Molecular Recognition Study on a Supramolecular System. 10.[1] Inclusion Complexation of Modified β-Cyclodextrins with Amino Acids: Enhanced Enantioselectivity for L/D-Leucine

Yu Liu,*[,†] Yi-Min Zhang,[†] Ai-Di Qi,[†,‡] Rong-Ti Chen,[†] Keiko Yamamoto,[§] Takehiko Wada,[§] and Yoshihisa Inoue*[,§]

Department of Chemistry, Nankai University, Tianjin 300071, China, and Department of Molecular Chemistry, Osaka University, Yamadaoka, Suita 565, Japan

Received August 20, 1996 (Revised Manuscript Received December 27, 1996[⊗])

The novel β-cyclodextrin (β-CD) derivatives bearing a *m*-toluidinyl or (9-fluorenyl)alkylamino moiety have been synthesized by a convenient method in 45% and 66% yields, respectively. The stability constants (K_s) and Gibbs free energy changes ($-\Delta G$) for inclusion complexation of mono-[6-(*m*-toluidinyl)-6-deoxy]-β-cyclodextrin **1** and mono-[6-[(9-fluorenyl)alkylamino]-6-deoxy]-β-cyclodextrin **2** with various L/D-amino acids have been examined by the fluorescence spectrum method in buffered aqueous solution (pH = 7.20) at 20−23 °C. The modified β-cyclodextrins, possessing a toluidinyl or fluorenyl moiety as fluorescent probe, can recognize not only the size and shape but also the chirality of L/D-amino acids, giving fairly good enantioselectivity up to 33 for L/D-leucine. β-Cyclodextrin derivative **1** gave the highest K_s for L-leucine and the lowest for D-leucine among the amino acid series, eventually showing the highest enantioselectivity for L/D-amino acids. The molecular recognition ability and enantioselectivity for amino acids of the modified β-cyclodextrins **1** and **2** are discussed from the viewpoint of the size/shape−fit relationship between the host cavity and the guest molecules.

Introduction

The inclusion complexation of guest molecules by the host cyclodextrins (CDs) and chemically modified cyclodextrins has been extensively studied in recent years as models of biological receptor−substrate interactions and is currently a significant topic in chemistry and biochemistry.[2−10] Consequently, a good deal of effort has been devoted to the synthesis of a wide variety of cyclodextrins derivatives in order to examine their molecular recognition ability.[11−15] For enantiomer separations, some modified cyclodextrins have been employed successfully in several areas of science and technology.[16−21] Our recent study on the molecular recognition thermodynamics of some naphthalene derivatives with natural cyclodextrins and various cyclodextrin derivatives serves our understanding of the cooperation of several weak forces working between receptor and substrate, which include dipole−dipole, electrostatic, van der Waals, hydrogen bonding, and hydrophobic interactions.[22,23] More recently, we have reported that the biological chiral recognition of aromatic amino acids by binuclear copper(II)−cyclodextrin complexes.[8] Cyclodextrins have been found to show selectivity for L-amino acids, while the binuclear copper(II)−cyclodextrin complexes favor D-amino acids.[8] The elucidation of the inclusion mechanism is also helpful for our further understanding of the multiple recognition mechanism and the induced-fit interaction hypothesis proposed for the selective binding of specific substrate by the biological receptor. However, the effects of the size of aromatic chromophore and the chain length between the chromophore and cyclodextrin on inclusion behavior have not been investigated systematically, and the enantioselection mechanism for L/D-amino acid is left unanswered.

In the present study, we synthesized mono-[6-(*m*-toluidinyl)-6-deoxy]-β-cyclodextrin **1** and mono-[6-[[(9-fluorenylamino)ethyl]amino]-6-deoxy]-β-cyclodextrin **2** and investigated their inclusion complexation behavior with L/D-amino acids (Ala, Ser, Val, Leu) in buffered aqueous

† Nankai University.
‡ On the leave from Tianjin College of Traditional Chinese Medicine.
§ Osaka University.
⊗ Abstract published in *Advance ACS Abstracts,* February 15, 1997.
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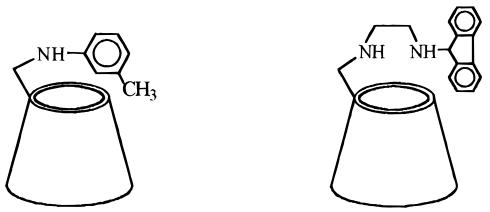
S0022-3263(96)01625-8 CCC: $14.00

Chart 1



(1) (2)

solution (pH = 7.20) using fluorescence spectrometry. The chromophoric fluorene originally attaching on the edge of β-cyclodextrin must suffer substantial conformational change upon guest inclusion and therefore functions as a fluorescent probe to determine complex stability constants in differential fluorescence spectrometry.

A series of L/D-amino acids were chosen as the guest molecules for this study in order to examine the effects of size, shape, and chirality of the guest molecule upon inclusion complexation. From the results of such investigations we can discuss the molecular recognition ability and enantioselectivity of host compounds **1** and **2** for amino acids from the viewpoint of the size-fit and the geometrical complement between the receptor (host) and substrate (guest).

Experimental Section

General Procedure. Elemental analyses were performed on a Perkin-Elmer-240 instrument. ^{1}H NMR spectra were recorded at 400 MHz in [$^2H_6$]-dimethyl sulfoxide (DMSO-d_6) on a Bruker AM400 spectrometer or at 750 MHz on a Variant UNITYplus 750 instrument. IR and UV spectra were obtained on a Nicolet FT-IR 5DX and Shimadzu UV-240 spectrometer, respectively. Fluorescence spectrometry data were obtained on a Shimadzu RF-5000 spectrometer. Circular dichroism (CD) spectra were recorded on a JASCO J-720 spectropolarimeter.

Materials. Commercially available amino acids (Tianjin Chemical Reagent Plant) were without further purification. β-Cyclodextrin of reagent grade (Suzhou Monosodium Glutamate Works) was recrystallized twice from water and dried for 12 h in vacuo at 100 °C. *N*,*N*-Dimethylformamide (DMF) was dried over calcium hydride for two days and then distilled under reduced pressure prior to use. 9-Bromofluorene was used without further purification. Alkylamines and *m*-toluidine were distilled under a reduced pressure before use. Disodium hydrogen phosphate and sodium dihydrogen phosphate were dissolved in distilled, deionized water to make a 0.1 M phosphate buffer solution of pH 7.20 for fluorescence spectral titration.

Synthesis of Mono-[6-(*m*-toluidinyl)-6-deoxy]-β-cyclodextrin 1. Mono-[6-O-(*p*-tolylsulfonyl)]-β-cyclodextrin (6-OTs-β-CD) was prepared by a reaction of β-cyclodextrin with *p*-toluenesulfonyl chloride in dry pyridine.[24] Compound **1** was prepared by the reaction of 6-OTs-β-CD (2 g) with *m*-toluidine (10 mL) in *N*,*N*-dimethylformamide (20 mL) at 85 °C with stirring for 12 h under N_2. The reaction mixture was evaporated in vacuo at 40 °C to dryness. The residue was dissolved in water, and acetone was added to the resulting solution to give a gray precipitate. After drying, the gray precipitate was purified by repeated recrystallization from water and dried in vacuo to give a pure sample (45% yield). IR (KBr)/cm^{-1}: 3386, 2927, 2106, 1639, 1606, 1417, 1638, 1335, 1245, 1155, 1081, 941, 851, 753, 704, 581, 531; ^{1}H NMR (DMSO-d_6, TMS, ppm): 1.14 (s, 3H), 3.1−4.8 (m, 70H), 6.5 (s, 1H), 6.8−6.9 (d, 1H), 7,41−7.44 (d, 1H), 7.73−7.75 (t, 1H). Anal. Calcd for $C_{49}H_{77}O_{34}N\cdot 3H_2O$ C: 46.05%, H: 6.05%, N: 1.10%. Found C: 45.55%, H: 6.74%, N: 1.08%.

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Synthesis of Mono-[6-[[(9-fluorenylamino)ethyl]amino]-6-deoxy]-β-cyclodextrin 2. A solution of mono-[6-[(2-aminoethyl)amino]-6-deoxy]-β-cyclodextrin[25] (1.5 mmol) and 9-bromofluorene[26] (0.6 g, 2 mmol) in dry *N*,*N*-dimethylformamide (30 mL) was stirred for 10 min under nitrogen. A solution of triethylamine (10 mL) was added dropwise into the clear solution over 1 h with magnetic stirring under N_2. The solution was allowed to warm up and stirred for 6 h at room temperature, and then the resultant mixture was evaporated under a reduced pressure, leaving a white solid. The residue was dissolved in water, and then acetone was added to the solution to give a yellow precipitate. The crude product (0.5 g) was purified by column chromatography over Sephadex G-25 with the distilled, deionized water to give a pure sample (0.2 g).[27] IR (KBr)/cm^{-1}: 3386, 2927, 1704, 1639, 1409, 1368, 1335, 1302, 1245, 1204, 1155, 1081, 1032, 941, 859, 761, 704, 580. ^{1}H NMR (DMSO-d_6, TMS, ppm): 2.7−2.9 (m, 4H), 3.6−4.4 (m, 72H), 7.30−7.32 (d, 2H), 7.33−7.35 (d, 2H), 7.61−7.63 (d, 2H), 7.76−7.79 (d, 2H). Anal. Calcd for $C_{57}H_{84}O_{34}N_2\cdot 3H_2O$, C: 49.07%, H: 6.46%, N: 2.01%. Found C: 48.72%, H: 6.88%, N: 1.94%.

Spectral Measurements. The NMR spectra of mono-[6-[[(9-fluorenylamino)ethyl]amino]-6-deoxy]-β-cyclodextrin **2** were measured in D_2O at 750 MHz in the absence/presence of L-leucine, by using a Varian UNITYplus 750 instrument. However, practically no changes were observed in chemical shift or coupling pattern of both aromatic and cyclodextrin protons even in the presence of 3 equiv of the added guest. We therefore evaluated the complexation behavior of modified β-cyclodextrins **1** and **2** with some selected amino acid biological molecules by the differential fluorescence spectrometry. The fluorescence spectral titration of a series of solutions containing β-cyclodextrin derivatives **1** and **2** (5×10^{-5} mol dm^{-3}) was carried out in buffered aqueous solution at 20−23 °C with excitation at 294.1 and 277.0 nm, respectively.

Results and Discussion

Synthesis. Modified β-cyclodextrins were synthesized in satisfactory yields by using the 6-*O*-monotosylate of β-cyclodextrin as the starting material (Scheme 1) according to the following scheme.

UV and CD Spectra. The electronic spectra of modified β-cyclodextrin **1** in aqueous solution showed a strong negative Cotton effect peak, corresponding to the ^1L_a band at 216 nm ($\Delta\epsilon = -1.59$) and a strong positive Cotton effect for the ^1L_b band at 245 nm ($\Delta\epsilon = 1.46$). According to the sector rule proposed by Kajtar,[28] the Cotton effects observed for the ^1L_a and ^1L_b bands indicate that the *m*-toluidinyl moiety penetrates only shallowly into the hydrophobic cavity of cyclodextrin.[29−31] For the modified β-cyclodextrin **2** (0.099 mmol dm^{-3}), the CD spectra show a major positive Cotton effect peak ($\Delta\epsilon = 1.12$) at 213.8 nm and a weak negative Cotton effect peak ($\Delta\epsilon = -0.163$) at 231.4 nm, which enables us to elucidate the conformation of the (9-fluorenyl)alkylamino moiety in **2**, although no further information about the detailed structure of **2** could be obtained from the UV spectra. According to the ICD spectrum of **2** in buffer solution at

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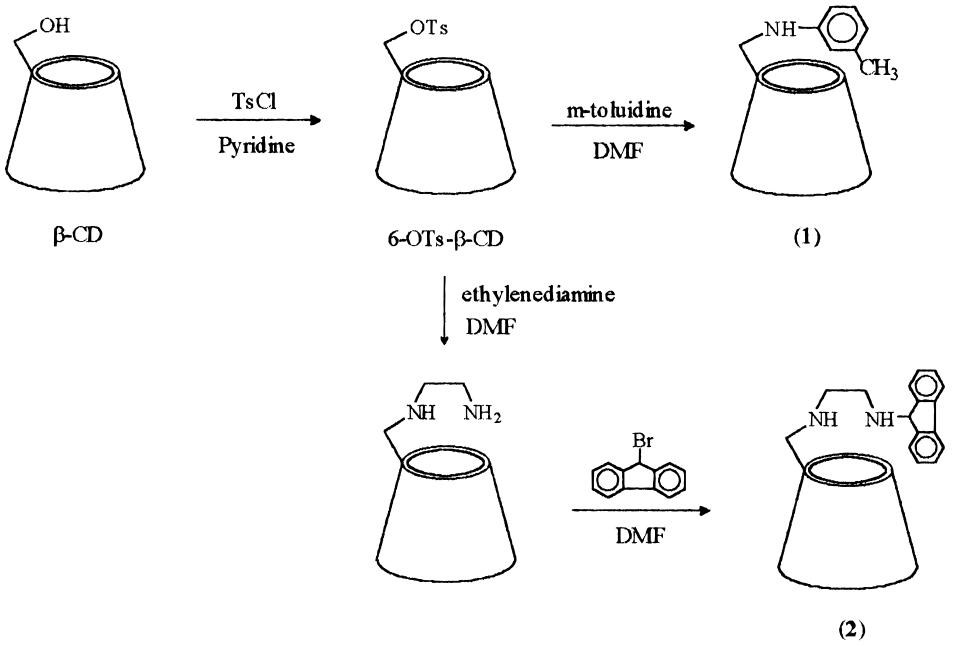
Scheme 1



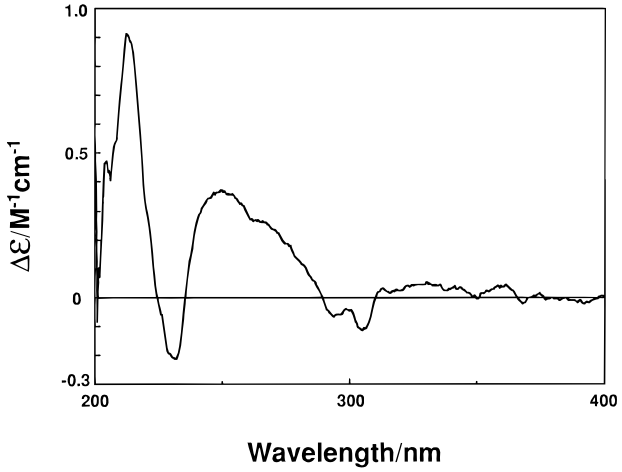


Figure 1. Circular dichroism spectra of β-cyclodextrin derivative **2** (0.099 mmol dm^{-3}) in buffer solution (pH 7.20) at room temperature.

pH 7.20 shown in Figure 1, the (9-fluorenyl)alkylamino group, originally attached to the edge of the cyclodextrin cavity, may include into the cavity of β-cyclodextrin in the longitudinal direction[22,29−31] and must suffer substantial conformational change upon guest inclusion. This substantial conformational change is used to determine complex stability constants.

Fluorescence Spectral Titrations. In the titration experiments using fluorescence spectrometry, the fluorescence intensity of the chromophoric group, originally attaching on the edge of β-cyclodextrin cavity, gradually increased for **1** and decreased for **2** upon the inclusional complexation with the addition of a varying concentration of amino acids, respectively. The inclusion phenomena is consistent with the result obtained by Ueno.[15b] The fluorescence behaviors of modified β-cyclodextrins **1** and **2** upon addition of amino acids further confirm the geometric conformation of **1** and **2** inferred by ICD. Typical fluorescence spectral changes upon addition of amino acid to modified cyclodextrin solution are shown in Figure 2. These results indicate that the inclusion complex formed by modified cyclodextrins complexation

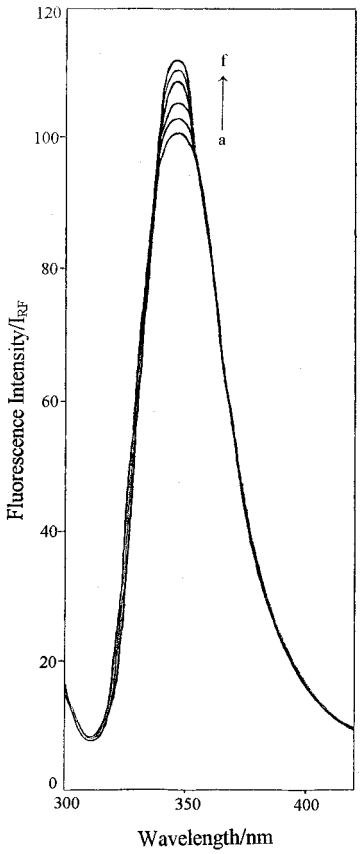


Figure 2. Fluorescence spectra of (**1**) (5.49 × 10^{-5} mol dm^{-3}) in the presence of L-valine (mmol dm^{-3}), (a) 0; (b) 1.512; (c) 3.024; (d) 4.536; (e) 5.670; (f) 6.804; with excitation at 294.1 nm.

with amino acids. With the assumption of a 1:1 stoichiometry, the inclusion complexation of amino acids (G) with β-cyclodextrin derivatives (H) is expressed by eq 1.

$$H + G \overset{K_S}{\rightleftharpoons} G{\cdot}H \qquad (1)$$

Under the conditions employed, the concentration of

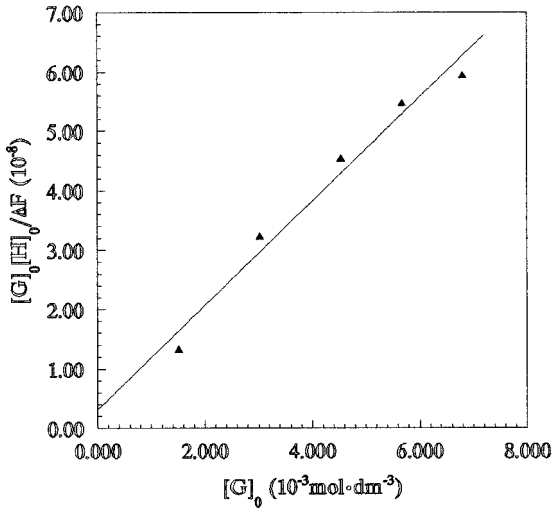


Figure 3. Typical plot of $[G]_0[H]_0/\Delta F$ versus $[G]_0$ for the inclusion complexation of cyclodextrin derivative **1** with L-valine in phosphate buffer solution (pH = 7.20) at 20−23 °C.

β-cyclodextrin derivatives is much smaller than the concentration of amino acids, i.e. $[H]_0 \ll [G]_0$. Therefore, the stability constant (K_s) of the inclusion complex formed by the host and guest can be calculated according to the modified Hildebrand and Benesi eq 2.[32,33] where $[G]_0$

$$\frac{[G]_0[H]_0}{\Delta F} = \frac{1}{K_s \Delta \epsilon} + \frac{[G]_0}{\Delta \epsilon} \qquad (2)$$

denotes the total concentration of amino acids, $[H]_0$ refers to the total concentration of β-cyclodextrin derivatives, $\Delta\epsilon$ is the difference of molar extinction coefficients for free and complexed β-cyclodextrin derivatives, and ΔF denotes the changes in the fluorescence intensity of β-cyclodextrin derivatives upon addition of guest amino acid. For all host compounds examined, the plots of calculated $[G]_0[H]_0/\Delta F$ values as a function of $[G]_0$ give good straight lines. Typical plots shown in Figure 3 for the inclusion complexation of cyclodextrin derivative **1** with L-valine, where the calculated $[G]_0[H]_0/\Delta F$ values are plotted against the $[G]_0$ to give an excellent linear relationship ($r = 0.986$) with a slope of 8.76×10^{-6} and an intercept of 3.142×10^{-9}. The stability constants (log K_s) and the free energy change ($-\Delta G$) calculated from the slope and the intercept are listed in Table 1, along with enantioselectivity ($\Delta\Delta G$) calculated from ΔG for inclusion complexation of L/D-amino acids by the modified β-cyclodextrins.

Complex Stability. Extensive studies of molecular recognition by cyclodextrins have shown that an important characteristic of the complexation is simultaneous operation of several weak forces working between the guest and host, which determine how the size and shape of a guest molecule fit into the host cavity. As can be seen from Table 1, modified β-cyclodextrin **1** shows a much higher enantioselectivity ($\Delta\Delta G$) for all of the amino acids examined than **2** does, although both hosts possess roughly comparable complexing abilities. The size-fitted combination gives strong inclusion complexation. This seems reasonable since the lipophilic fluorenyl group,

Table 1. Stability Constants (log K_s) and the Gibbs Free Energy Changes ($-\Delta G$) for the Including Complexation of Amino Acids with β-Cyclodextrin Derivatives 1 and 2 in 0.1 mol dm^{-3} Phosphate Buffer Solution (pH = 7.20) at 20−23 °C[a]

host	guest	log K_s	$-\Delta G$/kJ mol^{-1}	$-\Delta\Delta G$/lJ mol^{-1}
1	L-Ala	2.83	16.0	2.7
	D-Ala	3.31	18.7	
	L-Ser	2.50	14.1	5.0
	D-Ser	3.38	19.1	
	L-Val	3.44	19.5	−3.2
	D-Val	2.89	16.3	
	L-Leu	4.19	23.6	−8.5
	D-Leu	2.67	15.1	
2	L-Ala	2.85	16.1	1.0
	D-Ala	3.02	17.1	
	L-Ser	2.51	14.1	4.6
	D-Ser	3.31	18.7	
	L-Val	2.82	15.9	−0.1
	D-Val	2.80	15.8	

[a] Excitation and emission are at 294.1 and 343.8 nm, respectively, for β-cyclodextrin derivative **1**, and for **2** are at 277.0 and 293.0 nm.

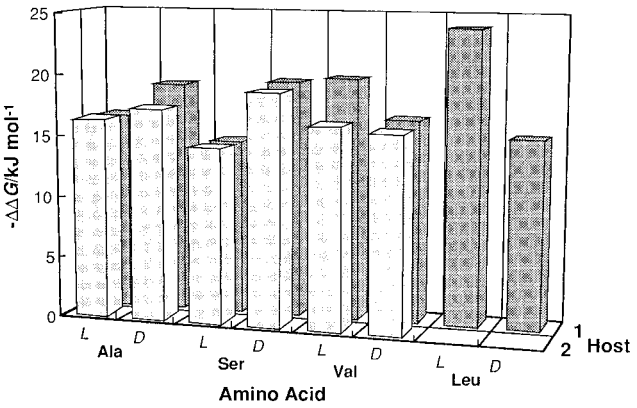


Figure 4. Gibbs free energy changes ($-\Delta G$) as a function of amino acids for the including complexation of modified β-cyclodextrin **1** and **2** with amino acids in phosphate buffer solution at 20−23 °C.

originally attached to the edge of cyclodextrin cavity, is embedded into the cavity of cyclodextrin, making the inclusion difficult for guest amino acid molecules. The stability of the inclusion complex with modified cyclodextrin should depend on the condition of strict size-fit between host and guest.

Molecular Recognition and Enantioselectivity. In order to visualize the inclusion complexation behavior of modified β-cyclodextrins **1** and **2** with amino acids, the changing profiles of free energy change ($-\Delta G$) on complexation of **1** and **2** are plotted as a function of chain length or size of amino acids in Figure 4.

Mono-[6-(*m*-toluidinyl)-6-deoxy]-β-cyclodextrin (1). As can be seen from Figure 4, the free energy change ($-\Delta G$) for inclusion complexation with **1** is highly sensitive to the chain length and shape of the alkyl group in amino acids and increases monotonically with increasing number of the methylene group in all of the examined cases of L-amino acids, i.e. L-Ala < L-Val < L-Leu. The unit increment in ΔG is much larger for L-leucine ($\Delta\Delta G = 7.8$ kJ mol^{-1}) than for L-valine ($\Delta\Delta G = 3.5$ kJ mol^{-1}). In sharp contrast to L-amino groups, the free energy change ($-\Delta G$) for D-amino acids decreases monotonically with increasing chain-length or molecular size. D-Alanine affords more stable complexes with **1** than do the D-valine and D-leucine ($-\Delta\Delta G = 2.4-3.7$ kJ mol^{-1}) as

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shown in Table 1 and Figure 4. These reduced stabilities for D-isomers and the enhanced enantioselectivity for L-isomer may be attributed to molecular chirality of the guests, since there must be the strict geometrical complementary relationship between the β-cyclodextrin cavity and amino acids.

As can be seen from Table 1 and Figure 4, modified β-cyclodextrins can recognize not only the size but also the chirality of the amino acids. The enantiomeric chiral isomers of L/D-amino acids show substantially different K_s values and free energy change ($-\Delta G$), yielding fairly good chiral recognition; the L/D enantioselectivities calculated from the K_s values are 3.6 for L/D-valine and enhanced to 33 for L/D-leucine. The free energy change ($-\Delta G$) for inclusion complexation of L-leucine with **1** is much higher (by 8.5 kJ mol^{-1}) than that for D-isomer, while a smaller free energy difference (3.2 kJ mol^{-1}) is observed for L- and D-valine. However, the both D-alanine and D-serine afford more stable complexes with **1** than does the corresponding L-isomer, giving fairly good D/L-enantioselectivities of 3.0 ($\Delta\Delta G = 2.7$ kJ mol^{-1}) and 7.6 ($\Delta\Delta G = 5.0$ kJ mol^{-1}), respectively.

Mono-[6-[(9-fluorenyl)alkylamino]-6-deoxy]-β-cyclodextrin (2). As shown in Table 1 and Figure 4, a tendency analogous to β-cyclodextrin derivative **1** is seen in the free energy change ($-\Delta G$) and the L/D-enantioselectivities of the modified β-cyclodextrin **2**. Somewhat unexpectedly, the host compound **2**, possessing a more bulky, hydrophobic fluorene chromophore, did not improve the complex stability for both enantiomers of all amino acids examined. Thus, the molecular recognition ability and the enantioselectivity of L/D-amino acids by β-cyclodextrin derivatives increase in the order **1** > **2**. These results indicate that the large chromophoric group linked to the edge of the cyclodextrin cavity through a longer chain is embedded in the cavity, which probably interferes upon inclusion complexation of amino acids to give the low binding abilities. In fact, in addition to the size and shape of the guest molecule, the microstructural change of the host molecule apparently governs the complexation phenomena to some extent.[8] Hence, the induced-fit and the geometrical complement between the host and the guest play a crucial role in the chiral recognition of amino acid molecules.

Acknowledgment. This work was supported by the National Outstanding Youth Fund, Natural Science Foundation of China, and Transcentury Qualified Personal Foundation of the State Education Commission of China, which are gratefully acknowledged.

Supporting Information Available: NMR spectra of **2** in the absence/presence of L-leucine (3 pages). This material is contained in libraries of microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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