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Spacer Effect of Appended Moieties for Molecular Recognition in Doubly-Sodium Anthranilate Modified γ-Cyclodextrin

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Abstract. γ-Cyclodextrin with two sodium anthranilate moieties (**1**) has been prepared as a sensor for detecting organic compounds including terpenoids and steroids. Compound **1** shows a pure monomer fluorescence whose intensity is increased or decreased upon addition of the guest species examined. In this system, the sodium anthranilate moieties act either as a spacer, which enables the cyclodextrin to form a 1 : 1 host–guest complex by narrowing the γ-cyclodextrin cavity, or as a hydrophobic cap. **1** recognizes steroids with much higher sensitivity than terpenoids, in which the appended moieties act as a hydrophobic cap for terpenoids and a spacer for steroids, respectively.

Key words: Modified cyclodextrin, spacer effect, steroid sensor, fluorescence spectroscopy.

1. Introduction

Cyclodextrins are torus-shaped cyclic oligomers of D-glucopyranose having the forms α-, β-, and γ-cyclodextrin, representing the hexa-, hepta-, and octamers, respectively. The compounds can undergo host–guest complexation with a variety of organic compounds in their cavities in aqueous solution [1]. When the inclusion phenomena of cyclodextrins are investigated, spectroscopically active guests should be used because cyclodextrins are basically inert with respect to optical spectroscopy. Cyclodextrins can, however, become spectroscopically active com-

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pounds by modification with chromophores, and spectroscopically inert guests can probably be recognized by the spectral change of modified cyclodextrins upon addition of guest. Ueno *et al.* have reported several modified cyclodextrins with chromophores in the last decade, and suggest the possibility of their use as sensors or indicators of molecules [2, 3]. Recently we reported the sensory system and molecular recognition properties of anthranilate-modified β-cyclodextrin (**2**), in which the anthranilate moiety acts as a spacer that enables the cyclodextrin to form a 1 : 1 complex with small molecules such as (–)-borneol by narrowing the large β-cyclodextrin cavity [4]. This induced-fit type of space regulation by the appended moiety for inclusion of a guest has never been observed before for the modified β-cyclodextrin reported by us [5]. We have also reported the synthesis and host–guest complexation behaviors of spectroscopically active γ-cyclodextrins that contain chromophores such as naphthalene, anthracene, and pyrene [6]. These cyclodextrin derivatives show remarkable variations in their circular dichroism, absorption, and fluorescence spectra associated with the formation of inclusion complexes. Here we would like to report the fluorescence sensory ability as well as the system of molecular recognition of γ-cyclodextrin modified with bis(sodium anthranilate) (**1**), which shows higher recognition ability for the steroidal compounds than does compound **2**.

2. Experimental Section

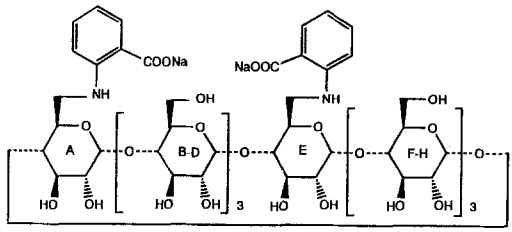
2.1. PREPARATION OF BIS(SODIUM ANTHRANILATE MODIFIED)γ-CYCLODEXTRIN (**1**)

A mixture of (*trans*-azobenzene-4,4′-disulfonyl)-γ-cyclodextrin (1.124 g, 0.74 mM) [6] and sodium anthranilate [4] (0.32 g, 2.01 mM) in DMF (30 mL) was heated at 80°C for 23 h under a nitrogen atmosphere. After cooling, the reaction mixture was poured into 300 mL of acetone. The resulting precipitates were filtered and dried. The crude product was purified with a Sephadex G-15 column (3 × 90 cm) to afford 0.181 g (16% isolated yield) of pure **1**. R_f 0.46 (1-butanol : ethanol : water 5 : 4 : 3 by volume). ^{1}H-NMR(DMSO-d_6) = 3.3–3.9 (48H, m, C_2–C_6H of cyclodextrin), 4.3–4.9 (14H, m, O_6H, C_1H), 5.81 (16H, s, O_2H, O_3H), 6.55 (1H, t, J=7.9 Hz, aromatic-H), 6.6 (1H, s, NH), 6.75 (1H, t, J=7.9 Hz, aromatic-H), 7.24 (1H, t, J=7.9 Hz, aromatic-H), 7.73 (1H, d, J=7.9 Hz, aromatic-H).

$C_{62}H_{88}O_{42}N_2Na_2 \cdot H_2O$ *Calcd.* for C, 46.16 H, 5.65 N, 1.75. *Found:* C, 46.75 H, 5.59 N, 1.6. MS(FAB): 1578([M+H$^+$]).

2.2. MEASUREMENT

Fluorescence and circular dichroism spectra were measured with a Hitachi U-2000 spectrophotometer, a HitachiF-3010 Fluorescence Spectrophotometer, and a JASCO J-700 spectropolarimeter, respectively. The excitation wavelength of the fluorescence spectra was 330 nm. Excitation and emission slits were 5 nm. 5μL



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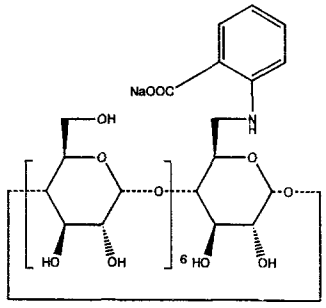

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of guest species (0.5, 0.05, 0.01, and 0.001 M) dissolved in dimethylsulfoxide (DMSO) or methanol were injected into a solution of **1** and **2** in aqueous solution (2.5 mL) to make sample solutions with a host concentration of 1×10^{-6} M and guest concentrations of 0.002, 0.02, 0.1, and 1.0 mM.

3. Results and Discussion

Compound **1** was prepared from *trans*-azobenzene-capped γ-cyclodextrin treated with sodium anthranilate as shown in Figure 1. It has been reported that *trans*-azobenzene-sulfonyl γ-cyclodextrin is a mixture of A–E and A–D isomers in a

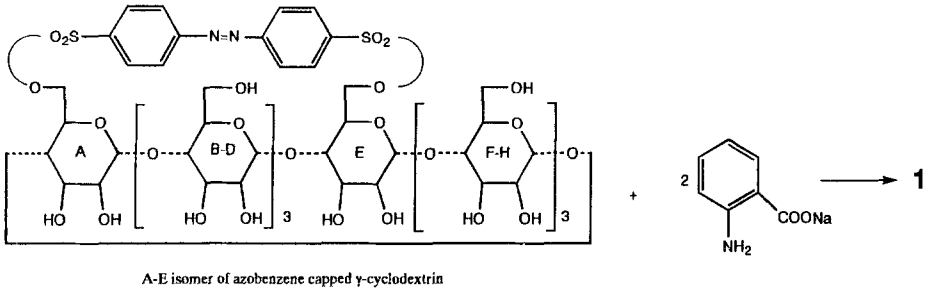


Fig. 1. Preparation of **1** from azobenzene capped γ-cyclodextrin.

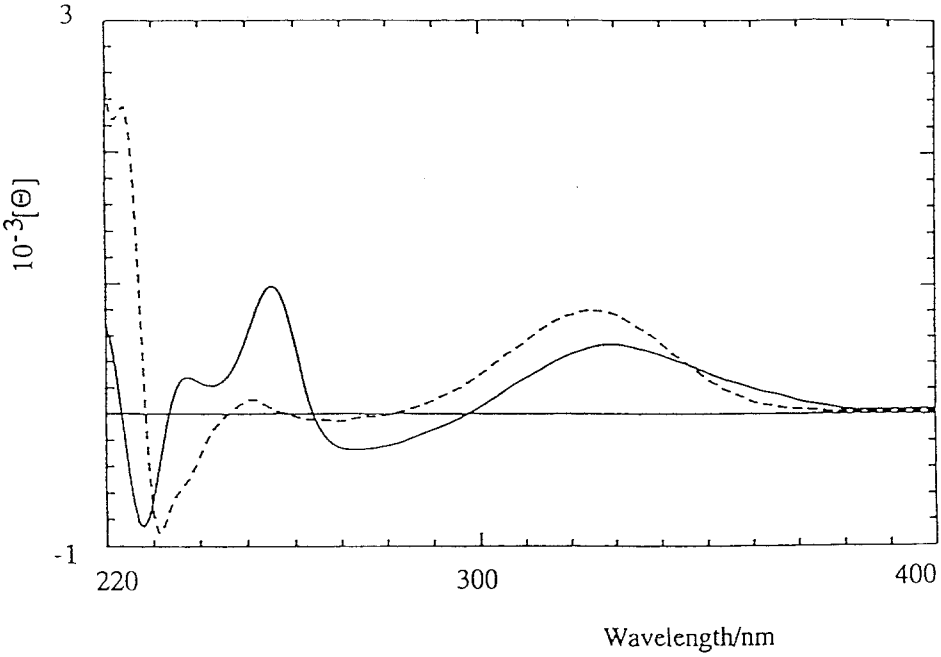


Fig. 2. Induced circular dichroism spectra of **1** at 25°C in aqueous solution (1×10^{-4} M) in the absence (——) and the presence of (- - - - - - -) ursodeoxycholic acid (1×10^{-4} M).

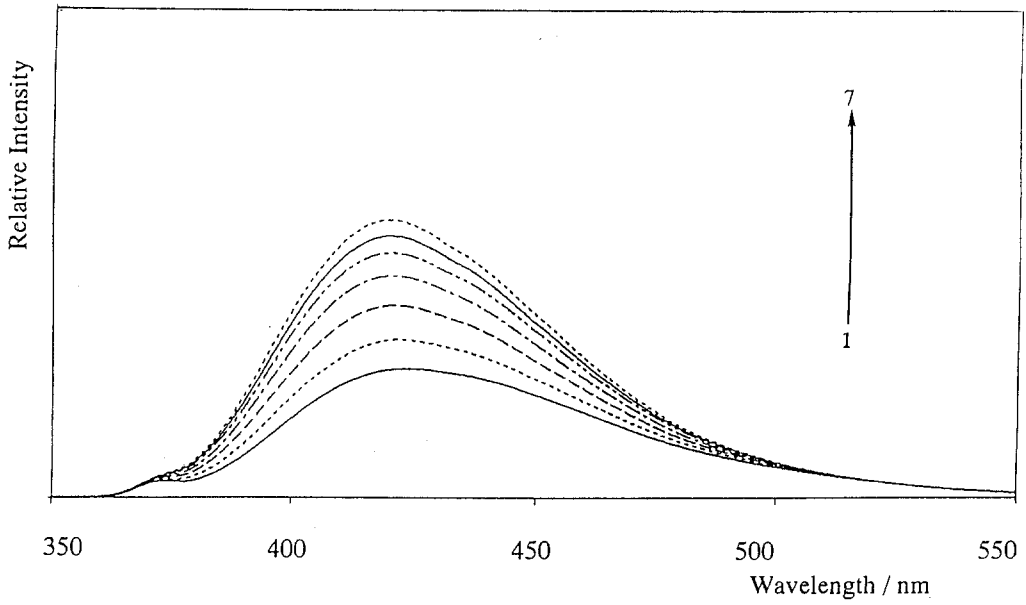


Fig. 3. Fluorescence spectra of **1** (1×10^{-6} M) in aqueous solution at various ursodeoxycholic acid (1, 0; 2, 2.00×10^{-6}; 3, 6.00×10^{-6}; 4, 1.20×10^{-5}; 5, 1.90×10^{-5}; 6, 3.00×10^{-5}; 7, 4.00×10^{-5} M).

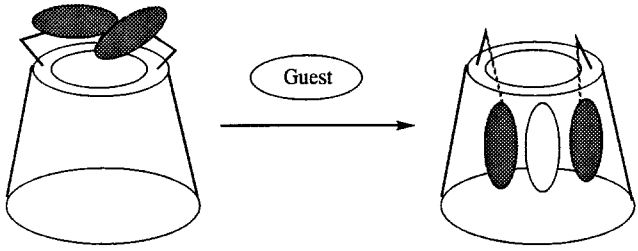


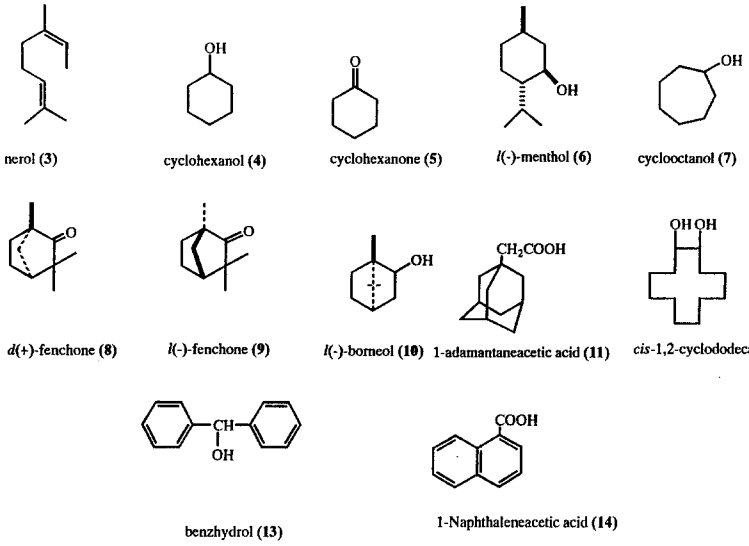
Fig. 4. Induced-fit type of space regulation by the bis-appended moieties for inclusion of a guest molecule in the cyclodextrin cavity.

ratio of 94 : 6 [7], which suggests that compound **1** should also exist as a mixture with a ratio of 94 : 6. However, no indication of the existence of two isomers of **1** is obtained from the experimental data, such as those of TLC or ^{1}H-NMR experiments. This means that one isomer of **1** is formed exclusively. The isolated yield of **1** from *trans*-azobenzene-γ-cyclodextrin is 16%, which means that the A–E isomer was obtained. Figure 2 shows the circular dichroism spectra (CD) of **1** in aqueous solution. The CD spectrum of **1** shows positive bands at ca. 250 nm and 325 nm. When ursodeoxycholic acid was added as a guest, the [Θ] value of the band at ca. 325 nm increases and the others at ca. 255 nm and 270 nm decrease. This suggested that two sodium anthranilate moieties are involved in the cavity of **1**. Figure 3 shows fluorescence spectra of **1** in aqueous solution in the presence and absence of ursodeoxycholic acid. The guest-induced fluorescence enhancement suggests that the sodium anthranilate moieties move from the outside of the cyclodextrin cavity toward the inside of the cavity, as shown in Figure 4, because it has been reported that the fluorescence enhancement of the anthranilate moiety of β-cyclodextrin is caused by the movement of the sodium anthranilate moiety from the bulk water environment outside of the cyclodextrin cavity toward the interior of the hydrophobic cavity. This fluorescence enhancement has been observed when steroidal compounds, except for cortisone and hydrocortisone, were used as a guest. On the other hand, the fluorescence intensities were decreased when smaller molecules such as terpenoids were used as a guest. The fluorescence enhancement upon guest addition in the γ-cyclodextrin with two appended moieties has only been observed before for the bis(2-napthylsulfonyl)-γ-cyclodextrin derivatives [8]. However, this fluorescence enhancement has been shown with all guests examined, including terpenoids and steroidal compounds. To calculate the sensory ability of modified cyclodextrins in pure water, the $\Delta I/I^0$ value (= sensitivity factor) was used as reported previously [4]. Here, ΔI is $I^0 - I$, where I^0 is the fluorescence intensity for the host alone, and I is for a mixture of host and guest. The $\Delta I/I^0$ values of **1** and **2** obtained with 12 guests, including terpenoids and aromatic compounds, are listed in Table I [9]. For **1**, all the guests were examined at 1.0 mM. For **2**, the guests were added at 1.0 mM (M=mol dm^{-3}), except for

TABLE I. The sensitivity factor of **1** and **2** for small guests.

Guest	1: $\Delta I/I^0$	2: $\Delta I/I^0$
3	0.06	–0.26
4	0.01	–0.45
5	0.05	–0.51
6	–0.02	–0.32
7	0.09	–0.73
8	0.05	–0.72
9	0.04	–0.73
10	0.12	–0.47
11	0.05	–0.38
12	0.11	–0.52
13	–0.09	–0.10
14	0.00	–0.09

Measured at 25°C. Excitation wavelength was 330 nm. The concentration of **1** and **2** was 1.0×10^{-6} M.



nerol (**3**) cyclohexanol (**4**) cyclohexanone (**5**) *l*(-)-menthol (**6**) cyclooctanol (**7**)

d(+)-fenchone (**8**) *l*(-)-fenchone (**9**) *l*(-)-borneol (**10**) 1-adamantaneacetic acid (**11**) *cis*-1,2-cyclododecanediol (**12**)

benzhydrol (**13**) 1-Naphthaleneacetic acid (**14**)

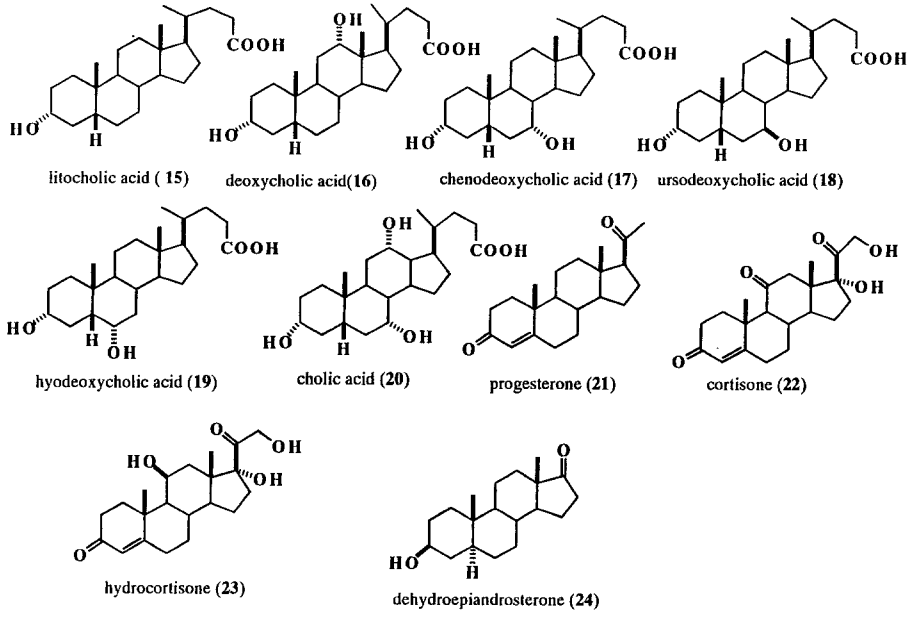
1-adamantaneacetic acid (**11**) and *cis*-1,2-cyclododecanol (**12**), which were examined at 0.1 mM because 1.0 mM of these guests are not soluble in pure water. The signs of the $\Delta I/I^0$ values obtained from **1** and **2** are opposite, except when (–)-menthol (**6**) and benzhydrol (**13**) are used as guests. When cyclohexanol (**5**) and **11** were used as guests for **1**, the $\Delta I/I^0$ value is negative, which means the fluorescence intensity increases upon addition of guest.

When the other guests are used, negative $\Delta I/I^0$ values are obtained, suggesting that sodium anthranilate moieties are excluded to the outside of the cyclodextrin cavity associated with guest binding [4]. The $\Delta I/I^0$ values range from -0.089 to

TABLE II. The sensitivity factor of **1** and **2** for steroidal compounds.

Guest	**1**: $\Delta I/I^0$	**2**: $\Delta I/I^0$
15	−1.80	−0.12
16	−0.47	−0.10
17	−1.42	−0.23
18	−1.37	0.03
19	−1.83	−0.33
20	−0.52	−0.06
21	−0.18	0.22
22	0.13	0.16
23	0.10	0.17
24	−0.83	−0.04

Measured at 25°C. Excitation wavelength was 330 nm. The concentration of **1** and **2** was 1.0×10^{-6} M.



0.107. This suggests that the behavior of **1** on complexation with a guest molecule was affected by the molecular structure and size. However, **1** shows poor sensitivity for the guests in comparison to **2**. This suggests that the cavity of γ-cyclodextrin is too big to undergo complexation with the guest molecules examined. Steroids are biologically important substances, and it seems to be interesting to investigate how they are detected by **1**. The $\Delta I/I^0$ values of **1** and **2** obtained with 10 steroids are shown in Table II. For **1**, because of the solubility in pure water of guest molecules, lithocholic acid (**15**) was used at 0.002 mM, chenodeoxycholic

acid (**17**), ursodeoxycholic acid (**18**), and hyodeoxycholic acid (**19**) were used at 0.02 mM and the other guests were used at 0.1 mM. For **2**, all the guests were examined at 0.1 mM. Among the steroidal molecules, the $\Delta I/I^0$ values of **1** for **22** and **23** and **2** for **18**, **21**, **22**, and **23** are positive, which means the fluorescence intensity decreases upon guest addition. Ueno *et al.* reported the sensory system of pyrene-appended γ-cyclodextrin, in which some steroids having an α,β-unsaturated ketone system, such as **21**, **22**, and **23**, act as a quencher, decreasing the intensity of the fluorescence and causing an anomaly in the fluorescence intensity of pyrene-modified γ-cyclodextrin [10]. As in the above cases, those guests probably act as quencher in systems containing **1** and **2**, which cause **22** and **23** to be detected with positive $\Delta I/I^0$ values by **1** and **2**. However, progesterone (**21**) is detected with a negative $\Delta I/I^0$ value by **1**, which means that the fluorescence intensity increases upon addition of guest.

The $\Delta I/I^0$ values of **1** for the steroids examined range from 0.134 to −1.798. This means that **1** recognizes steroidal compounds with very high sensitivity even at one-fiftieth or one-fifth concentration at which compound **2** recognizes them. Compound **1** recognizes lithocholic acid (**15**) and hyodeoycholic acid (**19**) with the greatest sensitivity. Chenodeoxycholic acid (**17**) and ursodeoxycholic acid (**18**), which bear an extra hydroxy group compared with lithocholic acid, were detected with the next highest sensitivity. However, deoxycholic acid (**16**), which bears the same number of hydroxy groups, but in different positions than **17** and **18**, was detected with lower sensitivity. Cholic acid (**20**), which bears an additional hydroxy group compared to **16**, was detected with almost the same sensitivity as **16**. This fact suggests that the existence of a hydroxy group at C(12) of the steroidal framework seems to vary the effect of the sensory ability of this system. The binding constant (K) of **1** for these steroids (apart from **21**, **22**, and **23**) were obtained by the analysis of the guest-induced fluorescence variations using Equation 1 [11]:

$$\frac{1}{I_f - I_{f_0}} = \frac{1}{(b-a)} + \frac{1}{(b-a)\,[CD]_0\,K}\,\frac{1}{[G]_0} \tag{1}$$

Here, I is the fluorescence intensity at 424 nm (I_f for the complex, I_{f_0} for the host alone), $[CD]_0$ is the total host concentration, $[G]_0$ is the total guest concentration, a and b are constants. The binding constants of **1** for these steroidal compounds are listed in Table III. The sensitivity of **2** to steroids decreases in the sequence: lithocholic acid > hyodeoxycholic acid > ursodeoxycholic acid, chenodeoxycholic acid, which is almost the same tendency obtained from the $\Delta I/I^0$ values.

4. Conclusion

The sensory system of **1** for steroidal compounds has been investigated. It is interesting that steroidal compounds, which are biologically significant substances, were detected by this system with high sensitivities at very low concentration. It is recognized that the appended moieties of **1** act as a spacer in collaboration with

TABLE III. Binding constants (K) of **1** in aqueous solution at 25°C.

Guest	K($mol^{-1} \cdot dm^3$)
lithocholic acid (**15**)	1400000 ± 90000
deoxycholic acid (**16**)	76000 ± 14000
chenodeoxycholic acid (**17**)	78000 ± 2100
ursodeoxycholic acid (**18**)	95000 ± 2200
hyodeoxycholic acid (**19**)	190000 ± 5200
cholic acid (**20**)	15000 ± 1500
dehydroepiandrosterone (**24**)	26000 ± 610

each other to produce a 1 : 1 host–guest complexation, which is a different host–guest complexation pattern from that reported previously. Further work with many other guests is needed to clarify the relationships between guest and sensitivity of this system.

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