PERKIN 2 COMMUNICATION

Discrimination between deoxycholic acid epimers by fluorescence of excimer-forming $6^A,6^E$-*O*-bis[2-(1-naphthyl)propanoyl]-γ-cyclodextrins

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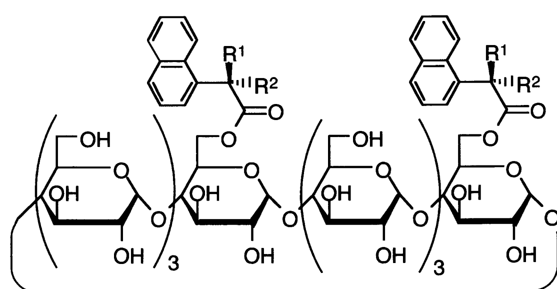
The bisnaphthyl-γ-CyD, (*R,R*)-1, which had (*R*)-2-(1-naphthyl)propanoyl moieties at A and E glucose units, showed marked capability of discriminating between chenodeoxycholic and ursodeoxycholic acids on the basis of fluorescence output.

Introduction

Cyclodextrins (CyDs) are widely known to form host–guest complexes with a wide variety of organic compounds in aqueous solution.[1] When CyDs are modified with aromatic residues either at the primary or secondary hydroxy face, the modified CyDs become spectroscopically active hosts with which monoterpenes and steroids can be detected on the basis of absorption,[2] fluorescence[3] and circular dichroism[4] variations induced by guest binding. The variations in spectroscopic properties of the modified CyDs upon guest binding are caused by their conformational changes. When enantiomeric residues are introduced to CyD, resulting CyD diastereomers are expected to show different self complexation and guest binding behaviour.[5] The difference may be emphasized if CyDs are modified with two or more enantiomeric residues. Here we wish to show very different behaviour in intramolecular excimer formation associated with self-complexation of γ-CyD bearing two (*R*)- or (*S*)-2-(1-naphthyl)propanoyl moieties at the primary hydroxy face [(*R,R*)-**1** or (*S,S*)-**1**, respectively]. These, especially (*R,R*)-**1**, were excellent hosts in discriminating between chenodeoxycholic acid and its epimer ursodeoxycholic acid.



(*R,R*)-**1**: $R^1 = CH_3$, $R^2 = H$
(*S,S*)-**1**: $R^1 = H$, $R^2 = CH_3$

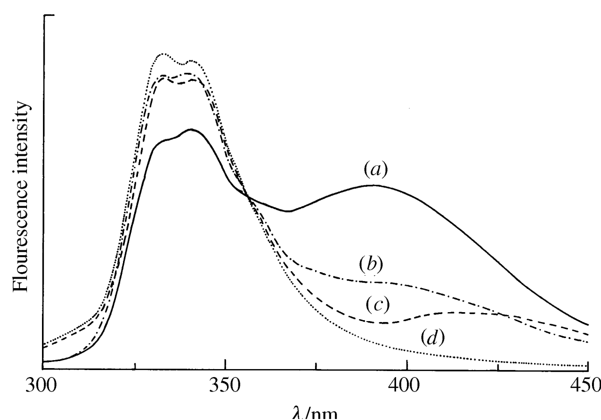


Fig. 1 Fluorescence spectra of (*R,R*)-**1** and (*S,S*)-**1** excited at 280 nm: (*a*) (*R,R*)-**1** in a 10% aqueous ethylene glycol solution; (*b*) (*S,S*)-**1** in a 10% aqueous ethylene glycol solution; (*c*) (*R,R*)-**1** in a 10% ethylene glycol methanol solution; (*d*) (*S,S*)-**1** in a 10% ethylene glycol methanol solution. Concentrations of (*R,R*)-**1** and (*S,S*)-**1** were *ca.* 3.7×10^{-5} mol dm^{-3} and the spectra were normalized at 356 nm

Experimental

Syntheses of (*R,R*)-1 and (*S,S*)-1

A mixture of $6^A,6^E$-di-*O*-tosyl-γ-CyD (150 mg, 0.093 mmol) and (*R*)- or (*S*)-2-(1-naphthyl)propanoic acid sodium salt (100 mg, 0.45 mmol) in DMSO (1 cm^3) was heated at 80 °C for 20 h. After being cooled, the mixture was poured into acetone (50 cm^3) and the precipitate formed was collected. The crude mixture was purified by HPLC (YMC S-343-15 ODS column, 20 × 250 mm; flow rate, 10 cm^3 min^{-1}). After step gradient elution (10% aqueous MeOH and 30% aqueous MeOH, each 100 cm^3), linear gradient elution was applied (50% aqueous MeOH–80% aqueous MeOH, 0.5% min^{-1}). (*R,R*)-**1**: 39 mg (23%); eluted at 59% aqueous MeOH; *m/z* (FAB) 1661 [$(M + H)^+$]; δ_H[500 MHz, $(CD_3)_2SO$] 1.38–1.55 (m, 6H), 3.02 (t, *J* 9.3 Hz, 2H), 3.3–4.1 (m, overlapped with H_2O), 4.32–4.47 (m, 6H), 4.50–4.94 (m, 8H), 5.47–5.68 (m, 16H), 7.38–8.08 (m, 14H) (Found: C, 49.93; H, 6.13. Calc. for $C_{74}H_{100}O_{42}{\cdot}7H_2O$: C, 49.72; H, 6.43%). (*S,S*)-**1**: 30 mg (18%); eluted at 62% aqueous MeOH; *m/z* (FAB) 1661 [$(M + H)^+$]; δ_H[500 MHz, $(CD_3)_2SO$] 1.44–1.58 (m, 6H), 3.3–4.2 (m, overlapped with H_2O), 4.39–4.42 (m, 6H), 4.77–4.92 (m, 8H), 5.50–5.70 (m, 16H), 7.36–7.98 (m, 14H) (Found: C, 47.95; H, 6.23. Calc. for $C_{74}H_{100}O_{42}{\cdot}10H_2O$: C, 48.26; H, 6.57%).

Results and discussion

In 10% aqueous ethylene glycol solutions, (*R,R*)-**1** and (*S,S*)-**1** showed excimer fluorescence with a peak maximum at 390 nm, together with monomer fluorescence around 330–340 nm (Fig. 1). Since no concentration dependence (7.5×10^{-5}–3.8×10^{-6} mol dm^{-3}) on fluorescence, UV absorption or circular dichroism spectra was observed, the excimer fluorescence was attributed to intramolecular excimer fluorescence. The intensity of the excimer fluorescence of (*R,R*)-**1** was greater than that of (*S,S*)-**1**, indicating that the naphthyl moieties of (*R,R*)-**1** are in a more favourable position to form an excimer than those of (*S,S*)-**1**. The naphthyl moieties of (*R,R*)-**1** were probably fully accommodated in the hydrophobic cavity whereas those of (*S,S*)-**1** were accommodated only partly in the cavity. In the UV absorption spectra of (*R,R*)-**1** and (*S,S*)-**1** (data not shown), the ^1B_b transition of (*R,R*)-**1** appeared at 221 nm ($\varepsilon = 1.09 \times 10^5$ dm^3 mol^{-1} cm^{-1}) with a shoulder at *ca.* 224 nm in a 10% aqueous ethylene glycol solution. The ^1B_b transition of (*R,R*)-**1** was observed at 224 nm in 10% ethylene glycol MeOH solution,

Table 1 Binding constants (K_g), changes in excimer and normal fluorescence intensity ($\Delta I/I^0$), and shift in excimer fluorescence ($\Delta\lambda$) of (*R*,*R*)-**1** and (*S*,*S*)-**1** in 10% aqueous ethylene glycol solutions at 25 °C

Host	Guest	$K_g{}^a$/dm³ mol⁻¹	Conc.^{b}/mol dm⁻³	$\Delta I_{ex}/I_{ex}{}^{0\,c}$	$\Delta I_n/I_n{}^{0\,d}$	$\Delta\lambda^e$/nm
(*R*,*R*)-**1**	BL	6 440	1.0	0.073	−0.099	0
	CDCA	65 300	0.1	0.099	−0.123	+8
	UDCA	7 180	0.1	−0.337	−0.007	+3
(*S*,*S*)-**1**	BL	11 600	1.0	0.108	−0.051	—
	CDCA	74 300	0.1	−0.443	0.135	—
	UDCA	25 000	0.1	−0.500	0.020	—

^{a} K_g values were obtained from guest-induced fluorescence variations. ^{b} Guest concentration at which the $\Delta I/I^0$ values were collected. ^{c} $\Delta I_{ex} = I_{ex} - I_{ex}{}^0$; I_{ex} and $I_{ex}{}^0$ represent the intensity in the excimer fluorescence (390 nm) before and after the addition of guests. ^{d} $\Delta I_n = I_n - I_n{}^0$; I_n and $I_n{}^0$ represent the intensity in the normal fluorescence (340 nm) before and after the addition of guests. ^{e} $\Delta\lambda$ = (peak maximum of initial excimer fluorescence) − (peak maximum of excimer fluorescence at the given guest concentration). The values for (*S*,*S*)-**1** could not be obtained accurately because the peak maximum of the excimer fluorescence was obscured.

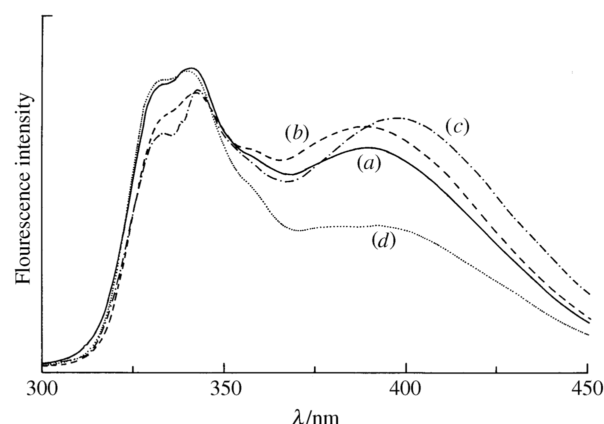
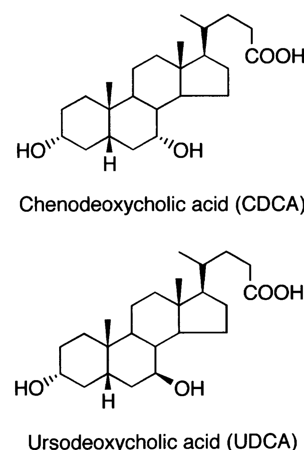


Fig. 2 Fluorescence spectra of (*R*,*R*)-**1** in 10% aqueous ethylene glycol solutions, (*a*) alone (3.67 × 10⁻⁵ mol dm⁻³); or in the presence of (*b*) (−)-borneol (1.0 × 10⁻³ mol dm⁻³); (*c*) chenodeoxycholic acid (1.0 × 10⁻⁴ mol dm⁻³) and (*d*) ursodeoxycholic acid (1.0 × 10⁻⁴ mol dm⁻³)



where neither intramolecular nor intermolecular complexation was expected for CyD derivatives. The hypsochromism observed for (*R*,*R*)-**1** in a 10% aqueous ethylene glycol solution indicates that the two naphthyl moieties of (*R*,*R*)-**1** strongly interacted with each other, even in the ground state. In contrast to (*R*,*R*)-**1**, the ^1B_b transition of (*S*,*S*)-**1** was observed at 224 nm ($\varepsilon = 1.09 \times 10^5$ dm³ mol⁻¹ cm⁻¹) with a shoulder at 220 nm in a 10% aqueous ethylene glycol solution. The difference between (*R*,*R*)-**1** and (*S*,*S*)-**1** is only the configuration around the C-2 position of the 2-(1-naphthyl)propanoyl groups. Therefore, the configuration at this position seems to determine the efficiency of the intramolecular excimer formation of $6^A,6^E$-bis[2-(1-naphthyl)propanoyl]-γ-CyDs. Note that, as seen in Fig. 1, (*R*,*R*)-**1** was able to form an excimer even in a 10% ethylene glycol MeOH solution where (*S*,*S*)-**1** did not show excimer fluorescence. A peak maximum of the excimer fluorescence of (*R*,*R*)-**1** in a 10% ethylene glycol MeOH solution appeared at 414 nm, shifting from 390 nm which was observed in a 10% aqueous ethylene glycol solution. This indicates that the two naphthyl groups of (*R*,*R*)-**1** adopt an intrinsically favourable conformation to form the excimer even when they exist outside the cavity.

The shift in the maximum of excimer fluorescence peak would promise (*R*,*R*)-**1** to be used for a molecular detection system because upon guest binding the peak maximum position was expected to change, depending on the type of guest species. This is a great difference from other molecular detection systems based on modified CyDs because they exhibited only a change in the intensity of fluorescence, absorption and circular dichroism.[2–4] Indeed, the peak maximum of the excimer fluorescence varied from guest to guest as seen in Fig. 2, where typical fluorescence spectra of (*R*,*R*)-**1** obtained in the presence of (−)-borneol (BL), chenodeoxycholic acid (CDCA) and ursodeoxycholic acid (UDCA) in 10% aqueous ethylene glycol solutions are presented. In addition to the excimer fluorescence, the monomer fluorescence intensity was also different from guest to guest. The guest-induced fluorescence intensity changes of (*R*,*R*)-**1** and (*S*,*S*)-**1** for several guests were summarized in Table 1 together with the binding constants and the shifts in the peak position of the excimer fluorescence.

A small guest relative to the γ-CyD cavity such as BL increased the intensity of the excimer fluorescence of (*R*,*R*)-**1** and decreased the monomer fluorescence intensity, while the peak maximum of the excimer fluorescence was unchanged. Thus, accommodation of BL by (*R*,*R*)-**1** may result in the enhancement in efficiency of the excimer formation between the naphthyl moieties, presumably at the rim of the primary hydroxy side. CDCA increased the excimer fluorescence intensity of (*R*,*R*)-**1** and the monomer fluorescence intensity decreased at a guest concentration of 0.1 mmol dm⁻³. The position of the peak maximum of the excimer fluorescence was shifted by 9 nm toward a longer wavelength region. This suggests that the excimer formed in the presence of CDCA has a different conformation from that induced by BL. The inconsistency in the spectral shape change induced by BL and CDCA is also indicative of excitation energy migration between the naphthyl moieties.[7] In contrast to BL and CDCA, UDCA, which is an epimer of CDCA at the C-7 position on the steroidal framework, strongly suppressed the excimer formation. The monomer fluorescence intensity was enhanced by UDCA. The small shift (3 nm) in the peak maximum of the excimer fluorescence was caused by the presence of 0.1 mmol dm⁻³ UDCA, indicating that although UDCA prohibited the naphthyl residues of (*R*,*R*)-**1** from forming the excimer, UDCA could induce another type of excimer.

The binding constants of (*R*,*R*)-**1** and (*S*,*S*)-**1** on 1:1 complexation of the guests were estimated from guest-induced

fluorescence variations, the values of (*R*,*R*)-**1** being 6440, 65 300 and 7180 dm^3 mol^{-1} and those of (*S*,*S*)-**1** being 11 600, 74 300 and 25 000 dm^3 mol^{-1} for BL, CDCA and UDCA, respectively (Table 1). According to these values, (*S*,*S*)-**1** for which the less intense excimer fluorescence was observed had larger binding constants than (*R*,*R*)-**1**. This may be because (*R*,*R*)-**1** can form a stable intramolecular complex in which the two naphthalene rings are accommodated in the cavity. The stability of the intramolecular complex of (*R*,*R*)-**1** prohibits the insertion of other external guests in the cavity. It is interesting that (*R*,*R*)-**1** binds CDCA nine times as strong as UDCA and (*S*,*S*)-**1** binds CDCA three times as strong as UDCA. The only difference existing between CDCA and UDCA is the stereochemistry around the C-7 position; CDCA has a 7α hydroxy group whereas UDCA has a 7β hydroxy group. Therefore, the hosts, especially (*R*,*R*)-**1**, recognize the configuration of the hydroxy groups and the recognition directly reflects their fluorescence responses. A difference in binding affinity for UDCA between (*R*,*R*)-**1** and (*S*,*S*)-**1** is also significant (the ratio in the binding constants is 1 : 3.5).

In summary, stereochemistry of the pendant moieties of the modified γ-CyDs were found to influence the intramolecular excimer formation of the pendant moieties and the guest binding behaviour. The results obtained here would demonstrate great potential of CyD derivatives with plural chiral moieties on molecular recognition and applicability toward molecular sensing systems.

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