we modified (+)-**2** and (–)-**2** by introducing a pyridinium moiety into each bridging component to afford cationic cage-type peptide cyclophanes [(+)-**3** and (–)-**3**] (refs. 4, 5). Non-cage cyclophanes were also synthesized by introducing the identical L- and D-amino acid residues into a rigid tetraaza[6.1.6.1]paracyclophane skeleton [(+)-**4** and (–)-**4**, respectively] as references in order to characterize the specific molecular recognition feature of the cage molecules.



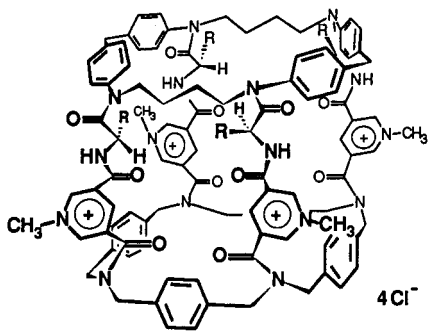
1



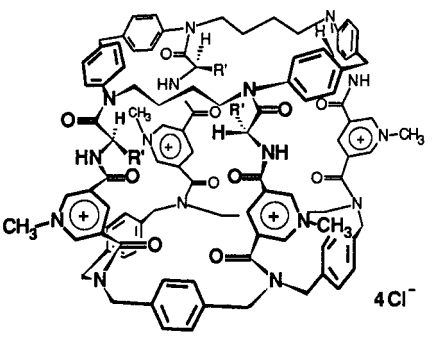
(+)-**2** : R = $CH(CH_3)_2$



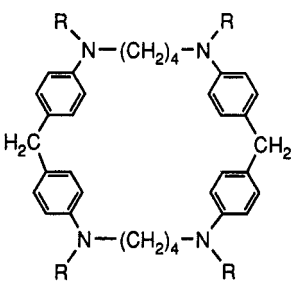
(−)-**2** : R' = $CH(CH_3)_2$

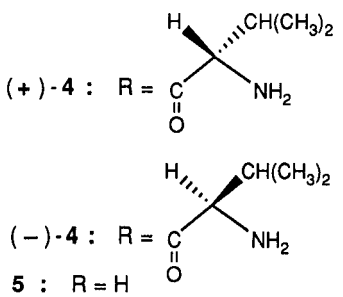


(+)-**3** : R = $CH(CH_3)_2$



(−)-**3** : R' = $CH(CH_3)_2$

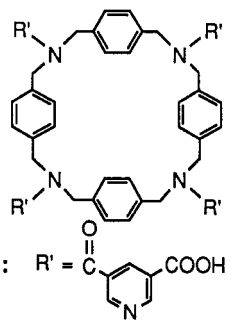




(+)-**4** : R =

(−)-**4** : R =

5 : R = H



6 : R' =

PREPARATION OF CHIRAL CYCLOPHANE HOSTS

Peptide cyclophanes

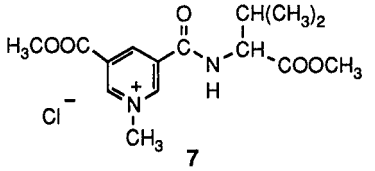
A peptide cyclophane bearing L-valine moieties, *N,N',N'',N'''*-tetrakis(2-aminoisovaleryl)-1,6,20,25-tetraaza[6.1.6.1]paracyclophane [(+)-**4**], was prepared by condensation of 1,6,20,25-tetraaza [6.1.6.1]paracyclophane (**5**) (ref. 3) with *tert*-butoxycarbonyl-L-valine in the presence of dicyclohexylcarbodiimide (DCC), followed by removal of the protecting groups. The use of *tert*-butoxycarbonyl-D-valine in place of *tert*-butoxycarbonyl-L-valine afforded the corresponding peptide cyclophane bearing D-valine moieties [(−)-**4**].

Cage-type peptide cyclophanes

A novel cage-type peptide cyclophane bearing chiral binding sites provided by L-valine residues [(+)-**2**] was synthesized by condensation of *N,N',N'',N'''*-tetrakis(5-carboxynicotinoyl)-2,11,20,29-tetraaza[3.3.3.3]paracyclophane (**6**) with (+)-**4** in the presence of diethyl cyanophosphonate (DECP) under high dilution conditions at 0°C (ref. 3). A water-soluble cage-type peptide cyclophane [(+)-**3**] was derived from (+)-**2** by a reaction with methyl iodide and the subsequent replacement of the counterion iodide with chloride (ref. 4). The use of (−)-**4** in place of (+)-**4** afforded the corresponding cage-type peptide cyclophane bearing chiral binding sites provided by D-valine residues [(−)-**2**], and (−)-**2** was converted to (−)-**3** after the method applied to the preparation of (+)-**3** (ref. 5).

ASYMMETRIC CHARACTER OF CAGE-TYPE PEPTIDE CYCLOPHANES

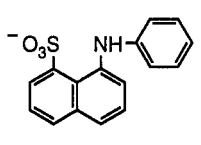
The asymmetric character of the present hosts was examined by means of circular dichroism (CD) spectroscopy and computer-aided molecular modeling study. Cage-type hosts (+)-**3** and (–)-**3** show CD bands in aqueous 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) buffer (0.01 mol dm^{-3}, pH 7.0, μ 0.1 with KCl) at 30 °C; [Θ] +1.04 x 10^5 and –1.10 x 10^5 deg cm^2 $dmol^{-1}$ for (+)-**3** and (–)-**3**, respectively, at their respective CD peak wavelengths of 242 and 244 nm. (refs. 4, 5); see Fig. 1 for (+)-**3**. On the other hand, peptide cyclophanes (+)-**4**, (–)-**4** and **7** (a bridging component analogue of (+)-**3**) do not show any detectable CD bands in a relatively wide wavelength range. These results suggest that the four pyridinium moieties bound to the chiral valine residues in the bridging segments of (+)-**3** and (–)-**3** approach close to each other and are twisted in the same direction [see Fig. 2(a)]. Such helical conformations of (+)-**3** and (–)-**3** seem to be caused by the chirality of the valine residues in the bridging segments in the light of minimum energy conformations for these hosts in the gas phase, as obtained on the basis of molecular mechanics and dynamics [BIOGRAF, Dreiding-I and Dreiding-II (ref. 6)] calculations on an IRIS-4D/220GTX workstation (Silicon Graphics). Moreover, the twisted direction of bridging components in (+)-**3** is opposite to that evaluated for (–)-**3**, so that (+)-**3** furnishes an internal cavity different from that of (–)-**3** for chiral recognition. A similar asymmetric character in the internal cavities of hosts (+)-**2** and (–)-**2** was also confirmed by the identical methods (ref. 3).



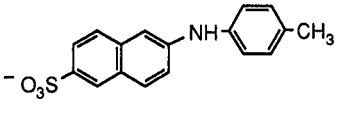
7

INCLUSION BEHAVIOR TOWARD ACHIRAL GUEST MOLECULES IN AQUEOUS MEDIA

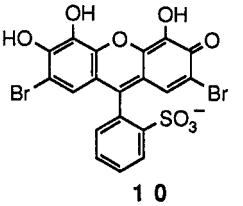
The guest-binding behavior of azaparacyclophanes toward various hydrophobic molecules was examined by electronic absorption and fluorescence spectroscopy in the following aqueous buffers (0.01 mol dm^{-3}, μ 0.10 with KCl) at 30 C°: acetate and HEPES for pH 4.1 and 7.0, respectively. The following hydrophobic probe were adopted as guest molecules: 8-anilinonaphthalene-1-sulfonate (**8**), 6-*p*-toluidinonaphthalene-2-sulfonate (**9**), 5,5'-dibromopyrogallolsulfonphthalein (**10**), 2,4-dinitro-1-naphthol-7-sulfonate (**11**), 1-phenylazo-2-naphthol-6,8-disulfonate (**12**), 2,7-bis[(4-methyl-2-sulfophenyl)azo]-1,8-dihydroxynaphthalene-3,6-disulfonate (**13**), 1-(2-pyridylazo)-2-naphthol (**14**), *N*-phenyl-1-naphthylamine (**15**), *N,N*-dimethyl-*p*-phenylazoaniline (**16**), [[1-(dimethylamino)naphthalene-5-sulfonamido]ethyl]trimethylammonium (**17**) and 2-[4-(dimethylamino)-styryl]-1-ethylquinolinium (**18**).



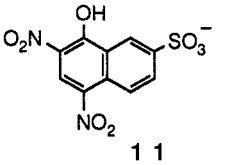
8



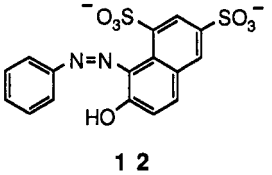
9



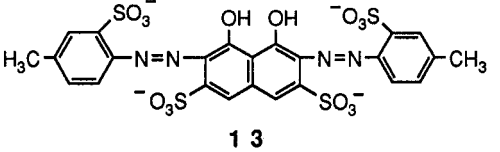
10



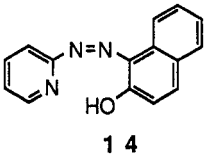
11



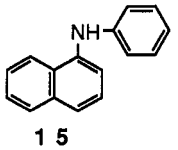
12



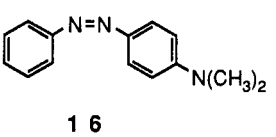
13



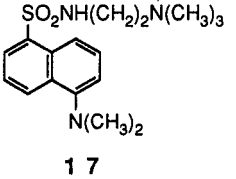
14



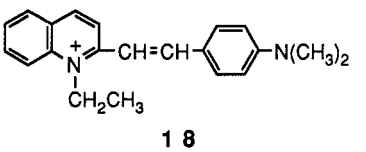
15



16



17



18

In general, electronic and fluorescence spectra of the guest molecules were measured by changing the concentration of each host. Binding constants for the formation of inclusion complexes of the hosts with

various guest molecules in a 1:1 molar ratio (K) were evaluated on the basis of the Benesi-Hildebrand relationship (ref. 7) in a manner as described previously (ref. 8), and are summarized in Table 1. The K values for the cage-type hosts with anionic and nonionic guests are greater by 1–2 orders of magnitude than the corresponding values for peptide cyclophane (+)-**4**, due to an enhanced hydrophobic effect given out by the former hosts. In addition, the electrostatic interaction between these host and the guest molecules is another effective recognition factor so that the cationic guests can not be incorporated into the cationic hosts.

TABLE 1. Binding constants (K/dm^3 mol^{-1}) for host–guest complexes of cyclophanes with various guests in aqueous media at 30 °C.

Guest	Method[a]	Host[b]		
		(+)-**2**	(+)-**3**	(+)-**4**
8	F	2.8×10^4		5.4×10^2
9	F	5.8×10^4		
10	E	5.8×10^4	2.5×10^5	
11	E	5.5×10^4	3.7×10^5	4.0×10^3
12	E		1.2×10^5	
13	E	3.8×10^4	3.4×10^5	5.0×10^3
14	E		1.0×10^6	
15	F	2.0×10^4		5.7×10^2
16	E		6.5×10^5	
17	F	–[c]		–[c]
18	E		–[c]	–[c]

[a] F, fluorescence spectroscopy; E, electronic absorption spectroscopy. [b] pH values for measurements: (+)-**2** and (+)-**4**, 4.1; (+)-**3**, 7.0. [c] Complex formation was not detected.

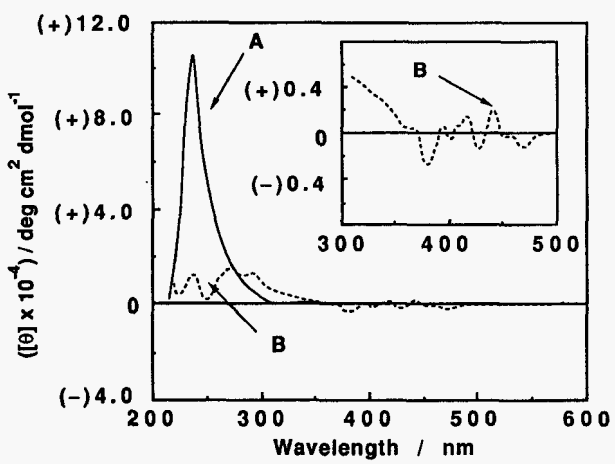


Fig. 1. CD spectra of (+)-**3** (7.0×10^{-5} mol dm^{-3}) in aqueous HEPES buffer at 30.0 °C: A, without any guest; B, in the presence of **11** (2.1×10^{-4} mol dm^{-3}).

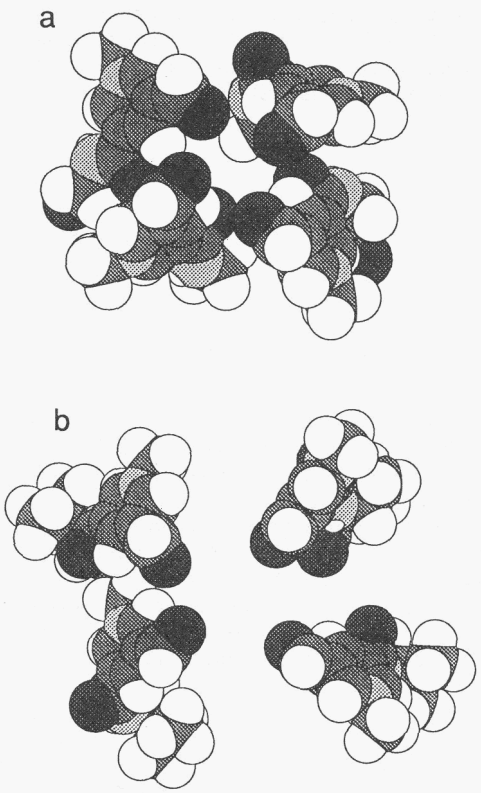


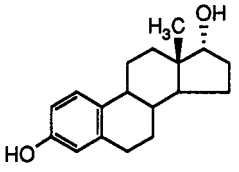
Fig. 2. Space-filling models optimized conformationally for (+)-**3** (a, top) and a complex of (+)-**3** with **11** (b, bottom); two macrocyclic rings of the host and **11** are removed for simplicity to show conformational changes in the bridging moieties of (+)-**3**.

The formation of inclusion complexes of (+)- and (–)-**3** and the hydrophobic guest molecules cited above was also detected by CD spectroscopy. Upon addition of hydrophobic molecules, such as **11**, **12**, **13** and **14**, to an aqueous HEPES buffer (0.01 mol dm^{-3}, pH 7.0, μ 0.10 with KCl) containing (+)-**3**, the CD band originated from the host was weakened in intensity along with appearance of induced CD bands in the absorption ranges of the guests (Fig. 1). The decrease in CD band intensity at 242 nm is attributable to conformational changes around the pyridinium moieties of (+)-**3** upon complexation with the guest molecules to form thermodynamically stable complexes. The computational evaluation reveals that the pyridinium moieties in the bridging components of (+)-**3** are separated from each other as the guest molecule is incorporated into the internal cavity of the host [Fig. 2(b)]. As a consequence, the CD

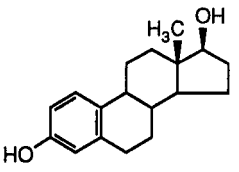
phenomena are induced in the incorporated guest molecule through its stereochemical interaction with the chiral host cavity.

MOLECULAR DISCRIMINATION TOWARD STEROID HORMONES IN AQUEOUS MEDIA

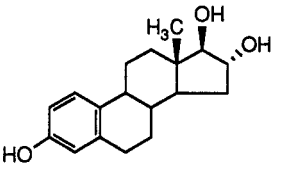
Specific guest-binding abilities of water-soluble cage-type peptide cyclophanes (+)-**3** and (−)-**3** and peptide cyclophanes (+)-**4** and (−)-**4** toward the following steroid hormones were examined in order to characterize their chirality-based molecular discrimination behavior: estrogens such as α-estradiol (**19**), β-estradiol (**20**) and estriol (**21**); androgen such as testosterone (**22**). The molecular recognition of these hosts toward the various guests was investigated by means of ^{1}H NMR spectroscopy in D_2O/CD_3OD (3:1 v/v) at 300 K.



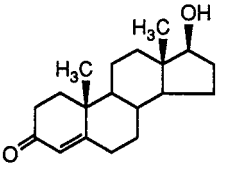
19



20



21



22

Upon addition of the hosts to each of solutions of individual guests, all the ^{1}H NMR signals due to the guests were subjected to substantial upfield shifts, except for **22**, reflecting formation of the host–guest complexes. The present hosts were found to undergo complexation with the guest in a 1:1 molar ratio of host to guest as confirmed by the Job's continuous variation method (ref. 9). The binding constants (*K*) for 1:1 host–guest complexes and complexation-induced shifts (CIS), the shifts of NMR signals for the guests upon complete complexation (ref. 10), were evaluated by means of the computer-aided least-squares curve fitting method applied to NMR titration data. The evaluated *K* values are listed in Table 2. Hosts **3** and **4** apparently include the estrogens having an aromatic moiety, such as **19**, **20** and **21**. The CIS values obtained for **20** upon complexation with (+)-**4** prove that the aromatic moiety of the guest is incorporated into the internal macrocyclic cavity of peptide cyclophane (+)-**4** (refer to Fig. 3) in a manner similar to those exercised by various cyclophanes reported up to the present time (ref. 11). The CIS values for **20** upon complexation with (+)-**3** are relatively smaller than those for the identical guest upon complexation with (+)-**4** (refer to Fig. 3). Consequently, the guest is undoubtedly placed in the three-dimensional cavity provided intramolecularly by the two macrocyclic rings and the four bridging components of host (+)-**3**. On the other hand, the present hosts show no capacity of binding a fully aliphatic guest, **22**. This means that hosts (+)- and (−)-**3** as well as (+)- and (−)-**4** are the potent hydrophobic hosts showing unique selectivity toward guest molecules through hydrophobic and π–π interactions.

TABLE 2. Binding constants (K/dm^3 mol^{-1}) for host–guest complexes of cyclophanes with various steroid hormones in D_2O/CD_3OD (3:1 v/v) at 27 °C.

Guest	Host			
	(+)-**3**	(−)-**3**	(+)-**4**	(−)-**4**
19	460	1300	540	600
20	760	700	620	610
21	360	520	360	340
22	–[a]	–[a]	–[a]	–[a]

[a] Complex formation was not detected by ^{1}H NMR spectroscopy

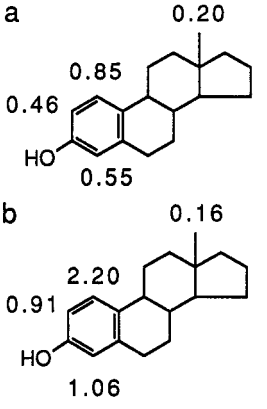


Fig. 3. Selected CIS values (ppm; minus sign is omitted) for **20** upon complexation with (+)-**3** (a, top) and (+)-**4** (b, bottom).

Moreover, hosts (+)- and (−)-**3** show chiral recognition behavior toward **19** and **20** through stereochemical interactions of the asymmetric host cavities with these guests. As is obvious from the data in Table 2, the *K* values for complexation of (−)-**3** with **19** is greater than the corresponding value with **20**, whereas (+)-**3** show a larger affinity for **20** relative to that for **19**. On the other hand, peptide

cyclophanes (+)- and (–)-**4** show no capacity of performing effective diastereo-selectivity toward **19** and **20**. It now becomes apparent that the three-dimensionally extended hydrophobic cavity with the chiral binding sites provided by the cage-type host effectively performs the sophisticated and chiral recognition toward various guests to afford diastereo-selective complexes. The computational evaluations reveal that total molecular energies (E_{total}) for the lowest energy conformations are 2113.71 and 2128.96 kJ mol^{-1} for the (–)-**3•19** and (–)-**3•20** complexes, respectively (ref. 12). The hydrogen-bonding interaction between the guest and the chiral valine residues of (–)-**3** seems to be much favored for **19** relative to **20** [E_{hb} = –32.49 and 0.00 kJ mol^{-1} for (–)-**3•19** and (–)-**3•20**, respectively], and this effect must cause an apparent difference in stability between these diastereomeric complexes. A similar difference in stability between (+)-**3•19** and (+)-**3•20** was also evidenced by a computer-aided molecular modeling study.

CONCLUSION

Cage-type peptide cyclophanes **2** and **3** were synthesized on the basis of molecular design that allows to connect two rigid macrocyclic skeletons with four bridging components. The helically twisted and globular hydrophobic cavities provided by the hosts are suitable for chiral recognition toward diastereomeric guests through mutual stereochemical interactions in aqueous media. We believe that our concept on molecular design provides a useful guidepost for preparation of multifunctional receptor models that are capable of performing chirality-based molecular discrimination.

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.

REFERENCES

1. Y. Murakami, *J. Phenomena and Molecular Recognition*, ed. J. L. Atwood, pp. 107–117, Plenum Press, New York (1990); Y. Murakami, J. Kikuchi, Y. Hisaeda, and T. Ohno, *Frontiers in Supramolecular Organic Chemistry and Photochemistry*, ed. H.-J. Schneider and H. Dürr, pp. 145–166, VCH Verlagsgesellschafts, Weinheim (1991); J.-M. Lehn, *Angew. Chem., Int. Ed. Engl.* **29**, 1304–1319 (1990).
2. Y. Murakami, J. Kikuchi, T. Ohno, T. Hirayama, Y. Hisaeda, H. Nishimura, J.Snyder, and K. Steliou, *J. Am. Chem. Soc.* **113**, 8229–8242 (1991).
3. Y. Murakami, T. Ohno, O. Hayashida, and Y. Hisaeda, *J. Chem. Soc., Chem. Commun.* 950–952 (1991).
4. Y. Murakami, T. Ohno, O. Hayashida, and Y. Hisaeda, *Chem. Lett.* 1595–1598 (1991).
5. Y. Murakami, O. Hayashida, T. Ito, and Y. Hisaeda, *Chem. Lett.* 497–500 (1992).
6. L. Mayo, B. D. Olafson, and W. A. Goddard III, *J. Phys. Chem.* **94**, 8897–8909 (1990).
7. H. A. Benesi, J. H. Hildebrand, *J. Am. Chem. Soc.* **71**, 2703–2707 (1949).
8. Y. Murakami, J. Kikuchi, M. Suzuki, and T. Matsuura, *J. Chem. Soc., Perkin Trans. 1* 1289–1299 (1988).
9. Y. Kikuchi, Y. Kato, Y. Tanaka, H. Toi, and Y. Aoyama, *J. Am. Chem. Soc.* **113**, 1349–1354 (1991)
10. H.-J. Schneider, K. Rüdiger, S. Suetlana, and S. Ulrich, *J. Am. Chem. Soc. 110*, 6442–6448(1988).
11. D. R. Carcanague and F. Diederich, *Angew. Chem., Int. Ed. Engl.* **29**, 769–771 (1990); K. Odashima, A. Itai, Y. Iitaka, Y. Arata, and K. Koga, *Tetrahedron Lett.* **21**, 4351–4354(1980).
12. The total molecular energy (E_{total}) is expressed as an energy sum of bonded and non-bonded interactions, and a conformation with the lowest molecular energy is regarded to be the most favorable one for a diasteromeric complex (Eq. 1).

$$E_{total} + E_b + E_\theta + E_\varphi + E_i + E_{vdw} + E_{el} + E_{hb} \quad (1)$$

The bonded interactions consist of bond stretching (E_b), bond angle bending (E_θ), dihedral angle torsion ($E\varphi$) and inversion (E_i) terms, while the non-bonded interactions are composed of van der Waals (E_{vdw}), electrostatics (E_{el}) and hydrogen bond (E_{hb}) terms.