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Chiral recognition and enantiomer assays of *N*-(3,5-dinitrobenzoyl)amino acid derivatives using electrospray ionization–mass spectrometry

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ABSTRACT

A pair of pseudoenantiomers, anilide derivatives of *N*-pivaloylproline were prepared and used as chiral selectors for enantiomer discrimination of amides or esters of *N*-(3,5-dinitrobenzoyl)amino acids in single-stage electrospray ionization/mass spectrometric experiments. Addition of a chiral analyte to a solution of the two pseudoenantiomeric chiral selectors affords selector–analyte complexes in the electrospray ionization mass spectrum where the ratio of these complexes is dependent on the enantiomeric composition of the analyte. The relationship between the ratio of the selector–analyte complexes in the electrospray ionization mass spectrum and the enantiomeric composition of the analyte can be used to relate the extent of the measured enantioselectivity and for quantitative enantiomeric composition determinations. Effects of the added cationic ions (H^+, Li^+, Na^+ and K^+) and instrument conditions on the selector–analyte ion intensity and the enantioselectivity (α_{MS}) were investigated. The percent ratio of the sum of the selector–analyte ion counts and the total ion counts decreases accordingly with the increase of the desolvation temperature for H^+, Na^+ and K^+. The ratio for Li^+ kept almost constant. The best α_{MS} was observed at a desolvation temperature of 200 °C with the added H^+. The cone voltage has little effects on the α_{MS} values though the intensities of selector–analyte complexes are decreased at higher cone voltages. The observed MS enantioselectivities are comparable to the HPLC enantioselectivities and the sense of chiral recognition by MS is consistent with what is observed chromatographically. Quantitative enantiomeric composition determinations for five different samples of *N*-(3,5-dinitrobenzoyl)leucinyl butylamide at four different concentrations were performed. The average % difference between the HPLC and MS enantiomer determinations is 6.8% and 3.7% for the calibration lines constructed at a concentration of the analyte of 125 μM and 12.5 μM, respectively.

1. Introduction

Methods that use only mass spectrometry (MS) for the determination of enantiomeric composition are receiving increasing attention given the need for robust and rapid enantiomer assays that are generally applicable [1–5]. This is particularly evident in the area of combinatorial asymmetric catalysis, whereby libraries of potential chiral catalysts are produced in parallel and each must be independently evaluated [6–10]. The limiting step in this process is typically the time required to determine the enantiomeric composition of the product yielded from each chiral catalyst [11–17]. Electrospray ionization (ESI)–MS appears to be well suited for this task given its broad analyte scope, high sensitivity, easy identification of interests based on unique masses, and potential for rapid analysis. Additionally, since solutions containing the analyte can be directly introduced into the spectrometer, sample handling is straightforward and has the potential to be automated [18,19].

Enantiomers will, under identical conditions, afford identical mass spectra. It is only through the influence of a second chiral agent (i.e., chiral selector or chiral derivatizing reagent) that the enantiomers can be differentiated via diastereomer formation (non-covalent or covalent). The MS methods where chiral recognition has been observed, and where quantitative enantiomer assays have been demonstrated can be grouped into three categories:

(1) Ionized non-covalent selector–analyte (or host–guest) complexes are generated and observed in a single-stage mass spectrum. Mass-labeling of the enantiomeric chiral selectors affords pseudoenantiomers (where each pseudoenantiomer has the opposite stereochemistry, but a slightly different mass due to labeling of one enantiomer at a remote position). Complexation of the analyte with the pseudoenantiomeric chiral selectors allows mass-differentiated (pseudo)diastereomeric

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complexes to be observed in the mass spectrum, the relative amounts of which will depend on the enantiomeric composition of the analyte should chiral recognition be observed. Since the first reported observation of chiral recognition in a chemical ionization mass spectrum by Fales and Wright [20], a number of observations of MS chiral recognition have been reported using a variety of ionization methods, including fast-atom bombardment [21–26], matrix assisted laser desorption [27], and ESI [21,28–37]. Some progress, including our own work, has been made in developing methods for quantitative enantiomer assays, using single-stage MS experiments [33–35,38–40].

(2) Tandem methods (MS/MS) rely on isolating a specific ion and allowing this ion to react with another reagent, or observing the collision-induced dissociation (CID) of the complex. The first type of tandem measurement has mainly been applied to cyclodextrin–analyte complexes [41–44], and chiral crown ether–chiral ammonium cation complexes [45,46]. The rate at which the analyte exchanges for an achiral reagent gas in the host–guest complex is used as a metric for determining the stereochemical composition of the analyte. For the other type of tandem experiments, higher order complexes are mass-selected and allowed to undergo CID, and the observed relative branching ratios are related to the enantiomeric composition by the kinetic method (KM) of Cooks and co-workers [47–64]. KM greatly reduces matrix effects that are usually seen in the single-stage MS measurements, whereas it loses information of the equilibrium distribution of species in solution phase. This information should allow straightforward comparison between MS selectivities and HPLC selectivities.

(3) Derivatization of a chiral analyte with a mixture of mass-labeled, pseudoenantiomeric chiral reagents affords covalent derivatives that can be discriminated by mass spectrometry [65–67]. As long as kinetic resolution is observed in the derivatization step, the relative amounts of the derivatives can be related back to the enantiomeric composition of the analyte. Of course, one major drawback to this method is the requirement to derivatize the analyte prior to analysis.

We have been interested in developing enantiomer assays of the first type [33–35]. In addition to the high-throughput screening applications that will become possible via this type of analysis, such chiral analyses could also be used for the discovery and optimization of novel chiral selectors by combinatorial methods. Instead of measuring the enantiomeric composition, one would instead be using mass spectrometry to directly measure the relative binding of analyte enantiomers to potential chiral selectors.

Herein, we report the use of soluble analogues of **CSP 1**, i.e., **2** and **3**, as pseudoenantiomeric chiral selectors for the enantiomeric analyses of *N*-(3,5-dinitrobenzoyl)amino acid derivatives (Fig. 1). The optimization of enantioselectivity with respect to instrumental parameters and additives, an analyte survey with comparisons of the mass spectrometric enantioselectivities with the enantioselectivities observed by chiral HPLC, and the use of soluble analogues of **CSP 1** for enantiomer assays is detailed below.

2. Experimental

The chiral stationary phase, **CSP 1** (4.6 mm × 250 mm column), was available from previous studies [68,69]. All solvents used were HPLC grade and used without further purification. The chiral selectors and analytes used herein have been previously reported [33–35].

All mass spectra were obtained on a Micromass Quattro Micro™ (Beverly, MA) triple quadrupole mass spectrometer with electrospray ionization running in the positive ion mode. Solutions were introduced either by flow injection analysis (FIA), or by direct infusion (DI). FIA: solutions were flow injected into the electrospray ionization source through a 10 μL injection loop with mobile phase running at a flow rate of 200 μL/min. The full positive ion spectrum was recorded every 0.7 s. All scans for which a significant total ion count was observed were averaged together to afford the final spectrum, typically requiring approximately 10 s per injection. DI: for data collected by direct infusion with a syringe pump (8 μL/min), scans collected over every 20-s period were averaged together to afford the final spectrum. Each sample was repeated four times in order for the calculation of percent relative standard deviation (RSD%). Spectrometer conditions are as follows: capillary voltage 3.5 kV; extractor voltage, 1.0 V; RF lens, 0.5 V; source temperature, 80 °C; cone gas flow, 61 L/h; desolvation gas flow, 409 L/h. The cone voltage and desolvation temperature are given in the data tables.

3. Results and discussion

3.1. Chiral recognition

Observations of chiral recognition, using ESI–MS, were detailed between the pseudoenantiomeric chiral selectors (*S*)-**4** and (*R*)-**15** (Fig. 1), which differ only by the length of their *N*-alkyl chains, using **3** as the analyte, in a previous report [33]. In order to observe the ionized selector–analyte complexes in the mass spectrum, lithium chloride was added to the chiral selector/analyte solutions. Though highly effective for ionization, the lithium cations undoubtedly reduced the extent of chiral recognition by interfering with the selector–analyte interactions. As has been demonstrated through a number of studies [70–74], the primary interactions between *N*-(3,5-dintrobenzoyl)amino acid derivatives and proline derivatives such as **3** are: (1) a π-stacking interaction between the electron-rich aromatic ring of **3** and the electron-poor dinitrobenzamide, (2) a hydrogen bond between the benzamide proton and the pivaloyl carbonyl of **3**, and (3) a hydrogen bond between the amide proton of **3** and the amino acid carbonyl group.

For our initial experiments, we set out to determine whether chiral recognition would be observed in the reciprocal sense, i.e., using pseudoenantiomeric chiral selector derived from **3**, and analytes similar to **4** and **15**. The pseudoenantiomeric chiral selectors that were prepared, (*S*)-**2** and (*R*)-**3**, differ only by substitution about the aromatic ring, which is likely to have very little effect on chiral recognition. It has been previously demonstrated [33,35], that this type of substitution will typically afford only a minor perturbation of the extent of observed chiral recognition, so that in our MS experiments, the observed enantioselectivity should be intermediate to the "true" enantioselectivities of the two chiral selectors.

Fig. 2 presents the ESI-mass spectrum of a solution containing the pseudoenantiomeric chiral selectors, (*S*)-**2** and (*R*)-**3**, and racemic **4**, with added lithium chloride in methanol/water. The lithiated selectors and analyte are observed at *m*/*z* 295, 309, and 387, along with the methanol adducts at *m*/*z* 327, 341, and 419, for compounds **2**, **3**, and **4**, respectively. The lithiated selector dimers are observed at *m*/*z* 583 $[\mathbf{2}_2 + \mathrm{Li}]^+$, 597 $[\mathbf{2} + \mathbf{3} + \mathrm{Li}]^+$, and 611 $[\mathbf{3}_2 + \mathrm{Li}]^+$. The lithiated selector analyte complexes are observed at *m*/*z* 675 $[\mathbf{2} + \mathbf{4} + \mathrm{Li}]^+$ and 689 $[\mathbf{3} + \mathbf{4} + \mathrm{Li}]^+$.

Fig. 3 presents the portion of the mass spectrum showing the selector–analyte complexes that are observed at three different enantiomeric compositions of analyte **4**. It can clearly be seen that the relative intensity of the selector–analyte complexes changes regularly with the enantiomeric composition of analyte **4**. It is apparent from the figure that (*R*)-**4** complexes to a greater extent with the (*R*)-enantiomer of the chiral selector, and that (*S*)-**4** preferentially binds to the (*S*)-enantiomer of the chiral selector. This observed sense of chiral recognition is consistent to what

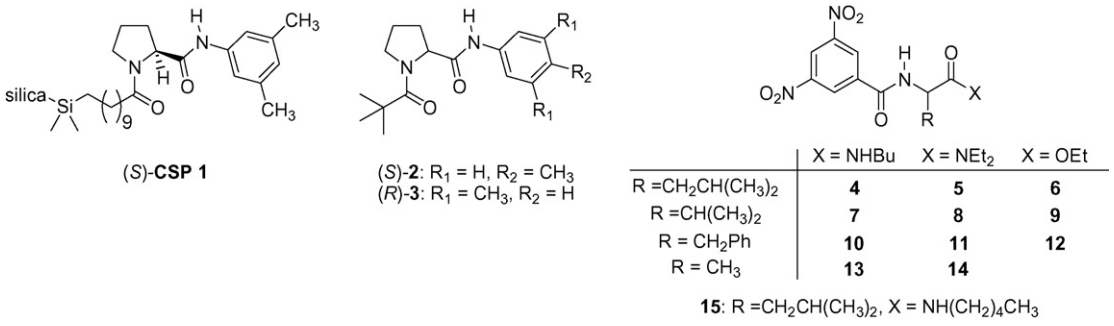

Fig. 1. Structures of the chiral stationary phase, the chiral selectors, and the chiral analytes.

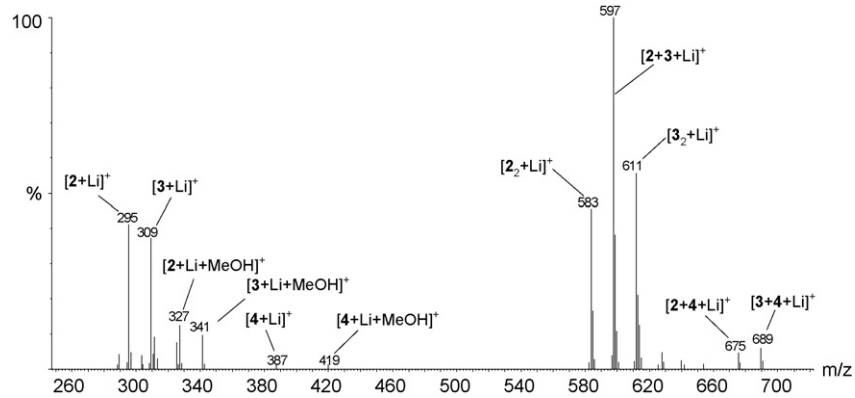

Fig. 2. Electrospray ionization mass spectrum of a solution containing psuedoenantiomeric chiral selectors (*S*)-**2** and (*R*)-**3** (1.0 mM), racemic analyte **4** (0.50 mM), and lithium chloride (5.0 mM) in methanol/water (1:1).

is observed chromatographically. The elution order for analyte **4**, using (*S*)-**CSP 1**, is (*R*) then (*S*).

Having clearly demonstrated chiral recognition, and that the sense of chiral recognition is consistent with chiral chromatography, we set out to determine how the extent of enantioselectivity compares between our MS experiments and chiral chromatography. We have previously shown that the enantiomeric composition of the analyte can be related to MS observables by Eq. (1) [35], where CIF is the complex intensity fraction (i.e., the ion counts of one selector–analyte complex divided by the sum of the ion counts for both selector–analyte complexes), X_R is the mole fraction of the (*R*)-enantiomer of the analyte, and α_{MS} is the observed MS enantioselectivity.

$$\mathrm{CIF} = \frac{(\alpha_{MS} - 1)}{(\alpha_{MS} + 1)} X_R + \frac{1}{(\alpha_{MS} - 1)} \quad (1)$$

From previous works [33–35], it has been shown that one CH_2 unit difference between the two pseudoenantiomeric selectors does not significantly perturb the ionization efficiency as well as the chiral recognition. In most chiral recognition systems, differences in binding energy between selectors and analytes are small, so that abundances of the complexes in solution are very close. When these complexes are transferred to the gas phase via ESI, the sum of the ion counts for both selector–analyte complexes varies only slightly with different enantiomeric excess as long as the concentrations of selectors and analyte are held constant throughout. This hypothesis can be manifested by the linearity of the plot of CIF vs mole fraction. In fact, for all analytes studied in this work, the correlation coefficient values are beyond 0.997.

However, it should be mentioned here that this assumption would not be true in extreme chiral recognition systems, where each chiral selector exclusively binds with one enantiomer of the analyte (i.e., *R*-selector binds mostly with *R*-analyte; whereas *S*-selector binds mostly with *S*-analyte). Also, an appreciable change in ionization efficiency caused by mass labeling will have large impact on chiral recognition. A thorough investigation of the influence of mass labeling on ionization efficiency would undoubtedly be beneficial to the study of chiral recognition by MS.

By determining the CIF value at (a minimum of) two differing analyte enantiomeric compositions, the α_{MS} value can be evaluated from a plot of CIF vs X_R. Additionally, this linear plot can be used for subsequent enantiomer assays, whereby the CIF is measured using ESI–MS of a solution that contains the pseudoenantiomeric chiral selectors and the analyte, and Eq. (1) is used to determine X_R.

Table 1 presents the α_{MS} values obtained for *N*-(3,5-dinitrobezoyl)amino acid derivatives **4**–**14**, using pseudoenantiomeric chiral selectors (*S*)-**2** and (*R*)-**3**, with added lithium chloride. Also presented in Table 1 are the chromatographic separation factors (α_{HPLC}) for these same analytes, using **CSP 1** under reverse-phase conditions. It is apparent, for the majority of analytes that the α_{HPLC} value is much greater than the observed α_{MS} value.

3.2. Optimization of enantioselectivity

Given the disparity between the enantioselectivities observed by MS and HPLC, it was reasoned that the enantioselectivity was being diminished during the MS experiment, and that the actual solution-state enantioselectivity was likely much closer to the α_{HPLC} value than the α_{MS} value. Therefore, we set out to determine the effect that the instrumental parameters, desolvation temperature and cone voltage, as well as the additive, have on the magnitude of the observed α_{MS} value.

Fig. 4 presents enantioselectivity data using pseudoenantiomeric chiral selectors (*S*)-**2** and (*R*)-**3**, analyte **4**, with added hydrochloric acid, lithium chloride, sodium chloride, or potassium chloride, as a function of desolvation temperature. Ionized

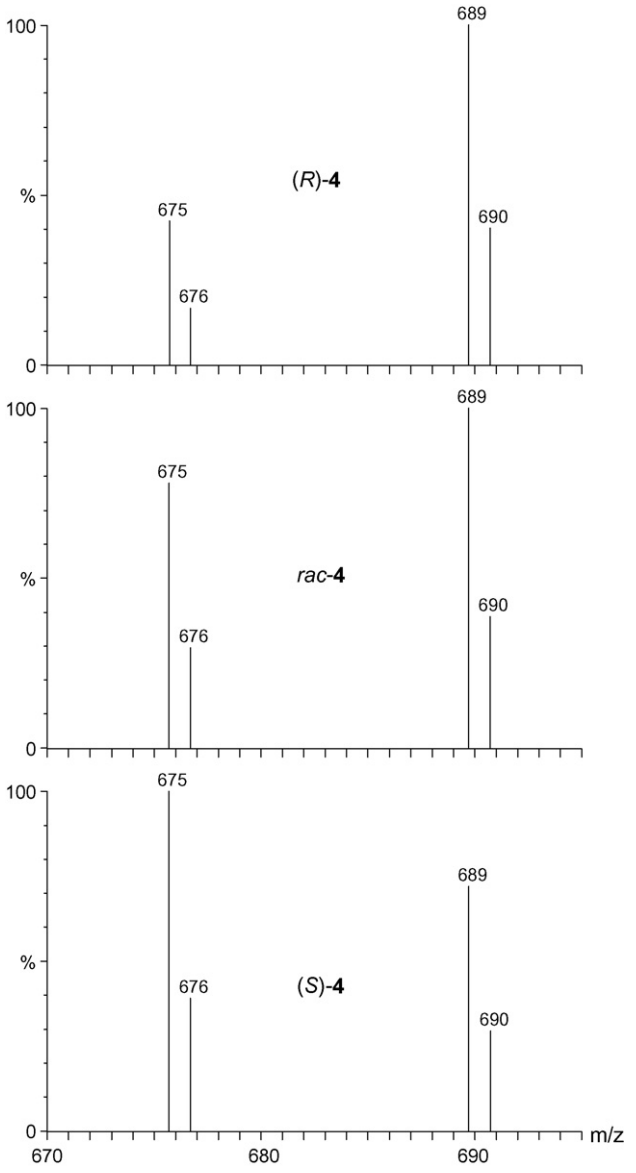

Fig. 3. Electrospray ionization mass spectra of solutions containing pseudoenantiomeric chiral selectors (*S*)-**2** and (*R*)-**3** (1.0 mM), analyte **4** (0.50 mM), and lithium chloride (5.0 mM) in methanol/water (1:1). Enantiomeric composition of analyte **4** is noted in the figure.

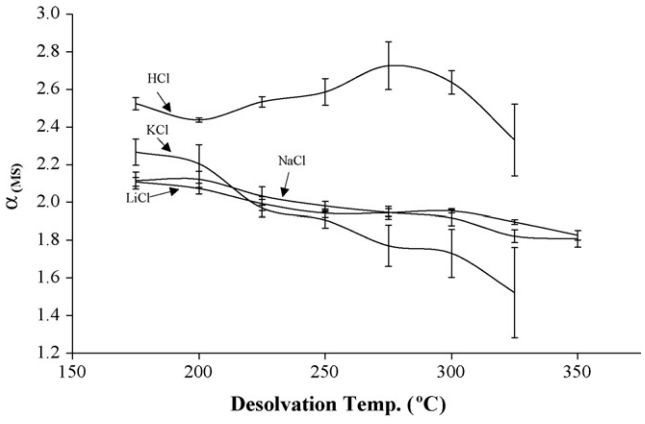

Fig. 4. Observed mass spectrometric enantioselectivity (α_{MS}) as a function of desolvation temperature and additive: [**2**] = [**3**] = 250 μM, [**4**] = 125 μM, [additive] = 5.0 mM, cone = 8 V.

Table 1
Comparison of the chromatographic separation factors (α_{HPLC}) for the enantiomers of analytes **4**–**14** on **CSP 1**[a], and observed mass spectrometric enantioselectivities (α_{MS}) using pseudoenantiomeric chiral selectors (*S*)-**2** and (*R*)-**3** with added lithium chloride for ionization.

Analyte	k_1[b]	α_{HPLC}[c]	α_{MS}[d]
4	2.79	3.53	1.69
5	2.31	4.80	1.28
6	2.99	1.63	1.58
7	2.14	2.77	1.53
8	1.95	2.71	1.07
9	2.14	1.39	1.42
10	3.18	2.86	1.28
11	2.79	3.23	1.07
12	3.31	1.41	1.09
13	1.72	1.93	1.54
14	1.45	3.25	1.21

[a] Eluent: CH_3CN/H_2O (60: 40), 1.0 mL/min.
[b] Retention factor for the first eluted enantiomer.
[c] Ratio of the retention factors for the analyte enantiomers.
[d] [**2**] = [**3**] = 1.0 mM; [analyte] = 0.5 mM; [LiCl] = 5.0 mM; MeOH/H_2O (1:1); desolvation temperature 325 °C, cone voltage 15 V.

selector–analyte complexes were too small to allow a determination of enantioselectivity above 325 °C with hydrochloric acid and potassium chloride as the additive. It should be noted that the data discussed previously were collected at a desolvation temperature of 350 °C. It is apparent from the figure that the α_{MS} value generally increases with decreasing desolvation temperature. Additionally, the additives afford the following orders: HCl > KCl > LiCl ∼NaCl below ∼210 °C and HCl > LiCl ∼NaCl > KCl above 225 °C for the α_{MS} values. Similar results have been observed by Schug et al. with the *tert*-butylcarbamoylquinine (tBuCQN)/dinitrobenzoyl-leucine (DNB-Leu) chiral recognition system [75].

In addition to the effect that the desolvation temperature and additive have on enantioselectivity, which is related to the relative intensities of the selector–analyte complexes, one must also consider the effect these parameters have on the intensity of the bimolecular complexes. Fig. 5 shows the percent ratio of the sum of the selector–analyte ion counts and the total ion counts as a function of desolvation temperature and additive. The ratios decrease accordingly with the increase of temperature for HCl, NaCl and KCl. The ratio for LiCl kept almost steady. Below 230 °C, an order in relative bimolecular abundances for additives was observed as: HCl > LiCl > NaCl > KCl; beyond that, the relative bimolecular abundance for HCl decreased sharply so that order was changed to: LiCl > NaCl > HCl > KCl.

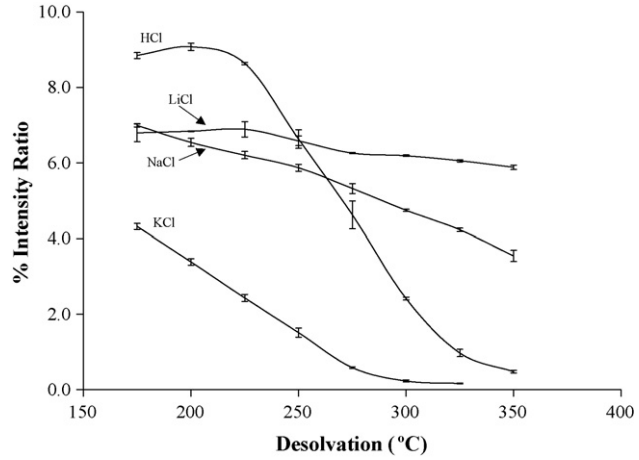

Fig. 5. Percent ratio of the sum of the selector–analyte ion counts and total ion counts as a function of desolvation temperature and additive: [**2**] = [**3**] = 250 μM, [**4**] = 125 μM, [additive] = 5.0 mM, cone = 8 V.

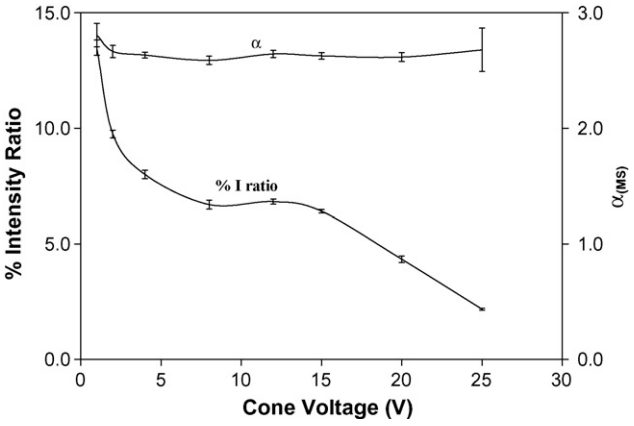

Fig. 6. Percent ratio of the sum of the selector–analyte ion counts and total ion counts as a function of cone voltage; observed mass spectrometric enantioselectivity as a function of cone voltage: [**2**] = [**3**] = 250 μM, [**4**] = 125 μM, [HCl] = 5.0 mM, desolvation temperature = 275 °C.

The final parameter investigated was the cone voltage, which is used to accelerate ions on their way to the mass analyzer. Higher cone voltage increases the possibility of the ion – cone gas (nitrogen gas in this case) collision that causes an increase of the internal energy of the ion. This will result in dissociation of ions, especially the ions formed based on the non-covalent bonding [76–78]. As can be seen from Fig. 6, this parameter does have a major effect on the relative intensity of the selector–analyte complexes, though it has almost no effect on the enantioselectivity. As the cone voltage increases, the relative intensity of the selector–analyte complexes (compared to the total ion counts) decreases. Although the absolute ion counts for all increase with increasing cone voltage, such that at cone voltages less than 5 V the ion counts were too low to be of practical utility.

Fig. 7 presents the ESI-mass spectrum of a solution containing the pseudoenantiomeric chiral selectors, (*S*)-**2** and (*R*)-**3**, and racemic **4**. Based on our optimization experiments, hydrochloric acid was used as the additive, the desolvation temperature was set to 200 °C, and the cone voltage was set to 8 V. The protonated selectors and analyte are observed at *m*/*z* 289, 303, and 381, for compounds **2**, **3**, and **4**, respectively. The protonated selector dimers are observed at *m*/*z* 577 $[\mathbf{2}_2 + H]^+$, 591 $[\mathbf{2} + \mathbf{3} + H]^+$, and 605 $[\mathbf{3}_2 + H]^+$. The protonated selector–analyte complexes are observed at *m*/*z* 669 $[\mathbf{2} + \mathbf{4} + H]^+$ and 683 $[\mathbf{3} + \mathbf{4} + H]^+$.

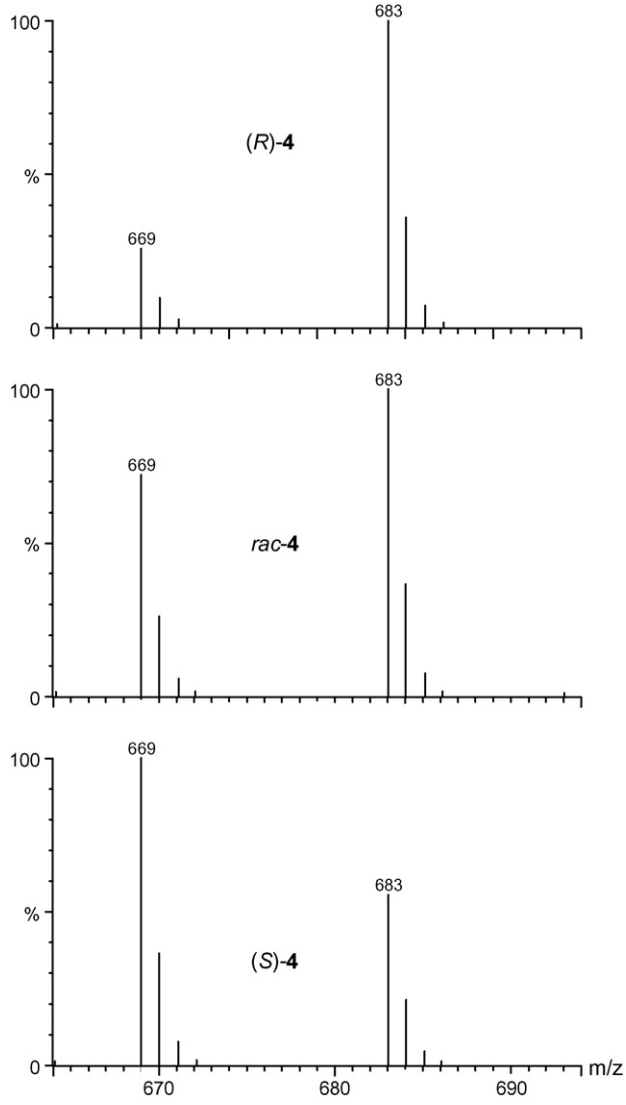

Fig. 8. Electrospray ionization mass spectra of solutions containing pseudoenantiomeric chiral selectors (*S*)-**2** and (*R*)-**3** (250 μM), analyte **4** (125 μM), and hydrogen chloride (5.0 mM) in acetonitrile/water (1:1). Enantiomeric composition of analyte **4** is noted in the figure.

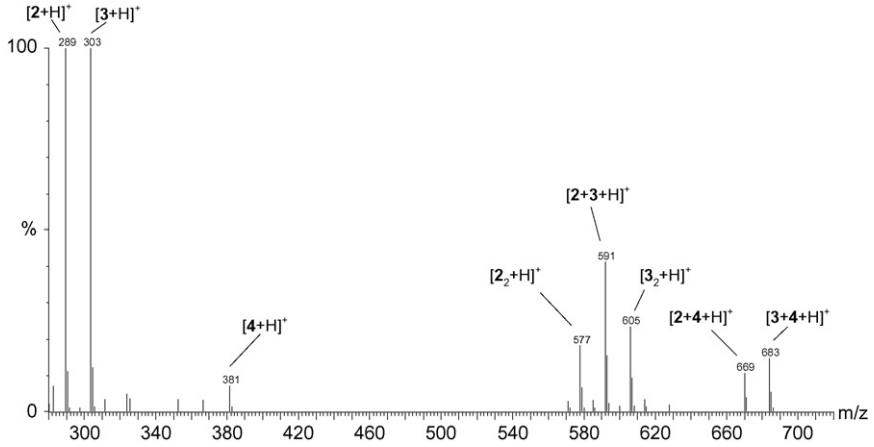

Fig. 7. Electrospray ionization mass spectrum of a solution containing psuedoenantiomeric chiral selectors (*S*)-**2** and (*R*)-**3** (250 μM), racemic analyte **4** (125 μM), and hydrogen chloride (5.0 mM) in acetonitrile/water (1:1).

Table 2
Observed mass spectrometric enantioselectivities (α_{MS}) using pseudoenantiomeric chiral selectors (*S*)-**2** and (*R*)-**3** with added hydrochloric acid for ionization.

Analyte	α_{MS}[a] (RSD%)
4	2.56 (1.19)
5	1.59 (1.05)
7	2.08 (0.85)
8	1.12 (1.01)
10	1.59 (1.23)
11	1.16 (1.29)
13	2.00 (1.18)
14	1.41 (1.02)

[a] [**2**] = [**3**] = 250 μM; [analyte] = 125 μM; [HCl] = 5.0 mM; CH_3CN/H_2O (1:1); desolvation temperature 200 °C, cone voltage 8 V, syringe pump 8 μL/min.

Fig. 8 presents the portion of the mass spectrum showing the protonated selector–analyte complexes that are observed at three different enantiomeric compositions of analyte **4**. It can clearly be seen that the relative intensity of the selector–analyte complexes changes regularly with the enantiomeric composition of analyte **4**, and that the sense of chiral recognition is consistent with the data shown previously and the chromatographic data. The α_{MS} value is 2.56, which is substantially increased from our initial conditions (*cf.* Figs. 3 and 8), though it is still less than the enantioselectivity observed by chiral HPLC (Table 1).

3.3. Analyte survey

The MS enantioselectivities, using pseudoenantiomeric chiral selectors (*S*)-**2** and (*R*)-**3** and our optimized conditions, for all of our tested analytes are presented in Table 2. It should be noted that for the ester derivatives, **6**, **9**, and **12**, no protonated selector–analyte complexes were observed in the mass spectrum. In every case where the selector–analyte complexes were observed in the ESI-mass spectrum, the α_{MS} values for the protonated complexes were greater than the α_{MS} values observed for the lithiated complexes, and in many cases (especially for the *N*-butyl amide derivatives) the α_{HPLC} values are comparable to the α_{MS} values (*cf.* Tables 1 and 2). The similarities between the enantioselectivities observed by these two methods portends the use of ESI–MS as a screening tool for chiral selector discovery.

3.4. Enantiomeric composition determinations

In order for this method to be practicable for quantitative enantiomer assays, the results of any assay should be independent of the absolute concentrations of the analyte inasmuch as possible. One can readily control the concentrations of the selectors and additives and the solvent composition by preparing a stock solution, where this same solution is used for the construction of the calibration curve and for subsequent enantiomer assays. Ideally, one would like to add a sample of the analyte to a small aliquot of this stock solution and record the electrospray ionization mass spectrum, without measuring the amount of analyte. Previously we demonstrated that the α_{MS} values are relatively invariant, as long as the concentrations of the chiral selectors are in excess of the analyte. In essence, each of our pseudoenantiomeric chiral selectors is acting as an internal standard for the other so that only the relative extent of binding is important, not the absolute extent of binding, in our enantiomer assays.

In order to test the validity of this method to accurately determine enantiomeric composition independent of analyte concentration, the enantiomeric compositions of five different samples were determined at four different concentrations, using calibration lines that were constructed at concentrations different from each of these. The calibration lines were constructed by fitting a plot of the CIF value vs mole fraction of (*R*)-**4** to a straight line. In each case three enantiomeric compositions of **4** were used to construct the plot: (*R*)-**4**, *rac*-**4**, and (*S*)-**4**. The concentrations of the selectors were 250 μM and the concentration of hydrochloric acid was 5.0 mM throughout, using acetonitrile and water as the solvents (1:1). Two calibration lines were constructed, one with an analyte concentration of 125 μM, the other with an analyte concentration of 12.5 μM.

Table 3
Determination of the enantiomeric compositions of five different samples of analyte **4** by mass spectrometry at four different concentrations using two calibration lines constructed at different analyte concentrations.

	Enantiomeric excess (*R*)-**4**		
[**4**] (μM)	HPLC[a]	MS[b,d] (RSD%)	MS[c,d] (RSD%)
200	0.97	0.96 (1.31)	0.89 (1.21)
	0.75	0.74 (2.87)	0.70 (2.62)
	0.55	0.56 (1.91)	0.53 (1.68)
	0.26	0.29 (2.97)	0.32 (2.37)
	0.04	0.04 (26.04)	0.10 (9.78)
100	0.97	1.01 (0.30)	0.93 (0.28)
	0.75	0.78 (1.56)	0.73 (1.43)
	0.55	0.59 (2.33)	0.57 (2.07)
	0.26	0.26 (2.10)	0.28 (1.64)
	0.04	−0.02 (30.05)	0.04 (13.56)
50	0.97	1.03 (2.42)	0.95 (2.26)
	0.75	0.77 (2.17)	0.72 (1.98)
	0.55	0.59 (2.50)	0.56 (2.23)
	0.26	0.23 (4.97)	0.26 (3.80)
	0.04	−0.08 (48.23)	−0.01 (458.18)
20	0.97	1.02 (1.50)	0.94 (1.41)
	0.75	0.81 (4.48)	0.77 (4.11)
	0.55	0.57 (0.68)	0.56 (0.60)
	0.26	0.23 (7.80)	0.26 (5.96)
	0.04	−0.01 (483.20)	0.06 (38.02)

[a] **CSP 1**; MeOH/water (82:18), 1.2 mL min^{-1}.
[b] Calibration line: [**4**] = 125 μM.
[c] Calibration line: [**4**] = 12.5 μM.
[d] [**2**] = [**3**] = 250 μM; [HCl] = 5.0 mM; CH_3CN/H_2O (1:1); desolvation temperature 200 °C, cone voltage 8 V, syringe pump 8 μL/min.

Table 3 presents the enantiomeric composition assays of samples of **4** at five different compositions. Each sample of **4** was analyzed at four different concentrations spanning an order of magnitude (20–200 μM). Each sample was also analyzed by chiral HPLC using **CSP 1** to allow comparisons between enantiomeric composition determinations by these two methods. As can be seen from the data, enantiomeric composition values that are accurate enough for screening applications are obtained. The majority of the data, using either calibration line, are within 0.05 of the mole fraction obtained by contemporary methods. In fact, the average % difference between the HPLC and MS enantiomer determinations is 6.8% and 3.7% for the calibration lines constructed at a concentration of analyte **4** of 125 μM and 12.5 μM, respectively.

4. Conclusions

Observations of chiral recognition in the electrospray ionization mass spectra, using anilide derivatives of *N*-pivaloylproline as chiral selectors, have been demonstrated for a number of *N*-(3,5-dinitrobenzoyl)amino acid analytes. Electrospray ionization of a solution of the analyte and a one-to-one mixture of mass-labeled pseudoenantiomeric chiral selectors affords selector–analyte complexes in the mass spectrum where the complex intensity fraction for either of the selector–analyte complexes varies linearly with

the enantiomeric composition of the analyte. This relationship provides a measure of the extent of enantioselectivity, and allows quantitative determination of the enantiomeric composition. The observed precision for enantiomeric composition determinations is more than adequate for most high-throughput analyses where one is often willing to trade some precision for analysis time.

Optimization of the chiral selectivity in the mass spectrometric experiments afforded, in a number of instances, enantioselectivities comparable to what is observed by chiral HPLC, when using a chiral stationary phase analogous to the pseudoenantiomeric chiral selectors. The chiral selectors used in this study were derived from an established Pirkle-type chiral stationary phase [68,69]. Given the correlation between the enantioselectivities observed chromatographically and by mass spectrometry, one would expect the scope of analytes that one can assay by this method should be comparable to scope of analytes that can be enantioresolved on the corresponding chiral stationary phase. It is also expected that this method will have utility as a screening method for the discovery of new chiral selectors.

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References

[1] M. Speranza, Int. J. Mass Spectrom. 232 (2004) 277.
[2] W.A. Tao, R.G. Cooks, Anal. Chem. 75 (2003) 25A.
[3] A. Filippi, A. Giardini, S. Piccirillo, M. Speranza, Int. J. Mass Spectrom. 198 (2000) 137.
[4] M. Sawada, Mass Spectrom. Rev. 16 (1997) 73.
[5] K.A. Schug, W. Lindner, J. Sep. Sci. 28 (15) (2005) 1932–1955.
[6] M.G. Finn, Chirality 14 (2002) 534.
[7] M.T. Reetz, Angew. Chem. Int. Ed. 41 (2002) 1335.
[8] M.T. Reetz, Angew. Chem. Int. Ed. 40 (2001) 284.
[9] M.T. Reetz, K.M. Kuhling, A. Deege, H. Hinrichs, D. Belder, Angew. Chem. Int. Ed. 39 (2000) 3891.
[10] W. Schrader, A. Eipper, D.J. Pugh, M.T. Reetz, Can. J. Chem. 80 (2002) 626.
[11] Y. Wang, L.H. Blum, T. Li, Anal. Chem. 72 (2000) 5459.
[12] L.H. Bluhm, Y. Wang, T. Li, Anal. Chem. 72 (2000) 5201.
[13] E. Brahmachary, F.H. Ling, F. Svec, J.M.J. Frechet, J. Comb. Chem. 5 (2003) 441.
[14] C.J. Welch, S.D. Pollard, D.J. Mathre, P.J. Reider, Org. Lett. 3 (2001) 95.
[15] C.J. Welch, G. Bhat, M.N. Protopopova, J. Comb. Chem. 1 (1999) 364.
[16] K. Lewandowski, P. Murer, F. Svec, J.M.J. Frechet, J. Comb. Chem. 1 (1999) 105.
[17] M.D. Weingarten, K. Sekanina, W.C. Still, J. Am. Chem. Soc. 120 (1998) 9112.
[18] D. Gördes, K. Thurow, JALA 11 (3) (2006) 128–133.
[19] K.A. Schug, Comb. Chem. High Throughput Screening 10 (5) (2007) 301–316.
[20] H.M. Fales, G.J. Wright, J. Am. Chem. Soc. 99 (1977) 2339.
[21] Y. Liang, J.S. Bradshaw, R.M. Izatt, R.M. Pope, D.V. Dearden, Int. J. Mass Spectrom. 185 (1999) 977.
[22] M. Swada, Y. Okumura, M. Shizuma, YoshioTakai, H. Yamada, T. Tanaka, T. Kaneda, K. Hirose, S. Misumi, S. Takahashi, J. Am. Chem. Soc. 115 (1993) 7381.
[23] M. Swada, Y. Takai, H. Yamada, S. Hirayama, T. Kaneda, T. Tanaka, K. Kamada, T. Mizooku, S. Takeuchi, K. Ueno, K. Hirose, Y. Tobe, K. Naemura, J. Am. Chem. Soc. 117 (1995) 7726.
[24] A. Dobo, M. Liptak, P. Huszthy, K. Vekey, Rapid Commun. Mass Spectrom. 11 (1997) 889.
[25] G. Pocsfalvi, M. Liptak, P. Huszthy, J.S. Bradshaw, R.M. Izatt, K. Vekey, Anal. Chem. 68 (1996) 792.
[26] M. Swada, Y. Takai, H. Yamada, J. Nishida, T. Kaneda, R. Arakawa, M. Okamato, K. Hirose, T. Tanaka, K. Naemura, J. Chem. Soc., Perkin Trans. 2 (1998) 701.
[27] M.P. So, T.S.M. Wan, T.W.D. Chan, Rapid Commun. Mass Spectrom. 14 (2000) 692.
[28] H. Nierengarten, E. Leize, C. Garcia, G. Jeminet, A.V. Dorsselaer, Analusis 28 (2000) 259.
[29] C. Czerwenka, N.M. Maier, W. Lindner, Anal. Bioanal. Chem. 379 (2004) 1039.
[30] M. Sawada, Y. Takai, H. Yamada, M. Yoshikawa, R. Arakawa, H. Tabuchi, M. Takada, J. Tanaka, M. Shizuma, H. Yamaoka, K. Hirose, K. Fukuda, Y. Tobe, Eur. J. Mass Spectrom. 10 (2004) 27.
[31] A. Mehdizadeh, M.C. Letzel, M. Klaes, C. Agena, J. Mattay, Eur. J. Mass Spectrom. 10 (2004) 649.
[32] J.L. Seymour, F. Turecek, A.V. Malkov, P. Kocovsky, J. Mass Spectrom. 39 (2004) 1044.
[33] M.E. Koscho, C. Zu, B.N. Brewer, Tetrahedron: Asymmetry 16 (2005) 801.
[34] B.N. Brewer, C. Zu, M.E. Koscho, Chirality 17 (8) (2005) 456–463.
[35] C. Zu, B.N. Brewer, B. Wang, M.E. Koscho, Anal. Chem. 77 (15) (2005) 5019–5027.
[36] K. Schug, P. Frycak, N.M. Maier, W. Lindner, Anal. Chem. 77 (11) (2005) 3660–3670.
[37] Y. Takai, K. Iguchi, H. Yamada, M. Shizuma, R. Arakawa, M. Sawada, J. Mass Spectrom. 41 (2) (2006) 266–268.
[38] M. Shizuma, H. Imamura, Y. Takai, H. Yamada, T. Takeda, S. Takahashi, M. Sawada, Int. J. Mass Spectrom. 210/211 (2001) 585.
[39] M. Sawada, H. Yamaoka, Y. Takai, Y. Kawai, H. Yamada, T. Azuma, T. Fujioka, T. Tanaka, Int. J. Mass Spectrom. 193 (1999) 123.
[40] M. Sawada, H. Yamaoka, Y. Takai, Y. Kawai, H. Yamada, T. Azuma, T. Fujioka, T. Tanaka, Chem. Commun. 15 (1998) 1569–1570.
[41] J. Ramirez, F. He, C.B. Lebrilla, J. Am. Chem. Soc. 120 (1998) 7387.
[42] J. Ramirez, S. Ahn, G. Grigorean, C.B. Lebrilla, J. Am. Chem. Soc. 122 (2000) 6884.
[43] G. Grigorean, J. Ramirez, S.H. Ahn, C.B. Lebrilla, Anal. Chem. 72 (2000) 4275.
[44] G. Grigorean, X. Cong, C.B. Lebrilla, Int. J. Mass Spectrom. 234 (2004) 71.
[45] D.V. Dearden, C. Dejsupa, Y. Liang, J.S. Bradshaw, R.M. Izatt, J. Am. Chem. Soc. 119 (1997) 353.
[46] I.H. Chu, D.V. Dearden, J.S. Bradshaw, P. Huszthy, R.M. Izatt, J. Am. Chem. Soc. 115 (1993) 4318.
[47] H. Bagheri, H. Chen, R.G. Cooks, Chem. Commun. 23 (2004) 2740–2741.
[48] L. Wu, E.C. Meurer, R.G. Cooks, Anal. Chem. 76 (2004) 663.
[49] C.T. Yu, Y.L. Guo, G.Q. Chen, Y.W. Zhong, J. Am. Soc. Mass Spectrom. 15 (2004) 795.
[50] L. Wu, R.G. Cooks, Anal. Chem. 75 (2003) 678.
[51] L. Wu, R.L. Clark, R.G. Cooks, Chem. Commun. 1 (2003) 136–137.
[52] D.V. Augusti, F. Carazza, R. Augusti, W.A. Tao, R.G. Cooks, Anal. Chem. 74 (2002) 3458.
[53] D.V. Augusti, R. Augusti, F. Carazza, R.G. Cooks, Chem. Commun. 19 (2002) 2242–2243.
[54] W.A. Tao, R.L. Clark, R.G. Cooks, Anal. Chem. 74 (2002) 3783.
[55] G. Fago, A. Filippi, A. Giardini, A. Lagana, A. Paladini, M. Speranza, Angew. Chem. Int. Ed. 40 (2001) 4051.
[56] W.A. Tao, R.G. Cooks, Angew. Chem. Int. Ed. 40 (2001) 757.
[57] W.A. Tao, L. Wu, R.G. Cooks, J. Med. Chem. 44 (2001) 3541.
[58] W.A. Tao, F.C. Gozzo, R.G. Cooks, Anal. Chem. 73 (2001) 1692.
[59] Z.P. Yao, T.S.M. Wan, K.P. Kwong, C.T. Che, Anal. Chem. 72 (2000) 5394.
[60] Z.P. Yao, T.S.M. Wan, K.P. Kwong, C.T. Che, Anal. Chem. 72 (2000) 5383.
[61] W.A. Tao, D. Zhang, E.N. Nikolaev, R.G. Cooks, J. Am. Chem. Soc. 122 (2000) 10598.
[62] W.A. Tao, L. Wu, R.G. Cooks, Chem. Commun. 20 (2000) 2023–2024.
[63] R.G. Cooks, P.S.H. Wong, Acc. Chem. Res. 31 (1998) 379.
[64] K. Vekey, G. Czira, Anal. Chem. 69 (1997) 1700.
[65] S. Yao, J.C. Meng, G. Siuzdak, M.G. Finn, J. Org. Chem. 68 (2003) 2540.
[66] D.D. Diaz, S. Yao, M.G. Finn, Tetrahedron Lett. 42 (2001) 2617.
[67] J. Guo, J. Wu, M.G. Finn, Angew. Chem. Int. Ed. Engl. 38 (1999) 1755.
[68] W.H. Pirkle, P.G. Murray, J. Chromatogr. A 719 (2) (1996) 299–305.
[69] W.H. Pirkle, M.E. Koscho, J. Chromatogr. A 840 (2) (1999) 151–158.
[70] W.H. Pirkle, P.G. Murray, S.R. Wilson, J. Org. Chem. 61 (1996) 4775.
[71] W.H. Pirkle, T.C. Pochapsky, J. Am. Chem. Soc. 108 (1986) 5627.
[72] W.H. Pirkle, A. Tsipouras, Tetrahedron Lett. 26 (1985) 2989.
[73] W.H. Pirkle, C.J. Welch, J. Org. Chem. 49 (1984) 138.
[74] W.H. Pirkle, S.R. Wilson, J. Am. Chem. Soc. 111 (1989) 9222.
[75] K.A. Schug, N.M. Maier, W. Lindner, Chem. Commun. (2006) 414–416.
[76] J.A. Loo, D.D. Holsworth, R.S. Root-Bernstein, Biol. Mass Spectrom 23 (1) (1992) 6–12.
[77] J.A. Loo, R.R.O. Loo, P.C. Andrews, Org. Mass Spectrom 28 (12) (1994) 1640–1649.
[78] J.A. Loo, H.R. Udseth, R.D. Smith, J.H. Futrell, Rapid Commun. Mass Spectrom. 2 (10) (1988) 207–210.