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Discrimination of Steroid Hormones by Novel Cage-type Azacyclophanes Bearing Chiral Binding Sites

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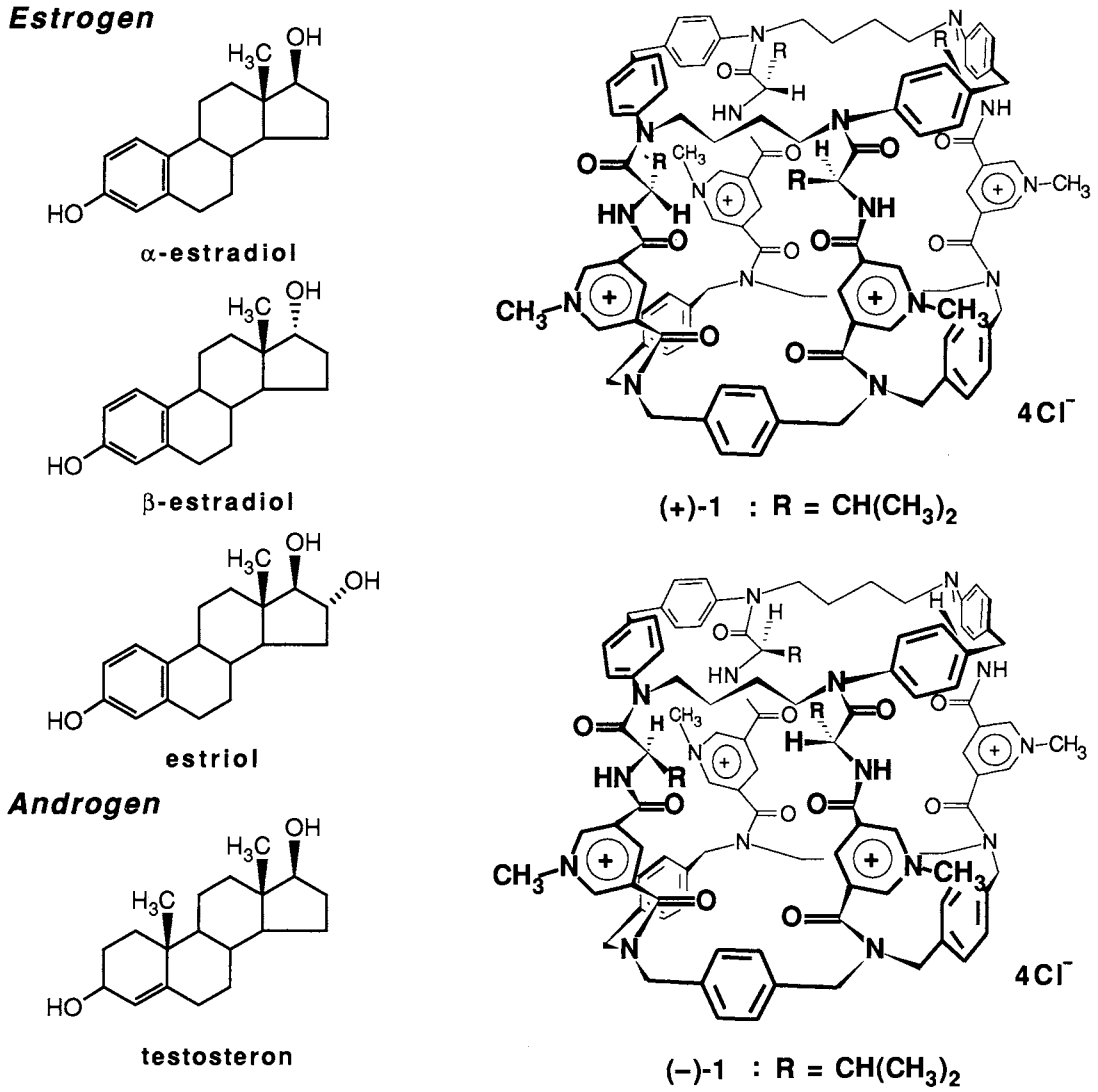
Novel cage-type azacyclophanes bearing chiral binding sites provided by L- and D-valine residues exhibited discrimination toward steroid hormones in D_2O-CD_3OD (3:1 v/v), as effected by hydrophobic and $\pi-\pi$ interactions. In addition, chirality-based discrimination of α- and β-estradiol was attributed to their different modes of hydrogen bonding with the hosts.

Various artificial hosts have been designed to mimic the molecular discrimination functions performed by receptors and enzymes in biological systems.[1] Recently, much attention has been focused on chiral recognition of guest molecules by macrocyclic hosts.[2] In this context, we became interested in the host-guest chemistry of cyclophanes which provide three-dimensionally extended hydrophobic cavities constructed with rigid macrocyclic skeletons.[3] We report here the preparation and unique guest recognition ability of cationic cage-type cyclophanes bearing chiral binding sites provided by L- and D-valine residues [(+)-**1**[4] and (−)-**1**, respectively] and their inclusion performance toward steroid hormones in D_2O/CD_3OD (3:1 v/v).

A cage-type cyclophane with D-valine residues [(−)-**1**] was prepared by a procedure identical with that applied to the synthesis of (+)-**1**:[4] mp 268–270 °C (decomp), $[\alpha]_D^{25}$ −118° (c 0.3, CH_3OH); 500 MHz ^1H NMR (DMSO-d_6, 343 K, TMS) δ = 0.8 (24H, br s, $CH(C\underline{H}_3)_2$), 1.3 (8H, m, $NCH_2C\underline{H}_2$), 1.4 (4H, m, $C\underline{H}(CH_3)_2$), 3.2 (8H, m, $NC\underline{H}_2CH_2$), 3.5 (12H, m, NCH_3), 4.0 (4H, br s, Ar-$C\underline{H}_2$-Ar), 4.1 (4H, m, CO-CH), 4.4 (16H, m, Ar-$C\underline{H}_2$-N), 7.1 (32H, m, Ar-H), 9.0 (4H, m, Py-H4), 9.2 (4H, m, Py-H2), and 9.5 (4H, m, Py-H6); IR (KBr) 2940 (CH) and 1640 (C=O) cm^{-1} ; MS (ESI) m/z 2104 (M^+). Found: C, 66.06; H, 6.18; N, 10.43%. Calcd for $C_{118}H_{128}N_{16}O_{12}Cl_4 \cdot 2H_2O$: C, 66.22; H, 6.22; N, 10.47%.

We have previously examined the asymmetric character of host (+)-**1** by means of a computer-aided molecular modeling study and circular dichroism (CD) spectroscopy.[4] In order to compare the chiral internal cavity of host (−)-**1** with that of host (+)-**1** for molecular recognition, we investigated in this work the asymmetric character of (−)-**1** by the identical methods. Host (−)-**1** shows a CD band at 244 nm with molecular ellipticity ([Θ], deg cm^2 $dmol^{-1}$) of -1.10×10^5 in an aqueous HEPES buffer [0.01 mol dm^{-3}, pH 7.0, μ 0.10 (KCl)] at 30.0°C. Optimized conformations of (−)-**1** in the gas phase were also examined by molecular mechanics and dynamics (BIOGRAF, Dreiding-I and Dreiding-II[5]) calculations on an IRIS-4D/220GTX workstations (Silicon Graphics). The result reveals that the four pyridinium moieties bound to the



chiral D-valine residues in the bridging components are twisted in the same direction. However, the twisted direction in (−)-**1** is opposite to that predicted for (+)-**1**, so that (−)-**1** furnishes an internal cavity different from that of (+)-**1** for chiral molecular recognition. In order to clarify specific guest-binding ability of **1**, we examined the chirality-based molecular discrimination behavior toward steroid hormones: estrogens such as α-estradiol, β-estradiol, and estriol; androgen such as testosteron. Complexation of the hormones by **1** was investigated by means of ^{1}H NMR spectroscopy in D_2O/CD_3OD (3:1 v/v) at 300 K, and the complexation-induced shifts (CIS)[6] of discrete signals of the aromatic and methyl protons of the guests were evaluated by the computer-aided least-squares curve fitting method applied to NMR titration data. Table 1 summarizes the binding constants (K)[7] and the free energies of formation ($-\Delta G$) for the diastereomeric complexes composed of the steroid hormones and the two optically active hosts. These hosts incorporate the steroid hormones having an aromatic moiety such as α-estradiol, β-estradiol, and estriol. The CIS values (Table 2) evaluated for complete complexation prove that the aromatic moiety of these guests is located in the internal cavities of **1**. On the other hand, both hosts show no capacity of binding a fully aliphatic guest, testosteron. This means that the

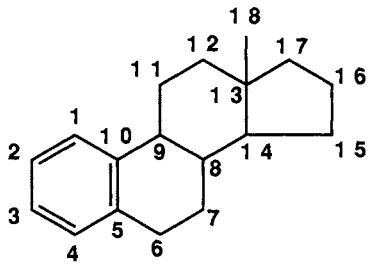
Table 1. Binding constants (K) and free energies of formation ($-\Delta G$) for the diastereomeric complexes of **1** formed with steroid hormones in D_2O/CD_3OD (3:1 v/v) at 300 K

Steroid	(+)-**1**		(−)-**1**		
	K[a]	$-\Delta G$	K[a]	$-\Delta G$	$\Delta(-\Delta G)$[b]
	$dm^3\ mol^{-1}$	$kJ\ mol^{-1}$	$dm^3\ mol^{-1}$	$kJ\ mol^{-1}$	$kJ\ mol^{-1}$
α-Estradiol	460	15.4	1300	17.9	−2.5
β-Estradiol	760	16.7	700	16.4	0.3
Estriol	360	14.8	520	15.6	−0.8
Testosteron	——[c]		——[c]		

a) Obtained by nonlinear least-squares curve fitting of 500-MHz ^1H NMR titration: guest, 5.0 x 10^{-4} mol dm^{-3}; **1**, 2.5 x 10^{-4} – 2.0 x 10^{-3} mol dm^{-3}. b) Calculated difference in stability between diastereomeric complexes. c) Complex formation was not detected by ^1H NMR spectroscopy.

Table 2. Complexation-induced shifts (CIS) for selected protons of guest molecules included in host **1**[a]

Host	Guest	CIS			
		CH(1)	CH(2)	CH(4)	CH_3(18)
(+)-**1**	α-Estradiol	-0.88	-0.50	-0.60	-0.29
(+)-**1**	β-Estradiol	-0.85	-0.46	-0.55	-0.20
(+)-**1**	Estriol	-0.85	-0.40	-0.51	-0.16
(−)-**1**	α-Estradiol	-0.74	-0.31	-0.40	-0.25
(−)-**1**	β-Estradiol	-0.86	-0.36	-0.48	-0.25
(−)-**1**	Estriol	-0.90	-0.42	-0.58	-0.24



Steroid framework

a) Refer to Table 1 for measurement conditions.

cage-type cyclophanes, (+) and (−)-**1**, are the potent hydrophobic hosts showing unique selectivity toward guest molecules through hydrophobic and π–π interactions. The selective recognition toward aromatic guests was also observed with other host molecules such as 1,6,20,25-tetraaza[6.1.6.1]paracyclophane;[8] one of the macrocyclic components of the present cage-type hosts. Moreover, the present hosts show chiral recognition behavior toward α- and β-estradiol through stereochemical interactions of the asymmetric host cavities with these guests. As is obvious from the data in Table 1, the binding constant for complexation of (−)-**1** with α-estradiol is greater than the corresponding value with β-estradiol, whereas (+)-**1** shows larger affinity for β-estradiol relative to that for α-estradiol. Optimized conformations of the host–guest complexes in the gas phase were examined by molecular mechanics and dynamics calculations[9] on an IRIS-4D/220GTX workstation. The result reveals that the total molecular energies (E_{total}) for the lowest energy conformations are 2113.71 and 2128.96 $kJ\ mol^{-1}$ for the (−)-**1**•α-estradiol and (−)-**1**•β-estradiol

complexes, respectively. The hydrogen-bonding interaction between the guest and the chiral valine residues of host (−)-**1** seems to be much favored for α-estradiol relative to β-estradiol [E_{hb} = −32.49 and 0.00 kJ mol^{-1} for (−)-**1**•α-estradiol and (−)-**1**•β-estradiol, respectively], and this effect must cause an apparent difference in stability between these diastereomeric complexes. A similar difference in stability between (+)-**1**•α-estradiol and (+)-**1**•β-estradiol was also evidenced by a computer-aided molecular modeling study [E_{total} = 2130.17 and 2108.05 kJ mol^{-1}; E_{hb} = 0.00 and −32.61 kJ mol^{-1} for (+)-**1**•α-estradiol and (+)-**1**•β-estradiol, respectively].

In conclusion, the present cage-type hosts provide hydrophobic internal cavities that exhibit chirality-based molecular discrimination toward steroid hormons and is expected to be utilized as a multifunctional receptor model.

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7) The binding constant (K) was calculated on the basis of the following equations. The observed chemical shift (δ_{obs}) appears as an average of those for the free guest (δ_{free}) and the complexed guest (δ_{comlex}). Under an assumption that a 1:1 host–guest complex is formed (H + G = H•G), δ_{obs} is expressed by Eq. 1, which is subsequently converted to Eq. 2.

$$\delta_{obs} = \delta_{free}([G]_0 - [H\bullet G])/[G]_0 + \delta_{complex}([H\bullet G])/[G]_0 \quad (1)$$

$$\delta_{obs} = \delta_{free} + [[H]_0 + [G]_0 + K^{-1} + \{([H]_0 + [G]_0 + K^{-1})^2 - 4[H]_0[G]_0\}^{1/2}](\delta_{complex} - \delta_{free})(2[G]_0)^{-1} \quad (2)$$

Here, $[G]_0$ and $[H]_0$ stand for total concentrations of the guest and the host, respectively. The K value was determined by the computer-aided nonlinear least-squares method based on Eq. 2.

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9) The total molecular energy (E_{total}) is expressed as an energy sum of bonded and non-bonded interactions, and a diastereomer complex with a lower energy value is regarded to be more stable relative to the other (refer to Eq. 3).

$$E_{total} = E_b + E_\theta + E_\varphi + E_i + E_{vdw} + E_{el} + E_{hb} \quad (3)$$

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

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