# Chiral amino acid recognition detected by electrospray ionization (ESI) and fast atom bombardment (FAB) mass spectrometry (MS) coupled with the enantiomer-labelled (EL) guest method

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**Chiral recognition of crown ethers toward amino acids and their esters is detected both by electrospray ionization (ESI) and by fast atom bombardment (FAB) mass spectrometry (MS) and then compared as a series of host (H)–guest (G) pairs. A racemic guest ($G_R^+$:[$^2H_n$]$G_S^+$ = 1:1, of which one enantiomer is deuterium-labelled), is mixed with the target host. The chiral amino acid recognition of the host is determined from the relative peak intensities of the corresponding diastereomeric host–guest complex ions, [eqn. (*a*)]:**

$$I[(H\cdot G_R)^+]/I[(H\cdot[^2H_n]G_S)^+] = IRIS. \qquad (a)$$

**For the complexation between chiral host 1 and guest MetOMe$^+$, FABMS gives *IRIS* = 5.0 (NBA matrix), which is practically equal to the corresponding equilibrium constant ratio ($K_R/K_S$) in solution. However, ESIMS gives *IRIS* = 1.5 for the same complexation (MeOH), which is a remarkable decrease in the *IRIS* value. Another complex between chiral host 8 and guest MetOMe$^+$, gives *IRIS* = 2.0 by FABMS but *IRIS* = 1.2 by ESIMS. Moreover, in a much simpler system, the amino ester ion selectivity, Leu′OMe$^+$/MetOMe$^+$, of host 18-crown-6 is depressed to such an extent that we must conclude that ammonium ion selectivity cannot be evaluated by ESIMS, but the metal ion selectivity, $K^+/Na^+$, of the same host 18-crown-6 gives a good qualitative evaluation of the relative concentrations of the corresponding H–G complex ions in solution. It is demonstrated that the *IRIS* values from the FABMS coupled with the enantiomer-labelled (EL) amino ester guest method are the most reliable and generally useful of the measures considered for the chiral amino acid recognition.**

## Introduction

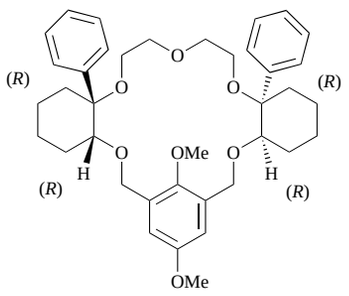
Chiral recognition of chiral amino acids by synthetic and natural host compounds is one of the most challenging subjects in modern host–guest chemistry.[1,2] To determine the chiral recognition of these hosts, various NMR, UV, LC and electrochemical methods have been used. However, mass spectrometry (MS) has never been used for this purpose, at least in a quantitative fashion. With recent advances in both hardware and software aspects of mass spectrometry,[3] new methodology for detecting chiral recognition behavior, making the best use of MS's merits (trace amount detection and rapid measurement) has been expected.[4]

In a previous paper, we have already reported a fast atom bombardment (FAB) mass spectrometric study, which, coupled with the enantiomer-labelled (EL) guest method, was used as a new detection method for chiral recognition of crown ethers toward amino acid esters.[5] Several fundamental features of the new methodology (abbreviated as FABMS–EL guest method) such as sample concentration effects, isotope effects, and correlations with solution equilibrium data, *etc*., have already been clarified. In this paper, we extend the use of the enantiomer-labelled guest method from amino esters to amino acids, and also, the use of mass spectrometry from FABMS (with matrix) to electrospray ionization (ESI)MS (with pure organic solution). We also compare the capabilities of FABMS with ESIMS for the present detection of chiral amino acid recognition and disclose the scope and limitations of each technique.

Until today, most of the reports of chiral recognition properties of synthetic chiral host compounds toward amino acid guests have been primarily of amino acid ester (abbreviated as 'amino ester') guests.[1b,5,6] There are very few reports of free amino acid recognition except for chiral separation by liquid chromatography, *etc*.[7] and pioneering experiments on extraction of racemic amino acids by bis-binaphthyl-type crown ethers and subsequent determinations of chiral recognition properties.[8]

In recent years, ESIMS has become widely applied in various fields of organic chemistry,[9] and its power has become rapidly realized by host–guest and supramolecular chemists.[10] Its most striking feature is that it can detect hydrogen-bonded adducts or host–guest complexes in a pure organic, or an aqueous organic solution. Here, with the use of ESIMS, we treat quantitatively chiral amino acid recognition behavior for the first time.[11]

Based on the comparison of four sets of experimental results from the same series of host–guest pairs, we demonstrate that the FABMS–EL amino ester guest method is the most useful for detecting chiral amino acid recognition. After screening trials of several chiral host compounds with the use of this type of FABMS, we show that a newly found host–guest pair provides a relatively high degree of chiral recognition ability.



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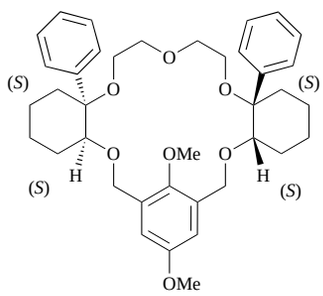

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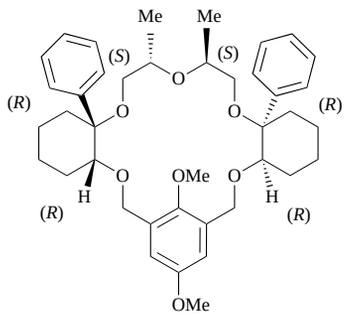

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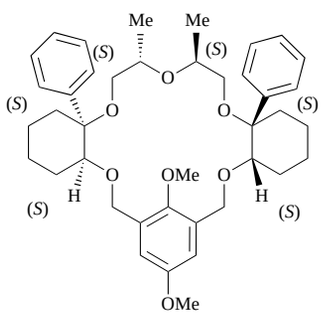

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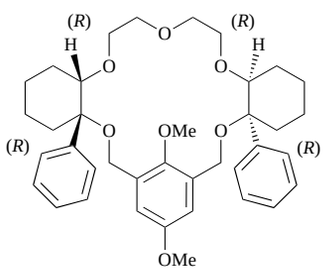

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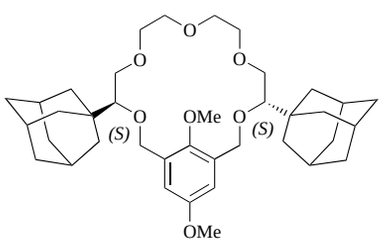

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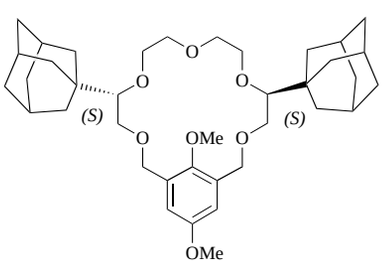

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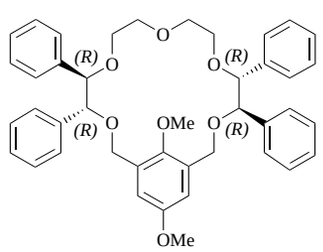

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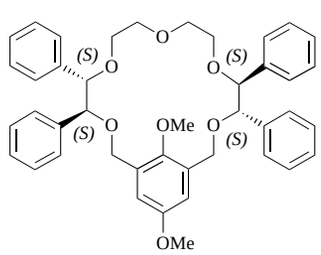

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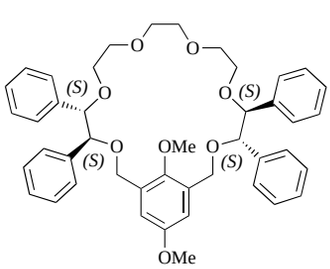


**10**

The hosts studied are shown (structures **1**–**17**); they include chiral crown ethers, disaccharides (permethylated α,α-treharose and sucrose), and acyclic (monencin methyl ester and lasalocid) and cyclic ionophores (valinomycin and nonactin). The guests studied in this paper are shown (structures **18**–**26**); they include salts of amino acids and their methyl, ethyl, and isopropyl esters.

## Results

### (1) Outline of experimental method

**(a) Enantiomer-labelled (EL) guest method.** In order to detect and evaluate quantitatively chiral recognition ability of a given host (H)–guest ($G^+$) complexation system, we used our newly developed EL guest method.[5] This method particularly requires isotopic labelling of one of the guest enantiomers and involves the complexation of a target host compound with a 1 : 1 mixture of the labelled and unlabelled enantiomer guests [reactions (1) and (2)]. For simplicity, we uniformly deuterium-labelled an (*S*)-enantiomer guest ($[^2H_n]G_S^+$) and used an NBA matrix.

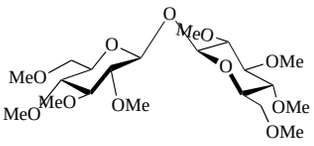
$$H + G_R^+ \rightleftharpoons (H \cdot G_R)^+ \quad (1)$$

$$H + [^2H_n]G_S^+ \rightleftharpoons (H \cdot [^2H_n]G_S)^+ \quad (2)$$

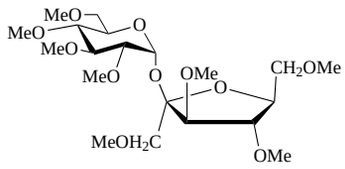
The peak intensity ratio, $I[(H \cdot G_R)^+]/I[(H \cdot [^2H_n]G_S^+)]$, of the diastereomeric H–G complex ions, which appeared simultaneously with *n* mass-unit difference in one FAB mass spectrum, was abbreviated as '*IRIS*' for short and adopted here as a critical measure for detecting chiral recognition ability [eqn. (3)] (see ref. 5).

$$|RT \ln(IRIS)| \leqslant |-\Delta\Delta G_{enan}| \quad (3)$$

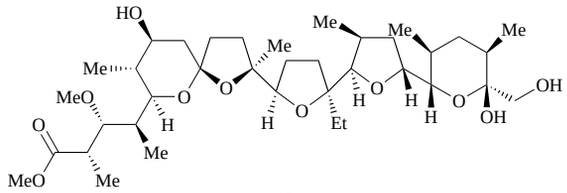
For an amino ester guest, we deuterium-labelled the alkyl part of the ester group (*i.e.*, $[^2H_3]$methyl, $[^2H_5]$ethyl, or $[^2H_7]$-isopropyl group). Alternatively, for an amino acid guest, we used terminal alkyl group labelling (*i.e.*, $[^2H_3]$methyl or $[^2H_5]$phenyl group) of the amino acid. When this EL guest method was coupled with FABMS, we called it the



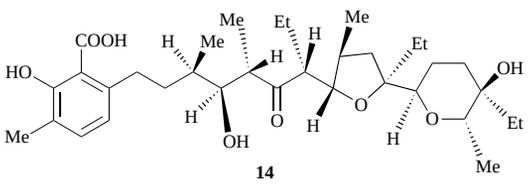
**11**
permethylated α,α-trehalose



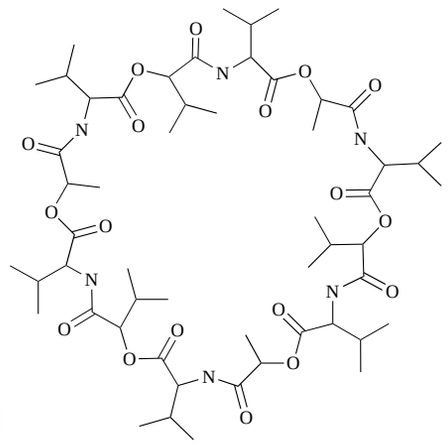
**12**
permethylated sucrose



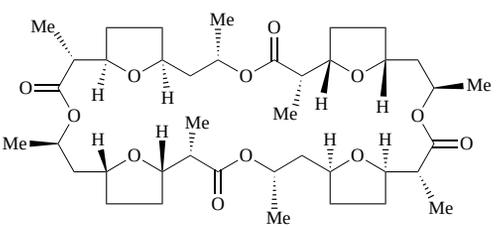
**13**
monencin methyl ester



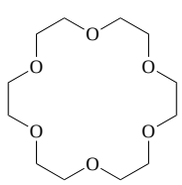
**14**
lasarosid



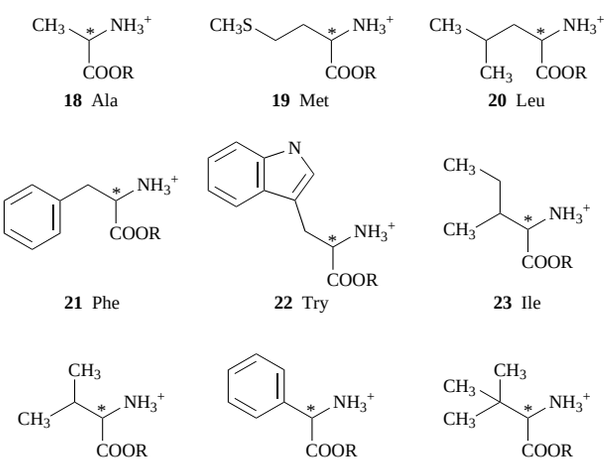
**15**
valinomycin



**16**
nonactin



**17**



**18** Ala **19** Met **20** Leu
**21** Phe **22** Try **23** Ile
**24** Val **25** PGly **26** *tert*-Leu

**a** R = $OCH_3$
**b** R = $OC_2H_5$
**c** R = $OCH(CH_3)_2$
**d** R = H

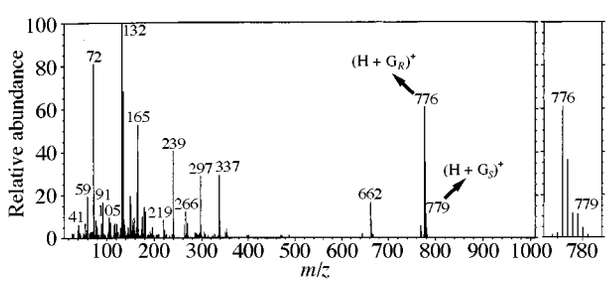



**Fig. 1** A FAB mass spectrum for the complexation between host **3** and guest **24a**, a 1 : 1 mixture of (*R*)-**24a** and (*S*)-[$^2H_3$]**24a** (a racemic mixture of $ValOMe^+Cl^-$, NBA mixture). A FABMS–EL amino ester guest method.

FABMS–EL guest method, and with ESIMS, the ESIMS–EL guest method.

**(b) Checking for a quantitative cross-chiral relationship.** If highly structured diastereomeric H–G complex ions are formed and detected by MS, the following quantitative cross-chiral relationship [eqn. (4)] between the *IRIS* values of a pair of

$$\{IRIS_{(\text{host 1})}\} \times \{IRIS_{(\text{host 2})}\} = 1.00 \qquad (4)$$

enantiomeric hosts (for example, **1** and **2**) should be satisfied for any different guests on purely stereochemical grounds: we called this experimental test the cross-chiral check.

Here, we used the experimental errors from eqn. (4) as another critical measure to evaluate the chiral amino acid recognition among the four sets of experimental methods: (1) FABMS–EL amino ester guest, (2) FABMS–EL amino acid guest, (3) ESIMS–EL amino ester guest and (4) ESIMS–EL amino acid guest methods.

### (2) *IRIS* Values detected by FABMS: chiral amino acid recognition of hosts

The *IRIS* values detected by the FABMS–EL guest method using hydrochloride salts of various amino esters are summarized in Table 1. The *IRIS* values by the FABMS–EL guest method using toluene-*p*-sulfonic acid salts of various amino acids are shown in Table 2. Representative FAB mass spectra are given in Figs. 1 and 2. The results of the cross-chiral check using an enantiomeric pair of hosts **1** and **2** are given in square brackets in the corresponding tables.

### (3) *IRIS* Values detected by ESIMS: chiral amino acid recognition of hosts

The *IRIS* values detected by the ESIMS–EL guest method using hydrochloride salts of various amino esters are summarized in Table 3. The *IRIS* values by the ESIMS–EL guest method using various amino acids (free amino acids, not HCl salts) are given in Table 4. Typical ESI mass spectra are given in Figs. 3 and 4. The results of the cross-chiral check are similarly shown in square brackets in the corresponding tables.

**Table 1** *IRIS* Values using the FABMS–EL amino ester guest method[a]

| Host | Guest | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | AlaOMe$^+$ (**18a**) | MetOMe$^+$ (**19a**) | LeuOMe$^+$ (**20a**) | PheOMe$^+$ (**21a**) | PheOEt$^+$ (**21b**) | PheOPr$^{i+}$ (**21c**) | TryOMe$^+$ (**22a**) | IleOMe$^+$ (**23a**) | ValOMe$^+$ (**24a**) | PGlyOMe$^+$ (**25a**) | PGlyOPr$^{i+}$ (**25c**) | Leu′OMe$^+$ (**26a**) |
| **1** | 4.00[b,d] | 5.35[b,c] | 3.16[b,d] | 4.37[b,c] | 5.03[b,f] | 3.66[b,c] | 3.49[b,c] | 3.62[b,d] | 5.03[b,d] | 1.15[b,c] | | ND[b,c] |
| | | 4.73[d] [1.04] | | 4.60[d] [1.01] | | | | | | 1.14[d] [1.04] | | |
| **2** | 0.26[d] | 0.22[d] | | 0.22[d] | | | | 0.26[d] | | 0.91[d] | | |
| **3** | 0.93[c] | | | 1.87[d] | 2.09[e] | 2.45[d] | | 3.36[d] | 6.93[e] | 1.33[d] | 1.36[e] | |
| **4** | 1.27[c] | | | 0.64[d] | 0.63[e] | 0.71[d] | 2.72[d] | 2.72[d] | 2.17[e] | 1.41[d] | 1.41[e] | |
| **5** | 1.10[d] | 1.01[d] | 1.14[d] | 1.28[d] | 1.34[f] | 1.34[c] | 1.21[d] | 0.60[d] | 0.72[d] | 0.70[d] | | ND[c] |
| **6** | | 1.60[b,c] | | 1.60[b,c] | | | | | | 1.40[b,c] | | |
| **7** | 0.72[d] | 0.99[d] | | 0.96[d] | | | | | | 0.84[d] | | ~0.9[c] |
| **11** | 0.93[c] | 1.04[d] | | 1.02[d] | 1.05[e] | | | | 1.00[e] | 1.04[d] | | |
| **12** | | ~1.0[d] | | ~1.0[d] | | | | | | ~1.0[d] | | |
| **13** | 0.75[b,d] | | | | 0.71[e] | 0.81[d] | | 0.81[b,d] | 0.85[e] | 0.54[b,d] | 0.61[e] | ND[b,d] |
| **14** | 0.95[c] | 0.96[c] | | 0.97[c] | 1.22[e] | | | | 0.97[e] | 0.74[c] | | |
| **15** | | | | | 1.05[e] | | | | | | | |
| **16** | | 1.02[b,d] | | 0.99[b,d] | 0.96[e] | | | | | 1.02[b,d] | | |
| **17** | 1.01[c] | 1.02[c] | 1.01[d] | 0.98[c] | 1.01[f] | 1.00[c] | 0.97[c] | 0.95[d] | 1.02[d] | 0.99[d] | 1.00[e] | 0.98[c] |
| | 0.98[d] | 1.03[d] | | 1.00[d] | 1.01[e] | 1.01[d] | | 0.99[d] | 0.95[e] | 1.02[d] | | |

[a] [ ] shows a value of the quantitative cross-chiral check on the basis of eqn. (4) under the same concentration conditions paired. ND (not detected). [b] Data are taken from ref. 5. [c] Concentration condition A. [d] Concentration condition B. [e] Concentration condition B′. [f] Concentration condition C.

**Table 2** *IRIS* Values using the FABMS–EL amino acid guest method[a]

| Host | Guest[b] Ala$^+$ (**18d**) | Met$^+$ (**19d**) | Leu$^+$ (**20d**)[c] | Phe$^+$ (**21d**)[c,d] | Val$^+$ (**24d**)[c] |
|---|---|---|---|---|---|
| **1** | 2.82 [1.02] | 3.14 [1.07] | | 3.1 [0.93] | >2 |
| **2** | 0.36 | 0.34 | | 0.3 | >0.2 |
| **8** | 1.71 | 1.97[f] | 1.69 | 1.97[g] | |
| **10** | 1.17 | 1.14 | 1.08 | 0.92 | (*ca.* 1.0) |
| **13**[e] | 0.7 | 0.7 | ND | 0.8 | *ca.* 0.8 |
| **15**[e] | 0.9 | 1.1 | 1.0 | (0.7) | *ca.* 1.0 |
| **17**[h] | 1.03 | 1.02 | 1.00 | 0.98 | 0.97 |

[a] Concentration condition C; see Experimental section. [ ] shows a value of the quantitative cross-chiral check on the basis of eqn. (4). [b] Amino acid toluene-*p*-sulfonic acid salt. [c] Because of solubility problems, the guest solution was warmed. [d] The peaks of $(H\cdot[^2H_5]G)^+$ and $(H\cdot[^2H_4]G)^+$ were summed for *IRIS* calculations. [e] FAB mass spectra were noisy because of the weak H–G complex peaks. [f] Average of 4 runs (±0.03). [g] Average of 3 runs (±0.10). [h] Average of 2–6 runs (within ±0.03).

**Table 3** *IRIS* Values using the ESIMS–EL amino ester guest method[a]

| Host | Solvent | Guest MetOMe$^+$ (**19a**) | PheOMe$^+$ (**21a**) | ValOMe$^+$ (**24a**) |
|---|---|---|---|---|
| **1** | MeOH | 1.47 [1.01] | 1.36 [0.98] | 1.13 [0.92] |
| | $CH_3CN$ | 1.67[b] [1.07] | | |
| | aq. MeOH(AcOH)[e] | 1.53[c] [1.06] | | |
| | MeOH–$CHCl_3$[e] | 1.52 [1.03] | | |
| **2** | MeOH | 0.69 | 0.72 | 0.81 |
| | $CH_3CN$ | 0.64[b] | | |
| | aq. MeOH(AcOH)[d] | 0.69[c] | | |
| | MeOH–$CHCl_3$[e] | 0.68 | | |
| **8** | MeOH | 1.22 [1.00] | 1.06 [1.07] | 1.24 [0.89] |
| **9** | MeOH | 0.82 | 1.01 | 0.72 |
| **13** | MeOH | 0.86 | 0.98 | 0.89 |
| **17** | MeOH | 1.06 | 1.06 | 0.93 |
| | MeOH–$CHCl_3$[e] | 0.96 | | |

[a] The *IRIS* values are not corrected by the theoretical (M + 3) ion distribution. Unless otherwise is noted, $[G^+]/[H] = 10/1 = 0.91/0.091$ mmol dm$^{-3}$. [ ] shows a value of the quantitative cross-chiral check based on eqn. (4). [b] $[G^+]/[H] = 20/1 = 1.82/0.091$ mmol dm$^{-3}$. [c] $[G^+]/[H] = 10/1 = 0.88/0.088$ mmol dm$^{-3}$. [d] Solvent composition (vol%): MeOH (96.5), $H_2O$ (1.75), AcOH (1.75). [e] Solvent composition (vol): MeOH–$CHCl_3$ (10 : 1).

**Table 4** *IRIS* Values using the ESIMS–EL amino acid guest method[a,b]

| Host | Guest Ala$^+$ (**18d**) | Met$^+$ (**19d**) | Leu$^+$ (**20d**) | Phe$^+$ (**21d**) | Val$^+$ (**24d**) | MetOMe$^+$ (**19a**) |
|---|---|---|---|---|---|---|
| **1** | 1.2 [0.8] | 1.5 [0.7] | 1.4 [0.9] | 1.8 [1.1] | 1.7 [1.2] | 1.53 [1.06] |
| **2** | 0.7 | 0.5 | 0.7 | 0.6 | 0.7 | 0.69 |
| **17** | 1.2 | 0.9 | 0.9 | 1.2 | 0.7 | 0.93 |

[a] $[G^+]/[H] = 0.877/0.0877$ mmol dm$^{-3}$ = 10 : 1. Solvent composition (vol%); MeOH (96.5), $H_2O$ (1.75), ACOH (1.75): see Experimental section. [b] [ ] shows a value of the quantitative cross-chiral check on the basis of eqn. (4).

## Discussion

### (1) *IRIS* Values detected by the FABMS–EL guest method

Chiral recognition abilities (*IRIS* values) of several chiral hosts toward several amino ester guests and amino acid guests are plotted in Figs. 5 and 6, respectively. Here the vertical axis is the *IRIS* value when *IRIS* is larger than unity (*IRIS* ≥ 1.00), or 1/*IRIS* value, when *IRIS* is smaller than unity (*IRIS* < 1.00). Because hosts **1** and **2** are enantiomers of each other, an expression 'upward' for host **1** corresponds to an expression 'downward' for host **2** with respect to each guest. This type of the upward–downward relation with an equal height is a graphical illustration of the cross-chiral check. As one can see in Table 1, the cross chiral-check concerning hosts **1** and **2** toward amino ester guests holds experimentally within a few percent accuracy

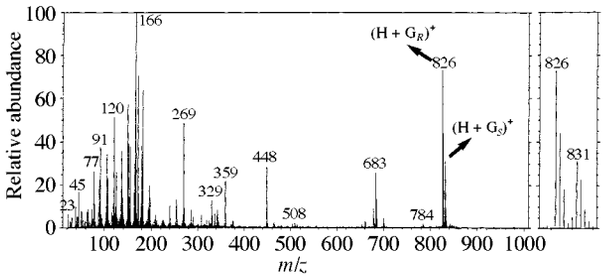



**Fig. 2** A FAB mass spectrum for the complexation between host **8** and guest **21d**, a 1 : 1 mixture of (*R*)-**21d** and (*S*)-[$^2H_5$]**21d** (a racemic mixture of Phe$^+$OTs$^-$, NBA matrix). A FABMS–EL amino acid guest method.

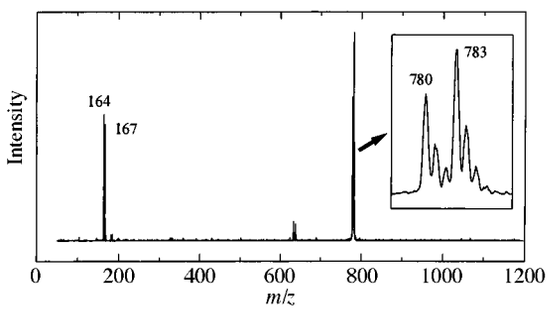



**Fig. 3** An ESI mass spectrum for the complexation between host **2** and guest **19a**, a 1 : 1 mixture of (*R*)-**19a** and (*S*)-[$^2H_3$]**19a** (a racemic mixture of MetOMe$^+$Cl$^-$, MeOH solvent). An ESIMS–EL amino ester guest method. *m/z* 780 = $(H\cdot G_R)^+$, *m/z* 783 = $(H\cdot[^2H_3]G_S)^+$.

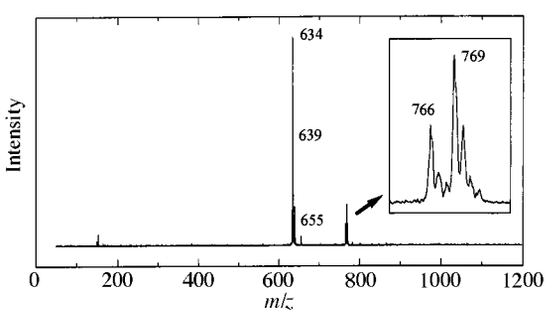



**Fig. 4** An ESI mass spectrum for the complexation between host **2** and guest **19d** (free amino acid), a 1 : 1 mixture of (*R*)-**19d** and (*S*)-[$^2H_3$]**19d** (a racemic mixture of Met, MeOH–$H_2O$–AcOH solvent). An ESIMS–EL amino acid guest method. *m/z* 766 = $(H\cdot G_R)^+$, *m/z* 769 = $(H\cdot[^2H_3]G_S)^+$, *m/z* 634 = $(H\cdot NH_4)^+$, *m/z* 639 = $(H\cdot Na)^+$.

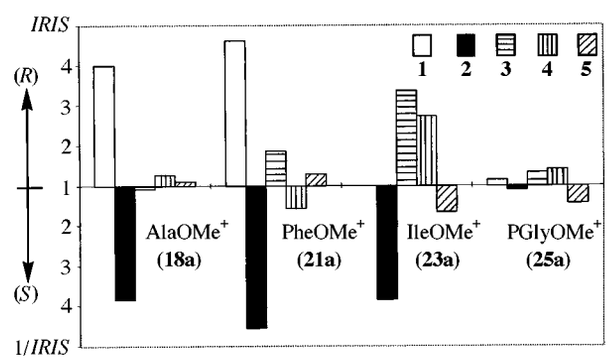



**Fig. 5** Variations of chiral recognition properties (*IRIS* values) determined by the FABMS–EL amino ester guest method. For the (*S*)-enantiomer guest preference, the value of 1/*IRIS* is plotted.

**Table 5** Variation in $-\Delta\Delta G_{enan}$ (kcal mol$^{-1}$) values[a] determined by the FABMS–EL guest method due to the variation from $-COOCH_3$ to $-COOH$ in the amino acid moiety

| | | Guest | | | | |
|---|---|---|---|---|---|---|
| Host | Guest's system | **18** | **19** | **20** | **21** | **25** |
| **1** | $COOCH_3$ | 0.83 | 0.97 | | 0.90 | |
| | COOH | 0.62 | 0.69 | | 0.68 | |
| | $\Delta$[b] | 0.21 | 0.28 | | 0.22 | |
| **8** | $COOCH_3$ | 0.27 | 0.27 | 0.14 | 0.39 | |
| | COOH | 0.32 | 0.41 | 0.31 | 0.41 | |
| | $\Delta$[b] | −0.05 | −0.14 | −0.17 | −0.02 | |
| $(Me)_2D(OEOEO)_2D$[c] | $COOCH_3$[d] | | | | | 1.67 |
| | COOH[e] | | | | | 1.38 |
| | $\Delta$[b] | | | | | 0.29 |

[a] Data are calculated from eqn. (3). [b] $\Delta = (-\Delta\Delta G_{enan})_{COOCH_3} - (-\Delta\Delta G_{enan})_{COOH}$. [c] Cram's bis-binaphthyl crown derivative (ref. 1*b*). [d] Ref. 1*b* ($ClO_4^-$). [e] Ref. 8 ($ClO_4^-$).

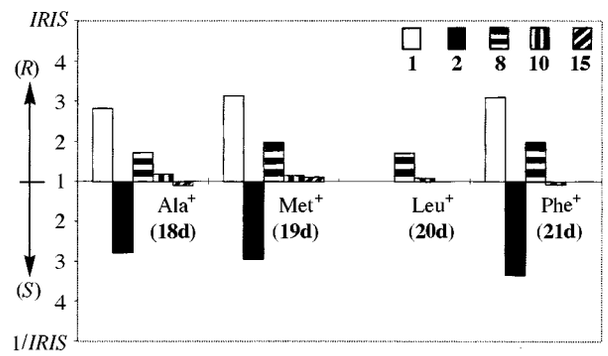



**Fig. 6** Variation of chiral recognition properties (*IRIS* values) determined by the FABMS–EL amino acid guest method. For the (*S*)-enantiomer guest preference, the value of 1/*IRIS* is plotted.

[cross-chiral check = 1.03 ± 0.02 (FABMS–EL amino ester guest method; $n = 3$)]. It is clear from Fig. 5 and Table 1 that *IRIS* values change dramatically for different combinations of chiral hosts and guests. For example, the *IRIS* value of host **1** changes from 5.0 to 1.2 with different guests. On the other hand, the *IRIS* values for guest $AlaOMe^+$ (**18a**) vary from 4.0 to 0.2 with different hosts. The structural difference in host **1** and host **5** (the position of the phenyl group) nearly causes the disappearance of (*R*)-guest preference or changes it to a weak (*S*)-guest preference. Addition of a methyl group to host **1** (giving hosts **3** and **4**) results in decreasing (*R*)-guest preference. These observations indicate that the *IRIS* values are highly sensitive to both host and guest structures, and that FABMS–EL is a practically and sensitive method of detecting their chiral recognition properties.

A recent report has pointed out that careful attention should be paid to information concerning relative extents of metal ion binding in FABMS analysis, derived from the relative peak intensities of the different complex ions, because of possible differences in ionization efficiencies.[4e] As mentioned previously in detail,[5] our FABMS–EL analysis has been based on the relative peak intensities of the diastereomeric complex ions, so their ionization efficiencies can be safely assumed to be equal.

Table 2 also satisfactorily shows the cross-chiral check (with hosts **1** and **2**) toward amino acid guests, reflecting a high degree of intermolecular structural dependence between a chiral host and a chiral guest. [cross-chiral check = 1.01 ± 0.07 (FABMS–EL amino acid guest method; $n = 3$)].

Compared with the FABMS–EL amino ester guest method, we can point out some characteristic features of the FABMS–EL amino acid guest method. (1) Generally the solubility of the amino acid salts used is poor, so it can be rather difficult to make the concentration of the guest high enough to obtain good quality spectra: for example, sometimes warming the solution is necessary. (2) There is rather limited availability of deuterium-labelled amino acids commercially and/or synthetically. (3) The COOH function of amino acids is much more strongly hydrogen-bonded with matrix NBA than the COOR function, and the host–guest complex ion peaks detected are much smaller. These points lead us to conclude that the FABMS–EL amino ester guest method can easily provide FAB mass spectra in good quality and is practically more suitable than the FABMS–EL amino acid guest method for reliably detecting chiral amino acid recognition properties.

We can determine a change in the *IRIS* value with respect to a change in the guest's function from COOMe to COOH and then estimate the change in the $-\Delta\Delta G_{enan}$ value for such a change. Table 5 shows the variation in $-\Delta\Delta G_{enan}$ values calculated using eqn. (3). The chiral recognition of host **1** [(*R*)-guest preference] toward amino ester guests $AlaOMe^+$ (**18a**), $MetOMe^+$ (**19a**) and $PheOMe^+$ (**21a**) is more favorable by 0.2–0.3 kcal mol$^{-1}$ unit (cal = 4.184 J) than that toward the corresponding amino acid guests $Ala^+$ (**18d**), $Met^+$ (**19d**) and $Phe^+$ (**21d**), respectively. This chiral recognition variation is in agreement with the previous Cram's data (0.3 kcal mol$^{-1}$)[1b,8] showing that a bis-binaphthyl crown host: amino ester recognition [$PGlyOMe^+$(**25a**)] was more favorable than amino acid recognition [$PGly^+$(**25d**)].

**(2) *IRIS* Values detected by the ESIMS–EL guest method**

Chiral recognition abilities (*IRIS* values) of hosts toward amino ester guests with the use of ESIMS are surprisingly shown to approach unity (Table 3): a substantial decrease in the magnitude of *IRIS* occurred on going from FABMS to ESIMS. For example, on a combination of chiral host **1** and guest $MetOMe^+$ (**19a**), the *IRIS* value detected in MeOH is 1.5, much smaller than the corresponding *IRIS* value with the use of FABMS (*IRIS* = 5.0, which is practically equal to the equilibrium constant ratio [$K_R/K_S = 4.9$) in MeOH–$CHCl_3$ (1 : 1) at 25 °C, as previously reported].[5] Further, on combination of chiral host **8** and guest $MetOMe^+$ (**19a**), the *IRIS* value is 1.2 in MeOH, showing virtual disappearance of the chiral recognition property (*IRIS* = 2.0 for FABMS).[5,11] This behavior is similarly observed with different guests and also with different solvents (Table 3). However, the cross-chiral check (hosts **1** and **2**) is still satisfied within experimental error, confirming structure-specific H–G complex ions.[11] [cross-chiral check = 1.00 ± 0.10 (ESIMS–EL amino ester guest method; $n = 10$)].

Chiral recognition abilities toward free amino acid guests detected by ESIMS are shown in Table 4. Here, we did not use the salts (*i.e.* toluene-*p*-sulfonic acid salts) of the amino acids but used the amino acids themselves and employed a standard technique for protonation *via* ESI process: that is, the addition of a small amount of AcOH to the solution as a proton donor. As was observed for the amino ester guests, the *IRIS* values by ESIMS for amino acid guests also show depressed *IRIS* values. Further, as seen in Fig. 4, the peak intensities of H–G complex

ions formed are inherently small and that of the host–$NH_4^+$ complex ion (*m*/*z* 634)[12] is large: errors in the *IRIS* values themselves become relatively large (*ca.* ±20%). With these results, the application of the data to the cross-chiral relationship showed the worst precision of the four methods we have considered, indicating that this type of *IRIS* could not give a valid quantitative measure of chiral amino acid recognition [cross-chiral check = 1.0 ± 0.3 (ESIMS–EL amino acid guest method; $n = 6$)].

In recent years, it has been reported that alkali-ion selectivity of crown ethers and cryptants can be reasonably detected by ESIMS, just like that observed in solution.[10n,13a] Further, it has been shown from the ESIMS studies of cyclohexyltriamides that a reliable qualitative order of alkali-ion complexing ability may be obtained under controlled conditions, although it seems doubtful that the ESI mass spectrum can represent directly the sample solution equilibria.[10p] The present experimental observation of depressed *IRIS* values detected by ESIMS seems to imply a guest-specific character essentially involved in the ESI process. It is important to realize that the present system, in which we compare the peak intensities of two diastereomeric H–G complex ions, is an ideal case, because the two can be compared without any corrections for the transferability of the two target ions from the solution to the gas phase. That is to say, the ratio of the peak intensities corresponds to the ratio of the concentrations of the diastereomeric H–G complex ions formed in solution.

To better understand the experimental results for the depressed chiral recognition and to further establish the capability of the direct reflection of the solution behavior, we selected two particularly simple competitive equilibrium systems and investigated the correlations between the peak intensities detected by ESIMS and the concentrations of the H–G complex ions, calculated under competitive thermodynamic equilibria in solution. It is very important to note that the relative concentration ratio calculated is strongly dependent upon not only the initial sample concentrations employed, but also the magnitude of the equilibrium constants ($K$) in the given systems under the competitive equilibrium conditions.[5] Here, it is worth noting that the concentrations of the neutral host are varied to keep constant the ionic strength of all solutions used under the competitive equilibrium conditions: the initial concentrations of the cationic guests are constant.

**(a) $K^+$/$Na^+$ metal ion selectivity of host 18-crown-6 (17).** Fig. 7 shows a plot of the ESIMS peak intensity ratio, $I[(\mathbf{17}\cdot K)^+]/I[(\mathbf{17}\cdot Na)^+]$, against the concentration of host **17**: $[K^+] = [Na^+] = 1.0$ mmol dm$^{-3}$, [**17**] = 0.1–5.0 mmol dm$^{-3}$ and solvent = MeOH. Here in this case, the experimental values (filled ellipses) are in good agreement with the calculated values indicated by the solid line. Here, the values $K = 1.32 \times 10^6$ mol$^{-1}$ dm$^3$ for the complexation between 18-crown-6 and $K^+$ (in MeOH at 25 °C)[14] and $K = 2.2 \times 10^4$ mol$^{-1}$ dm$^3$ between 18-crown-6 and $Na^+$ (in MeOH at 25 °C)[14] were employed and the values of the concentration ratio under competitive conditions were calculated.[5] For simplicity, the transferability (ESIMS response factor) difference between the two H–G ions was not corrected at all. With these results, it is now interesting that the metal ion selectivity of 18-crown-6 can be detected and evaluated at least qualitatively by ESIMS, supporting again the previous finding.[10n,p]

**(b) Leu′OMe$^+$/MetOMe$^+$ (26a/19a) amino ester ion selectivity of host 18-crown-6 (17).** Fig. 8 similarly shows a plot of the ESIMS peak intensity ratio, $I[(\mathbf{17}\cdot\mathbf{26a})^+]/I[(\mathbf{17}\cdot\mathbf{19a})^+]$, against the concentration of host **17**: $[\mathbf{26a}^+] = [\mathbf{19a}^+] = 1.0$ mmol dm$^{-3}$, [**17**] = 0.05–10 mmol dm$^{-3}$ and solvent = MeOH. In sharp contrast, the experimental values (filled ellipses) are not in agreement with the calculated values indicated by the solid line: the ESIMS peak intensity ratios appear almost constant, not reflecting the complex ion behavior in solution in this case. Here, the values $K = 2.7 \times 10^4$ mol$^{-3}$ dm$^3$ for the complexation

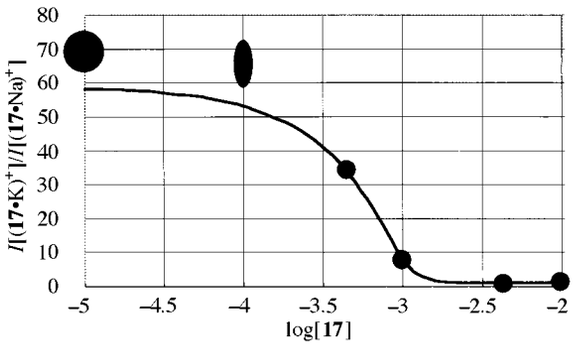



**Fig. 7** A plot of peak intensity ratio, $[(\mathbf{17}\cdot K)^+]/[(\mathbf{17}\cdot Na)^+]$, determined by ESIMS against concentration of host **17** (18-crown-6)

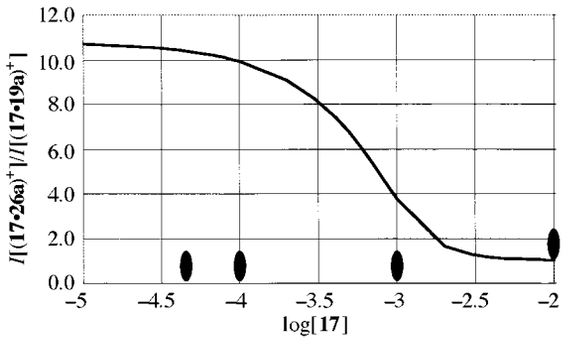



**Fig. 8** A plot of peak intensity ratio $[(\mathbf{17}\cdot\mathbf{26a})^+]/[(\mathbf{17}\cdot\mathbf{19a})^+]$, determined by ESIMS against concentration of host **17** (18-crown-6)

between 18-crown-6 and guest Leu′-$OMe^+$ and $K = 2.1 \times 10^3$ mol$^{-1}$ dm$^3$ between 18-crown-6 and guest $MetOMe^+$ were employed for calculation: these $K$ values (in MeOH at 25 °C) were newly determined using an NMR titration method.[15] Again the transferability difference between the two H–G complex ions was not corrected here for simplicity. The basic conclusion to be drawn from the results is that the amino ester ammonium ion selectivity cannot be correctly detected and evaluated by ESIMS. This is in line with the depressed *IRIS* values mentioned before.

The reason for the dramatic loss of selectivity detection in changing from metal ion to amino ester ammonium ion must lie in the process of ESI itself which proceeds *via* (1) formation of charged droplets, (2) solvent evaporation and (3) ion evaporation, in turn.[3b,9h,10p] There is little doubt that the major factors causing the observed effect are the dynamic processes of solvent evaporation and ion evaporation, where the original thermodynamic system will be particularly disturbed by intermolecular interactions (amino ester ammonium ion–ammonium ion interaction) under highly concentrated ion conditions in droplets. Therefore, it is noted that special attention should be given to the depressed ammonium ion selectivity detected by ESIMS.

**(3) Screening of various host–guest pairs by the FABMS–EL amino ester guest method**

The utility of the FABMS–EL amino ester guest method is best illustrated by straightforward application to specific host compounds. Chiral recognition properties of various hosts such as crown ethers, disaccharides and cyclic and acyclic ionophores toward various amino ester guests were extensively studied (Table 1). The disaccharides and the ionophores studied here did not show any particular ability for chiral guest recognition. Among the crown ethers studied, the combination of chiral host **3** and guest $ValOMe^+$ (**24a**) provided an *IRIS* value of 6.9, which is the highest degree of chiral amino acid recognition in the present paper. Using eqn. (3), $-\Delta\Delta G_{enan} \geqslant 1.2$ kcal mol$^{-1}$ (25 °C) was estimated. The FABMS–EL amino ester

guest method is thus established as operationally simple and straightforward.

## Conclusions

Further application of the methods described here to other types of chiral hosts may lead to discovery of new host families demonstrating a high degree of chiral amino acid recognition in the future. The entire results and discussion in this paper have dealt with mass spectrometric studies of chiral recognition behavior and have emphasized the use of the enantiomer-labelled guest method for the detection of chiral amino acid recognition properties by FABMS.

## Experimental

### Materials

**(a) Chiral hosts.** Chiral crown ethers were synthetic compounds which had been already reported elsewhere.[5,16] Disaccharide derivatives **11** and **12** were obtained by the methylation of α,α-treharose and sucrose *via* the Hakomori method.[17] Commercially available compounds of acyclic ionophores such as monencin methyl ester **13** (Calbiochem) and lasarosid sodium salt **14** (Sigma) and cyclic ionophores such as valinomycin **15** (Calbiochem) and nonactin **16** (Calbiochem) were employed without further purification.

**(b) Chiral guests.** All of the amino acid methyl ester hydrochlorides, ethyl ester hydrochlorides and isopropyl ester hydrochlorides used were synthesized and purified according to the standard method[5] from commercial D-(*R*)-amino acids and L-(*S*)-amino acids. For deuterium labelling, $[^2H_4]CH_3OH$ (99.8 atom% $^2H$, Isotec, Inc.), $[^2H_6]C_2H_5OH$ (99.5 atom% $^2H$, Isotec, Inc.), $[^2H_8](CH_3)_2CHOH$ (99.5 atom% $^2H$, C/D/N Isotopes) were employed. For the methyl-esterification of tryptophane, $[^2H_3]CH_3OH$ (99 atom% $^2H$, Aldrich) was particularly employed to avoid H/D exchange of the ring protons.

L-[$^2H_5$]*Ethyl phenylalaninate hydrochloride* [(S)-[$^2H_5$]–**21b**$^+$Cl$^-$]. Commercial L-phenylalanine (0.83 g, 5.0 mmol) was esterified by refluxing (24 h) with $[^2H_6]C_2H_5OH$ (5 g, 99.5 atom% $^2H$, Isotec, Inc.) in the usual manner.[5] After the standard workup, the desired compound {(*S*)-[$^2H_5$]**21b**, Cl$^-$} was obtained as a white solid (1.01 g, 86% yield, recrystallization from ethylacetate–chloroform): white needles, mp 150–153 °C; $[\alpha]_D^{25}$ +31.50° (*c* 1.0 $C_2H_5OH$); $\delta_H$(360 MHz, $^2H_2O$) 7.43–7.28 (m, 5H), 4.34 (m, 1H), 3.31 (dd, *J* 6.0, 14.5, 1H), 3.23 (dd, *J* 7.3, 14.5, 1H) (Calc. for $C_{11}H_{11}{}^2H_5NO_2Cl$: C, 56.28; H, 6.87; N, 5.97; Cl, 15.10. Found: C, 56.07; H, 6.81; N, 6.01; Cl, 14.96%).

Commercially available amino acid pairs such as (*R*)-unlabelled ones (Aldrich, Wako, Tokyo Kasei Kogyo Co. Ltd.) and (*S*)-deuterium labelled ones were used without purification: L-[$^2H_3$]alanine (99.1 atom% $^2H$, C/D/N Isotopes), L-[$^2H_3$]leucine ([$^2H_3$]methyl) (99 atom% $^2H$, C/D/N Isotopes), L-[$^2H_3$]methionine ([$^2H_3$]methyl) (99.1 atom% $^2H$, Isotec, Inc.), L-[$^2H_5$]phenylalanine (98.6 atom% $^2H$, C/D/N Isotopes), L-[$^2H_8$]valine (98.6 atom% $^2H$, Isotec, Inc.).

Toluene-*p*-sulfonic acid was recrystallized from ethyl acetate, dried *in vacuo* (*ca.* 1 mmHg) at 80 °C for 1 h and at room temperature for 12 h.

### FAB mass spectral measurements

FAB mass spectra in the positive ion mode were obtained with a JEOL JMS-DX 300 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 (Xe atom beam, 20 mA emission current, 6 kV acceleration). The source pressure was typically *ca.* $10^{-5}$–$10^{-6}$ Torr. Spectra were obtained with a magnet scan rate of 5 s scan$^{-1}$ (to *m*/*z* 1000) and the data were processed with a JEOL JMA 5000 data processing system.

### ESI mass spectral measurements

ESI mass spectra in the positive ion mode were obtained with a JEOL D 300 mass spectrometer which was equipped with a laboratory-made ESI interface.[18] A sample solution in a 100 ml microsyringe was sprayed at the tip of a needle (0.25 mm diameter) applied by 3.5 kV higher than a counter electrode. The flow rate of the solution was 2 μl min$^{-1}$ using a Harvard syringe pump. A heated $N_2$ gas (70 °C) flowing between the needle and the capillary electrode was used to aid desolvation of charged droplets sprayed. The ion translational energy was 2 keV. The source pressure was typically $2 \times 10^{-6}$ Torr. Data were acquired with a magnet scan rate of 5 s scan$^{-1}$ (to *m*/*z* 1200) into a personal computer and the averaged data of total 20 scans were recorded using a home-made program.

### Preparation of sample solutions for the FABMS–EL guest method

Generally a weighed sample was dissolved with an appropriate amount of solvent using a microsyringe or a digital micropipette. A FABMS solution was prepared by mixing the following three solutions using a microsyringe and an ultrasound vibrator (20 min). FABMS measurements were usually performed, after the solution had stood overnight, with a deposit of 1 μl aliquot of the mixed solution on a FAB probe tip.

**(a) Amino ester hydrochloride salt guest (concentration conditions A, B and C).** The three solutions were as follows: (1) 5 ml of a 1.33 mol dm$^{-3}$ MeOH solution of a 1:1 mixture of (*R*)-unlabelled and (*S*)-labelled ester guests, (2) 5 ml of a 0.20 mol dm$^{-3}$ $CHCl_3$ solution of a given host and (3) 15 ml of NBA matrix.

*Concentration condition A.*—These conditions are similar to those reported previously.[5] After evaporation of MeOH and $CHCl_3$ in the ion source, the concentrations in NBA were as follows: [$G^+$] = 0.05 mol dm$^{-3}$ ([$G_R^+$] = [$G_S^+$] = 0.025 mol dm$^{-3}$); [H] = 0.0083 mol dm$^{-3}$; [$G^+$]/[H] = 6.6.

*Concentration condition B.*—After evaporation of MeOH and $CHCl_3$ in the ion source, the concentrations in NBA were as follows: [$G^+$] = 0.444 mol dm$^{-3}$ ([$G_R^+$] = [$G_S^+$] = 0.222 mol dm$^{-3}$); [H] = 0.0667 mol dm$^{-3}$; [$G^+$]/[H] = 6.6.

*Concentration condition B′.*—As condition B, but using more MeOH to overcome solubility difficulties.

*Concentration condition C.*—As condition B, but using 30 ml rather than 15 ml NBA, resulting in the following final concentrations in NBA: [$G^+$] = 0.222 mol dm$^{-3}$ ([$G_R^+$] = [$G_S^+$] = 0.111 mol dm$^{-3}$); [H] = 0.0334 mol dm$^{-3}$; [$G^+$]/[H] = 6.6.

For every preparation of guest solutions, the 1:1 equivalency of the concentrations of (*R*)- and (*S*)-enantiomer guests was confirmed by checking that the *IRIS* value with an achiral host 18-crown-6 (**17**) was experimentally obtained as unity (1.00 ± 0.03).

Three relative peak intensity data of the diastereomeric host–guest complex ions obtained from the 10th, 20th, and 30th scan spectra were simply averaged ($n = 3$) and tabulated in Table 1 after the usual (M + 3) isotope correction.[5] As a typical example, the scan stability of the *IRIS* values observed in the combination between host **1** and guest PheOMe$^+$ (**21a**) provided a sufficient stability of the *IRIS* values (total 36 scans): [$IRIS^{obs}$ = 3.84 ± 0.10 (standard deviation)].

**(b) Amino acid toluene-*p*-sulfonic acid salt guest (concentration condition C).** Toluene-*p*-sulfonic acid (TsOH) was dissolved by $H_2O$–MeOH (1:1, volume) and a 0.667 mol dm$^{-3}$ solution was prepared. Both (*R*)-unlabelled and (*S*)-labelled amino acids were dissolved by the above aqueous MeOH solution of TsOH. Each 0.667 mol dm$^{-3}$ amino acid–TsOH salt solution prepared was mixed in a 1:1 (equal volume) fashion. (We shall call this guest solution **a** of amino acid–TsOH salt.)

A sample solution was made by mixing the following three solutions and a deposit of a 1 μl aliquot of the mixed solution was set on a FAB probe tip: (1) 5 ml of 0.667 mol dm$^{-3}$ aqueous MeOH guest solution **a**, (2) 5 ml of a 0.10 mol dm$^{-3}$ $CHCl_3$

solution of a given host, and (3) 15 ml of NBA matrix. After evaporation of the solvents, the concentrations in NBA were as follows: $[G^+] = 0.222$ mol dm$^{-3}$ ($[G_R{}^+] = [G_S{}^+] = 0.111$ mol dm$^{-3}$); $[H] = 0.033$ mol dm$^{-3}$; $[G^+]/[H] = 6.6$. This corresponds to the concentration conditions C in the amino ester guest series.

Some of the amino acids such as Leu, Phe and Val, were not soluble enough to allow preparation of the solutions. It was necessary in these cases to use a relatively lower concentration of the guest solution, or to warm the guest solution (see Table 2). Particularly, the *IRIS* value for guest $Phe^+$ (**21d**) was obtained by the use of the complex ion peaks combined with [$^2H_4$] and [$^2H_5$] labelling ones (see Table 2). As a typical example, the scan stability of the *IRIS* values observed in the combination between host **8** and guest $Met^+$ (**19d**) exhibited a sufficient stability of the *IRIS* values (total 31 scans): [$IRIS^{obs} = 1.74 \pm 0.05$ (standard deviation)].

### Preparation of sample solutions for the ESIMS–EL guest method

**(a) Amino ester hydrochloride salt guest.** A MeOH solution (1 mmol dm$^{-3}$) of the 1:1 mixed guest was prepared by mixing the three solutions: (1) 200 ml of 10 mmol dm$^{-3}$ MeOH solution of (*R*)-unlabelled amino ester hydrochloride guest, (2) 200 ml of 10 mmol dm$^{-3}$ MeOH solution of (*S*)-labelled one, and (3) 3.6 ml of MeOH. An ESIMS sample solution was made by the mixture of (i) 200 ml of 1 mmol dm$^{-3}$ MeOH solution of the above 1:1 mixed guest with (ii) 20 ml of 1 mmol dm$^{-3}$ MeOH solution of a given host. Accordingly, the concentrations in MeOH were as follows: $[G^+] = 0.909$; $[H] = 0.0909$ mmol dm$^{-3}$; $[G^+]/[H] = 10$. No correction of the observed *IRIS* values on the basis of the natural abundance of the (M + 3) isotope was performed.

**(b) Amino acid guest.** A typical ESIMS sample solution was prepared by mixing the following three solutions: (1) 200 ml of 1 mmol dm$^{-3}$ MeOH solution of the 1:1 mixed [(*R*)-unlabelled:(*S*)-labelled] guest, (2) 20 ml of 1 mmol dm$^{-3}$ MeOH solution of a given host, and (3) 8 ml of $H_2O$–AcOH (1:1 by volume) as a proton donor source. Accordingly, the concentrations were: $[G^+] = 0.877$; $[H] = 0.0877$ mmol dm$^{-3}$; $[G^+]/[H] = 10$. Volume percent of the mixed solvent was MeOH–AcOH–$H_2O$ = 96.5:1.75:1.75. Because of relatively large experimental error, any corrections of the observed *IRIS* values were not performed on the basis of the natural abundance of the (M + 3) isotope.

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## References

1 (*a*) D. J. Cram, *Angew. Chem., Int. Ed. Engl.*, 1988, **27**, 1009; (*b*) S. C. Peacock, L. A. Domeier, F. C. A. Gaeta, G. R. C. Helgeson, J. M. Timko and D. J. Cram, *J. Am. Chem. Soc.*, 1978, **100**, 8190.

2 (*a*) Y. Kuroda, Y. Kato, T. Higashioji, J. Hasegawa, S. Kawanami, M. Taskahashi, N. Shiraishi, K. Tanabe and H. Ogoshi, *J. Am. Chem. Soc.*, 1995, **117**, 10 950; (*b*) M. Asakawa, C. L. Brown, D. Pasini, J. F. Stoddart and P. G. Wyatt, *J. Org. Chem.*, 1996, **61**, 7234.

3 (*a*) J. R. Chapman, *Practical Organic Mass Spectrometry*, 2nd edn., John Wiley & Sons, New York, 1993; (*b*) *Biological Mass Spectrometry—Present and Future*, ed. T. Matsuo, R. M. Caprioli, M. L. Gross and Y. Seyama, John Wiley & Sons, New York, 1994; (*c*) M. L. Gross, *Acc. Chem. Res.*, 1994, **27**, 361; (*d*) K. Biemann and I. A. Papayannopoulos, *Acc. Chem. Res.*, 1994, **27**, 370; (*e*) F. W. McLafferty, *Acc. Chem. Res.*, 1994, **27**, 379.

4 (*a*) G. Hofmeister and J. A. Leary, *Org. Mass Spectrom.*, 1991, **26**, 811; (*b*) F. J. Winkler and J. S. Splitter, *Applications of Mass Spectrometry to Organic Stereochemistry*, ed. J. S. Splitter, F. Turecek, VCH Publishers Inc., 1994, ch. 16; (*c*) T. T. Dang, S. F. Pedersen and J. A. Leary, *J. Am. Soc. Mass Spectrom.*, 1994, **5**, 452; (*d*) G. Smith and J. A. Leary, *J. Am. Chem. Soc.*, 1996, **118**, 3293; (*e*) G. J. Langley, D. G. Hamilton and M. C. Grossel, *J. Chem. Soc., Perkin Trans. 2*, 1995, 929.

5 (*a*) M. Sawada, Y. Takai, H, Yamada, S. Hirayama, T. Kaneda, T. Tanaka, K. Kamada, T. Mizooku, S. Takeuchi, K. Ueno, K. Hirose, Y. Tobe and K. Naemura, *J. Am. Chem. Soc.*, 1995, **117**, 7726; (*b*) M. Sawada, *J. Mass Spectrom. Soc. Jpn.*, 1997, **45**, 439; (*c*) M. Sawada, *Mass Spectrom. Rev.*, 1997, **16**, 73.

6 For crown ether derivatives: (*a*) E. P. Kyba, J. M. Timko, L. J. Kaplan, F. de Jong, G. W. Gokel and D. J. Cram, *J. Am. Chem. Soc.*, 1978, **100**, 4555; (*b*) L. R. Sousa, G. D. Y. Sogah, D. J. Hoffman and D. J. Cram, *J. Am. Chem. Soc.*, 1978, **100**, 4569; (*c*) M. Newcomb, J. L. Toner, R. C. Helgeson and D. J. Cram, *J. Am. Chem. Soc.*, 1979, **101**, 4941; (*d*) R. B. Davidson, J. S. Bradshaw, B. A. Jone, N. K. Dalley, J. J. Christensen and R. M. Izatt, *J. Org. Chem.*, 1984, **49**, 353; (*e*) T. Shinbo, T. Yamaguchi, K. Nishimura, M. Kikkawa and M. Sugiura, *Anal. Chim. Acta*, 1987, **193**, 367; (*f*) K. Naemura, H. Miyabe, Y. Shingai and Y. Tobe, *J. Chem. Soc., Perkin Trans. 1*, 1993, 1073. For porphyrin derivatives: (*g*) K. Konishi, K. Yahara, H. Toshishige, T. Aida and S. Inoue, *J. Am. Chem. Soc.*, 1994, **116**, 1337; (*h*) T. Mizutani, T. Ema, T. Tomita, Y. Kuroda and H. Ogoshi, *J. Am. Chem. Soc.*, 1994, **116**, 4240; (*i*) Y. Kuroda, Y. Kato, T. Higashioji, J. Hasegawa, S. Kawakami, M. Takahashi, N. Shiraishi, K. Tanabe and H. Ogoshi, *J. Am. Chem. Soc.*, 1995, **117**, 10 950. For other derivatives: (*j*) K. Maruyama, H. Sohmiya and H. Tsukube, *J. Chem. Soc., Chem. Commun.*, 1989, 864; (*k*) R. Liu, E. J. Sanderson and W. C. Still, *J. Org. Chem.*, 1990, **55**, 5184; (*l*) H. Tsukube and H. Sohmiya, *J. Org. Chem.*, 1991, **56**, 875; (*m*) T. H. Webb and C. S. Wilcox, *Chem. Soc. Rev.*, 1993, 383; (*n*) T. Morozumi, S. Shinkai, *Chem. Lett.*, 1994, 1515; (*o*) K. Araki, S. Shinkai, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 72.

7 For LC separation of amino acids, (*a*) G. D. Sogah and D. J. Cram, *J. Am. Chem. Soc.*, 1979, **101**, 3035; (*b*) T. Shinbo, T. Yamaguchi, K. Nishimura and M. Sugiura, *J. Chromatogr.*, 1987, **405**, 145; (*c*) R. Corradini, A. Dossena, G. Impellizzeri, G. Maccarrone, R. Marchelli, E. Rizzarelli, G. Sartor and G. Vicchio, *J. Am. Chem. Soc.*, 1994, **116**, 10 267. For capillary electrophoresis: (*d*) M. Novotny, H. Soini and M. Stefansson, *Anal. Chem.*, 1994, **66**, 646A; (*e*) R. Kuhn, F. Erni, T. Berenter and J. Hausler, *Anal. Chem.*, 1992, **64**, 2815.

8 S. S. Peacock, D. M. Walba, F. C. A. Gaeta, R. C. Helgeson and D. J. Cram, *J. Am. Chem. Soc.*, 1980, **102**, 2043.

9 (*a*) M. Yamashita and J. B. Fenn, *J. Phys. Chem.*, 1984, **88**, 4451; (*b*) M. Yamashita and J. B. Fenn, *J. Phys. Chem.*, 1984, **88**, 4671; (*c*) J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. Whitehouse, *Science*, 1989, **246**, 64; (*d*) J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. Whitehouse, *Mass Spectrom. Rev.*, 1990, **9**, 37; (*e*) J. A. Loo, C. G. Edmonds and R. D. Smith, *Science*, 1990, **248**, 201; (*f*) R. D. Smith, J. A. Loo, C. G. Edmonds, C. J. Barinaga and H. R. Udseth, *Anal. Chem.*, 1990, **62**, 882; (*g*) R. D. Smith, J. A. Loo, R. R. O. Loo, M. Busman and H. R. Udseth, *Mass Spectrom. Rev.*, 1992, **10**, 359; (*h*) P. Kebarle and L. Tang, *Anal. Chem.*, 1993, **65**, 972A.

10 (*a*) T. Lippmann, H. Wilde, M. Pink, A. Schäfer, M. Hesse and G. Mann, *Angew. Chem., Int. Ed. Engl.*, 1993, **32**, 1195; (*b*) G. Siuzdak, Y. Ichikawa, T. J. Caulfield, B. Munoz, C. H. Wong and K. C. Nicolaou, *J. Am. Chem. Soc.*, 1993, **115**, 2877; (*c*) P. S. Bates, D. Parker and B. N. Green, *J. Chem. Soc., Chem. Commun.*, 1993, 693; (*d*) S. R. Wilson, J. Perez and A. Pasternak, *J. Am. Chem. Soc.*, 1993, **115**, 1994; (*e*) E. Lieze, A. van Dorsselaer, R. Krämer and J.-M. Lehn, *J. Chem. Soc., Chem. Commun.*, 1993, 990; (*f*) Y.-T. Li, Y.-L. Hsieh, J. D. Henion, T. D. Ocain, G. A. Schiehser and B. Ganem, *J. Am. Chem. Soc.*, 1994, **116**, 7487; (*g*) R. T. Alpin, C. V. Robinson, C. J. Schofield and N. J. Westwood, *J. Chem. Soc., Chem. Commun.*, 1994, 2415; (*h*) K. C. Russell, E. Leize, A. van Dorsselaer and J.-M. Lehn, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 209; (*i*) X. Cheng, Q. Gao, R. D. Smith, E. E. Simanek, M. Mammen and G. M. Whitesides, *Rapid Commun. Mass Spectrom.*, 1995, **9**,

312; (*j*) K. Wang, X. Han, R. W. Gross and G. W. Gokel, *J. Am. Chem. Soc.*, 1995, **117**, 7680; (*k*) M. Vincent, *J. Mass Spectrom.*, 1995, **30**, 925; (*l*) A. Marquis-Rigault, A. Dupont-Gervais, A. van Dorsselaer and J.-M. Lehn, *Chem. Eur. J.*, 1996, **2**, 1395; (*m*) X. Cheng, Q. Gao, R. D. Smith, E. E. Simanek, M. Mammen and G. M. Whitesides, *J. Org. Chem.*, 1996, **61**, 2204; (*n*) K. Wang and G. W. Gokel, *J. Org. Chem.*, 1996, **61**, 4693; (*o*) M. Przybylski and M. O. Glocker, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 806; (*p*) M. Goodall, P. M. Kelly, D. Parker, K. Gloe and H. Stephan, *J. Chem. Soc., Perkin Trans. 2*, 1997, 59.

11 M. Sawada, Y. Takai, T. Kaneda, R. Arakawa, M. Okamoto, H. Doe, T. Matsuo, K. Naemura, K. Hirose and Y. Tobe, *Chem. Commun.*, 1996, 1735.

12 K. Hiraoka, K. Murata and I. Kudaka, *J. Mass Spectrom. Soc. Jpn.*, 1995, **43**, 127.

13 (*a*) E. Leize, A. Jaffrezic and A. van Dorsselaer, *J. Mass Spectrom.*, 1996, **31**, 537. For gas phase characteristics: (*b*) I.-H. Chu, H. Zhang and D. V. Dearden, *J. Am. Chem. Soc.*, 1993, **115**, 5736; (*c*) S. Maleknia and J. Brodbelt, *J. Am. Chem. Soc.*, 1993, **115**, 2837.

14 E. M. Izatt, K. Pawlak and J. S. Bradshaw, *Chem. Rev.*, 1991, **91**, 1721.

15 M. Sawada, Y. Okumura, M. Shizuma, Y. Takai, Y. Hidaka, H. Yamada, T. Tanaka, T. Kaneda, K. Hirose, S. Misumi and S. Takahashi, *J. Am. Chem. Soc.*, 1993, **115**, 7381.

16 (*a*) K. Naemura, T. Mizooku, K. Kamada, K. Hirose, Y. Tobe, M. Sawada and Y. Takai, *Tetrahedron Asymmetry*, 1994, **5**, 1549; (*b*) K. Naemura, M. Asada, K. Hirose and Y. Tobe, *Tetrahedron Asymmetry*, 1995, **6**, 1873; (*c*) K. Naemura, K. Ueno, S. Takeuchi, K. Hirose, Y. Tobe, T. Kaneda and Y. Sakata, *J. Chem. Soc., Perkin Trans. 1*, 1996, 383; (*d*) K. Hirose, J. Fuji, K. Kamada, Y. Tobe and K. Naemuira, *J. Chem. Soc., Perkin Trans. 2*, 1997, 1649.

17 Y. Takai, Y. Okumura, T. Tanaka, M. Sawada, S. Takahashi, M. Shiro, M. Kawamura and T. Uchiyama, *J. Org. Chem.*, 1994, **59**, 2967.

18 (*a*) R. Arakawa, L. Jian, A. Yoshimura, K. Nozaki, T. Ohno, H. Doe and T. Matsuo, *Inorg. Chem.*, 1995, **34**, 3874; (*b*) R. Arakawa, S. Tachiyashiki and T. Matsuo, *Anal. Chem.*, 1995, **67**, 4133; (*c*) K. Kimura, R. Mizutani, M. Yokoyama, R. Arakawa, G. Matsubayashi, M. Okamoto and H. Doe, *J. Am. Chem. Soc.*, 1997, **119**, 2062.

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