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International Journal of Mass Spectrometry 210/211 (2001) 585–590

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Facile *ee*-determination from a single measurement by fast atom bombardment mass spectrometry: a double labeling method

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Received 28 November 2000; accepted 19 January 2001

Abstract

From a single fast atom bombardment mass spectrum, the optical purity (enantiomeric excess: *ee*) of chiral organic primary and secondary amine salts (guests) such as tryptophan 2-propyl ester hydrochloride and proline 2-propyl ester hydrochloride was easily determined with a high accuracy using both the deuterium-labeled/unlabeled enantiomeric host pair (DD-Gal2deg and LL-Gal2deg-d_{24}) and the corresponding deuterium-labeled internal standard guest (for example, the *S*-amino acid ester-d_m salt). (Int J Mass Spectrom 210/211 (2001) 585–590) © 2001 Elsevier Science B.V.

Keywords: FAB mass spectrometry; Enantiomeric excess; Deuterium labeling; Host-guest complexation; Optical purity

1. Introduction

Evaluating the optical purity (enantiomeric excess: *ee*) of chiral compounds is essential and indispensable in chiral chemistry. In general, the optical purity is evaluated using chromatographic [1] and capillary electrophoretic methods [2]. Recently, mass spectrometric methods have been paid significant attention because of their high sensitivity and short measurement time. Various techniques and methodologies were designed for the evaluation. For example, the *ee* values were evaluated by (1) gas phase equilibria of crown ether derivatives with amines using electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI FTICRMS) [3], (2) rates of amine-exchange reactions of cyclodextrin complexes using ESI FTICRMS [4], (3) kinetic resolution of alcohols and amines followed by electrospray ionization mass spectrometry (ESIMS) [5], etc. [6]. We have reported a method for determining the enantiomeric excess of amino acid ester salts by resorting to complex formation with a deuterium-labeled/unlabeled enantiomeric host pair and detection of the complexes by fast atom bombardment (FAB) mass spectrometry [the enantiomer labeled (EL) -host method] [7].

However, Tao et al. recently reported that the optical purity of α-amino acids can be determined with a high accuracy ($R^2 > 0.999$) based on the collision-induced dissociation (CID) of Cu-amino acid complex ions using ESIMS/MS (ion trap) [8].

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PII S1387-3806(01)00459-6

Therefore, we have demonstrated an improved EL-host method which provides a high accuracy with $R^2>0.999$ based on the use of a deuterium labeled internal standard. The latter approach enabled us to determine the optical purity of amino acid ester salts from a single measurement of a FAB mass spectrum with an increased accuracy.

Previously, in order to evaluate the *ee* value of an amino acid ester salt (guest, G^+), we introduced the use of a pair of deuterium-labeled/unlabeled (1/1) enantiomeric hosts (for example, H_{DD}, $H_{LL\text{-}dn}$, n: number of deuterium atoms) [9]. In the present method, we further introduced the use of the corresponding deuterium-labeled *S*- (or *R*-) enantiomer guest compound (for example, $G_{S\text{-}dm}$, m: number of deuterium atoms, $m<n$ or $m>n$) as the internal standard. This was then called the double labeling method. After mixing a given *ee*-unknown amine salt (guest, G) with (1) the host pair (H_{DD} and $H_{LL\text{-}dn}$) and (2) the corresponding labeled *S*-enantiomer guest salt ($G_{S\text{-}dm}$), the FAB mass spectra were measured in the 3-nitrobenzyl alcohol (NBA) matrix. The following four host-guest complex ion peaks then appeared: $(H_{DD}+G)^+$, $(H_{DD}+G_{S\text{-}dm})^+$, $(H_{LL\text{-}dn}+G)^+$, and $(H_{LL\text{-}dn}+G_{S\text{-}dm})^+$.

Here, the peak intensity excess (*Ie*) value resulting from the *ee*-unknown guest is defined as $Ie=[I(H_{DD}+G)^+ - I(H_{LL\text{-}dn}+G)^+]/[I(H_{DD}+G)^+ + I(H_{LL\text{-}dn}+G)^+]$, which was already stated in the previous papers [9,10]. Similarly, the Ie_{std} value resulting from the *ee*-100% standard (labeled) guest is defined as $Ie_{std}= [I(H_{DD}+ G_{S\text{-}dm})^+ - I(H_{LL\text{-}dn}+G_{S\text{-}dm})^+]/[I(H_{DD}+G_{S\text{-}dm})^+ + I(H_{LL\text{-}dn}+ G_{S\text{-}dm})^+]$. Therefore, the *ee* value can be obtained as the ratio of *Ie* to Ie_{std}: $ee\ (\%)=Ie/Ie_{std}\times100$ [9,10].

We previously demonstrated a linear (or a V-shaped) correlation between the relative peak intensity values $[I(H_{DD}+G)^+/I(H_{LL\text{-}dn}+G)^+ = IRIS$ value] of the complex ions and the *ee* values [7(c),(d)]. However, after considering the mathematical correlations with the *ee* values in detail, we reached the conclusion that the linear correlation of the *Ie* values versus the *ee* values should be adopted rather than that of the *IRIS* values versus the *ee* values [9,10].

2. Experimental

2.1. Materials

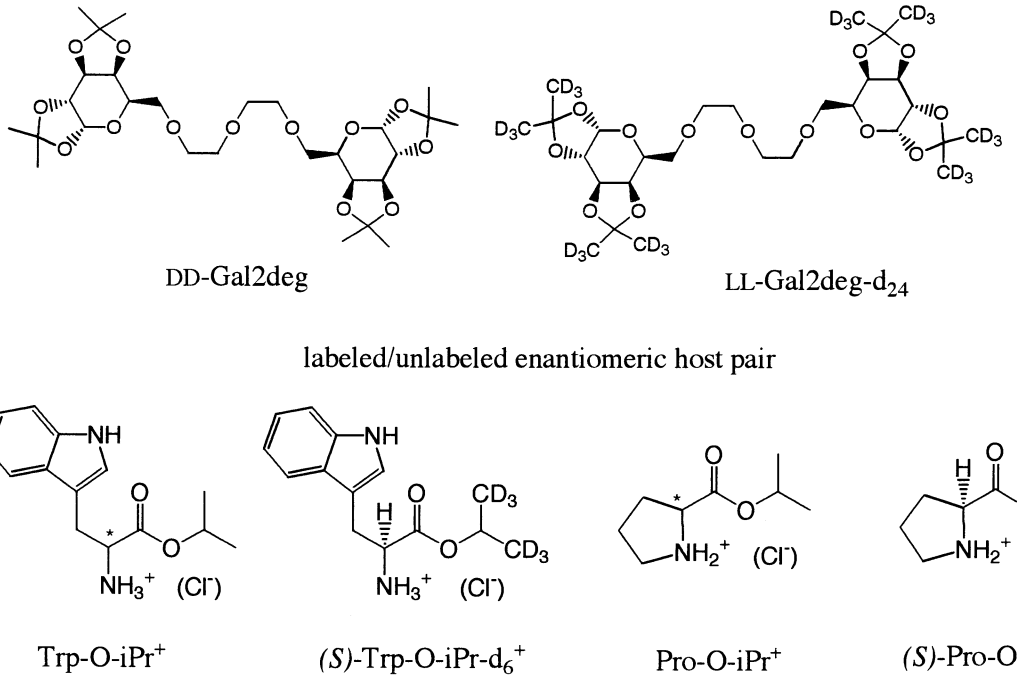
The previously synthesized host compounds (DD-Gal2deg and LL-Gal2deg-d_{24}) containing galactose-end groups capable of chiral recognition [9] were used in the present study (Scheme 1).

The D content of LL-Gal2deg-d_{24} was 98.8 D atom %. Tryptophan 2-propyl ester hydrochloride (Trp-O-iPr^+ Cl^-) and proline 2-propyl ester hydrochloride (Pro-O-iPr^+ Cl^-), which were used as the chiral guests, were prepared from commercial amino acids (Sigma Chemical Co., St. Louis, MO, USA) and 2-propanol (Wako Pure Chemical Industries, Ltd., Osaka, Japan) by use of standard methods [11]. The corresponding deuterium (-d_6 and -d_7) -labeled *S*-enantiomer guests (*S*-Trp-O-iPr-$d_6^+Cl^-$ and *S*-Pro-O-iPr-$d_7^+Cl^-$) as the internal standards were also prepared from the commercial *S*-amino acids (Sigma Chemical Co.) and deuterium-labeled 2-propanol (d_6 and d_8, respectively, CDN isotopes, Quebec, Canada).

In the double labeling method, the four complex ion peaks are independently observed in a single mass spectrum. If the number of deuteriums in the labeled host and the labeled internal standard are close to each other, the observed spectrum is not simple due to the inseparability of the complex ion peaks. Thus, $n>m$ (or $n< m$) is recommended and the present labeled enantiomeric host ($n=24$) and the internal standard labeled guest ($m=6$ or 7) satisfy this requirement.

2.2. Preparation of typical sample solutions

A sample solution was prepared by mixing the next three solutions and 15 μL of the matrix (NBA, Aldrich). The three solutions were as follows: (1) 5.0 μL of a 0.160 M $CHCl_3$ solution of a 1/1 mixture of unlabeled and labeled enantiomeric hosts ($[H_{DD}^+]=0.160$ M and $[H_{LL\text{-}d24}^+]=0.160$ M), (2) 2.5 μL of a 0.0200 M $CHCl_3$ solution of a given *ee*-unknown amino acid ester hydrochloride, and (3) 2.5 μL of a 0.0200 M $CHCl_3$ solution of the corresponding labeled *S*-amino acid ester hydrochloride. In order to minimize the concentration effect on the *Ie* value, it is important that the concentrations of the *ee*-un-



Scheme 1. The various *ee* guests and the corresponding internal standard labeled guests with deuteriums.

known guest and the internal standard guest should remain equal [10]. Several different *ee* solutions were prepared by mixing the two solutions of the optically pure enantiomers with the corresponding proportions. The FAB mass spectra were measured at room temperature with the deposit of a 1 μL aliquot of the final mixed solution, which had been left overnight to be homogenized. The concentration ratio in NBA after evaporating the solvents was as follows: $[H_{DD}]/[H_{LL\text{-}d24}]/[G]/[G_{S\text{-}dm}]=8/8/1/1$ (= 0.267 M/0.267 M/0.0333 M/0.0333 M).

2.3. Measurement conditions of FAB mass spectra

The FAB mass spectra (positive mode) were measured with a JEOL JMS-M600 mass spectrometer. 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 0.5 mA and an acceleration voltage of 3 kV. The ion source pressure was typically $\sim 1\text{–}2\times10^{-5}$ Torr. The data were processed using a JEOL data processing system. Calibration was carried out with CsI. The spectra were accumulated from the 30th to the 100th scan (n=70).

2.3.1. Condition A

The spectrometer was operated with an accelerating voltage of 3 kV and a mass range of *m/z* 20–2300. The spectra were obtained with a magnet scan rate of 5 s/scan over the mass range.

2.3.2. Condition B

The spectrometer was operated with an accelerating voltage of 3 kV and a mass range of *m/z* 800–900. The spectra were obtained with an electric scan rate of 7 s/scan over the mass range. The spectra were accumulated from the 30th to the 100th scan (n=70).

3. Results

3.1. FAB mass spectra of doubly-labeled compounds

The FAB mass spectra of the host pair with various *ee* values of Trp-O-iPr$^+$(Cl^-) containing the internal

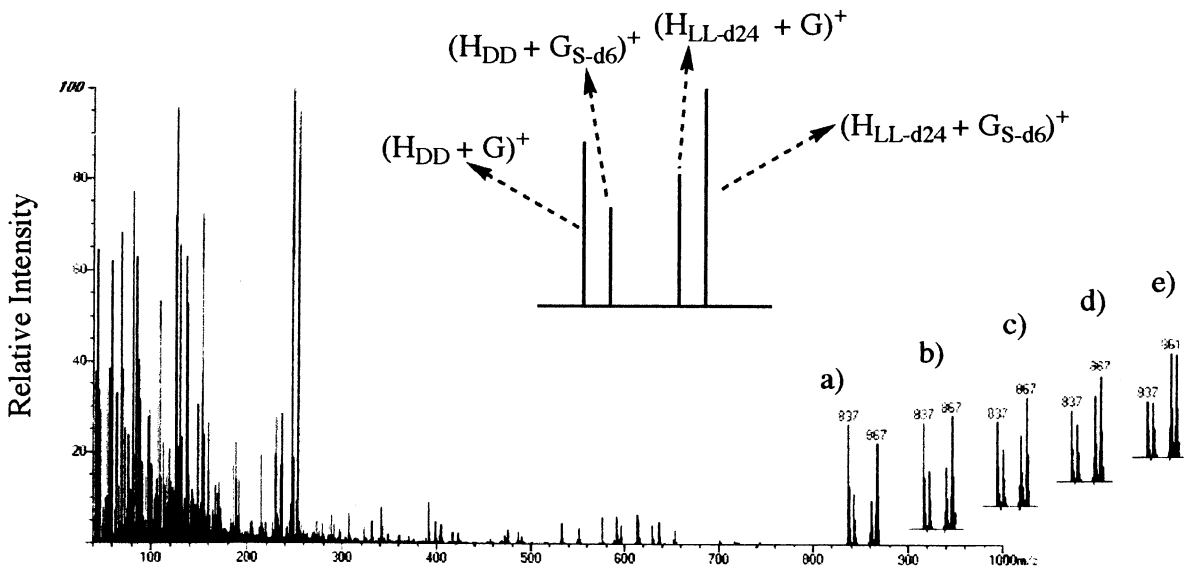


Fig. 1. FAB mass spectra of the deuterium-labeled/unlabeled enantiomeric host pair (H_{DD}, $H_{LL\text{-}d24}$) with various *ee* guests [G^+: Trp-O-iPr$^+$(Cl^-)] including the deuterium-labeled standard guest [$G^+_{S\text{-}d6}$: *S*-Trp-O-iPr-d_6^+(Cl^-)]. NBA matrix. (a) *R* 100% *ee*, (b) *R* 50% *ee*, (c) 0% *ee*, (d) *S* 50% *ee*, (e) *S* 100% *ee*. In the case of the spectrum (e), the two pairs of the peaks differing from six mass units show equal intensities because the concentrations of the *S* 100% guest and the deuterium-labeled *S* 100% guest (the standard) are equal.

standard [*S*-Trp-O-iPr-d_6^+(Cl^-)] are shown in Fig. 1. The four complex ions of $(H_{DD}+G)^+$, $(H_{DD}+G_{S\text{-}dm})^+$, $(H_{LL\text{-}dn}+G)^+$, and $(H_{LL\text{-}dn}+G_{S\text{-}dm})^+$ [n=24, m=6] were observed at *m/z* 837, 843, 861, and 867, respectively. In the case of Pro-O-iPr$^+$(Cl^-), the four peaks of the complex ions were also observed at *m/z* 748, 755, 772, and 779, respectively. The relative peak intensity of the complex ions changed depending on the *ee* of the amino acid ester hydrochlorides.

3.2. The estimated ee values

The complex ion peak intensities resulting from the LL-Gal2deg-d_{24} host, $I(H_{LL\text{-}d24}+G)^+$ and $I(H_{LL\text{-}d24}+G_{S\text{-}dn})^+$, were reduced because the D content (98.8%) of the above labeled host is slightly smaller than the 100 D atom %. Their peak intensities of the labeled host complex ions were statistically corrected based on the natural abundance of the complex ion peaks [12], and the corrected ones were used to calculate the *Ie* and Ie_{std} values.

The *ee* values were directly obtained by Ie/Ie_{std}. The obtained *ee* values were compared with the *ee* values of the prepared solutions (Table 1). In both cases of the Trp-O-iPr$^+$(Cl^-) and Pro-O-iPr$^+$(Cl^-), the *ee* values determined from the FAB mass spectrometry showed very good agreement with the *ee* values prepared. The correlation coefficients of the linear line were R^2=0.9992 (n=5) and 0.9990 (n=7), respectively (Fig. 2). For the measurements in the narrow mass range previously mentioned (measurement condition B), the correlation coefficient (Trp-O-iPr$^+$(Cl^-): R^2= 0.9990, n=5) was almost the same as that above (measurement condition A). As seen in Table 1, the *ee* values could be estimated within the error range of ~±1% for the primary amine case and ~±3% for the secondary amine case. Generally, the latter case gives a weaker binding between a host and a guest, so that the complex ion peaks become relatively smaller and the accuracy becomes slightly reduced.

Table 1
Ie and Ie_{std} values of the DD-Gal2deg/LL-Gal2deg-d_{24} enantiomeric host pair with chiral guests prepared to various optical purities (FABMS measurement condition A).

Chiral guest	Optical purity prepared (% *ee*)	*Ie*	Ie_{std}	Optical purity evaluated (% *ee*)
Trp-O-iPr$^+$(Cl$^-$)	*R* 100	0.396	−0.393	*R* 101
	R 50	0.190	−0.382	*R* 50
	0	0.002	−0.373	R 1
	S 50	−0.170	−0.371	*S* 46
	S 100	−0.369	−0.370	*S* 100
Pro-O-iPr$^+$(Cl$^-$)	*R* 100	0.319	−0.318	*R* 100
	R 75	0.193	−0.260	*R* 74
	R 50	0.132	−0.260	*R* 51
	R 25	0.063	−0.255	*R* 25
	0	0.005	−0.253	*R* 2
	S 25	0.080	−0.284	*S* 28
	S 50	0.137	−0.254	*S* 54
	S 75	0.197	−0.252	*S* 78
	S 100	0.252	−0.257	*S* 98

4. Discussion

In the case without a deuterium-labeled internal standard, the measurements of the *Ie* and the Ie_{100} values are required to evaluate the *ee* [9, 10]. The Ie_{100} value means the *Ie* value of *R* or *S* 100% *ee* guest. Therefore, the *ee* of the *ee*-unknown guest must be estimated by two successive measurements of the FAB mass spectra. Considering the accuracy, it is desirable to measure the spectrum of the *ee*-unknown guest and the *R* or *S* 100% *ee* standard guest at the same time [6,7(a),7(b)]. In fact, the correlation coefficient (R^2) of the previous measurements without the internal standard was 0.9970 (host pair: DD-Gal2deg/LL-Gal2deg-d_{24}; guest: 1-(1-naphthyl)-ethylammonium chloride) [9]. Measuring the *ee*-unknown and the standard compounds at the same time can reduce errors and so produce a result of higher accuracy. The

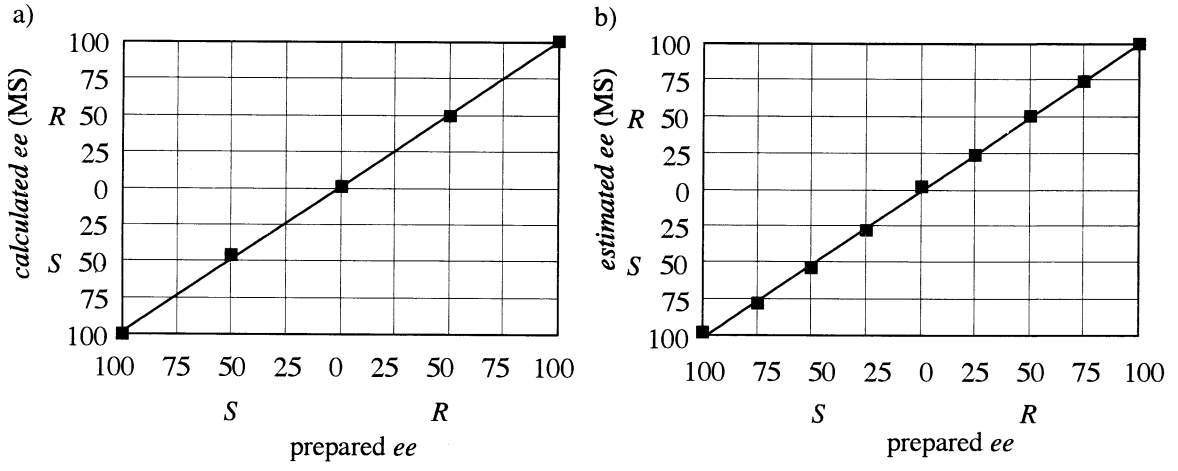


Fig. 2. Plots of the evaluated *ee* values from FAB mass spectra against the prepared *ee* values. (a) the primary amine salt guest, Trp-O-iPr$^+$(Cl$^-$); (b) the secondary amine salt guest, Pro-O-iPr$^+$(Cl$^-$). The correlation coefficients (R^2) were 0.9992 (n=5) and 0.9990 (n=9), respectively.

feasibility and the high accuracy obtained suggest the potential utility of the present double labeling method by FAB mass spectrometry.

5. Conclusions

In this paper, we have demonstrated a typical mass spectrometric methodology for the *ee*-determination of primary and secondary organic amine salts with the high accuracy of $R^2 > 0.999$. The method requires using (1) the deuterium-labeled/unlabeled enantiomeric host pair, and (2) the deuterium-labeled internal standard guest in FAB mass spectrometry. The merit of this double labeling method is that a single measurement of the FAB mass spectrum is enough to evaluate the optical purity without any calibration-line determinations. The accuracy of the evaluated *ee* is typically within the error range of the $\sim \pm 1\%$ *ee* level for the primary amines and the $\sim \pm 3\%$ *ee* level for the secondary amines. It is expected that chiral host pair compounds, specifically designed to be effective with various types of chiral target compounds, will be developed in the future.

The details of the complexing characteristics of the employed host compounds (chiral recognition abilities toward different amines, stability constants, thermodynamic parameters, structures of the complexes, molecular dynamics simulations, isotope effects, etc.) will be described in another paper.

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