Structural Requirement for Chiral Recognition of Amino Acid by (18-Crown-6)-tetracarboxylic Acid: Binding Analysis in Solution and Solid States

Hiroomi Nagata,*[1] Yoshio Machida,[1] Hiroyuki Nishi,[2] Miyoko Kamigauchi,[3] Katsuhiko Minoura,[4] and Toshimasa Ishida[4]

[1]Mitsubishi Tanabe Pharma Corporation, 3-16-89 Kashima, Yodogawa-ku, Osaka 532-8505

[2]Faculty of Pharmacy, Yasuda Women's University, 6-13-1 Yasuhigashi, Asaminami-ku, Hiroshima 731-0153

[3]Kobe Pharmaceutical University, 4-19-1 Motoyama-kitamachi, Higashinada-ku, Kobe 658-8558

[4]Osaka University of Pharmaceutical Sciences, 4-20-1 Nasahara, Takatsuki, Osaka 569-1094

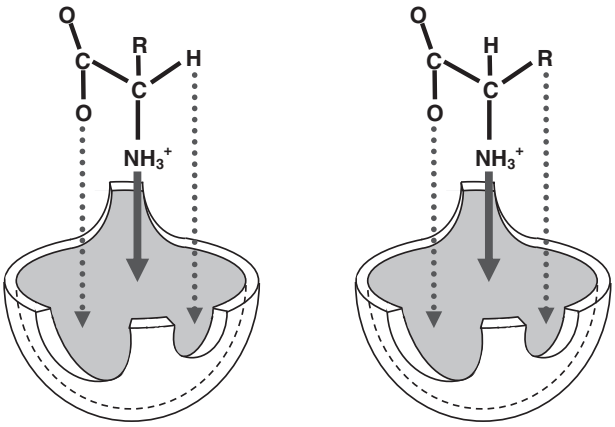
Received August 13, 2008; E-mail: nagata.hiroomi@md.mt-pharma.co.jp

(+)-(18-Crown-6)-tetracarboxylic acid (18C6H$_4$) is used as a chiral selector for various amino acids, where the L-isomer is usually eluted prior to the D-isomer in HPLC using 18C6H$_4$-linked column. To clarify the structural scaffold of (+)-18C6H$_4$ responsible for chiral separation of amino acids, we have previously investigated the interaction mode between (+)-18C6H$_4$ and amino acids using X-ray analysis. However, no conclusive results could be obtained to explain the reverse elution order in the case of serine and to establish a general separation rule of chiral amino acid by (+)-18C6H$_4$ in HPLC. Thus, to clarify the exceptional result obtained with serine and to set a general separation rule, interaction between (+)-18C6H$_4$ and α-amino-*n*-butyric acid, valine, and alanine, as methyl substitutes of the methyl groups for the hydroxy groups of serine and threonine, and the simplest chiral amino acid, respectively, was investigated both in solution and solid states. Consequently, it was found that an asymmetric bowl-like conformation of (+)-18C6H$_4$ is necessary for chiral separation. This conformation is constructed by chiral-specific interaction between the C_α–H groups of the amino acid and the polar oxygen atoms of (+)-18C6H$_4$. It was also found that the exceptional reverse elution observed with serine is due to additional interaction between the polar groups of the amino acid side chain and (+)-18C6H$_4$.

The preparation and evaluation of optically active compounds have become very important steps in the development of new drugs. Particularly, determination of new compounds optical purity is essential to guarantee safety, effectiveness, and quality.[1] Therefore, the pharmaceutical industry has a tremendous interest in techniques that allow chiral separation. Over the past few decades, various analytical methods, such as high-performance liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis (CE), and nuclear magnetic resonance (NMR), have been used to assess compounds optical purity.[2] Among these analytical methods, HPLC using chiral stationary phase (CSP) has become widely used due to its high separation efficiency and generality.[3] CSPs have been broadly categorized into the following types: inclusion (e.g., crown ether and cyclodextrin),[4] brush (e.g., Pirkle-type),[5] polysaccharide (e.g., cellulose),[6] affinity (e.g., protein),[7] and ligand exchange (e.g., Cu^{2+}–amino acid).[8] Crown ether, first introduced by Pedersen in 1967,[9] was shown by Cram et al.[10] to resolve enantiomers through host–guest complexation. In addition, the optically active crown ether derivative has been widely used for optical synthesis, resolution, and analysis of chiral amino compounds. Therefore, crown ether-linked CSP is suitable for chiral separation of racemic amino compounds. In fact various amino acids are effectively separated by CSP-18C6I column, which is chemically immobilized by (+)-(18-crown-6)-tetracarboxylic acid ((+)-18C6H$_4$)[11] on silica gel.[12]

As for separation of D/L-amino acids by CSP-18C6I column, the L-isomer is usually eluted prior to the D-isomer (the first elution rule of L-amino acid),[13] indicating that the D-isomer forms a more stable interaction with (+)-18C6H$_4$ than the L-isomer. To clarify the structural scaffold of (+)-18C6H$_4$ for D/L-separation of racemic amino acids, we have previously investigated the interaction modes between (+)-18C6H$_4$ and six amino acids (Tyr, Ile, Met, PheG, Ser, and Glu) by single-crystal X-ray analysis and discussed the possible relationship between interaction mode and (+)-18C6H$_4$ molecular conformation.[13,14] On the basis of the crystal structures of (+)-18C6H$_4$ co-crystallized with achiral guest molecules, we furthermore investigated the allowable conformational variation of (+)-18C6H$_4$ and its possible transition pathway from standard symmetric conformation to the asymmetric bowl-like one.[15] Consequently, we proposed that the structural requirement of (+)-18C6H$_4$ necessary for chiral separation is an asymmetric bowl-like conformation, and that optical separation is achieved by chiral-dependent interaction between the amino acid and (+)-18C6H$_4$: a model is schematically shown in Figure 1.

Published on the web February 13, 2009; doi:10.1246/bcsj.82.219



(a) L-amino acid (b) D-amino acid

Figure 1. Possible interaction model of (+)-18C6H4 with amino acid.[13] (+)-18C6H4 is depicted with a bowl-like shape with two hollows (big and small) and a lug on the rim.

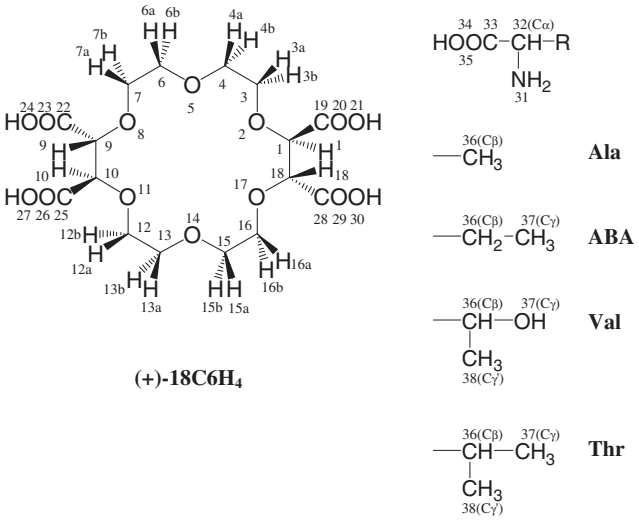


Figure 2. Chemical structure and atomic numbering of (+)-18C6H4 used in this work.

On the other hand, serine (Ser) does not obey the first elution rule of L-amino acid in HPLC, although both the structural requirement of (+)-18C6H4 and its interaction with Ser necessary for chiral separation are satisfied in the complex crystal.[14] Instead, it is suggested that the reversed elution order of racemic Ser is probably due to the hydrogen bond between the hydroxy group of L-Ser side chain and the carboxyl group of (+)-18C6H4, because such interaction is not formed for D-Ser.

To examine the structural origin of Ser reverse separation and to establish a universal rule for separation of chiral amino acids by (+)-18C6H4, interaction with (+)-18C6H4 in solution and solid states was investigated for five chiral amino acids i.e., Ser, threonine (Thr) having a hydroxy group at the same position as Ser, α-amino-*n*-butyric acid (ABA), valine (Val) having a methyl group instead of the hydroxy group of Ser or Thr, and Ala as the simplest chiral amino acid. On the basis of NMR solution and X-ray crystal analyses of these interaction pairs and the related interaction data reported so far, we report in this paper the conformational and interaction requirements of (+)-18C6H4 and amino acids necessary for chiral separation, together with the structural basis for the exception to the rule. The atomic numbering of (+)-18C6H4, Ala, ABA, Ser, Val, and Thr used in this work is shown in Figure 2.

Experimental

HPLC Analysis. Experiments were performed on a Shimadzu LC-10 system with a Shimadzu Chromatopac C-R7A plus a data processor, in which a CSP-18C6I column (2.0 mm i.d., length 15 cm) was used for separation: (+)-18C6H4 was chemically immobilized on 3-aminopropylsilanized silica gel according to the procedure described by Machida et al.[12a] Three columns connected in series were used for enantiomer separation. An aqueous solution containing 1 mM (1 M = 1 mol dm^{-3}) perchloric acid was used as the mobile phase. Chromatographic runs were performed at flow rate of 0.2 mL min^{-1} and temperature of 0 °C. The sample solution was prepared by dissolving the proper amount of racemic Ala, Ser, ABA, Thr, Val, and various amino acids: glutamic acid (Glu), glutamine (Gln), isoleusine (Ile), leusine (Leu), methionine (Met), cysteine (Cys), arginine (Arg), histidine (His), phenylalanine (Phe), tryptophane (Trp), tyrosine (Tyr), phenylglycine (PheG), and lysine (Lys) in 10 mM perchloric acid. Finally, 1-μL volume of the sample prepared was injected into the column, and the eluted solution was detected by absorbance at 200 or 254 nm.

NMR Measurement. All NMR spectra were measured on a Varian unity INOVA500 spectrometer. ^{1}H NMR spectra were measured in CD_3OD solution containing adequate DCl with tetramethylsilane (TMS) as an internal standard; The chemical shifts of various amino acids in CD_3OD solution were much more sensitive to interaction with (+)-18C6H4 than those in D_2O solution, though these shift patterns were common in CD_3OD and D_2O solutions.[16]

^{1}H NMR spectra were measured for a mixture solution of an equimolar amount (6 mM) of L- and D-enantiomers of each amino acid (Ala, ABA, Ser, Val, and Thr) and (+)-18C6H4 at 298 K. Proton assignments of each amino acid and (+)-18C6H4 were determined by the COSY, HSQC, and HMBC measurements. Furthermore, the chemical shift of C_α proton at the range of 273–323 K was measured for Ala, ABA, and Ser to investigate temperature dependence in the interaction with (+)-18C6H4. The stoichiometry between each amino acid (Ala, Ser, and ABA) and (+)-18C6H4 was determined by the continuous variation method (Job plot),[17] where the total concentration of both molecules was kept constant (10 mM) and the molar fraction of (+)-18C6H4 was varied in the range of 0.2–0.8 at 298 K. The binding constant (K_a) between each amino acid (Ala, Ser, and ABA) and (+)-18C6H4 at 298 K was estimated by Scott's modification[18] of Benesi–Hildebrand equation: $[(+)\text{-}18C6H_4]_t/\Delta\delta_{obs} = [(+)\text{-}18C6H_4]_t/\Delta\delta_c + 1/K_a\Delta\delta_c]$, where $[(+)\text{-}18C6H_4]_t$ is the molar concentration of (+)-18C6H4, $\Delta\delta_{obs}$ is the difference of the chemical shift of C_α proton of each amino acid observed for the given concentration of $[(+)\text{-}18C6H_4]_t$ from its free component, $\Delta\delta_c$ is the chemical shift difference of C_α proton of amino acid between a pure sample of complex and free component at the saturation. The concentration of amino acid was kept at 1 mM, while that of (+)-18C6H4 was varied from 0 to 4 mM.

X-ray Analysis. The complex crystals of (+)-18C6H4–L-Ala (L**1**-**1** and L**1**-**2**), (+)-18C6H4–D-Ala (D**1**-**1** and D**1**-**2**), (+)-

18C6H$_4$–L-ABA (L**2**), (+)-18C6H$_4$–D-ABA (D**2**), (+)-18C6H$_4$–L-Thr (L**3**), (+)-18C6H$_4$–D-Thr (D**3**), (+)-18C6H$_4$–L-Val (L**4**), and (+)-18C6H$_4$–D-Val (D**4**) in the form of colorless prisms, were prepared from aqueous solution or aqueous perchloric acid solution containing an equimolar amount of (+)-18C6H$_4$ and an enantiomer of each amino acids by slow evaporation at 293 K. X-ray data were collected with a Rigaku RINT RAPID/R diffractometer using graphite-monochromated Cu Kα radiation ($\lambda =$ 1.5418 Å) at 173 K. Details for cell parameter determination and data collection are summarized in Table 1. Intensity data within $2\theta \leqq 136.4°$ were measured using an imaging plate area detector. Each crystal structure was solved by the direct method using SIR-92.[19] In the solutions of L**1**-**1**, L**3**, and D**3**, disorder was encountered. Progress of Fourier refinement revealed the disordered two positions for the C13 atom of (+)-18C6H$_4$ in L**1**-**1** and L**3**, and for the water molecule in L**3** and D**3**. These final occupancies were determined as result of the refinement. Positional parameters of non-H atoms were refined by full-matrix least-squares with anisotropic temperature parameters using SHELXL-97.[20] The positions of H-atoms, except for the disorder sites, were determined by difference Fourier map. They were treated as riding with fixed isotropic displacement parameters and were not included as variables in the refinement. The final atomic coordinates, anisotropic temperature factors, and atomic coordinates of H atoms have been deposited with the following designations: D**1**-**1**: CCDC-698130, D**1**-**2**: CCDC-698131, D**2**: CCDC-698132, D**3**: CCDC-698133, D**4**: CCDC-698134, L**1**-**1**: CCDC-698135, L**1**-**2**: CCDC-698136, L**2**: CCDC-698137, L**3**: CCDC-698138, L**4**: CCDC-698139.

Results and Discussion

HPLC Analysis. Chiral separation parameters of Ala, Ser, ABA, Thr, and Val together with those of the references; Glu, Gln, Leu, Ile, Met, Cys, Arg, Lys, His, Phe, Trp, Tyr, and PheG, in HPLC analysis using CSP-18C6I column are given in Table 2. According to the first elution rule of L-amino acid in HPLC, the L-enantiomers of all amino acids, except for those of Ser and Thr, were eluted prior to the D-enantiomers. As ABA and Val are substitutes of the methyl groups and hydroxy groups of Ser and Thr side chains, respectively, it is obvious that Ser and Thr exception to the elution rule is due to the hydroxy groups of Ser and Thr side chains.

Interaction between (+)-18C6H$_4$ and Chiral Amino Acid in the Solution State. Binding Stoichiometry and Binding Constant: The stoichiometry between amino acid and (+)-18C6H$_4$ in solution at 298 K was investigated by Job plot for chemical shift of C$_\alpha$ proton of L- and D-enantiomers of Ala, Ser, and ABA. As shown in Figure 3, nearly symmetric bell-curves were obtained, indicating the formation of a 1:1 complex for all enantiomers.

The binding constant K_a for the 1:1 complex of each enantiomer and (+)-18C6H$_4$ at 298 K was evaluated from the chemical shift of C$_\alpha$ proton by Scott's modification of Benesi–Hildebrand equation. As shown in Figure 4, the slope of the plot is equal to $1/\Delta\delta_c$ and the intercept to $1/K_a\Delta\delta_c$, allowing estimation of K_a. The estimated values of $\Delta\delta_c$ and K_a are given in Table 3. The binding constants of the D-enantiomers were all larger than those of the L-enantiomers. In the case of Ala and ABA, which obey the elution rule, a positive correlation was observed between K_a value and the elution order in HPLC. A

Table 1. Details of Crystal Data, Intensity Collection, and Structure Refinement

	L1-1	L1-2	D1-1	D1-2	L2	D2	L3	D3	L4	D4
Formula	$C_{16}H_{24}O_{14}$ ·$C_3H_7NO_2$ ·$HClO_4$·H_2O	$C_{16}H_{24}O_{14}$ ·$C_3H_7NO_2$ ·3/$2H_2O$	$C_{16}H_{24}O_{14}$ ·$C_3H_7NO_2$ ·$HClO_4$·1/$2H_2O$	$C_{16}H_{24}O_{14}$ ·$C_3H_7NO_2$	$C_{16}H_{24}O_{14}$ ·$C_4H_9NO_2$ ·3/$2H_2O$	$C_{16}H_{24}O_{14}$ ·$C_4H_9NO_2$ ·1/$2H_2O$	$C_{16}H_{24}O_{14}$ ·$C_4H_9NO_2$ ·5/$2H_2O$	$C_{16}H_{24}O_{14}$ ·$C_4H_9NO_2$ ·$HClO_4$·5H_2O	$C_{16}H_{24}O_{14}$ ·$C_5H_{11}NO_2$ ·H_2O	$C_{16}H_{24}O_{14}$ ·$C_5H_{11}NO_2$ ·H_2O
Molecular weight	647.92	555.47	638.92	529.45	570.50	553.49	604.52	729.98	575.52	575.52
Crystal system	Orthorhombic	Monoclinic	Orthorhombic	Orthorhombic	Orthorhombic	Triclinic	Triclinic	Monoclinic	Triclinic	Orthorhombic
Space group	$P2_12_12_1$	$P2_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P1$	$P1$	$P2_1$	$P1$	$P2_12_12_1$
Unit cell dimensions										
a/Å	11.885(2)	8.8562(9)	9.740(4)	10.9105(9)	9.115(1)	9.480(2)	9.497(1)	10.845(2)	8.942(1)	9.561(1)
b/Å	14.246(2)	19.7092(4)	13.957(1)	11.241(1)	15.940(2)	9.855(2)	10.157(1)	10.292(3)	9.526(1)	16.369(2)
c/Å	16.255(2)	14.6031(5)	41.742(1)	19.905(2)	37.447(5)	14.419(3)	15.284(2)	14.576(3)	9.475(1)	16.812(2)
α/°	90	90	90	90	90	99.67(1)	81.761(6)	90	113.986(7)	90
β/°	90	91.245(5)	90	90	90	91.06(1)	84.419(6)	98.67(1)	93.221(8)	90
γ/°	90	90	90	90	90	109.01(1)	71.160(6)	90	110.378(7)	90
V/Å^3	2752.1(6)	2548.3(3)	5675(2)	2441.3(4)	5441(1)	1251.6(4)	1378.9(2)	1608.3(6)	672.6(2)	2631.1(5)
Z	4	4	8	4	8	2	2	2	1	4
D_x/g cm^{-3}	1.564	1.448	1.496	1.440	1.393	1.469	1.456	1.507	1.421	1.453
No. of reflections with $I > 2\sigma(I)$	4846	6864	10339	4212	9827	6980	7554	5185	3850	4693
R ($I > 2\sigma(I)$)	0.055	0.041	0.053	0.036	0.040	0.067	0.040	0.057	0.037	0.030
Rw ($I > 2\sigma(I)$)	0.149	0.112	0.146	0.095	0.103	0.173	0.106	0.149	0.110	0.074

Table 2. Enantiomer Separation Parameters of Various Amino Acids[a)]

	k_1[b)]	k_2[c)]
Ala	11.0 (L)	13.0 (D)
Ser	9.9 (D)	13.5 (L)
ABA	9.9 (L)	12.1 (D)
Thr	8.0 (D)	8.3 (L)
Val	8.8 (L)	9.5 (D)
Glu	10.6 (L)	12.8 (D)
Gln	10.1 (L)	12.7 (D)
Leu	11.3 (L)	15.6 (D)
Ile	10.7 (L)	12.2 (D)
Met	14.7 (L)	20.5 (D)
Cys	11.6 (L)	12.7 (D)
Arg	33.1 (L)	56.1 (D)
Lys	51.3 (L)	63.0 (D)
His	26.5 (L)	35.9 (D)
Phe	13.6 (L)	22.8 (D)
Trp	25.9 (L)	40.1 (D)
Tyr	14.5 (L)	21.7 (D)
PheG	17.1 (L)	33.2 (D)

a) The mobile phase was 1 mM perchloric acid, the column temperature was 0 °C. b) Retention time/min of the first eluted enantiomer. c) Retention time/min of the second eluted enantiomer.

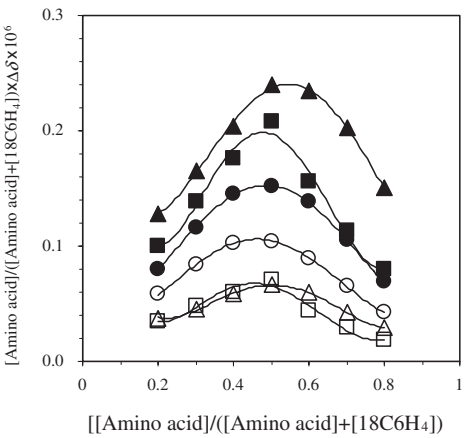


Figure 3. Job plots for L- and D-Ala, Ser, and ABA in solution with (+)-18C6H4; ● D-Ala, ○ L-Ala, ■ D-Ser, □ L-Ser, ▲ D-ABA, △ L-ABA.

similar positive correlation was observed for the enantiomers of 1-(1-naphthyl)ethylamine,[21] phenylglycine, and its methyl ester.[22] However, the K_a value estimated from C_α proton does not necessarily reflect the overall interaction, because a similar situation around C_α proton was also observed for Ser, which shows negative correlation with the elution order in HPLC.

On the other hand, $\Delta\delta_c$ values of the D-enantiomers of Ala, ABA, and Ser were larger than those of the L-enantiomers. It has been reported that $\Delta\delta_{obs}$ values of C_α protons of various chiral amino acids, such as acidic (Asp, Asn, Glu, and Gln), basic (Arg, His, and Lys), alkyl (Ala, Cys, Met, and Ser), and aromatic (Phe, Trp, Tyr, and DOPA) amino acids, are all larger for the D-enantiomers than the L-enantiomers in D_2O.[16] As these amino acids, except Ser, obey the first elution rule of

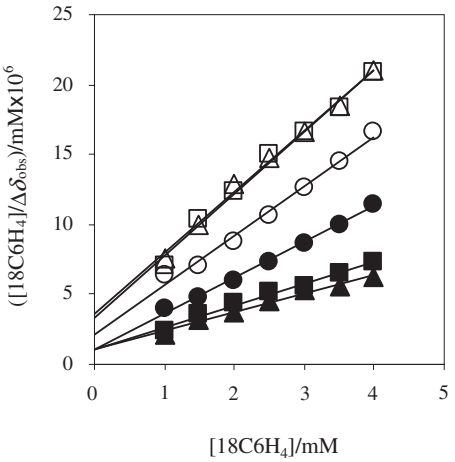


Figure 4. Scott plots for L- and D-Ala, Ser, and ABA in solution with (+)-18C6H4; ● D-Ala, ○ L-Ala, ■ D-Ser, □ L-Ser, ▲ D-ABA, △ L-ABA.

Table 3. Complex-Induced Chemical Shifts (ppm)[a)] at Saturation ($\Delta\delta_c$) and Apparent Binding Constants K_a/mol^{-1}

	$\Delta\delta_c$ (D)	$\Delta\delta_c$ (L)	K_a (D)	K_a (L)
Ala	0.39	0.28	2332	1642
ABA	0.74	0.23	1323	1236
Ser	0.64	0.23	1455	1323

a) All chemical shifts are reported in ppm relative to TMS in CD_3OD at 298 K.

L-amino acid in HPLC, it is suggested that the major driving force for chiral interaction is based on interaction between the backbone structure of the chiral amino acid and (+)-18C6H4, regardless of the sort of amino acid side chain. The exception of Ser to the elution rule, despite the fact that its K_a, $\Delta\delta_c$, and $\Delta\delta_{obs}$ values are similar to those of the other amino acids obeying the elution rule, is due to another factor such as the hydrogen bond between the side chain of L-serine and (+)-18C6H4 observed in the complex crystal.[6]

Chiral-Dependent Interaction of Amino Acid with (+)-18C6H4: The proton chemical shifts of Ala, Ser, ABA, Thr, Val, and (+)-18C6H4 at 298 K and their differences in the absence and presence of equimolar (+)-18C6H4 are summarized in Tables 4 and 5, respectively, and representative ^{1}H NMR spectra are shown in Figure 5.

As shown in Table 4, C_α protons of the D-enantiomers were more shifted toward the lower-field side than those of the L-enantiomers in the presence of (+)-18C6H4. Shift of C_α proton toward the lower-field side indicates participation of C_α–H group in the hydrogen bond or electrostatic interaction with the acceptor atom of (+)-18C6H4, and the large chemical shift change in the D-enantiomer as compared with the L-enantiomer suggests that interaction of the D-enantiomer with (+)-18C6H4 is stronger than that of the L-enantiomer. This agrees with the K_a values of these amino acids. Interestingly, no notable difference in the chemical shift patterns of C_α protons was observed between Ser and ABA or between Thr and Val. Again, this indicates that Ser and Thr exception to the elution rule is due to additional factors. In contrast, the chemical shifts

Table 4. ^{1}H Chemical Shifts (ppm) of Ala, Ser, ABA, Thr, and Val in the Absence or Presence of (+)-18C6H$_4$

	^{1}H chemical shifts of amino acids[a)]		
	C_αH	C_βH	C_γH
DL-Ala (Free)	4.02	1.54	—
L-Ala with (+)-18C6H$_4$	4.17	1.54	—
Δ_δ (L)	0.15	0.00	—
D-Ala with (+)-18C6H$_4$	4.28	1.58	—
Δ_δ (D)	0.26	0.04	—
$\Delta\Delta_\delta$ (D–L)	0.11	0.04	—
DL-Ser (Free)	4.04	3.99/3.95	—
L-Ser with (+)-18C6H$_4$	4.15	4.03/3.99	—
Δ_δ (L)	0.11	(NA)[b)]	—
D-Ser with (+)-18C6H$_4$	4.37	4.04/3.88	—
Δ_δ (D)	0.33	(NA)	—
$\Delta\Delta_\delta$ (D–L)	0.22	(NA)	—
DL-ABA (Free)	3.93	1.96	1.07
L-ABA with (+)-18C6H$_4$	4.04	1.98/1.94	1.06
Δ_δ (L)	0.11	0.02/−0.02	−0.01
D-ABA with (+)-18C6H$_4$	4.31	2.00/1.92	1.08
Δ_δ (D)	0.28	0.04/−0.04	0.01
$\Delta\Delta_\delta$ (D–L)	0.17	(NA)	0.00
DL-Thr (Free)	3.81	4.28	1.34
L-Thr with (+)-18C6H$_4$	3.86	4.25	1.34
Δ_δ (L)	0.05	−0.03	0.00
D-Thr with (+)-18C6H$_4$	3.93	4.25	1.34
Δ_δ (D)	0.12	−0.03	0.00
$\Delta\Delta_\delta$ (D–L)	0.07	0.00	0.00
DL-Val (Free)	3.85	2.31	1.09
L-Val with (+)-18C6H$_4$	3.98	2.26	1.15/1.05
Δ_δ (L)	0.13	−0.05	0.06/−0.04
D-Val with (+)-18C6H$_4$	4.24	2.19	1.17/1.08
Δ_δ (D)	0.39	−0.12	0.08/−0.01
$\Delta\Delta_\delta$ (D–L)	0.26	−0.07	(NA)

a) All chemical shifts are reported in ppm relative to TMS in CD$_3$OD at 298 K. b) Not available.

Table 5. ^{1}H Chemical Shifts (ppm) of (+)-18C6H$_4$ in the Absence or Presence of Amino Acid

	^{1}H chemical shifts of (+)-18C6H$_4$[a)]				
	CH[b)]	CH$_2$[c)]			
		(i)	(ii)	(iii)	(iv)
Free	4.79	3.80	3.62	3.64	3.60
With L-Ala	4.63	4.05	3.55	3.90	3.52
Δ_δ (with L)	−0.16	0.25	−0.07	0.26	−0.08
With D-Ala	4.62	4.02	3.54	3.84	3.51
Δ_δ (with D)	−0.17	0.22	−0.08	0.20	−0.09
$\Delta\Delta_\delta$ (with D–L)	0.01	−0.03	0.01	−0.06	0.01
With L-Ser	4.66	3.96	3.56	3.84	3.53
Δ_δ (with L)	−0.13	0.16	−0.06	0.20	−0.07
With D-Ser	4.66	4.02	3.53	3.86	3.49
Δ_δ (with D)	−0.13	0.22	−0.09	0.22	−0.11
$\Delta\Delta_\delta$ (with D–L)	0.00	0.06	0.03	0.02	0.04
With L-ABA	4.67	4.00	3.55	3.85	3.53
Δ_δ (with L)	−0.12	0.20	−0.07	0.21	−0.07
With D-ABA	4.64	4.04	3.51	3.87	3.48
Δ_δ (with D)	−0.15	0.24	−0.11	0.23	−0.12
$\Delta\Delta_\delta$ (with D–L)	0.03	0.04	0.04	0.02	0.05
With L-Thr	4.73	3.88	3.60	3.71	3.58
Δ_δ (with L)	−0.06	0.08	−0.02	0.07	−0.02
With D-Thr	4.73	3.87	3.59	3.70	3.57
Δ_δ (with D)	−0.06	0.07	−0.03	0.06	−0.03
$\Delta\Delta_\delta$ (with D–L)	0.00	−0.01	0.01	−0.01	0.01
With L-Val	4.68	3.99	3.58	3.84	3.53
Δ_δ (with L)	−0.11	0.19	−0.04	0.20	−0.07
With D-Val	4.67	4.00	3.53	3.83	3.50
Δ_δ (with D)	−0.12	0.20	−0.09	0.19	−0.10
$\Delta\Delta_\delta$ (with D–L)	0.01	0.01	0.05	−0.01	0.03

a) All chemical shifts are reported in ppm relative to TMS in CD$_3$OD at 298 K. b) Chemical shifts of H1, H9, H10, and H18 methine protons. c) Chemical shifts of methylene protons of (i) H3a, H7b, H12a, and H16b, (ii) H3b, H7a, H12b, and H16a, (iii) H4a, H6b, H13a, and H15b, and (iv) H4b, H6a, H13b, and H15a, respectively. Proton assignments were determined by the COSY, HSQC, and HMBC measurements.

of C_β or C_γ protons did not show such changes, but were randomly shifted toward the lower- or higher-field side due to interaction with (+)-18C6H$_4$, and their degrees were considerably small as compared with those of C_α protons, indicating the weak interaction of C_β or C_γ protons with (+)-18C6H$_4$ in solution state. The chemical shift changes of C_α, C_β, and C_γ protons suggest again that the major driving force for chiral interaction is based on interaction between the backbone structure of the chiral amino acid and (+)-18C6H$_4$. The chemical shifts of the methylene and methine protons of (+)-18C6H$_4$ were also changed by complexation with the amino acid. As shown in Table 5, only slight differences in the pattern of these shift changes were observed among the different guest compounds. It is believed that these differences are caused by difference of chiral interaction.

Temperature Dependency of Chiral Interaction between Amino Acid and (+)-18C6H$_4$: The chemical shifts of C_α protons of Ala, Ser, and ABA complexed with (+)-18C6H$_4$ at 273–323 K are shown in Figure 6. The chemical shift of the D-enantiomer increased with decreasing temperature, though that of the L-enantiomer showed little change. This suggests that the D- and L-enantiomers for each amino acid have different chiral interaction mode with (+)-18C6H$_4$. Furthermore, this finding indicates, as found with HPLC results,[12a] that NMR measurement at lower temperature is more effective for enantiomer separation.

Interaction between (+)-18C6H$_4$ and Chiral Amino Acid in the Crystal State. Structural Features of Complexes: The respective crystals consisted of the 1:1 complex of (+)-18C6H$_4$ and the amino acid. The crystals of L**1**-**2**, D**1**-**1**, L**2**, D**2**, and L**3** contained two crystallographically independent complexes per asymmetric unit, whereas others consisted of one complex. All crystals, except D**1**-**2**, included one (L**1**-**1**, D**1**-**1**, D**2**, L**4**, and D**4**), three (L**1**-**2** and L**2**), or five (L**3** and D**3**) independent water molecules, and one (L**1**-**1** and D**3**) or two (D**1**-**1**) perchloric ions per asymmetric unit, respectively. (+)-18C6H$_4$ took a neutral form (L**1**-**1** (Mol. B), L**2**, D**2** (Mol. B), L**3** (Mol. A), and D**3**), a dianionic form (L**1**-**1**

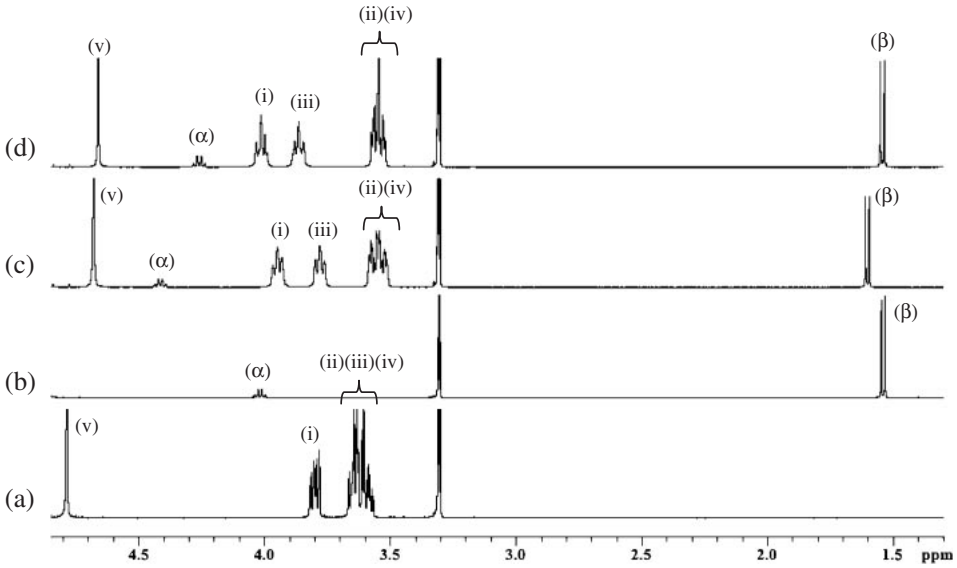


Figure 5. ^{1}H NMR spectra of Ala–(+)-18C6H$_4$ with equimolar mixtures (6 mM each); (a) (+)-18C6H$_4$, (b) DL-Ala, (c) L-Ala with (+)-18C6H$_4$ and (d) D-Ala with (+)-18C6H$_4$. (α): chemical shifts of C$_\alpha$ protons of Ala, (β): chemical shifts of C$_\beta$ protons of Ala, (i)–(iv): chemical shifts of methylene protons of (+)-18C6H$_4$; (i) H3a, H7b, H12a, and H16b, (ii) H3b, H7a, H12b, and H16a, (iii) H4a, H6b, H13a, and H15b, (iv) H4b, H6a, H13b, and H15a, and (v) chemical shifts of methine protons of (+)-18C6H$_4$: H1, H9, H10, and H18.

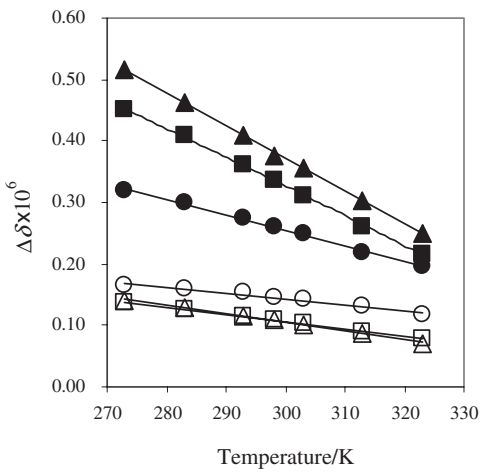


Figure 6. Temperature dependency of the chemical shift of C$_\alpha$ proton of Ala, Ser, and ABA complexed with (+)-18C6H$_4$; ● D-Ala, ○ L-Ala, ■ D-Ser, □ L-Ser, ▲ D-ABA, △ L-ABA.

(Mol. A) and D**2** (Mol. A)) or a monoanionic form (in others) depending on the protonation state of the carboxyl groups. The bond lengths and angles of (+)-18C6H$_4$ were accurate enough allowing us to equally compare the structural and conformational features with the other related complexes reported so far. All amino acids had the zwitterionic form (L-Ala (Mol. A) in L**1**-**2** and L-Thr (Mol. B) in L**3**) or monocationic form (others), and their bond lengths, angles, and molecular conformations were all in the usually observed range.[23–26] The interaction modes between amino acid and (+)-18C6H$_4$ observed in complexes are shown in Figure 7. Interaction patterns of the enantiomers of Ala, ABA, and Val with (+)-18C6H$_4$ were common to those of Tyr, Ile, Met, and PheG, which obey the first elution rule of L-amino acid in HPLC.[13] This clearly indicates that chiral interaction of the amino acid with (+)-18C6H$_4$ has the same mode regardless of the sort of side chain. Ala–(+)-18C6H$_4$ complex provides a representative model for chiral separation of amino acid by (+)-18C6H$_4$ (Figure 8). Furthermore, interaction patterns of D- and L-Thr with (+)-18C6H$_4$ are similar to those of Ser, both of which do not obey the first elution rule of L-amino acid in HPLC. This indicates that chiral interaction modes between amino acid and (+)-18C6H$_4$ are slightly affected by the packing force in complex crystals.

Conformation of (+)-18C6H$_4$ and Its Relation to Chiral Separation of Amino Acid: Previously, we have reported that although the molecular conformation of (+)-18C6H$_4$ can freely change within an allowable range, it preferentially takes one of the three different convex and asymmetric conformations; conformers I–III, when it forms a complex with chiral amino acid.[15] These conformers are classified according to the different orientation around the O11–C12–C13–O14–C15–C16–O17 bond sequence (Figure 9) and are dependent on the interaction modes of the guest molecule. In order to investigate the possible relation between conformation of (+)-18C6H$_4$ and chiral recognition of amino acid in more detail, X-ray structures of related complexes reported so far were surveyed. As shown in Table 6, the conformation of (+)-18C6H$_4$ did not depend on the charge, the structure, and chirality of the guest compound, or the presence of the counter ion. It is noteworthy that two independent (+)-18C6H$_4$ molecules in the complex with D-Ala (D**1**-**1**) and D-PheG[13] take two different conformers; conformer I and III (with D-Ala) or conformer I and II (with D-PheG). This means nearly equal stability of conformers I, II, and III. However, the ratio of each conformer in all complex crystals is 7:1:2 for conformer I:II:III, suggesting preference of conformation I for complexation with chiral amino acid in the

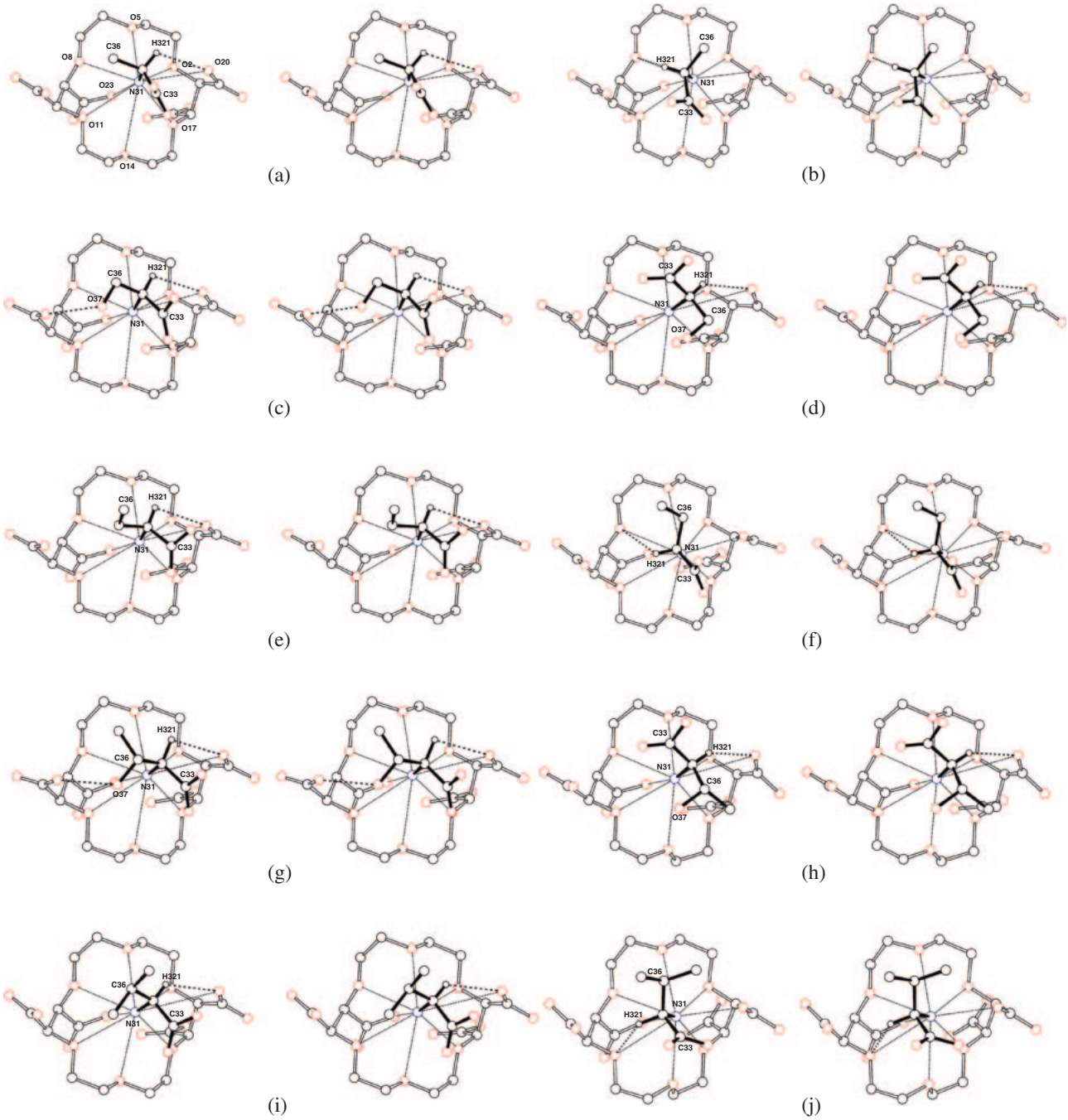


Figure 7. Stereoscopic views of representative molecular interactions of (a) L-Ala (L**1**-**1**), (b) D-Ala (D**1**-**1**, Mol. A), (c) L-Ser,[14] (d) D-Ser,[14] (e) L-ABA (L**2**, Mol. A), (f) D-ABA (D**2**, Mol. A), (g) L-Thr (L**3**, Mol. A), (h) D-Thr (D**3**), (i) L-Val (L**4**), and (j) D-Val (D**4**) with (+)-18C6H$_4$, viewed perpendicular to the crown ether ring. Amino acids and (+)-18C6H$_4$ are depicted with filled and open bonds, respectively. The thin dotted lines represent N–H···O/N···O interactions and the thick dotted lines represent C_α–H···O or O–H···O interactions.

solid state. Moreover, (+)-18C6H$_4$ (Mol. B) in L**2** exceptionally took the symmetric convex/C_{2s}-type conformation, as shown in Figure 10, though the conformations of other (+)-18C6H$_4$ molecules complexed with amino acids were all asymmetric (conformer I–III). This indicates that these asymmetric and symmetric conformations of (+)-18C6H$_4$ are energetically similar.

Structural Scaffold for Chiral Separation of Amino Acid by (+)-18C6H$_4$: In order to clarify the structural scaffold of (+)-18C6H$_4$ for making chiral separation of racemic amino acids possible, the noteworthy difference between the interaction modes of (+)-18C6H$_4$ with L- and D-amino acids was surveyed for hitherto analyzed complexes. The results are summarized in Figures 11–13.

In all complexes, the amino group of chiral amino acids was located near the center of the crown ether ring and formed hydrogen bonds or electrostatic interactions with the eight oxygen atoms of the ring and carboxyl groups. As for the chiral

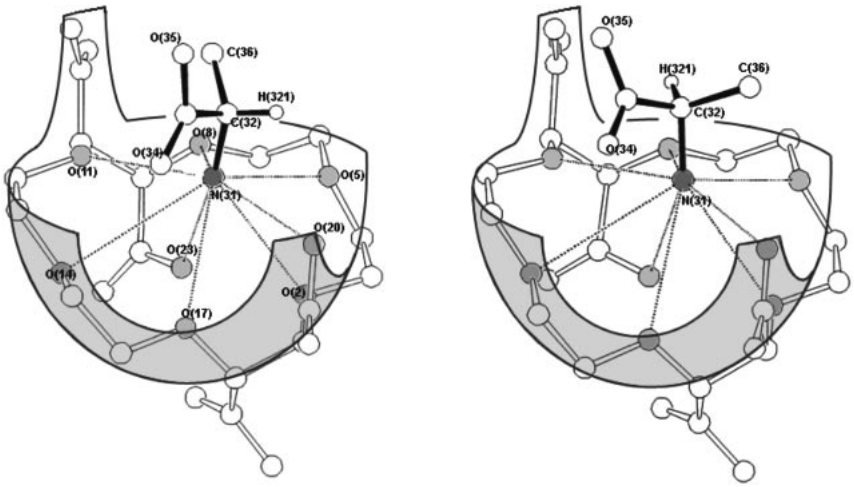


Figure 8. Schematic interaction patterns of (a) L-Ala (L**1**-**1**) and (b) D-Ala (D**1**-**1**, Mol. A) with (+)-18C6H4. The dotted lines represent N–H···O/N···O interactions. The conformation of (+)-18C6H4 is roughly depicted with a bowl-shaped figure with two concave and one convex rim.

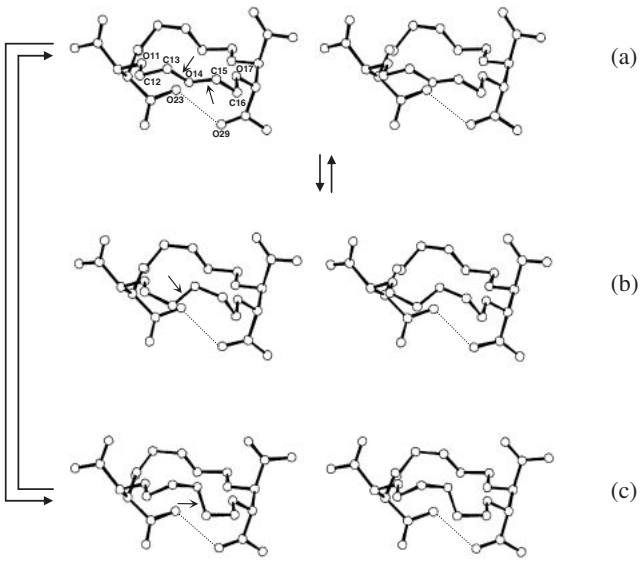


Figure 9. Stereoscopic views of the three different conformers of (+)-18C6H4 in the crystal state; (a) conformer I, (b) conformer II, and (c) conformer III. Conformer I could be converted into an equilibrium state with conformers II and III by successive rotation around the C13–O14 or O14–C15 bond as shown by the arrows in (a)–(c).[15] The dotted lines represent intramolecule hydrogen bond between O23 and O29 of the carboxyl groups.

separation of amino acid, NH···O interaction appears to be equally formed between the L- and D-amino acids but is not a driving force for chiral separation, because there is no clear difference between D- and L-amino acids concerning the number, average distance, and angle of hydrogen bonds, as shown in Figure 11.

The carboxyl group of the amino acid did not directly interact with the polar atoms of partner's (+)-18C6H4 in all complexes reported so far. Instead, the carboxyl group was hydrogen bonded to the donor or acceptor atom of neighboring

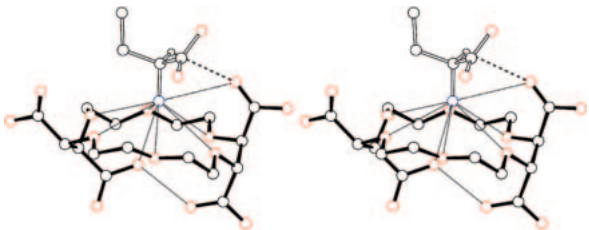


Figure 10. Stereoscopic view of the convex/C_{2s}-type conformation[15] of (+)-18C6H4 in L**2** (Mol. B). (+)-18C6H4 and L-ABA are depicted with filled and open bonds, respectively. The dotted lines represent hydrogen bond or electrostatic interaction.

(+)-18C6H4, water or counter ion, and the interaction mode was not significantly different between the L- and D-amino acid, as shown in Figure 12. However, the carboxyl group of the amino acid may assume a key role in chiral separation, because it determines the spatial location of each enantiomer with respect to (+)-18C6H4 leading to chiral separation (Figure 1).

On the other hand, C_α–H···O interaction of the amino acid with O atom of (+)-18C6H4 was significantly different between the L- and D-amino acids. As for C_α–H···O interaction, the distance of C_α···O is generally shorter than the sum of their van der Waals radii (3.22 Å) and the angle of C–H···O is greater than 110°,[27] although these geometric parameters are far from the usual hydrogen-bonding criteria.[28] The distance and angle of C_α–H···O interaction in the D-amino acid were meaningfully shorter and larger than those in the L-amino acid, respectively, as shown in Figure 13: C_α–H···O = 2.53 (D) and 2.76 Å (L), ∠O–H···O = 129 (D) and 105° (L) in average. This means that C_α–H···O interaction force of the D-amino acid with (+)-18C6H4 is stronger than that of the L-amino acid, though the distance of C_α···O is almost the same between the L- and D-amino acid. Therefore, it is reasonable to suggest that the major factor for separating the L- and D-amino acids is the difference in C_α–H···O interaction force, which is created by the position

Table 6. Conformer of (+)-18C6H$_4$ in Complex Crystal with Chiral Amino Acid

Conformer	Charge	Complex with	Counter ion	Crystal
I	(+)-18C6H$_4$	L-Ala	ClO_4^-	L**1-1**
I	(+)-18C6H$_2^{2-}$	L-Ala	—	L**1-2** (Mol. A)
I	(+)-18C6H$_4$	L-Ala	—	L**1-2** (Mol. B)
III	(+)-18C6H$_4$	D-Ala	ClO_4^-	D**1-1** (Mol. A)
I	(+)-18C6H$_4$	D-Ala	ClO_4^-	D**1-1** (Mol. B)
I	(+)-18C6H$_4$	D-Ala	—	D**1-2**
I	(+)-18C6H$_4$	L-Ser	ClO_4^-	(Ref. 6)
I	(+)-18C6H$_3^-$	D-Ser	—	(Ref. 6)
I	(+)-18C6H$_3^-$	L-ABA	—	L**2** (Mol. A)
(convex/C_{2s})	(+)-18C6H$_3^-$	L-ABA	—	L**2** (Mol. B)
I	(+)-18C6H$_2^{2-}$	D-ABA	—	D**2** (Mol. A)
I	(+)-18C6H$_4$	D-ABA	—	D**2** (Mol. B)
I	(+)-18C6H$_4$	L-Thr	—	L**3** (Mol. A)
I	(+)-18C6H$_3^-$	L-Thr	—	L**3** (Mol. B)
III	(+)-18C6H$_4$	D-Thr	ClO_4^-	D**3**
I	(+)-18C6H$_3^-$	L-Val	—	L**4**
I	(+)-18C6H$_3^-$	D-Val	—	D**4**
III	(+)-18C6H$_3^-$	L-Tyr	—	(Ref. 7)
I	(+)-18C6H$_4$	D-Tyr	ClO_4^-	(Ref. 7)
II	(+)-18C6H$_3^-$	L-Ile	—	(Ref. 7)
I	(+)-18C6H$_3^-$	D-Ile	—	(Ref. 7)
I	(+)-18C6H$_3^-$	L-Met	—	(Ref. 7)
II	(+)-18C6H$_3^-$	D-Met	—	(Ref. 7)
I	(+)-18C6H$_3^-$	L-PheG	—	(Ref. 7)
I	(+)-18C6H$_3^-$	D-PheG	—	(Ref. 7)
II	(+)-18C6H$_3^-$	D-PheG	—	(Ref. 7)
III	(+)-18C6H$_3^-$	L-Glu	—	(Ref. 6)
III	(+)-18C6H$_3^-$	D-Glu	—	(Ref. 6)

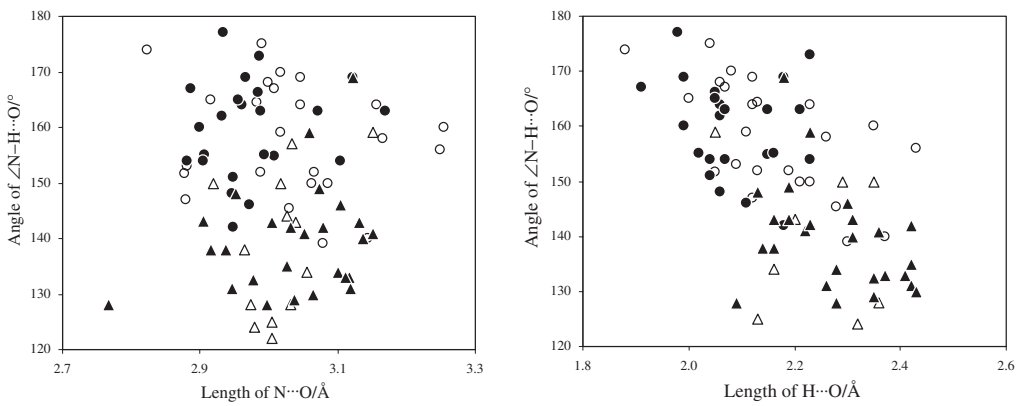


Figure 11. Distribution charts describing the geometry of N–H···O interactions of various amino acids with (+)-18C6H$_4$ in complex crystals. The vertical line shows the angle of N–H···O, and the horizontal line shows the length of N···O (a) and that of H···O (b); ● D-enantiomer (straight interaction type), ▲ D-enantiomer (branched interaction type), ○ L-enantiomer (straight interaction type), and △ L-enantiomer (branched interaction type).

of each residue of the amino acid with respect to two hollows of (+)-18C6H$_4$: the carboxyl groups of L- and D-amino acids complexed with (+)-18C6H$_4$ are both located in the larger hollow of the symmetric bowl, as shown in Figure 1.

On the other hand, the OH groups of L-Ser and L-Thr side chains formed hydrogen bonds with the carboxyl oxygen atoms of (+)-18C6H$_4$: L**3** Mol. A: O(37)(Accepter)···O(26)(Donor) = 2.691(2) Å, O–H···O = 1.69 Å, ∠O–H···O = 167°; L**3** Mol. B: O(37)(D)···O(26)(A) = 3.059(4) Å, O–H···O = 2.07 Å, ∠O–H···O = 144°; L-Ser-(+)–18C6H$_4$: O(37)(D)···O(26)(A) = 2.714(3) Å, O–H···O = 1.72 Å, ∠O–H···O = 173°.[14] This is in contrast with the other cases, because the side chains of D-Ser, D-Thr, and all other chiral amino acids did not form any specific interaction with (+)-18C6H$_4$. Especially, it is interesting to note that the side chains of ABA and Val, which have a methyl group instead of the hydroxy group of Ser and Thr and obey the first elution rule of L-amino acid in HPLC, did not form hydrogen bonds with the carboxyl groups of (+)-18C6H$_4$

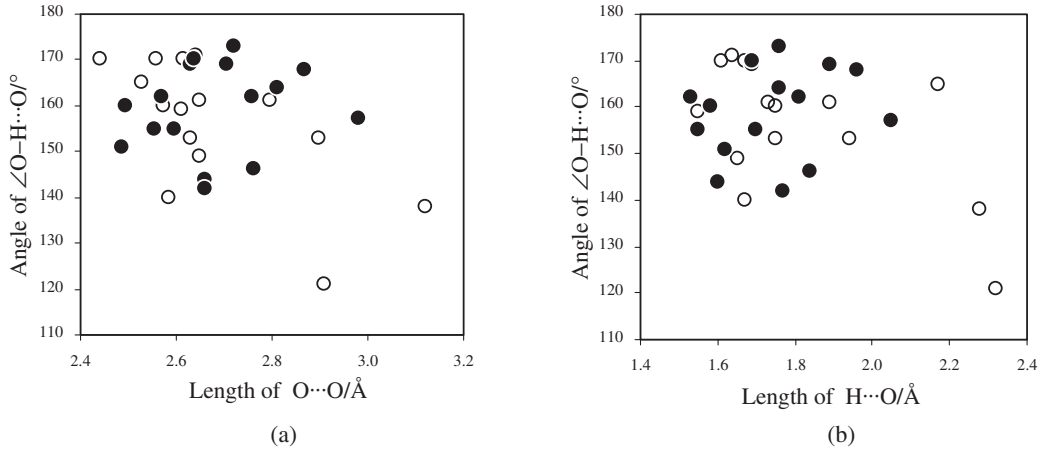


Figure 12. Distribution charts describing the geometry of O–H···O interactions between the carboxyl groups of amino acids and the O atoms of neighboring molecules in complex crystals. The vertical line shows the angle of O–H···O, and the horizontal line shows the length of O···O (a) and that of H···O (b); ● D-enantiomer and ○ L-enantiomer.

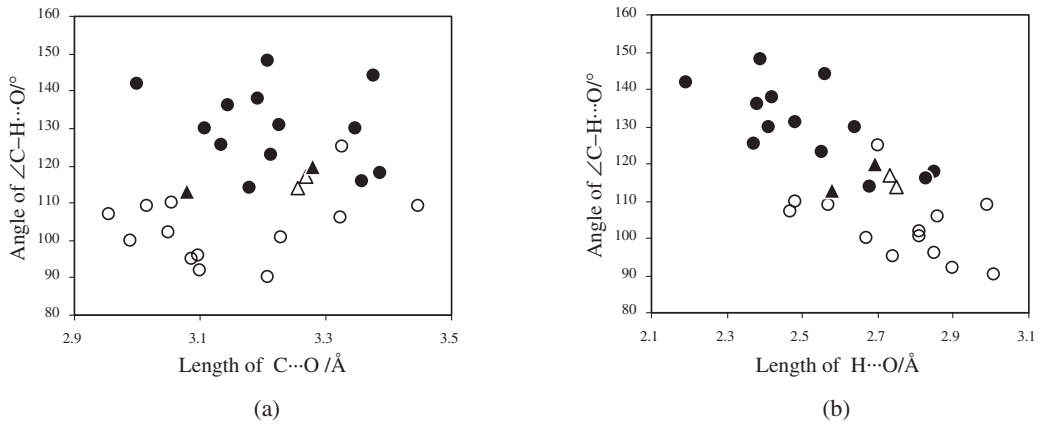


Figure 13. Distribution charts describing the geometry of C_α–H···O interactions of various amino acids with (+)-18C6H4 in complex crystals. The vertical line shows the angle of C_α–H···O, and the horizontal line shows the length of C_α···O (a) and that of H···O (b); ● D-enantiomer (straight interaction type), ▲ D-enantiomer (branched interaction type), ○ L-enantiomer (straight interaction type), and △ L-enantiomer (branched interaction type).

in complex crystals. This obviously indicates that hydrogen-bond formation between the OH group of L-Ser or L-Thr side chain and the carboxyl groups (+)-18C6H4 in solution state is the cause for the exception from the first elution rule of L-amino acid in HPLC.

Conclusion

From the present results of molecular conformation of (+)-18C6H4 and its interaction mode with guest molecule in solid and solution states, the mechanism of chiral separation of amino acids by (+)-18C6H4 could be summarized as follows. For chiral separation, (+)-18C6H4 takes an asymmetric bowl-like conformation with two hollows (big and small). (+)-18C6H4 anchors the amino acid by hydrogen bonds between the amino residue of the amino acid and the O atoms of (+)-18C6H4. These hydrogen bonds are equally formed between the L- and D-enantiomers of the amino acid and do not become a major driving force for chiral separation. The location of the amino acid anchored by (+)-18C6H4 is different between the D- and L-enantiomers, due to the different size of the two hollows (big and small) of (+)-18C6H4. The carboxyl group of the amino acid assumes an important role in positioning each enantiomer with respect to (+)-18C6H4, though it does not directly interact with the big hollow of (+)-18C6H4. Chiral separation is then achieved by different C_α–H···O interaction between the D- and L-amino acid and (+)-18C6H4, the force of which is superior in the case of the D-amino acid in both of the solid and solution states. This leads to the first elution rule of the L-isomer in HPLC using 18C6H4-linked column. On the other hand, the exceptional reversed elution order in the case of Ser and Thr is due to the hydrogen bond between the side chain OH of the L-enantiomer and (+)-18C6H4.

Supporting Information

Detailed data of C_α–H···O interaction (Table S1), conformation of amino acid (Table S2) and intermolecular hydrogen bond (Tables S3 and S4), and stereoscopic views of host–guest interaction (Figures S1-1–S1-3) and crystal packing (Figure S2), in complex crystals of amino acid with (+)-18C6H4. This material

is available free of charge on the web at http://www.csj.jp/journals/bcsj/.

References

1 ICH (International Conference on Harmonization of Technical Requirement of Pharmaceuticals for Human Use) guideline, topics Q6A: establishment of specification and testing method.

2 a) N. R. Srinivas, *Biomed. Chromatogr.* **2004**, *18*, 207. b) O. McConnell, A. Bach, II, C. Balibar, N. Byrne, Y. Cai, G. Carter, M. Chlenov, L. Di, K. Fan, I. Goljer, Y. He, D. Herold, M. Kagan, E. Kerns, F. Koehn, C. Kraml, V. Marathias, B. Marquez, L. McDonald, L. Nogle, C. Petucci, G. Schlingmann, G. Tawa, M. Tischler, R. T. Williamson, A. Sutherland, W. Watts, M. Young, M.-Y. Zhang, Y. Zhang, D. Zhou, D. Ho, *Chirality* **2007**, *19*, 658.

3 a) Y. Zhang, D.-R. Wu, D. B. Wang-Iverson, A. A. Tymiak, *Drug Discov. Today* **2005**, *10*, 571. b) G. Cancelliere, I. D'Acquarica, F. Gasparrini, M. Maggini, D. Misiti, C. Villani, *J. Sep. Sci.* **2006**, *29*, 770. c) C. Ravelet, E. Peyrin, *J. Sep. Sci.* **2006**, *29*, 1322.

4 a) L. R. Sousa, G. D. Y. Sogah, D. H. Hofmann, D. J. Cram, *J. Am. Chem. Soc.* **1978**, *100*, 4569. b) T. Shinbo, T. Yamaguchi, H. Yanagishita, D. Kitamoto, M. Sugiura, *J. Chromatogr., A* **1992**, *625*, 101. c) S. C. Chang, G. L. Reid, III, S. Chen, C. D. Chang, D. W. Armstrong, *TrAC, Trends Anal. Chem.* **1993**, *12*, 144.

5 a) W. H. Pirkle, M. H. Hyun, A. Tsipouras, B. C. Hamper, B. Banks, *J. Pharm. Biomed. Anal.* **1984**, *2*, 173. b) O. W. Griffith, E. B. Campbell, W. H. Pirkle, A. Tsipouras, M. H. Hyun, *J. Chromatogr., A* **1986**, *362*, 345. c) M. Schleimer, W. H. Pirkle, V. Schurig, *J. Chromatogr., A* **1994**, *679*, 23. d) W. H. Pirkle, P. G. Murray, S. R. Wilson, *J. Org. Chem.* **1996**, *61*, 4775.

6 a) Y. Okamoto, M. Kawashima, R. Aburatani, K. Hatada, T. Nishiyama, M. Masuda, *Chem. Lett.* **1986**, 1237. b) Y. Okamoto, Y. Kaida, H. Hayashida, K. Hatada, *Chem. Lett.* **1990**, 909. c) K. Tachibana, A. Ohnishi, *J. Chromatogr., A* **2001**, *906*, 127.

7 a) S. Allenmark, B. Bomgren, H. Borén, *J. Chromatogr., A* **1983**, *264*, 63. b) J. Haginaka, C. Seyama, H. Yasuda, K. Takahashi, *J. Chromatogr., A* **1992**, *598*, 67. c) J. Haginaka, *J. Chromatogr., A* **2001**, *906*, 253.

8 a) S. Lam, *J. Chromatogr. Sci.* **1984**, *22*, 416. b) Y. Yuki, K. Saigo, H. Kimoto, K. Tachibana, M. Hasegawa, *J. Chromatogr., A* **1987**, *400*, 65. c) V. A. Davankov, *J. Chromatogr., A* **2003**, *1000*, 891.

9 a) C. J. Pedersen, *J. Am. Chem. Soc.* **1967**, *89*, 2495. b) C. J. Pedersen, *J. Am. Chem. Soc.* **1967**, *89*, 7017.

10 L. R. Sousa, G. D. Y. Sogah, D. H. Hofmann, D. J. Cram, *J. Am. Chem. Soc.* **1978**, *100*, 4569.

11 a) J.-P. Behr, J.-M. Girodeau, R. C. Hayward, J.-M. Lehn, J.-P. Sauvage, *Helv. Chim. Acta* **1980**, *63*, 2096. b) J.-P. Behr, J.-M. Lehn, P. Vierling, *Helv. Chim. Acta* **1982**, *65*, 1853.

12 a) Y. Machida, H. Nishi, K. Nakamura, *J. Chromatogr., A* **1998**, *805*, 85. b) M. H. Hyun, J. S. Jin, W. Lee, *J. Chromatogr., A* **1998**, *822*, 155. c) M. H. Hyun, H. J. Koo, J. S. Jin, W. Lee, *J. Liq. Chromatogr. Relat. Technol.* **2000**, *23*, 2669. d) R. Berkecz, A. Sztojkov-Ivanov, I. Ilisz, E. Forro, F. Fülöp, M. H. Hyun, A. Péter, *J. Chromatogr., A* **2006**, *1125*, 138.

13 H. Nagata, H. Nishi, M. Kamigauchi, T. Ishida, *Org. Biomol. Chem.* **2004**, *2*, 3470.

14 H. Nagata, H. Nishi, M. Kamigauchi, T. Ishida, *Chem. Pharm. Bull. (Tokyo)* **2006**, *54*, 452.

15 H. Nagata, H. Nishi, M. Kamigauchi, T. Ishida, *Chirality* **2008**, *20*, 820.

16 Y. Machida, M. Kagawa, H. Nishi, *J. Pharm. Biomed. Anal.* **2003**, *30*, 1929.

17 P. Job, *Ann. Chim.* **1928**, 113.

18 R. L. Scott, *Rec. Trav. Chim* **1956**, *75*, 787.

19 A. Altomare, G. Cascarano, C. Giacovazzo, A. Guagliardi, M. C. Burla, G. Polidori, M. Camalli, *J. Appl. Cryst.* **1994**, *27*, 435.

20 G. M. Sheldrick, *SHELXL97, Program for the Refinement of Crystal Structures*, University of Göttingen, Germany, **1997**.

21 Y. Machida, H. Nishi, K. Nakamura, *J. Chromatogr., A* **1998**, *810*, 33.

22 E. Bang, J.-W. Jung, W. Lee, D. W. Lee, W. Lee, *J. Chem. Soc., Perkin Trans. 2* **2001**, 1685.

23 a) M. S. Lehmann, T. F. Koetzle, W. C. Hamilton, *J. Am. Chem. Soc.* **1972**, *94*, 2657. b) M. Subha Nandhini, R. V. Krishnakumar, S. Natarajan, *Acta Crystallogr., Sect. C* **2001**, *57*, 614.

24 a) T. Ichikawa, Y. Iitaka, *Acta Crystallogr., Sect. B* **1968**, *24*, 1488. b) F. Dahan, O. Lefebvre-Soubeyran, *Acta Crystallogr., Sect. B* **1976**, *32*, 2859.

25 K. Torii, Y. Iitaka, *Acta Crystallogr., Sect. B* **1970**, *26*, 1317.

26 J. Janczak, D. Zobel, P. Luger, *Acta Crystallogr., Sect. C* **1997**, *53*, 1901.

27 a) T. Steiner, *J. Chem. Soc., Perkin Trans. 2* **1995**, 1315. b) T. Steiner, *Angew. Chem., Int. Ed.* **2002**, *41*, 48.

28 J. R. Yates, T. N. Pham, C. J. Pickard, F. Mauri, A. M. Amado, A. M. Gil, S. P. Brown, *J. Am. Chem. Soc.* **2002**, *124*, 11315.