Enantioselective recognition of amino acids by β-cyclodextrin 6-*O*-monophosphates[1]

Yu Liu,* Bin Li, Bao-Hang Han, Yu-Mei Li and Rong-Ti Chen

Department of Chemistry, Nankai University, Tianjin, 300071, PR China

Mono-(6-*O*-diphenoxyphosphoryl)-β-cyclodextrin 1 and mono-(6-*O*-ethoxyhydroxyphosphoryl)-β-cyclodextrin 2 have been synthesized by a convenient method in 40 and 35% yields, respectively. The stability constant (K_s) and Gibbs free energy change ($-\Delta G°$) for the 1:1 inclusion complexation of the two β-cyclodextrin 6-*O*-monophosphates with some selected L/D-amino acids have been examined by means of differential UV spectrometry in buffered aqueous solution (pH = 7.20) at 25 °C. The results obtained indicate that the modified β-cyclodextrin compound 1 is favourable for complexation with D-amino acids except for alanine, giving fairly good enantioselectivity of up to 3.6 for D/L-serine, with a molecular selectivity of up to 12.9 for D-leucine/D-alanine. The molecular recognition ability and enantioselectivity for amino acids of the modified β-cyclodextrins 1 and 2 are discussed from the viewpoints of the size/shape-fit relationship and the multipoint recognition mechanism. The binding constant (K_s) and Gibbs free energy change ($-\Delta G°$) for the modified β-cyclodextrins (host) inclusion complexation with L/D-amino acids (guest) may more explicitly be understood in terms of the induced-fit interaction and the complementary geometrical relationship between the host and the guest.

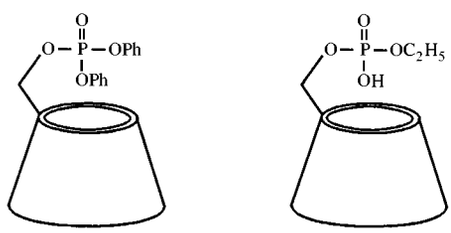
Introduction

The study of inclusion complexes formed by the selective binding of model substances with natural cyclodextrins (CDs) and chemically modified cyclodextrins as molecular receptors is currently a significant topic in chemistry and biochemistry.[1–10] Consequently, a wide variety of cyclodextrin derivatives have been synthesized in order to compare and examine the molecular recognition ability of cyclodextrins and modified cyclodextrins,[11–21] including multipoint and chiral recognition, and to gain insight into factors governing inclusion complexation from the viewpoint of induced-fit interaction and the complementary geometrical relationship between the molecular receptor and substrate. Possessing amino groups, carboxy groups and hydrophobic α-R groups, amino acids are excellent model substances for studying inclusion complexation with natural and modified cyclodextrins.

We have recently reported the chiral recognition of aromatic amino acids by binuclear copper(II)–cyclodextrin complexes.[8a] Cyclodextrins have been found to show enantioselectivity for L-amino acids, while binuclear copper(II)–cyclodextrin complexes[8b] favour D-amino acids.[8a] More recently, we have demonstrated that the modified β-cyclodextrin possessing one chromophoric *m*-toluidino moiety as a fluorescent probe can recognize not only the size of amino acids, but also the chirality of L/D-amino acids, giving fairly good enantioselectivity up to 33 for L/D-leucine.[22]

These results indicated that, in addition to the size and shape of amino acids, microstructural change in the cyclodextrins apparently governs the inclusion complexation phenomena to some extent. Therefore, the elucidation of the inclusion mechanism is also helpful for our further understanding of multipoint recognition and the induced-fit interaction hypothesis proposed for the selective binding of specific substrates by biological receptors.

Here, we report our study on the syntheses of mono-(6-*O*-diphenoxyphosphoryl)-β-cyclodextrin **1**, and mono-(6-*O*-ethoxyhydroxyphosphoryl)-β-cyclodextrin **2**, and their inclusion complexation behaviour with selected amino acids in buffered aqueous solution (pH = 7.20) at 25 °C using UV–VIS spectrometry. The phosphate group originally perched on the edge of β-cyclodextrin, and must suffer substantial conformational change upon guest inclusion, and therefore functions as a probe for differential UV spectrometry to determine the complex stability constant. A series of amino acids are employed as the guest molecules in order to examine the possible participation of several weak forces working between receptor (host) and substrate (guest). Under such circumstances, we can discuss the molecular recognition ability and enantioselectivity of amino acids by β-cyclodextrin derivatives **1** and **2** bearing a single phosphate group from the viewpoints of the size-fit and the complementary geometrical relationship between the host cyclodextrins and guest amino acids.



1 **2**

Experimental

General procedure

Melting points were measured with a Yanaco MP-21 apparatus and are uncorrected. Elemental analyses were performed on a Perkin-Elmer-240 instrument. NMR spectra were recorded at 200 MHz in $(CD_3)_2SO$ on a Bruker AM200 spectrometer. IR and UV spectra were obtained on a Nicolet FTIR 5DX and Shimadzu UV-2401PC spectrometer, respectively. Circular dichroism (CD) spectra were recorded on a JASCO J-720 spectropolarimeter.

Materials

Commercially available amino acids (Tianjin Chemical Reagent Plant) were used 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 cal-

cium hydride for 2 d and then distilled under a reduced pressure prior to use. Diphenyl phosphorochloridate (DojinDo) was used without further purification. Phosphorus oxychloride was distilled under 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 UV–VIS spectral titration.

Synthesis of mono-(6-*O*-diphenoxyphosphoryl)-β-cyclodextrin 1[23]

Diphenyl phosphorochloridate (1.4 g, 5.0 mmol) in 20 ml of pyridine was added dropwise to the solution of dried β-cyclodextrin (15.0 g, 13.2 mmol) in 200 ml of pyridine at room temp. The solution was stirred for 5 d, then 10 ml of water was added to quench the reaction, and the mixture was evaporated to dryness at 35 °C. Then diethyl ether (500 ml) was added to the residue with stirring for 12 h. The solid was isolated by filtration and was purified by five recrystallizations from 600 ml of boiling water, then dried to afford 3.0 g of the product as a white powder (42%), mp 222–223 °C. FTIR(KBr)ν/cm^{-1}: 3352.5, 2910.5, 1630.7, 1589.0, 1486.2, 1396.9, 1366.0, 1244.3, 1072.0, 941.5, 849.7, 750.0, 697.8. UV–VIS: 260.5 nm. δ_H[$(CD_3)_2SO$ 90 MHz, Me_4Si]: 2.8–4.1 (m, 42H, C^2-H, C^3-H, C^4-H, C^5-H, C^6-H), 4.1–5.2 (m, 33H, O^6-H, C^1-H, O^2-H, O^3-H, H_2O), 7.2 (s, 10H, Ar-H). δ_C[$(CD_3)_2SO$ 200 MHz]: 130.14 (*ipso*), 125.62 (*m*), 120.01 (*o*,*p*), 102.06 (C-1), 81.64 (C-4), 73.12 (C-3), 72.48 (C-2), 72.16 (C-5), 60.02 (C-6). Found: C, 45.01; H, 5.57. Calc. for $C_{54}H_{79}O_{38}P\cdot 4H_2O$: C, 45.07; H, 6.09%.

Synthesis of mono-(6-*O*-ethoxyhydroxyphosphoryl)-β-cyclodextrin 2

Ethyl phosphorodichloridate was prepared by the reaction of phosphorus oxychloride with ethanol at 5 °C with stirring for 3 h under N_2 in 83% yield. A solution of ethyl phosphorodichloridate (2.0 g, 12.3 mmol) in 100 ml of pyridine was added dropwise to a solution of dried β-cyclodextrin (15.0 g, 13.2 mmol) in 200 ml of pyridine at 5 °C with stirring over 2 d under N_2. The solution was allowed to warm and stirred for 2 d at room temperature, then the mixture was evaporated under reduced pressure to dryness at 35 °C. The residue was dissolved in water (50 ml), and the solution stirred for 5 h at 100 °C. The resultant mixture was evaporated under reduced pressure to dryness, and diethyl ether (100 ml) was added to the residue with stirring for 24 h. The crude product obtained after filtration was purified by column chromatography over Sephadex G-25 with distilled deionized water as the eluent to give a pure sample (5.4 g) in 35% yield, mp 221–222 °C. FTIR(KBr) ν/cm^{-1}: 3377.5, 2907.5, 2344.0, 1632.2, 1395.1, 1335.6, 1298.0, 1145.8, 1017.0, 936.6. UV–VIS λ/nm: 245.5, 212.7. δ_H[$(CD_3)_2SO$ 200 MHz, Me_4Si]: 1.6 (t, 3H, CH_3), 3.1–4.3 (m, 52H, $-CH_2-$, C^2-H, C^3-H, C^4-H, C^5-H, C^6-H, O^6-H, H_2O), 4.8 (s, 7H, C^1-H), 5.1–6.0 (m, br, 14H, O^2-H, O^3-H). Found: C, 41.89; H, 6.09. Calc. for $C_{44}H_{75}O_{38}P\cdot H_2O$: C, 41.91; H, 6.15%.

Spectral measurements

The complex stability constants of modified β-cyclodextrins **1** and **2** with some selected amino acid biological molecules were determined by UV spectrometry. Spectral titrations of a series of solutions containing β-cyclodextrin derivatives **1** and **2** (5×10^{-5} mol dm^{-3}) were performed in buffered aqueous solution at 25 °C.

Results and discussion

Synthesis

Modified β-cyclodextrins **1** and **2** were synthesized in satisfactory yields according to Scheme 1.

CD spectra

As can be seen from Fig. 1, the induced circular dichroism (ICD) spectrum of modified β-cyclodextrin **1** in aqueous solution showed two weak positive Cotton effect peaks for ^1L_a at 223 nm ($\Delta\varepsilon = 0.31$ dm^3 mol^{-1} cm^{-1}) and for ^1L_b at 291.6 nm ($\Delta\varepsilon = 0.31$ dm^3 mol^{-1} cm^{-1}), respectively. According to the sector rule proposed by Kajtar,[26] the Cotton effect observed for the ^1L_a and ^1L_b bands indicate that the phosphate moiety is not embedded into the hydrophobic cavity of cyclodextrin,[27,28] which may favour inclusion complexation with a guest molecule.

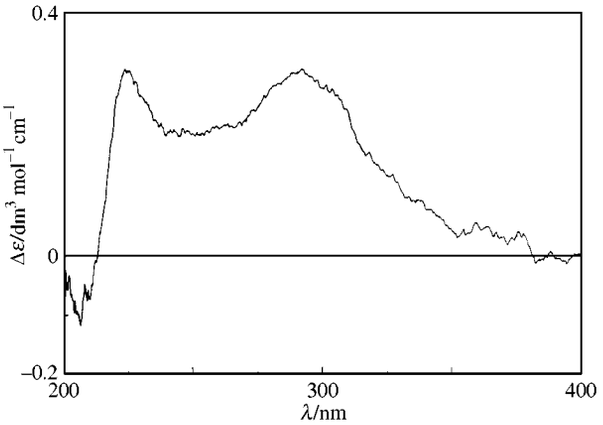
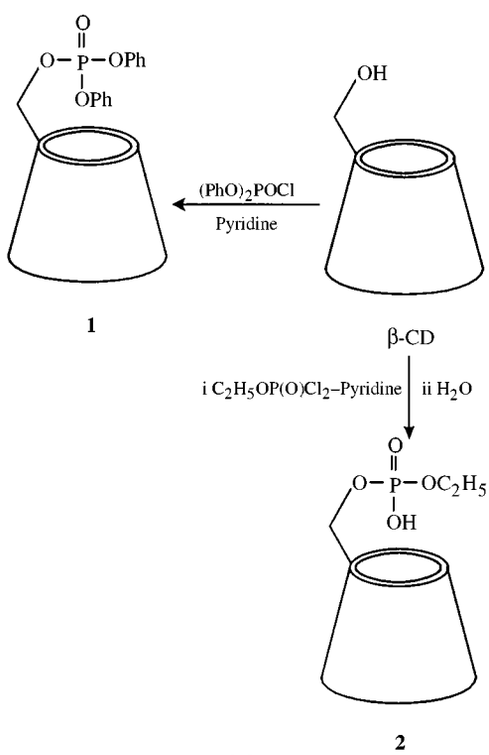


Fig. 1 Circular dichroism spectrum of β-cyclodextrin derivative **1** (0.096 mmol dm^{-3}) in phosphate buffer solution (pH 7.20) at room temperature



Scheme 1

UV spectral titrations

In the titration experiments using UV spectrometry, the absorption maximum of the modified cyclodextrins **1** and **2** gradually increased upon the addition of varying concentration of amino acids. Typical UV–VIS spectral changes upon addition of amino acid to modified cyclodextrin solution are shown in Fig. 2. These results indicated that inclusion complexes had formed by complexation of the modified cyclodextrins 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 eqn. (1).

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

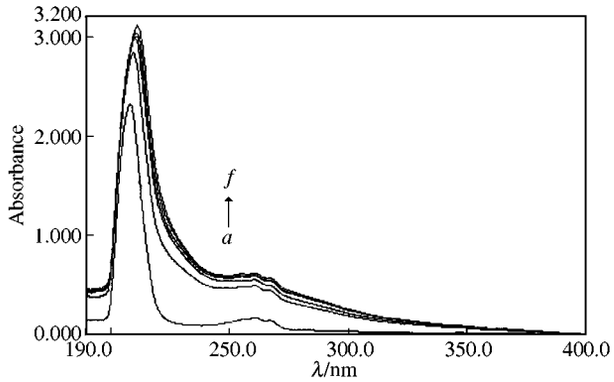


Fig. 2 UV–VIS spectra of **1** (2.02×10^{-4} mol dm^{-3}) in the presence of L-aspartic acid: (*a*) 0 mmol dm^{-3}; (*b*) 3.970; (*c*) 5.955; *d*) 7.940; (*e*) 9.925; (*f*) 11.91; with λ_{max} at 260.8 nm

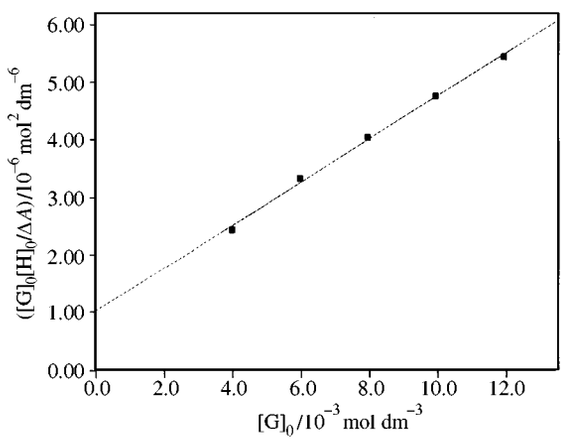


Fig. 3 Typical plot of $[G]_0[H]_0/\Delta A$ *vs.* $[G]_0$ for the inclusion complexation of cyclodextrin derivative **1** with L-aspartic acid in phosphate buffer solution (pH = 7.20) at 25 °C

Under the conditions employed, the concentration of β-cyclodextrin derivatives is much smaller than those 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–Benesi eqn. (2),[24,25]

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

where $[G]_0$ and $[H]_0$ refer to the total concentration of amino acids and β-cyclodextrin derivatives, respectively, $\Delta\varepsilon$ is the difference between the molar extinction coefficient for free and complexed β-cyclodextrin derivatives, and ΔA denotes the changes in the UV–VIS intensity of β-cyclodextrin derivatives upon addition of guest amino acids. For all guest compounds examined, the plots of calculated $[G]_0[H]_0/\Delta A$ values as a function of $[G]_0$ give good straight lines. A typical plot is shown in Fig. 3 for the inclusion complexation of cyclodextrin derivative **1** with L-aspartic acid, where the calculated $[G]_0[H]/\Delta A$ values are plotted against $[G]_0$ to give an excellent linear relationship ($r = 0.999$) with a slope of 3.745×10^{-4} mol dm^{-3} and an intercept of 1.023×10^{-6} mol^2 dm^{-6}. The stability constant (log K_s) and the free energy change ($-\Delta G°$) calculated from the slope and the intercept are listed in Table 1, along with the enantioselectivity ($\Delta\Delta G°$) calculated from $\Delta G°$ for inclusion complexation of L/D-amino acids by the modified β-cyclodextrins. The results obtained verified the 1 : 1 stoichiometry of complexation as assumed above.

Molecular recognition and enantioselectivity

Extensive studies of molecular recognition by cyclodextrins have shown that an important characteristic of the complexation is the simultaneous operation of several weak forces working between receptor (host) and substrate (guest), which should depend on how the size and shape of a guest molecule fit the host cavity. In order to visualize the inclusion complexation behaviour of modified β-cyclodextrins **1** and **2** with amino acids, the changing profiles of the Gibbs 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 Fig. 4. Molecular recognition ability and relative enantioselectivity of β-cyclodextrin 6-*O*-monophosphates **1** and **2** for amino acids will be discussed below from the viewpoints of size/shape-fit and induced-fit relationship between the host cavity and the guest molecule, respectively.

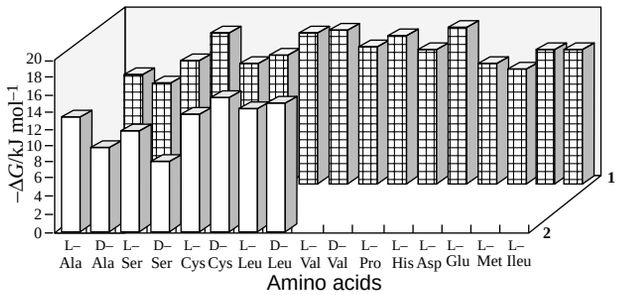


Fig. 4 Gibbs free energy change ($-\Delta G°$) as a function of amino acids for the inclusion complexation of modified β-cyclodextrins **1** and **2** with amino acids in phosphate buffer solution at 25 °C

Table 1 Stability constants (log K_s) and Gibbs free energy changes ($-\Delta G°$) for the inclusion complexation of amino acids with β-cyclodextrin derivatives **1** and **2** in 0.1 mol dm^{-3} phosphate buffer solution (pH 7.20) at 25 °C[a]

Host	Guest	log K_s	$-\Delta G°$/kJ mol^{-1}	$-\Delta\Delta G°$/kJ mol^{-1}
1	L-Ala	2.34	13.3	1.2
	D-Ala	2.12	12.1	
	L-Ser	2.61	14.9	−3.2
	D-Ser	3.17	18.1	
	L-Val	2.87	16.4	−1.6
	D-Val	3.15	18.0	
	L-Leu	3.15	18.0	−0.4
	D-Leu	3.23	18.4	
	L-Cys	2.52	14.4	−1.1
	D-Cys	2.71	15.5	
	L-Pro	2.82	16.1	
	L-His	3.26	18.6	
	L-Asp	2.56	14.6	
	L-Glu	2.40	13.7	
	L-Met	2.80	16.0	
	L-Ileu	2.82	16.1	
2	L-Ala	2.37	13.5	3.5
	D-Ala	1.75	10.0	
	L-Ser	2.09	11.9	3.5
	D-Ser	1.48	8.43	
	L-Leu	2.58	14.7	−0.7
	D-Leu	2.69	15.4	
	L-Cys	2.43	13.9	−1.9
	D-Cys	2.77	15.8	

[a] The log K_s values are the average of two or three independent runs: error < 5% of the reported value. $\Delta\Delta G°$ signifies the difference of free energy changes for the complexation behaviour with L/D-amino acids ($\Delta\Delta G° = \Delta G°_D - \Delta G°_L$).

Mono-(6-*O*-diphenoxyphosphoryl)-β-cyclodextrin 1

As can be seen from Fig. 4, the Gibbs free energy change ($-\Delta G°$) for inclusion complexation with modified cyclodextrin **1** is highly sensitive to the chain length and shape of the alkyl group in amino acids and increases with increasing numbers of methylene groups in some L/D-amino acids examined, *i.e.* L-Ala < L-Val < L-Leu < L-His, D-Ala < D-Val < D-Leu. Inclusion complexation of compound **1** with D-leucine/D-alanine gives the highest molecular selectivity (12.9) of all the amino

acids examined. This seems reasonable, since the induced-fit interaction between the hydrophobic cavity of the host compound and the side chain of guest amino acids enhances the complex stability of D-leucine compared with D-alanine. It is interesting that β-cyclodextrin 6-*O*-monophosphates can recognize not only the size but also the chirality of the amino acids. The natural cyclodextrins possess the higher selectivity for L-amino acids, while compound **1** favours D-amino acids, except for D-alanine. As can be seen from Table 1 and Fig. 4, the enantiomers of L/D-amino acids show substantially different K_s values and Gibbs free energy changes ($-\Delta G°$), yielding fairly good chiral recognition. For the smallest amino acid, alanine, as was the case with the chiral cavity of natural β-cyclodextrin,[1,8] modified β-cyclodextrin **1** is capable of chiral recognition for L/D-alanine; the L/D enantioselectivity calculated from the K_s values is 1.6 ($\Delta\Delta G° = 1.2$ kJ mol^{-1}). Somewhat unexpectedly, all D-amino acids except for D-alanine afford more stable complexes with **1** than the corresponding L-isomer, giving fairly good D/L enantioselectivity, *i.e.* 3.6 ($\Delta\Delta G° = 3.2$ kJ mol^{-1}) for serine, 1.9 ($\Delta\Delta G° = 1.6$ kJ mol^{-1}) for valine, 1.2 ($\Delta\Delta G° = 0.4$ kJ mol^{-1}) for leucine and 1.6 ($\Delta\Delta G° = 1.1$ kJ mol^{-1}) for cysteine.

Examination of CPK molecular models indicates that the enhanced enantioselectivity for D-amino acids may be attributed to the molecular chirality of the guests and the phosphate group originally perching on the edge of the β-cyclodextrin cavity, since the phosphate group in the host compound might be expected to enhance complex stability through electrostatic interaction between phosphorus possessing a positive centre lacking an electron and the amino acid anion accommodated in the cyclodextrin cavity, and there must be a strict complementary geometrical relationship between the β-cyclodextrin cavity and amino acids. In fact, in addition to the electrostatic interaction between host and guest, the microstructural change of the modified cyclodextrin apparently governs the chiral recognition of amino acid molecules to some extent.[8]

Mono-(6-*O*-ethoxyhydroxyphosphoryl)-β-cyclodextrin 2

It should be noted from Table 1 and Fig. 4, for alanine, cysteine and leucine, a tendency analogous to β-cyclodextrin derivative **1** is seen in Gibbs free energy changes ($-\Delta G°$) and the L/D-enantioselectivities of the modified β-cyclodextrin **2**. Since the phosphorus centre of compound **2** is less positive than that of compound **1**, the stability constants for inclusion complexation of most of the amino acids with **1** are higher than those with **2**. Because the phosphate group of modified cyclodextrin **2** bear a hydroxy group, deprotonated in pH 7.20 phosphate buffer, which may be taken as a new recognition point for inclusion complexation with amino acids, the enantioselectivity for L/D-amino acids of **2** is higher than that of **1**, *i.e.* 4.2 ($\Delta\Delta G° = 3.5$ kJ mol^{-1}) for alanine, 2.2 ($\Delta\Delta G° = -1.9$ kJ mol^{-1}) for cysteine, 1.3 ($\Delta\Delta G° = -0.7$ kJ mol^{-1}) for leucine. It is interesting that inclusion complexation of compound **2** with L/D-serine affords more stable complexes with L-serine than D-serine; the L/D enantioselectivity calculated from the K_s values is 4.1 ($\Delta\Delta G° = 3.50$ kJ mol^{-1}). In contrast with inclusion complexation of compound **2** with L/D-serine, compound **1** displays D/L enantioselectivity for serine; the D/L enantioselectivity calculated from the K_s values is 3.6 ($\Delta\Delta G° = -3.2$ kJ mol^{-1}).

One possible explanation for the drastic variation of compounds **1** and **2** in both L- and D-serine enantioselectivity would be intermolecular hydrogen bonding, since the $(C_2H_5O)P(O)O^-$ group of compound **2** may form an intermolecular hydrogen bond with the hydroxy group of serine upon inclusion complexation. These results indicate that the phosphate groups of modified cyclodextrins **1** and **2** possess induced-fit interaction and multipoint recognition mechanism in molecular chirality recognition. Hence, the induced-fit interaction and the complementary geometrical relationship between the host and guest play a crucial role in the inclusion complexation of amino acid molecules.

Acknowledgements

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