Chiral Recognition in Host-Guest Complexation Determined by the Enantiomer-Labeled Guest Method Using Fast Atom Bombardment Mass Spectrometry

Masami Sawada,*,† Yoshio Takai,† Hitoshi Yamada,† Shigeki Hirayama,‡ Takahiro Kaneda,† Toshikazu Tanaka,‡ Kimiko Kamada,§ Takashi Mizooku,§ Sachiko Takeuchi,§ Koji Ueno,§ Keiji Hirose,§ Yoshito Tobe,§ and Koichiro Naemura*,§

Contribution from The Institute of Scientific and Industrial Research, Osaka University, Ibaraki, Osaka 567, Japan, Department of Applied Chemistry, Osaka Institute of Technology, Asahi-ku, Osaka 535, Japan, and Department of Chemistry, Faculty of Engineering Science, Osaka University, Toyonaka, Osaka 560, Japan

Received February 27, 1995[⊗]

Abstract: Chiral recognition in host-guest complexations between crown ether hosts (H) and amino acid ester ammonium ion guests (G^+) has been evaluated by fast atom bombardment (FAB) mass spectrometry (*m*-nitrobenzyl alcohol matrix). The method uses a 1/1 mixed (for example, $G_R{}^+$ and $G_{S-d_n}{}^+$) solution of the guest whose enantiomer is isotopically (deuterium) labeled. Chiral recognition of a given host is simply measured with a given guest from the peak intensity ratio of the two diastereomeric host-guest complex ions as $I[(H + G_R)^+]/I[(H + G_{S-d_n})^+] \equiv I_R/I_{S-d_n}$. Both the degree and the direction of chiral recognition are characterized by the I_R/I_{S-d_n} values in the range from 0.5 to 5.4 (I_R/I_{S-d_3}) for the present host-guest combination systems studied. Among several synthetic chiral crown ethers and related natural host compounds, it has been found that host **5** possesses remarkably large guest dependence upon the chiral recognition properties: (1) toward primary amino acid ester guests **14-21**, a high degree of *(R)*-enantiomer preference (I_R/I_S = 3.2-5.4), (2) toward phenylglycine ester guest **22**, almost no enantiomer recognition (I_R/I_S = 1.1), and (3) toward secondary amino acid ester guest **24**, a weak *(S)*-enantiomer preference (I_R/I_S = 0.7). It is also shown that the I_R/I_S values measured with the present concentrations are reasonably correlated with the relative thermodynamic stabilities in the corresponding host-guest equilibria in solution ($I_R/I_S \leq K_R/K_S$) for three typical host-guest combination systems selected (**1-22, 4-16,** and **5-16**). Accordingly, the present FABMS/EL (enantiomer-labeled guest) method can be proposed as a new and practically useful technique for determining chiral recognition properties in the highly structured chiral host-chiral guest complexations.

Introduction

Chiral recognition is one of the fundamental processes in living systems. A lot of synthetic model compounds such as chiral crown ethers have been synthesized as host compounds.[1] Until today, marvelous host-guest combination systems, which show high degrees of chiral recognition, have been developed and their recognition mechanism has been gradually made clear as some combined effects of charge-dipole, hydrogen-bonding, hydrophobic, and π-π interactions, and steric complementarity, etc.[2] Crown ethers,[3-7] cyclophanes,[8] cyclodextrins,[9] calixarenes,[10] porphyrins,[11] etc.[12-14] have nowadays become well-known as representatives of host molecules of chiral recognition.

Various methods were used for determining chiral recognition of these hosts. Examples are the methods of extraction/NMR,[3a] extraction/polarimetry,[3a] titration NMR,[15] variable temperature NMR,[6a] nuclear Overhauser effect (NOE),[13b] titration UV-vis,[7b] induced circular dichromism,[10] liquid chromatography (LC),[13d] capillary electrophoresis,[16] transport,[3d] membrane electrode,[4b,17] etc. Mass spectrometry is highly sensitive but not generally considered as informative and facile for detecting chiral recognition properties because of the absence of mass differences between diastereomeric isomers.

† The Institute of Scientific and Industrial Research, Osaka University.
‡ Osaka Institute of Technology.
§ Faculty of Engineering Science, Osaka University.
⊗ Abstract published in *Advance ACS Abstracts,* July 1, 1995.

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Recently, fast atom bombardment (FAB) mass spectrometry has been applied very often to the field of host–guest complexation chemistry.[18-20] This trend has come with general recognition for easy and direct detection of the target products, the host–guest complex ions in question. Mass spectrometric observations of the chirality effect were previously reported in a few cases of (1) aggregation systems with chemical ionization (CI) MS,[21] FABMS,[22] and Fourier transform ion cyclotron resonance (FTICR) MS[23] and (2) metal coordination systems with FABMS/MS or FABMS/kinetic energy release (KER).[24]

In the host–guest complexation systems, there appeared only two observations of chiral recognition for (1) (dimethoxyphenyl)crown hosts with FABMS[25] and (2) pyridylcrown hosts with FTICRMS.[26] The former treats two FAB mass spectra with an internal standard host method (previously called the relative peak intensity (RPI) method),[25,27] and the latter compares two relative equilibrium constants, of course, using an internal standard host. Both approaches inherently require two successive measurements and comparisons. Therefore, they are not recognized as a direct (operationally simple) detection method, which is a major feature of the new directions currently needed for rapid screening of the chiral recognition ability of various synthetic and biologically important host compounds.

In this paper, we describe a novel and direct approach for chiral recognition of chiral crown ethers toward amino acid ester ammonium ion guests. The hosts and guests studied are shown in Charts 1 and 2, respectively. First, it has been described that both the degree and the direction of chiral recognition can be directly, easily, and reliably determined using FAB mass spectrometry, which is coupled with an enantiomer-labeled guest (EL) method.[28] Second, it has also been described that the results obtained by the present methodology are reasonably correlated with the thermodynamic relative stabilities for the corresponding host–guest complexation equilibria in solution.

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Chart 1

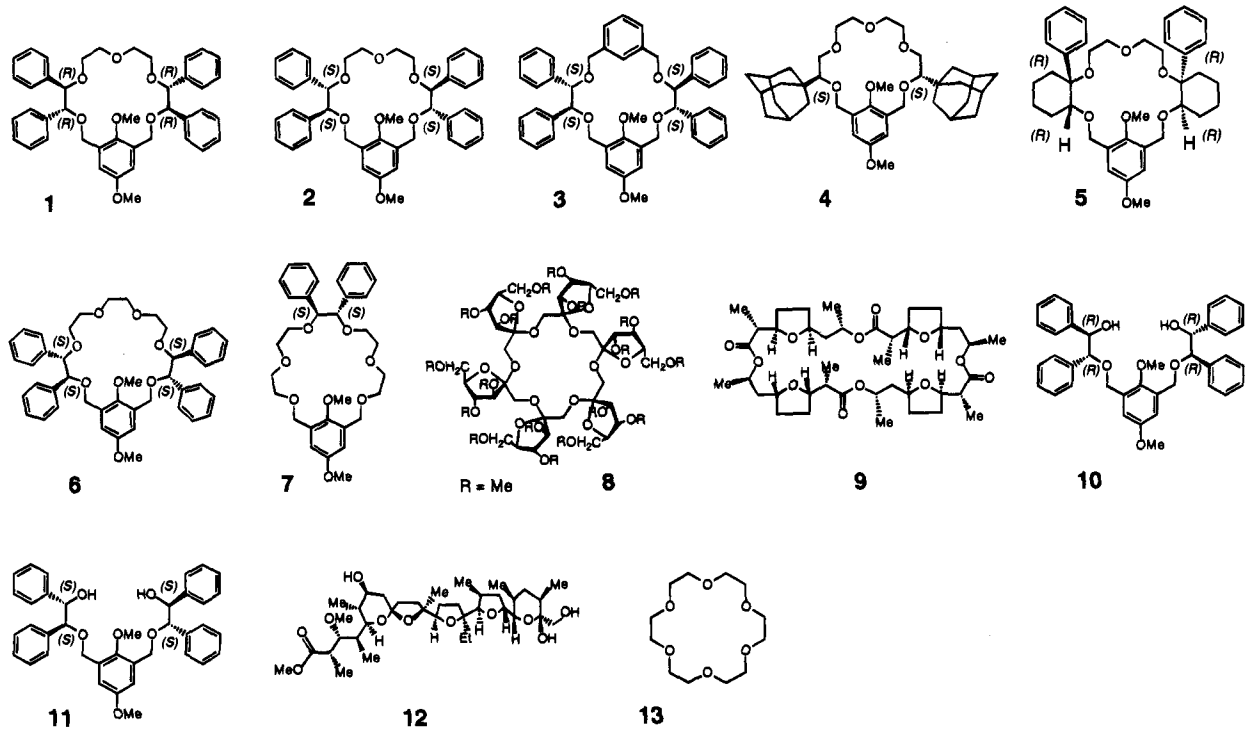
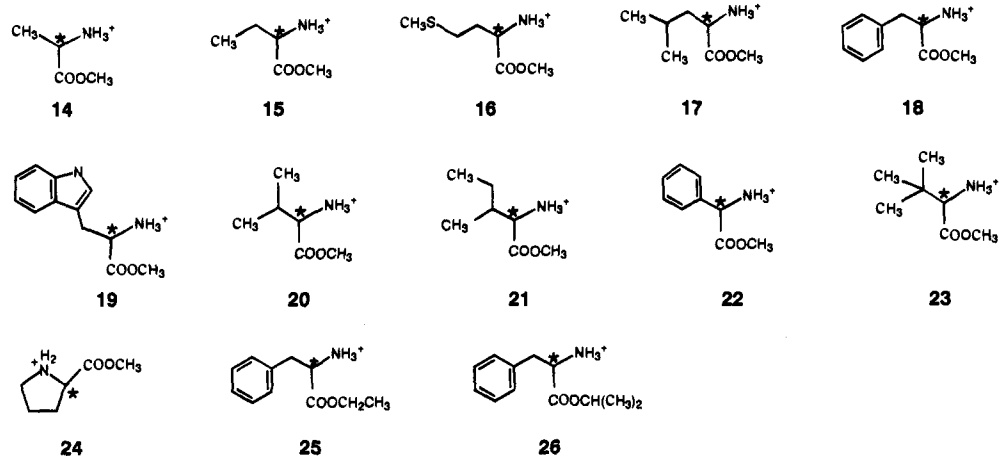


Chart 2



Accordingly, this type of FAB mass spectrometry has been proposed to be a new class of detection methods for chiral recognition properties, which has been abbreviated as the FABMS/EL method.

Results

Basic Concept and General Methodology of the FABMS/EL Method. A fundamental feature of this method is the use of an isotopically labeled enantiomer of a selected guest to distinguish the diastereomeric host–guest complex ions in one FAB mass spectrum. A 1/1 mixture of a labeled and an unlabeled guest enantiomer is complexed with a target chiral host. The enantiomer-labeled method was first adopted by Fale et al., and at the time the chirality effect was observed from the relative peak intensities for the protonated dimer ions of tartaric acid esters using CIMS: I[(homodimer + H)$^+$] > I[heterodimer + H)$^+$].[21a] We applied this enantiomer-labeled method to the highly structured host–guest complexation systems and used various guests of labeled and unlabeled amino acid methyl ester ammonium ions. Our principal aim was to detect the peak intensity difference of the diastereomeric ions, that is, to detect chiral recognition, in the highly structured systems of chiral host–chiral guest complexations.

In this paper, we treated mainly the methyl esters, and labeled the ester group (CD_3 ester; G_{S-d_3}) of L-amino acids ((*S*)-amino acids),[29] and then used 1/1 mixtures of G_R^+ and $G_{S-d_3}^+$ (G = guest) as the guests. That is, we systematically considerd the competitive equilibrium system of eqs 1 and 2 in an *m*-

$$H + G_R^+ \overset{K_R}{\rightleftharpoons} (H + G_R)^+ \quad (1)$$

$$H + G_{S-d_3}^+ \overset{K_S}{\rightleftharpoons} (H + G_{S-d_3})^+ \quad (2)$$

nitrobenzyl alcohol (NBA) matrix solution (H = chiral host). Therefore, the peak intensity ratio, I[(H + G_R)$^+$]/I[(H + G_{S-d_3})$^+$], of the diastereomeric host–guest complex ions, which appear simultaneously with 3 mass-unit differences in one FAB mass spectrum, is expected to become a measure of chiral recognition

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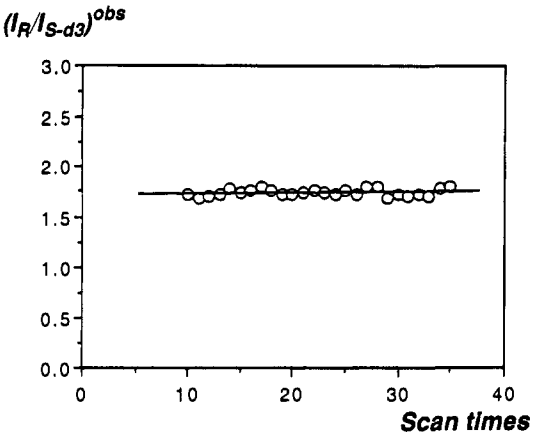

Figure 1. A plot of $(I_R/I_{S-d_3})^{obs}$ values against scan times for the complexation between host **1** and guest **22** (concentration condition A).

properties: for short expression, this ratio is abbreviated as I_R/I_{S-d_3} here on.

(1) $I_R/I_{S-d_3} > 1$ means that a given chiral host binds more strongly an (*R*)-enantiomer of a given guest [(*R*)-enantiomer preference]. The larger the I_R/I_{S-d_3} value from unity, the higher the degree of chiral recognition of the host.

(2) In contrast, $I_R/I_{S-d_3} < 1$ means that a given chiral host binds more strongly an (*S*)-enantiomer of a given guest [(*S*)-enantiomer preference].

(3) $I_R/I_{S-d_3} = 1.0 \pm 0.1$ means that a given chiral host cannot differentiate the chirality of a given guest. Of course, if a selected host is an achiral one such as host **13**, this relation should hold.

Here, we employed fundamentally two types of concentration conditions for measuring sample solutions. One is a relatively diluted one with NBA (concentration condition A) for overcoming solubility problems, and the other is a relatively concentrated one with NBA (concentration condition B) for getting high-quality FAB mass spectra (see Experimental Section).

For every preparation of 1/1 mixed guest solutions, the 1/1 equivalency for the concentrations of (*R*)/(*S*)-enantiomer guests should be checking by determining whether the I_R/I_{S-d_3} value with a standard achiral host, such as host **13**, can be experimentally obtained as unity (1.00 ± 0.03). This type of control experiment should be performed before the chiral recognition experiments for given chiral hosts are tested by the FABMS/EL method.

Scan Stability and Reproducibility of I_R/I_{S-d_3} Values. Figure 1 is a plot of I_R/I_{S-d_3} values observed against scan times for the complexation between host **1** and guest **22** as a typical case (concentration condition A). A statistical treatment gave $I_R/I_{S-d_3} = 1.74 \pm 0.033$ (standard deviation) ($n = 26$ from scan no. 10 to no. 35), showing satisfactory constancy of the value. Analogous situations were observed in other combinations between hosts and guests. Accordingly, in this paper a simple average of three experimental I_R/I_{S-d_3} values at the 10th, 20th, and 30th scan times was employed for easy data-handling as the observed I_R/I_{S-d_3} value.

Different concentrations of the sample solutions prepared by different persons, in maintaining a constant ratio of [H]/[G] of 1/6 (concentration conditions A, B′, and B), gave $(I_R/I_{S-d_3})^{obs} = 1.83 \pm 0.10$ ($n = 5$). The reproducibility of I_R/I_{S-d_3} values is then typically expected to be less than ±10%.

Concentration Effects on I_R/I_{S-d_3} Values. Table 1 shows the change in I_R/I_{S-d_3} values due to the change in host/guest concentration ratio values for the complexation between host **1** and guest **22**. The observed I_R/I_{S-d_3} value appreciably increases from 1.7 to 2.0 with the decreasing change in the [H]/[G] value.

Table 1. Concentration Effects on I_R/I_{S-d_3} Values (Host **1** and Guest **22**)

amt of host soln used (μL)[a]	concn ratio [H]/[G]	I_R/I_{S-d_3}[b]	calcd concn ratio of diastereomeric complex ions in NBA[c] A	B	C
5.0[d]	1/6[e]	1.73 (1.87)	1.81	1.91	1.98
3.0	0.6/6	1.77 (1.91)	1.87	1.94	1.99
2.0	0.4/6	1.82 (1.97)	1.92	1.96	1.99
1.0	0.2/6	1.86 (2.02)	1.96	1.98	2.00
0.5	0.1/6	ca. 2.1	1.98	1.99	2.00
0.2	0.04/6	ca. 2.0	1.99	2.00	2.00

[a] Sample preparation method for FABMS (see Experimental Section). [b] The observed I_R/I_{S-d_3} value. The corrected value is in parentheses, $(I_R/I_{S-d_3})^{corr}$ (see Experimental Section). [c] Calculated concentration ratio, $[(H + G_R)^+]/[(H + G_S)^+]$, based on the competitive equilibrium system. Here, $K_R/K_S = 2.0$ is assumed: (A) $K_R = 200$, $K_S = 100$ M^{-1}; (B) $K_R = 20$, $K_S = 10$ M^{-1}; (C) $K_R = 2$, $K_S = 1$ M^{-1}. [d] Corresponds to concentration condition A (see Experimental Section). [e] [H] = 0.0083 M in NBA, [G] = 0.05 M in NBA ($[G_R^+] = 0.025$ M and $[G_S^+] = 0.025$ M).

These effects are expected to be interpreted as a change in the concentrations of diastereomeric host–guest complex ions which are produced in the matrix solution (see Discussion).[18]

I_R/I_{S-d_3} Values Determined by the FABMS/EL Method. I_R/I_{S-d_3} values of various chiral hosts toward various chiral amino acid methyl ester guests were determined by the enantiomer-labeled guest method using FAB mass spectrometry (abbreviated as FABMS/EL). The $(I_R/I_{S-d_3})^{corr}$ values, which are corrected by the natural abundance of the (M + 3) isotope, are summarized in Table 2. Four typical FAB mass spectra are shown in Figures 2–5. Figures 2 and 3 are from a pair of cross-chiral (host) experiments. Figures 4 and 5 are from a pair of cross-label (guest) experiments. From these figures and table, one can directly identify both the degree and the direction of chiral recognition for a selected combination between a given host and a given guest.

Cross-Chiral Correlations. Hosts **1** and **2** are enantiomers. If those host–guest complex ions are highly structured ones (for example, see Figure 9), the complex ion between host **1** and guest (*R*)-**16** and the complex ion between host **2** and guest (*S*)-**16** are enantiomers of each other. Therefore, the cross-chiral relationship should hold: that is, the degree of (*R*)-enantiomer preference of host **1** should be equal to the degree of (*S*)-enantiomer preference of host **2** (cross-chiral experiments). This relationship is expressed by the following equation for each guest employed:

$$[(I_R/I_{S-d_3})_1][(I_R/I_{S-d_3})_2] = 1.00 \quad (3)$$

Using the $(I_R/I_{S-d_3})^{corr}$ values in Table 2, application of eq 3 was tested (concentration condition A):

guest **16** $1.56 \times 0.66 = 1.03$

guest **18** $1.90 \times 0.92 = 0.99$

guest **22** $1.99 \times 0.51 = 1.01$

Indeed, we found that three experimental cross-chiral relationships hold satisfactorily. These findings indicate the appropriateness of the present methodology and assure the quantitative nature of the $(I_R/I_{S-d_3})^{corr}$ values. Further, they confirm the highly structured host–guest complex ions which are generated and observed.

Isotope Effects. In order to determine certain effects of deuterium labeling on the FAB mass spectral intensities of the corresponding host–guest complex ions, we used here a 1/1

Table 2. $(I_R/I_{S-d_3})^{corr}$ Values Determined by the Enantiomer-Labeled Guest Method Using FABMS[a]

	guest										
host	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
1	1.58[b]	1.71[c]	1.57	1.27[b]	1.93	1.53	2.69[b]	2.07[b]	1.94	2.77	0.89[d]
			1.55		1.86				1.87		
									1.99[b]		
									2.06		
									2.06[d]		
2			0.65		0.52				0.48		
			0.67		0.52				0.53		
									0.51[b]		
									0.46[d]		
3			ca. 0.8		ca. 0.8				ca. 0.8		
									0.84[b]		
4			1.61		1.61				1.41		
			1.59		1.58				1.38		
5	4.00[b]	5.44[c]	5.35	3.16[b]	4.37	3.49	5.03[b]	3.62[b]	1.15	ND	0.65[d]
									1.17[d]		
6	1.59[b]	1.33[c]	1.50	1.39[b]	1.04	1.35	1.14[b]	1.12[b]	1.11	1.12	1.02[d]
			1.49		1.02				1.11		
									1.15[b]		
7			0.79		0.84				0.79		
			0.80		0.82				0.82		
8					1.0[b]				1.1[b]		
9			1.02[b]		1.02[b]				0.99[b]		
10			ca. 1.0		ca. 1.0				ca. 1.0		
11									0.97[b]		
12	0.75[b]	0.69[c]						0.81[b]	0.54[b]		ND
13	0.98[b]	0.97[c]	1.01	1.01[b]	0.98	0.97	1.02[b]	0.95[b]	0.99	0.98	0.98[d]
			1.01		0.99				0.98		
			1.04[b]		1.02[b]				0.98[b]		
									1.02[d]		
									0.99[b]		

[a] The values are corrected by the natural abundance of the corresponding (M + 3) isotope (see Experimental Section). Concentration condition A unless otherwise noted. ND means the host–guest complex ion peaks were not detected. [b] Concentration condition B. [c] Concentration condition A'. [d] Concentration condition B'.

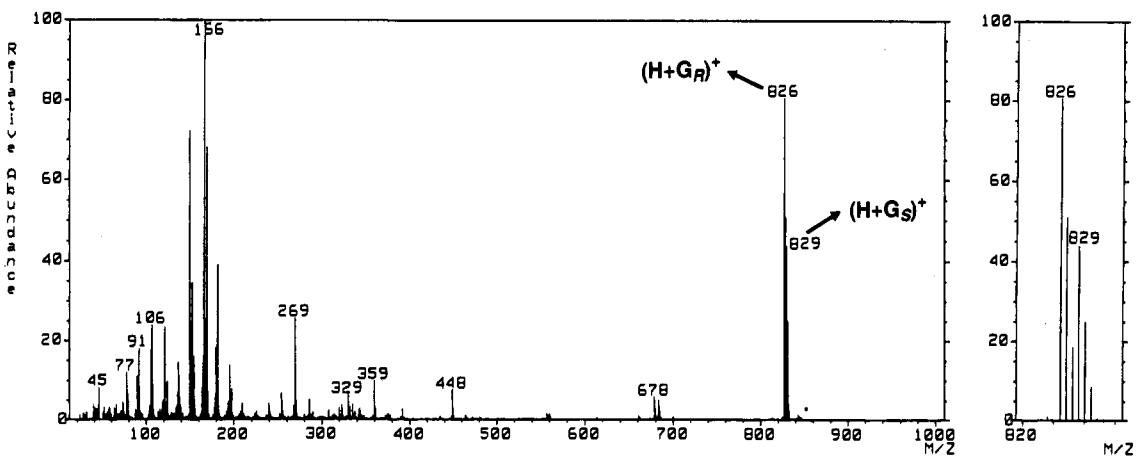

Figure 2. A FAB mass spectrum for the complexation between host **1** and guest **22** (1/1 mixture of (*R*)-**22** and (*S*)-**22**-d_3) (NBA matrix (condition B)).

mixture of a pair of labeled (*R*)- and unlabeled (*S*)-enantiomer guests and evaluated the I_{R-d_3}/I_S values specifically in this section (cross-label experiments).

In the case of the complexation between host **1** and a 1/1 mixture of guests (*R*)-**22**-d_3 and (*S*)-**22**, the value of $(I_{R-d_3}/I_S)^{corr}$ was 2.01 (concentration condition B). On the other hand, the complexation between host **1** and a 1/1 mixture of guests (*R*)-**22** and (*S*)-**22**-d_3 gave an experimentally equal value (1.99) of $(I_R/I_{S-d_3})^{corr}$:

$$[(I_{R-d_3}/I_S)]_1 = [(I_R/I_{S-d_3})]_1$$

The agreement indicates that there is no detectable deuterium isotope effect on I_R/I_{S-d_3} values at least in this host–guest complexation system and at this label position.

Further, alternative experiments were performed to check the presence or absence of isotope effects in the deuterium labeling. For the complexation of host **5** with a 1/1 mixture of guests (*R*)-**16** and (*R*)-**16**-d_3, the value of $(I_R/I_{R-d_3})^{corr}$ was practically unity (1.01) (concentration condition B'). The results for these two sets of label experiments demonstrate that it is not necessary to think about the contribution of isotope effects on the I_R/I_{S-d_3} values in the present cases.

Equilibrium Constants in a $CDCl_3$ or a Related Solution Determined by the Titration ^{1}H-NMR Method. (a) Determination of Equilibrium Constants (K_R and K_S) in a Competitive Equilibrium System. ^{1}H-NMR spectral changes in $CDCl_3$ at 25 °C are shown in Figure 6, where a stock solution of host **4** has been successively added into a racemic solution

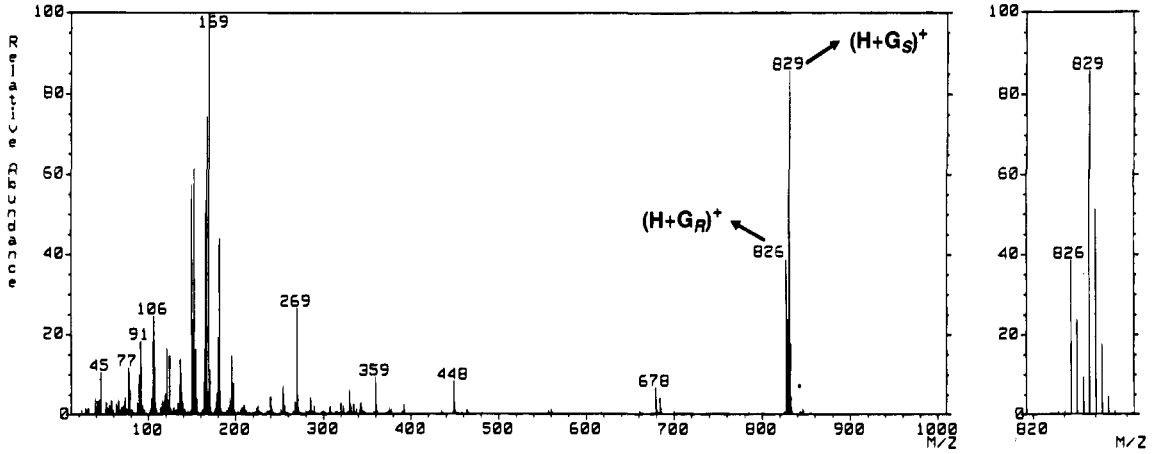

Figure 3. A FAB mass spectrum for the complexation between host **2** and guest **22** (1/1 mixture of (*R*)-**22** and (*S*)-**22**-d_3) (NBA matrix (condition B)).

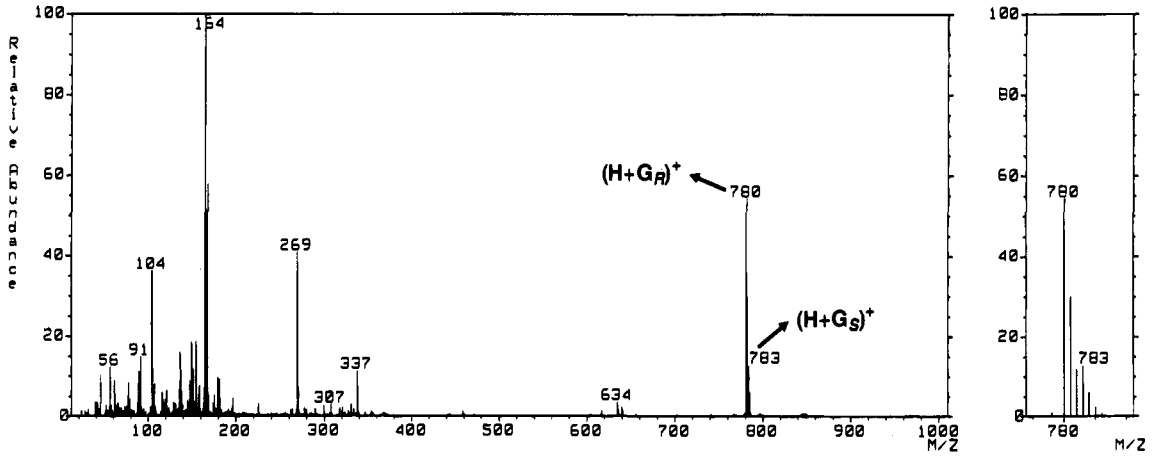

Figure 4. A FAB mass spectrum for the complexation between host **5** and guest **16** (1/1 mixture of (*R*)-**16** and (*S*)-**16**-d_3) (NBA matrix (condition B)).

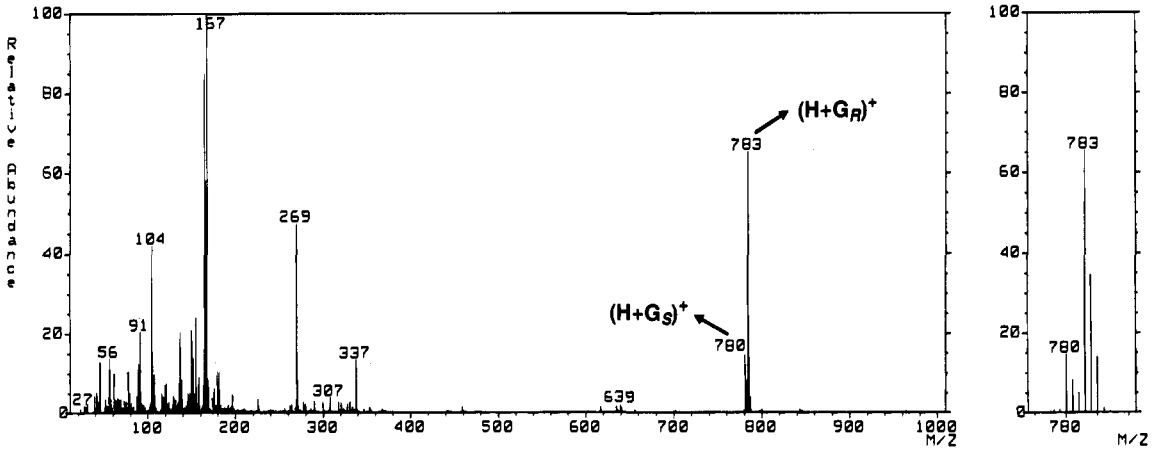

Figure 5. A FAB mass spectrum for the complexation between host **5** and guest **16** (1/1 mixture of (*R*)-**16**-d_3 and (*S*)-**16**) (NBA matrix (condition B)).

of guest **16** (ClO_4^- salt). Triplet signals (δ = 4.43 ppm, 1H, t) of the CH proton of guest **16** shifted gradually upfield as host **4** was added and separated into two sets of triplet signals. This separation indicates that the two diastereomeric host–guest complex ions produced, host **4**–guest (*R*)-**16** and host **4**–guest (*S*)-**16** ions, can be distinguished from each other in the ^{1}H-NMR spectral ground. Assignment of the two triplets was simply performed by further addition of (*S*)-**16**, resulting in an increase in the intensities of the corresponding triplet: the CH proton of the former ion accompanied by guest (*R*)-**16** shifted more upfield.

Two equilibrium constants, K_R and K_S, for the complexation between host **4** and guest **16** were determined by the nonlinear titration (^{1}H-NMR) method which was provided from a plot of induced shifts (Hz) observed versus host/guest concentration ratios prepared ([H]/[G]) on the basis of the equation derived from the competitive equilibrium system (see Experimental Section). The results are summarized in Table 3.

(b) Determination of Equilibrium Constants (K_R or K_S) in a Simple (Noncompetitive) Equilibrium System. The equilibrium constants (K_R or K_S) in simple equilibrium systems for the complexations between host **1** and guest **22** (host **5** and

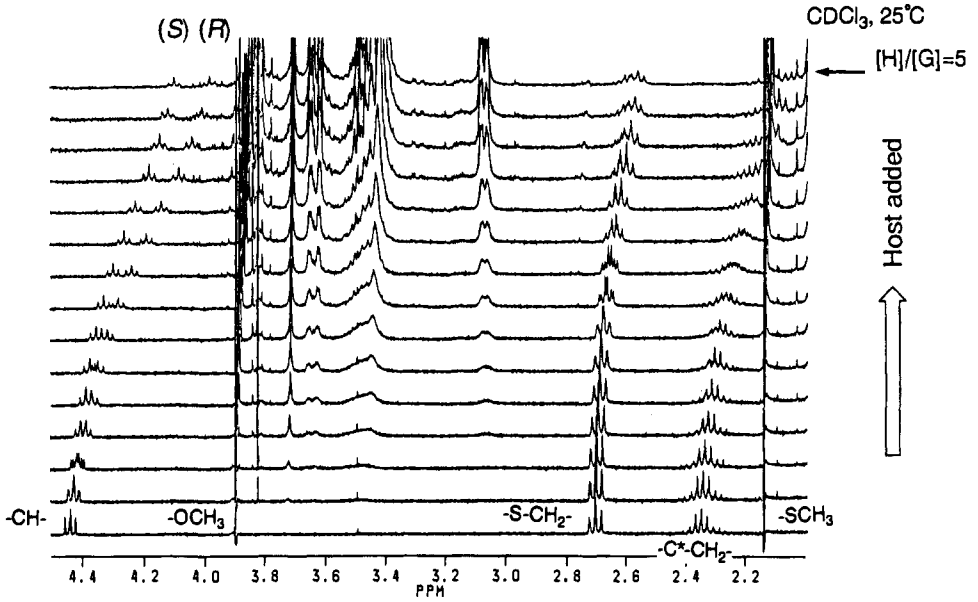

Figure 6. ^{1}H-NMR spectral changes for the complexation between host **4** and racemic guest **16** (ClO_4^-) in $CDCl_3$ at 25 °C. A stock solution of host **4** was successively added.

Table 3. Equilibrium Constants and Limiting Shifts Determined by the Titration ^{1}H-NMR Method under the Competitive Equilibrium System[a]

probe proton	K_R (M^{-1})	K_S (M^{-1})	$\Delta\delta_R^{lim}$ (ppm)	$\Delta\delta_S^{lim}$ (ppm)
$-\underset{\vert}{CH}-$[b]	150	120	−0.95	−0.77
$-COOCH_3$[c]	170	120	−0.14	−0.14
$-SCH_2-$[d]	180	110	−0.19	−0.36
$-SCH_3$[e]	(100)[f]		(−0.08)[f]	

[a] The host−guest complexation between host **4** and racemic guest **16** (ClO_4^- salt) in $CDCl_3$ at 25 °C. [b] δ = 4.43 ppm, 1H, t. [c] δ = 3.89 ppm, 3H, s. [d] δ = 2.7 ppm, 2H, t. [e] δ = 2.13 ppm, 3H, s. [f] No peak separation observed.

guest **16**) in $CD_3OD/CDCl_3$ (10/1, vol %) at 25 °C were determined by the usual nonlinear titration (^{1}H-NMR) method which had been reported previously[20,25a] and are summarized in Table 4.

Discussion

Correlation between I_R/I_{S-d_3} and K_R/K_S Values. Peak intensities of molecular ions or related ions in FAB mass spectra are generally governed by many factors: for example, molecular weight, hydrophobicity/hydrophilicity, basicity/complexation ability, etc., as well as matrix and sample concentration employed.[30] Therefore, particular attention should be paid to their quantitative comparisons.[18,25,31] The primary difficulty seems to come from different transferabilities of different ions from a matrix solution. In early quantitative experiments, certain normalization using the transferabilities of two selected ions had been carried out in an 18-crown-6−K^+ complexation study.[18] However, as far as peak intensities of the two diastereomeric host−guest complex ions are compared, the two transferabilities can be assumed to be equal, and then the relative peak intensity (I_R/I_{S-d_3}) may be expected to reflect quantitatively the concentration ratio in the matrix solution.

Variations in I_R/I_{S-d_3} with the concentration ratio of [H]/[G] are highly informative. When the K_R and K_S values in NBA are given, the concentrations of the corresponding diastereomeric host−guest complex ions under competitive equilibrium conditions can be calculated. The results in the three typical cases are shown in Table 1, together with the experimental I_R/I_{S-d_3} values, where K_R/K_S = 2.0 is assumed.[32] Both the degree and the direction of the variations in the concentration ratios, especially in case B, are remarkably reproducible with those of the I_R/I_{S-d_3} values: the degree of variations depends upon the magnitude of K. Therefore, actual I_R/I_{S-d_3} values seem to be correlated with the ratios of the corresponding complex ions produced in the matrix solution, $[(H + G_R)^+]/[(H + G_S)^+]$.

Figure 7 shows a computer-simulated plot of $[(H + G_R)^+]/[(H + G_S)^+]$ against [H], where [G] is kept constant ($[G] = [G_R^+] + [G_S^+] = 0.05$ M and $[G_R^+] = [G_S^+]$). If [H] becomes small enough when compared with [G], the concentration ratio reaches the K_R/K_S value (=2.0).[33] Our experimental concentration condition corresponds to the position marked by an arrow in Figure 7 ([H] = 0.008 M; concentration condition A). The I_R/I_{S-d_3} values are then very close to the K_R/K_S value with errors of 2% (case A), 10% (case B), and 20% (case C) (see also Table 1). Analogous situations are also simulated in the case of K_R/K_S = 5.0 which corresponds to the case of the complexation between host **5** and guest **16**. The correlation is then expressed as the following:

$$I_R/I_{S-d_3} \leq K_R/K_S$$

$$-RT\ln(I_R/I_{S-d_3}) \geq -RT\ln(K_R/K_S) = \Delta\Delta G_{enan}$$

Importantly, the smaller the magnitude of K_R (or K_S), the closer I_R/I_{S-d_3} is to K_R/K_S. These findings suggest that the I_R/I_{S-d_3} value determined by the FABMS/EL method can be regarded as a new measure of relative thermodynamic stability in solution, if K is on the order of about 10^2 or less.

Table 4 provides experimental evidence for such parallelism between I_R/I_{S-d_3} and K_R/K_S values. These two values, which were derived from completely different methods (FABMS in NBA and ^{1}H-NMR in $CDCl_3$ or $CD_3OD/CDCl_3$ (10/1)), agree well within experimental error. At present we cannot predict

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(31) Caprioli, R. M. In *Mass Spectrometry in Biomedical Research*; Gaskell, S., Ed.; John Wiley: Chichester, 1986; Chapter 4, pp 41−59.
(32) See Table 4 (K_R/K_S = 2.0).
(33) (a) Goldberg, I. *J. Am. Chem. Soc.* **1977**, *99*, 6049−6057. (b) Knobler, C. B.; Gaeta, F. C. A.; Cram, D. J. *J. Chem. Soc., Chem. Commun.* **1988**, 330−333. (c) Hunter, C. A.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1990**, *112*, 5525−5534. (d) Schneider, H.-J. *Angew. Chem., Intl. Ed. Engl.* **1991**, *30*, 1417−1436.

Table 4. K_R/K_S Values Determined by the Titration ^{1}H-NMR Methods

host	guest (counterion)	solvent	temp (°C)	method[a]	K_R (M^{-1})	K_S (M^{-1})	K_R/K_S	$(I_R/I_{S-d_3})^{corr}$
4	**16** (ClO_4^-)	$CDCl_3$	25	A[b]	150	120	1.3	1.6
1	**22** (Cl^-)	$CD_3OD/CDCl_3$ (10/1)	25	B[c]	2.0	1.0	2.0	2.0
5	**16** (Cl^-)	$CD_3OD/CDCl_3$ (10/1)	25	B[d]	78.6[e]	15.9[f]	4.9	5.4

[a] Key: A, the competitive equilibrium method (see Experimental Section); B, the noncompetitive equilibrium method. [b] The CH proton probe in the guest (see Table 3). [c] The OCH_3 proton probe in the host (δ = 4.58 ppm, 3H, s). [d] The Ph–H(*m*) proton probe in the host (δ = 7.32 ppm, 2H, s). [e] 78.6 ± 8.4 (n = 5). [f] 15.9 ± 0.7 (n = 4).

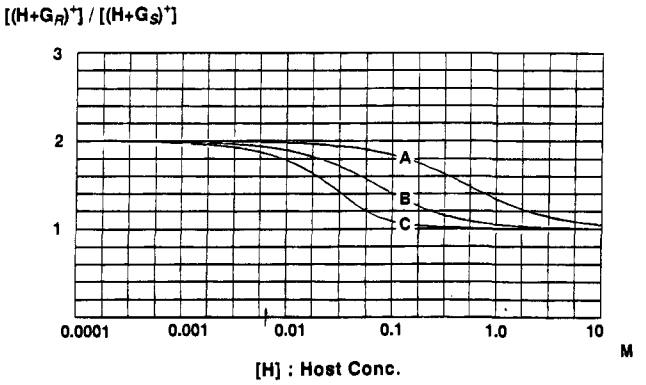


Figure 7. A computer-simulated plot of $[(H + G_R)^+]/[(H + G_S)^+]$ against [H]: (A) K_R = 2.0, K_S = 1.0 M^{-1}, (B) K_R = 20, K_S = 10 M^{-1}, (C) K_R = 200, K_S = 100 M^{-1}. $[G] = [G_R^+] + [G_S^+]$ = 0.05 M and $[G_R^+] = [G_S^+]$ are assumed. The concentration of the host under our measuring conditions (FABMS) is shown by an arrow.

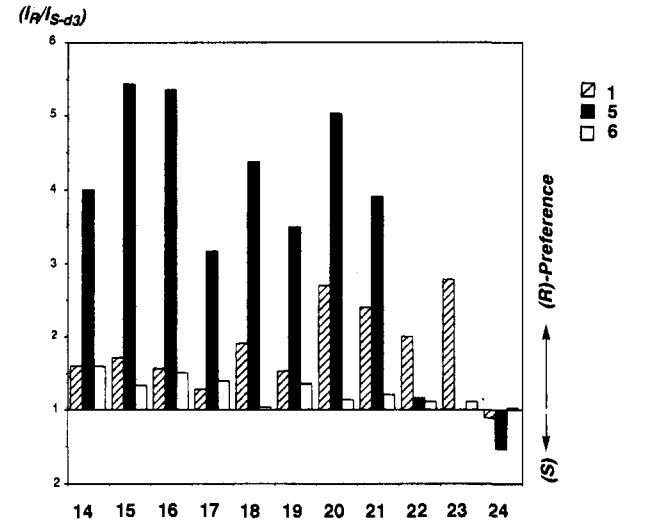


Figure 8. Variations of chiral recognition properties, $(I_R/I_{S-d_3})^{corr}$, for three typical hosts, **1**, **5**, and **6**, toward a series of guests (**14**–**24**). For the (*S*)-enantiomer preference, the value of $1/(I_R/I_{S-d_3})^{corr}$ is plotted.

how much the degree of chiral recognition is influenced by a change in solvent. Only one example is seen in the host–guest complexation between a chiral pyridylcrown host and a (1-naphth-1-ylethyl)ammonium ion guest, where the K_R/K_S value has been reported to be 2.6 in MeOH[6a] and 4.4 in the gas phase without solvent.[26] Although the corresponding K_R/K_S values in NBA are not available, we can assume tentatively that the different solvents employed here in Table 4 do not provide serious effects on the degree of chiral recognition. We will be able to predict reasonably K_R/K_S values of new host compounds toward various guests, information which is needed by organic chemists, on the basis of the corresponding I_R/I_{S-d_3} values by the present FABMS/EL approach.

Comparisons of Chiral Recognition by Different Chiral Hosts. Figure 8 illustrates three typical hosts (**1**, **5**, **6**) of guest dependencies upon chiral recognition. As seen in Figure 8, it

Table 5. I_R/I_{S-d_n} Values Determined by the Enantiomer-Labeled Guest Method Using FABMS

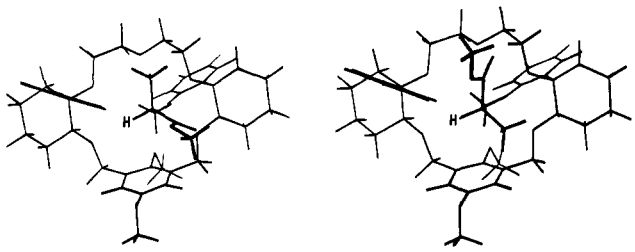
	guest	
host	**25** $(I_R/I_{S-d_5})^{obs}$ [a]	**26** $(I_R/I_{S-d_7})^{obs}$ [b]
1	2.25	1.67
5	5.03	3.66
6	1.04	1.16
13	1.01	1.00

[a] Ethyl-d_5-labeled ester was employed for the (*S*)-enantiomer guest. Concentration condition between A′ and B′: [H] = 0.033 M, [G] = 0.222 M. [b] Isopropyl-d_7-labeled ester was employed for the (*S*)-enantiomer guest. Concentration condition A because of solubility: [H] = 0.0083 M, [G] = 0.05 M.

is clear that host **5** has a remarkably high degree of chiral recognition toward various guests. Here, an amino ester ammonium ion is expressed as $RCH(COOMe)NH_3^+$. Host **5** shows a characteristic pattern where the I_R/I_{S-d_3} values are classified into four groups: (1) a high degree of (*R*)-enantiomer preference (I_R/I_S = 3.2–5.4) when R is a primary or secondary alkyl group (guests **14**–**21**), (2) almost no enantioselectivity (I_R/I_S = 1.1) when R is a phenyl group (guest **22**), (3) a low degree of (*S*)-enantiomer preference (I_R/I_S = 0.7) when the guest is a secondary ammonium ion (guest **24**), and (4) almost no complexation (I_R/I_S = ND (not detected)) when R is a tertiary alkyl group (guest **23**). The highest chiral recognition is observed for host **5** complexing guest (*R*)-**16** by a factor of 5.4 better than guest (*S*)-**16** (I_R/I_{S-d_3} = 5.4; $-\Delta\Delta G_{enan}$ = 1.0 kcal/mol).

On the other hand, host **1** shows a different structure-dependent pattern: (1) a moderate degree of (*R*)-enantiomer preference (I_R/I_S = 1.5–2.8) for primary ammonium ions (guests **14**–**23**) and (2) a weak (*S*)-enantiomer or almost no enantiomer preference (I_R/I_S = 0.9) for a secondary ammonium ion (guest **24**). It is noteworthy that the I_R/I_S values of host **1** become larger from primary to secondary or tertiary alkyl groups of R. This variation is in sharp contrast to that of host **6** where weak (*R*)-enantiomer preference reaches almost unity with a similar change. These changes in the degree and the direction of chiral recognition should be ascribed in terms of (1) complementarity on steric grounds and/or (2) secondary attractive interaction on electrostatic grounds[33] for the complex stabilization between various substituents attached to the host and the guest employed.

The primary binding force is attributable to the intermolecular hydrogen bonding and the charge–dipole interaction between RNH_3^+ and the host oxygens. The secondary binding interaction is believed to clarify such chiral recognition behavior. This must be π-acid and π-base interaction between the COOR group in the guest and the dimethoxyphenyl group in the host.[3a,33] As shown in Table 5, toward guests **25** and **26**, both hosts **1** and **5** complex the (*R*)-enantiomer better than the (*S*)-enantiomer guest. However, their chiral recognition ability toward guest **26**, which has a bigger and a more branching alkyl group, drops to about 70% when compared with that toward guest **25**. Such a decreasing effect with a change in the degree of branching size of the alkyl group may be attributed to a weaker (less effective)



a b

Figure 9. Structures of the diasteromeric host–guest complex ions between host **5** and guest **14** estimated from PM3 calculations (a top view): (a) host **5** plus guest (*R*)-**14**, (b) host **5** plus guest (*S*)-**14**.

secondary interaction on steric grounds and may lead to such lesser chiral recognition.

Comparisons among guests **17**, **21**, and **23** are also interesting where the alkyl group at the position located next to the asymmetric carbon in the guest corresponds to a series of primary, secondary, and tertiary groups, respectively. Chiral recognition of host **1** increases from 1.3 to 2.8 successively. On the other hand, host **5**, unlike host **1**, exhibits almost no complexation specifically toward guest **23**, which has the most highly branching *t*-Bu group. Therefore, host **5** should have the most limited complexing space which the R alkyl group of guest (*R*)-**23** is allowed to occupy and should provide a high degree of chiral recognition toward various guests with a less branching alkyl group.

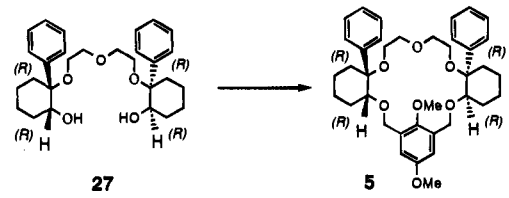
The switch of a guest's enantioselectivity was observed in both hosts **1** and **5**. The resulting (*S*)-enantiomer preference toward guest **24** is also predictable from Corey–Pauling–Koltun (CPK) model examinations if it is assumed that the interaction between the π-acid and the π-base functions is effective as the secondary attractive intermolecular binding force.

Acyclic natural host **12** shows characteristic (*S*)-enantiomer preference for guest **22** (I_R/I_S = 0.5), but cyclic natural host **9** shows nonselectivity toward the same guest (I_R/I_S = 1.0). These observations are in good agreement with the results derived from the ion selective electrode method.[12d]

Estimated Structures of the Host–Guest Complex Ions. The origin of the high chiral recognition for the complexation between host **5** and guest **14** has been estimated on the basis of complex ion structures. As expected from CPK model examinations[7f] and PM3 calculations,[37] the phenyl group attached to the cyclohexyl group is located almost vertically relative to the macrocyclic ring plane. Since host **5** is expected to be conformationally less mobile than host **1**, the phenyl group will effectively block the complexation space, and serve as an efficient chiral barrier for the complexations.

Figure 9 shows the two complex ion structures predicted from PM3 calculations between host **5** and guest (*R*)-**14** or (*S*)-**14**. Each hydrogen (a small size group) which is attached to the asymmetric carbon of the guest is initially set to occupy the most crowded space between the phenyl and the dimethoxyphenyl groups of host **5**. Such a position of the hydrogen after 100% complexation is deduced by the ^{1}H-NMR limiting shift values derived from the complexation between analogous host **4** and guest **16** mentioned before (Table 3). That is, in the two diastereomeric complex ions, the higher upfield shifts obtained (−0.95 and −0.77 ppm) are interpreted by the ring current effect of the neighboring dimethoxyphenyl group: the CH proton is located within the shielding region of the aromatic group. In this situation, in the case of the complexation with guest (*R*)-**14**, the O=COC plane of the COOMe group lies closely parallel to the phenyl plane of the dimethoxyphenyl group. The attractive π-acid and π-base combination is believed to serve as further stabilization for the present chiral recognition to a certain extent.[33] Crystal structures determined by X-ray crystallography in the future study, however, will provide evidence for the complex ion structures.

Scheme 1



Conclusions and Future Applications. In summary, a novel method for determining chiral recognition behavior in the highly structured host–guest complexations has been proposed. The method uses routine FAB mass spectrometry but specifically uses a 1/1 mixture of unlabeled and enantiomer-labeled guests with a given chiral host. The relative peak intensity of the diastereomeric host–guest complex ions observed, which is denoted as $I_R/I_{S\text{-}d_n}$, provides straightforwardly both the degree and the direction of chiral recognition properties. The operations are simple. The results are reliable and closely parallel to the thermodynamic properties in solution. If stock solutions of a series of 1/1 mixtures of unlabeled and enantiomer-labeled guests are prepared, one can carry out rapid screening of chiral recognition for many new chiral hosts qualitatively and/or quantitatively.

The FABMS/EL method will, of course, apply to various hosts such as cyclodextrins, cyclophanes, calixarenes, etc. as well as crown ethers for detecting highly structured chiral recognition behavior. Applicable guests are not limited to amino acid ester ammonium ions. Free amino acids and (1-phenylethyl)ammonium ions are the next potential candidates, whose applications will be described in the next paper of this series. Here, we have clarified the fundamental features and the representative applications of the novel method proposed. A new host, which has exhibited a high degree of chiral recognition, will be developed for practical use of chromatography, etc.

Experimental Section

Materials. Chiral Hosts. Except for host **5**, chiral hosts **1**–**12** were synthetic compounds which had already been reported[20,25a,34] or commercially available compounds [hosts **9** (nonactin) and **12** (monensin methyl ester) are from Calbiochem]. Host **5** was the synthetic intermediate which was modified into the corresponding azophenolic derivatives as reported in the previous communication.[7g] The synthetic procedure of host **5** from diol **27** (Scheme 1) and the characterization are as follows.

(4*R*,9*R*,17*R*,22*R*)-9,17-Diphenyl-27,29-dimethoxy-3,10,13,16,23-pentaoxatetracyclo[23.3.1.0^{4,9}.0^{17,22}]nonacosa-1(28),25(29),26(27)-triene (Host 5). A solution of (−)-**27**[7f] (1.60 g, 3.50 mmol) and 2,6-bis(bromomethyl)-1,4-dimethoxybenzene (1.15 g, 3.50 mmol) in dry THF (370 mL) was added dropwise to a boiling mixture of NaH (320 mg, 13.3 mmol), potassium tetrafluoroborate (443 mg, 3.50 mmol), and dry THF (180 mL) over a 10 h period, and then the mixture was refluxed for an additional 20 h under dry argon. After the reaction mixture was cooled in an ice bath, a small amount of water was slowly added to the chilled reaction mixture and the solvent was evaporated under reduced pressure. The residue was taken up in chloroform, washed with water, and dried ($MgSO_4$). After removal of the solvent, the residue was chromatographed on silica gel using hexane/diethyl ether (4/1) as an eluent to give host **5** (1.74 g, 80% yield) as a white glass: $[\alpha]^{24}_D$ −97.1° (*c* 1.0 $CHCl_3$); IR (KBr) ν_{max} (cm^{-1}) 3050 (w),

(34) Naemura, K.; Mizooku, T.; Kamada, K.; Hirose, K.; Tobe, Y.; Sawada, M.; Takai, Y. *Tetrahedron:Asymmetry* **1994**, *5*, 1549–1558.

3020 (w), 2950 (s), 2860 (m), 1610 (m), 1490 (m), 1450 (m), 1320 (m), 1250 (m), 1120 (m), 1100 (s), 760 (m), 700 (m); ^{1}H-NMR (360 MHz, $CDCl_3$) δ 7.21–7.44 (m, 10H, ArH), 6.76 (s, 2H, $Ar(OCH_3)_2$), 4.74 (d, J = 10.1 Hz, d, $ArCH_2$), 4.39 (d, J = 10.1 Hz, 2H, $ArCH_2$), 4.02 (m, 2H, CH), 3.99 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 2.97–3.23 (m, 8H, OCH_2), 1.25–2.09 (m, 16H, CH_2); FABMS (NBA) m/z 616 (M^+). Anal. Calcd for $C_{38}H_{48}O_7$: C, 74.00; H. 7.84. Found: C, 73.75; H, 7.61.

Chiral Guests. Commercial samples (Sigma) of D-methyl methionate hydrochloride (D-**16**, Cl^-), D-methyl phenylalaninate hydrochloride (D-**18**, Cl^-), and D- and L-methyl tryptophanate hydrochlorides (D-**19**, Cl^-, and L-**19**, Cl^-) were used without purification. All other amino acid methyl ester hydrochlorides were synthesized according to the standard method[3a] from commercial D-amino acids and L-amino acids (Sigma, Wako, Tokyo Kasei, and Aldrich) with methanol or methanol-d_4. Typical esterification procedures and characterizations are as follows.

L-Methyl-d_3 Methionate Hydrochloride (L-16-d_3, Cl^-). Into a suspension of L-methionine (1.5 g, 0.01 mmol) in 20 mL of dry (stored over molecular sieves 3A) CH_3OH-d_4 (99.8 atom % D, Isotec, Inc.) was bubbled dry HCl gas at room temperature until dissolution was completed. The solution was refluxed for 6 h, cooled, and evaporated to dryness. The residue was dissolved in ca. 5 mL of water, ca. 10 mL of CH_2Cl_2 was added, and enough aqueous NH_4OH was added to give pH 10. After extraction with CH_2Cl_2, the organic layer containing the free amino ester was dried over anhydrous $MgSO_4$, and dry HCl gas was bubbled in to precipitate the corresponding hydrochloride salt. After filtration, washing, and drying, the desired L-methyl-d_3 methionate hydrochloride (L-**16**-d_3, Cl^-, then (*S*)-**16**-d_3, Cl^-)[29] was obtained as a white solid (1.6 g, 79% yield): mp 145–148 °C; $[\alpha]^{25}_D$ +26.0° (*c* 1.0, CH_3OH); ^{1}H-NMR (360 MHz, D_2O) δ 4.31 (t, 1H), 2.69 (t, 2H), 2.36–2.15 (m, 2H), 2.11 (s, 3H). Anal. Calcd for $C_6H_{11}D_3N_1S_1O_2Cl_1$: C, 35.55; H, 6.96; N, 6.91. Found: C, 35.41; H. 6.93; N, 6.51.

L-Isopropyl-d_7 Phenylalaninate Hydrochloride (L-26-d_7, Cl^-). Similar to the method described above, commercial L-phenylalanine (0.66 g, 4 mmol) was esterified during 24 h of refluxing with 10 mL of $(CH_3)_2CHOH$-d_8 (99.5 atom % D, C/D/N Isotopes). After workup similar to that above, the desired compound (L-**26**-d_7, Cl^-, then (*S*)-**26**-d_7, Cl^-) was obtained as a white solid (0.95 g, 95% yield, recrystallization from ethyl acetate/methanol): mp 222–224 °C; $[\alpha]^{25}_D$ + 19.50° (*c* 1.0 CH_3OH); ^{1}H-NMR (360 MHz, D_2O) δ 7.44–7.28 (m, 5H), 4.23 (m, 1H), 3.26 (dd, J = 6.3 and 14.4 Hz, 1H), 3.19 (dd, J = 7.2 and 14.4 Hz, 1H). Anal. Calcd for $C_{12}H_{11}D_7N_1O_2Cl_1$: C, 57.47; H, 7.24; N, 5.59. Found: C, 57.28; H, 7.07; N, 5.52.

General Procedures. ^{1}H-NMR spectra (360 MHz) were taken with a Bruker AM360 spectrometer. TMS (in $CDCl_3$) and 3-(trimethylsilyl)propionic acid sodium salt (TSP) (in D_2O) were used as the internal standards, respectively. FTIR (or IR) spectra were recorded with an Analect RFX65 (or Hitachi IR345) spectrometer. Elemental analyses were performed using a Perkin-Elmer 2400 at the Material Analysis Center, The Institute of Scientific and Industrial Research, Osaka University. Melting points were measured with a Yanaco micro melting point apparatus, and specific rotations were taken with a Horiba SEPA300 polarimeter. Liquid column chromatography was carried out on a Yamazen LC apparatus under appropriate medium pressure. A Mettler AT261 balance was employed for weighing various host and guest compounds, and an EYELA FD-80 freeze dryer was employed for drying perchlorate salts.

FAB Mass Spectra. FAB mass spectra (positive mode) were obtained with a JEOL DX300 mass spectrometer operating at an accelerating voltage of 3 kV with a mass range of m/z 20–1000. The instrument was equipped with a standard JEOL FAB source and an ion gun. Xenon was used as the atom beam with an emission current of 20 mA and an acceleration of 6 kV. The source pressure was typically ca. 10^{-6}–10^{-5} Torr. Spectra were obtained with a magnet scan rate of 5 s/scan (to m/z 1000), and the data were processed with a JEOL JMA 5000 data processing system.

Preparation of Sample Solutions for the FABMS/EL Method. A sample solution was prepared by mixing three solutions: microsyringes and a vibrator were used. FABMS measurements were performed, after the solution stood overnight, with a deposit of a 1/μL aliquot of the mixed solution on a FAB probe tip.

(a) Concentration Condition A (or A'). The three solutions were as follows: (1) 5 μL of a 0.30 M MeOH solution of a 1/1 mixture of (*R*) unlabeled and (*S*) labeled ester guests ($[G_R^+]$ = 0.15 M and $[G_S^+]$ = 0.15 M), (2) 5 μL of a 0.05 M $CHCl_3$ solution of a given host, and (3) 30 μL of NBA matrix (the use of 15 μL corresponds to the concentration condition A'). In the concentration conditions after evaporation of MeOH and $CHCl_3$ solvents in the ion source, the concentrations in NBA were calculated to [H] = 0.0083 M, [G] = 0.05 M ($[G_R^+]$ = 0.025 M, $[G_S^+]$ = 0.025 M), and then [H]/[G] = 1/6 ($[H]/[G_R^+]/[G_S^+]$ = 1/3/3).

The accuracy of the 1/1 equivalent concentration of (*R*)- and (*S*)-enantiomer guests was confirmed by checking whether the I_R/I_{S-d_3} value with an achiral host **13** was experimentally obtained as unity (1.00 ± 0.03) or not for every preparation of guest solutions (see Table 2).

(b) Concentration Condition B (or B'). In order to obtain high-quality FAB mass spectra, a relatively lesser amount of NBA was employed, and then relatively higher concentrations of samples and ions were achieved. The three solutions were as follows: (1) 5 μL of a 1.33 M MeOH solutions of a 1/1 mixture of (*R*) unlabeled and (*S*) labeled ester guests, (2) 5 μL of 0.20 M $CHCl_3$ solution of a given host, and (3) 15 μL of NBA (the use of 20 μL corresponds to the concentration condition B'). After evaporation of the solvents, the concentrations in NBA were calculated to [H] = 0.0677 M and [G] = 0.444 M, and then [H]/[G] = 1/6.6. Compared with the condition A, [H] and [G] were 8 times higher. Accordingly, the quality of the FAB mass spectra improved, but the amounts of the host and guest samples consumed increased.

Three relative intensity data obtained from the 10th, 20th, and 30th scan spectra were simply averaged and tabulated after the (M + 3) isotope correction (see later) in Table 2.

Corrections of the Observed (I_R/I_{S-d_3}) Values on the Basis of the Natural Abundance of the (M + 3) Isotope. The observed peak intensity of a host plus (*S*)-guest complex ion (I_{S-d_3}) inevitably contains a contribution from an amount of the (M + 3) natural abundant isotope derived from the peak intensity of a host plus (*R*)-guest complex ion (I_R). Therefore, the corrected value, $(I_R/I_{S-d_3})^{corr}$, was derived from eq 4. Here, C is the theoretical (M + 3) ion distribution (%) of the

$$(I_R/I_{S-d_3})^{corr} = (I_R/I_{S-d_3})^{obs}/[1 - (I_R/I_{S-d_3})^{obs}C/100] \quad (4)$$

corresponding host–guest complex ion. For a typical example, in the combination between host **1** and guest **18**, the molecular equation of the complex ion is $C_{52}H_{58}N_1O_9$ (m/z 840) and then the theoretical (M + 3) ion (m/z 843) distribution is derived as 4.48%. Therefore, the value of $(I_R/I_{S-d_3})^{obs}$ = 1.78 is corrected as $(I_R/I_{S-d_3})^{corr}$ = 1.93 = 1.78/[1 − (1.78 × 0.0445)].

Thermodynamic Behaviors of Host–Guest Complexation Equilibria in Solutions. (a) Determination Procedures of K_R and K_S for the Competitive Equilibrium System. The equilibrium constants (K_R and K_S) for the 1/1 complexations between a chiral host and (*R*)- and (*S*)-enantiomer guests were determined by the titration ^{1}H-NMR method using a racemic guest solution. The equations applying to the competitive equilibrium system, which had been reported by Popov et al.,[35] were used:

$$H + G_R^+ \overset{K_R}{\rightleftharpoons} (H + G_R)^+ \quad (5)$$

$$H + G_S^+ \overset{K_S}{\rightleftharpoons} (H + G_S)^+ \quad (6)$$

$$K_RK_S[L]^3 - \{K_RK_S([H]_o - [G_R^+]_o - [G_S^+]_o) - K_R - K_S\}[L]^2 - [K_R([H]_o - [G_R^+]_o) + K_S([H]_o - [G_S^+]_o) - 1][L] - [H]_o = 0 \quad (7)$$

$$\Delta\delta_R^{obs} = \{K_R[L]/(1 + K_R[L])\}\Delta\delta_R^{lim} \quad (8)$$

$$\Delta\delta_S^{obs} = \{K_S[L]/(1 + K_S[L])\}\Delta\delta_S^{lim} \quad (9)$$

Equations 5 and 6 are in competitive conditions. Therefore, the third-order equation for [L] is derived. Here, [L] is the concentration of the free chiral host which does not complex with the two guests. $[H]_o$, $[G_R^+]_o$, and $[G_S^+]_o$ are the initial concentrations prepared for H, G_R^+,

(35) Boss, R. D.; Popov, A. I. *Inorg. Chem.* **1985**, *24*, 3660–3664.

and G_S^+, respectively. For the present experiments, since a racemic solution of the guest is used, the equation of $[G_R^+]_o = [G_S^+]_o$ holds exactly. Chemical shift changes ($\Delta\delta_R^{obs}$ and $\Delta\delta_S^{obs}$) for a target proton of the diastereomeric host–guest complex ions, which are observed by successive additions of a chiral host solution, are expressed by eqs 8 and 9. The values of $\Delta\delta_R^{lim}$ and $\Delta\delta_S^{lim}$ show the limiting chemical shifts at 100% complexation.[36]

A guest solution was prepared by mixing 0.237 mg of a racemic methyl methionate perchlorate salt (racemic guest **16**, ClO_4^-) and 500 μL of $CDCl_3$ ($[G_{RS}^+] = 1.80 \times 10^{-3}$ M). A stock host solution, which was prepared by mixing 5.27 mg of host **4** and 150 μL of $CDCl_3$, was successively added (A^{total}, μL) to the above guest solution using a microsyringe: A^{total} = 0, 1, 2, 4, 6, 8, 11, 15, 20, 26, 33, 43, 53, 63, or 73 μL (n = 15). The different solutions in the NMR tube were allowed to stand ca. 10 min to approach and maintain the probe temperature (298 K). The corresponding ^{1}H-NMR spectra in the selected region of 2–4 ppm are shown in Figure 6. Volume corrections were done for the calculation of host and guest concentrations: the final concentrations were $[H] = 7.16 \times 10^{-3}$ M and $[G_{RS}^+] = 1.57 \times 10^{-3}$ M. Equilibrium constants were determined using a home-made computer program (nonlinear least squares method). The errors are typically estimated within ±10%.

(b) Determination Procedures of K_R or K_S for the Usual (Noncompetitive) Complexation System. Equilibrium constants (K_R or K_S) in the usual (noncompetitive) complexation system were determined using the reported titration ^{1}H-NMR (non-linear least squares) method[20,25a] where the guest solution was added successively. Commercial samples of CD_3OD (Nihon Sanso, 99.8 atom % D) and $CDCl_3$ (Aldrich, 99.8 atom % D) were used without purification. Because of solubility problems, a $CD_3OD/CDCl_3$ (10/1, vol %) mixed solvent was employed. For example, at the highest guest concentration studied for the complexation between host **5** and guest **16** (Cl^-), the chemical shift differences were $\Delta\delta_{Ph-H(m)}^{max} = 20.41$ Hz (for (*R*)-**16**) and $\Delta\delta_{Ph-H(m)}^{max} = 10.48$ Hz (for (S)-**16**): $[H] = 5.6 \times 10^{-4}$ M and $[G_R^+] = 4.32 \times 10^{-2}$ M (in the former case), and $[H] = 5.3 \times 10^{-4}$ M and $[G_S^+] = 9.24 \times 10^{-2}$ M (in the latter case).

PM3 Calculations. Calculations were carried out using the PM3 semiempirical molecular orbital procedure[37] as implemented in the MOPAC 7 program package. An ANCHOR2 system on a Fujitsu S-4/2 workstation was employed.[38]

Acknowledgment. This work was supported in part by the Nishida Research Fund for Fundamental Organic Chemistry (M.S.). We are grateful to Mrs. Fusako Fukuda (Material Analysis Center, The Institute of Scientific and Industrial Research, Osaka University) for elemental microanalyses and to Professors Soichi Misumi, Terukiyo Hanafusa, Shigetoshi Takahashi, and Ikuo Ueda (The Institute of Scientific and Industrial Research, Osaka University) for stimulative encouragement. We are indebted to Dr. F. J. Winkler (Technische Universität München) for information about the commercial availability of (*R*)- or (*S*)-*tert*-leucine.

JA950647X

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