Determination of enantiomeric excess for amino acid ester salts using FAB mass spectrometry

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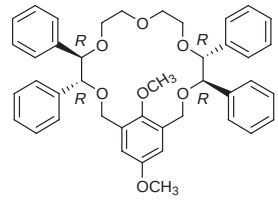
The enantiomeric excess of α-amino acid ester hydrochlorides is determined for the first time using FAB mass spectrometry coupled with enantiomer-labelled host method.

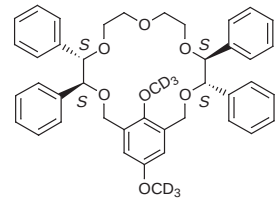
The simple determination of enantiomeric excess using FAB mass spectrometry, without chromatographic separation of the enantiomers,[1,2] has been demonstrated for the first time using *R*/*S* mixtures of α-amino acid esters or, more generally, organic primary amine hydrochlorides. The principle of this method is based on the FAB mass spectrometric detection (nitrobenzyl alcohol matrix)[3–6] of enantiomeric recognition of primary amines by chiral macrocyclic host compounds.[7,8] This method uses a 1:1 mixture [for example, (*R*)-nonlabelled/(*S*)-deuterium-labelled] of chiral crown ethers [for example, (*RRRR*)-**1** and [$^2H_6$]-(*SSSS*)-**1**], and is known as the enantiomer-labelled (EL) host method. In other words, the hosts are utilized as a pair of specific reagents for determining the ee of organic primary amines.

The relative peak intensity of the diastereomeric host–guest complex ions, which are produced from the complexation between a 1:1 mixture of the enantiomeric hosts (H_{RRRR}):[^2H_n]-(H_{SSSS}) and the primary amine salt guest (G^+), is taken as a quantitative measure; *n* is the number of deuterium labels [eqn. (1)].

$$I[(H_{RRRR} + G)^+]/I[[^2H_n]\text{-}(H_{SSSS} + G)^+] = I_R/I_S = IRIS \text{ (abbreviation)} \quad (1)$$



(*RRRR*)-**1**



[$^2H_6$]-(*SSSS*)-**1**

The fundamental concept of this methodology is schematically shown in Fig. 1, where the diastereomeric host–guest complex ion peaks are given. For the conceptual data shown in Fig. 1, the (*R*)-guest complexes the (*RRRR*)-host by an arbitrary factor of 2.0 better than the (*SSSS*)-host (run 1, *IRIS* = 2.0). Accordingly, the (*S*)-guest should complex the (*SSSS*)-host by a factor of 2.0 better than the (*RRRR*)-host (run 2, *IRIS* = 0.50) because of the mirror image relationship between the host–guest complex ions produced. Furthermore, the racemic (*RS*)-guest should provide a pair of equal peak intensities (run 3, *IRIS* = 1.0) because of the net compensation of a racemic host–racemic guest combination. Therefore, in the case of a given guest with unknown ee, one can expect to determine the percent enantiomeric excess from the relationship between the *IRIS* and the ee values.

The (*RRRR*)-**1** host was prepared by a route previously reported by Kaneda, Hirose and Misumi.[9,10] The corresponding

Run	Host	Guest	Pattern of H–G diastereomeric complex ion peaks	I_R/I_S
A:	**FABMS/EL host method**			
1	*RRRR* / [^2H_n]-*SSSS*	*R* (*R*)100% ee		2.0
2	*RRRR* / [^2H_n]-*SSSS*	*S* (*S*)100% ee		0.5
3	*RRRR* / [^2H_n]-*SSSS*	*R* / *S* 1:1 racemic	^2H_n	1.0
4	*RRRR* / [^2H_n]-*SSSS*	*R/S* unknown	Various	Various
B:	**FABMS/EL guest method**			
5	*RRRR*	*R* / [^2H_n]-*S* 1:1 racemic		2.0
6	*SSSS*	*R* / [^2H_n]-*S* 1:1 racemic	^2H_n	0.5

Fig. 1 FAB mass spectrometry with the enantiomer-labelled host and guest methods

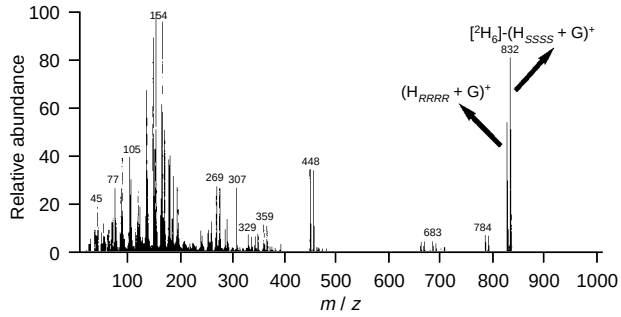


Fig. 2 A typical FAB mass spectrum (nitrobenzyl alcohol matrix) using the enantiomer-labelled host method [host: (*RRRR*)-**1** and [$^2H_6$]-(*SSSS*)-**1**; guest: (*S*)-PglyOMe•HCl (80% ee)]

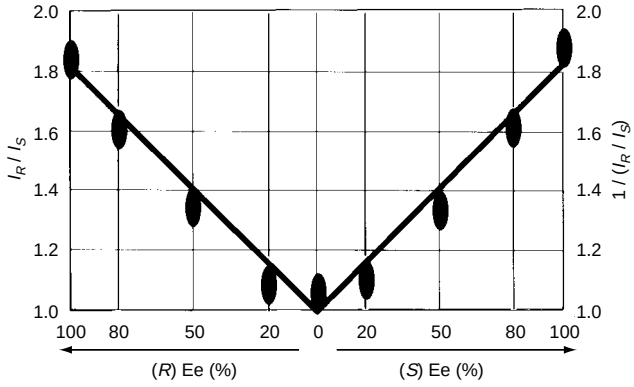


Fig. 3 A plot of $I[(H_{RRRR} + G)^+]/I[[^2H_6]\text{-}(H_{SSSS} + G)^+]$ *vs.* ee

enantiomer-labelled [$^2H_6$]-(*SSSS*)-**1** was similarly prepared *via* 2,6-bis(bromomethyl)hydroquinone [$^2H_6$]dimethyl ether.

A FABMS solution was prepared by mixing the following three solutions: (i) 5 μl of a 1:1 mixture of a 0.16 mmol dm^{-3} $CHCl_3$ solution (25 μl) of (*RRRR*)-**1** and a 0.16 mmol dm^{-3} $CHCl_3$ solution (25 μl) of [$^2H_6$]-(*SSSS*)-**1**, (ii) 5 μl of a 0.08 mmol dm^{-3} MeOH solution of a given guest, and (iii) 30 μl of NBA matrix. After evaporation of MeOH and $CHCl_3$ in the ion source, the concentrations in NBA were calculated to $[H_{RRRR}] = [H_{SSSS}] = [G] = 0.0133$ mmol dm^{-3}. Several guest solutions with different ees were prepared by appropriate mixing of solutions of both the (*R*)- and (*S*)-phenylglycine methyl ester (PglyOMe) hydrochloride salts, and the *IRIS* values were determined by FAB mass spectrometry (Table 1). A typical FAB mass spectrum is shown in Fig. 2. In order to see the characteristic relationship, the *IRIS* value is plotted on the ordinate when the (*R*)-guest is in excess, and the reciprocal of the *IRIS* value is plotted when the (*S*)-guest is in excess (Fig. 3). The *IRIS* value varies in a linear fashion with the ee quantity and produces a symmetric V-shaped plot. Therefore, it is clear that the ee value of a given guest with unknown ee can be determined from the *IRIS* values obtained for both the guests with unknown and known (100%) ee. As mentioned previously,[5] there exist, in general, weak concentration effects of the host and guest solutions upon the *IRIS* values, so it is necessary to measure the FAB mass spectra under fixed sample concentration conditions.

The *IRIS* value of run 1 (or run 2) (the EL host method) should be equivalent to the *IRIS* value of run 5 (or run 6) (the EL guest method)[5] for the mirror image relationship between the complex ions. Therefore, using the EL host method with a series of enantiomerically pure guests (run 1 or run 2), we can determine the chiral recognition abilities of this host toward a series of guests using the same *IRIS* scale derived from the EL guest method (run 5 or run 6). Table 1 shows several *IRIS* values

Table 1 The $I[(H_{RRRR} + G)^+]/I[[^2H_6]\text{-}(H_{SSSS} + G)^+]$ values toward various primary amine hydrochlorides

Amine	*IRIS* values[a]
GlyOMe	1.00
(*R*)-PglyOMe	1.84
(*S*)-PglyOMe	0.53
(*R*)-PglyOEt	1.98
(*R*)-PglyOPr^i	2.00
(*S*)-AspOMe[b]	0.37
(*S*)-AsnOMe	0.51
(*S*)-PheOMe	0.51
(*S*)-ValOEt	0.35
(*R*)-1-Phenylethylamine	0.95
(*R*)-1-Phenyl-2-hydroxyethylamine	0.62
(*R*)-1-(*p*-Nitrophenyl)ethylamine	1.03

[a] Averaged values of 10th, 20th, 30th and 40th scans ($n = 4$). The standard deviations were within ±5%. [b] (*S*)-Aspartic acid dimethyl ester hydrochloride.

obtained experimentally using the EL host method with a series of hydrochloride salts of enantiomerically pure amino acid esters and organic primary amines. These data have shown which type of guest is suitable to the ee determination. Since the *IRIS* values of the PglyOR$^+$, AspOR$^+$, AsnOR$^+$ and ValOR$^+$ guests show relatively high degrees of chiral recognition character [*IRIS* $\geqslant$ *ca.* 2.0 for (*RRRR*)-host preference or *IRIS* $\leqslant$ 0.5 for (*SSSS*)-host preference], these guests are applicable to determine the ee quantities. On the other hand, the *IRIS* values of the 1-phenylethylamine hydrochloride guests are close to unity, so these are not appropriate for such a purpose. These differences strongly suggest the importance of the COOR function in the guest part for higher chiral recognition ability in the present system.

We have described a conceptually novel method for ee determination of amino acid ester salts using FAB mass spectrometry (positive mode). The methodology is potentially applicable to other host–guest systems with relatively high degrees of chiral recognition.

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Notes and References

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