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# Extension of chromatographically derived chiral recognition systems to chiral recognition and enantiomer analysis by electrospray ionization mass spectrometry

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**Abstract**—Chiral recognition by positive ion electrospray ionization (ESI) mass spectrometry is demonstrated through the adaptation of chromatographically derived chiral recognition systems. Solutions of soluble analogues of chiral selectors used in Pirkle-type chiral stationary phases, when mixed with a chiral analyte, whose enantiomers are known to be resolved on the analogous chiral stationary phase, are shown to afford selector–analyte complexes in the mass spectrum. Pseudo-enantiomeric chiral selectors, where each pseudo-enantiomer has a different mass and a higher affinity for the opposite analyte enantiomer of its pseudo-antipode, were prepared. When mixed with a chiral analyte, solutions of these pseudo-enantiomeric selectors afford selector–analyte complexes in the ESI-mass spectrum where the relative intensities of the selector–analyte complexes are dependent on the enantiomeric composition of the analyte. Additionally, the sense of the observed chiral recognition is in agreement with the sense of chiral recognition observed chromatographically.


## 1. Introduction

The ability to produce single-enantiomer products is of paramount importance to a number of industries, particularly the pharmaceutical industry due to its direct impact on human health. It has long been known that the enantiomers of a chiral drug can have very different and even dangerous side effects.[1,2] This realization has served as the catalyst for the development of single-enantiomer pharmaceuticals. The development of single-enantiomer products requires not only efficient asymmetric synthetic methods, but also the ability to identify and quantify enantiomeric mixtures. With the advent of combinatorial asymmetric catalysis, whereby libraries of potential asymmetric catalysts are produced in parallel and each catalyst is screened for its ability to produce a product of high enantiomeric purity, it has become necessary to develop high throughput enantiomer assays. Typically, the size of combinatorial catalyst libraries has not been limited by the imagination of the chemist, but rather by the time needed to evaluate large libraries using contemporary methods for enantiomer analysis.[3–5]

Mass spectrometry (MS) appears to be a well-suited method for the development of high throughput enantiomer assays, considering its broad analyte scope, high sensitivity, tolerance to impurities and its potential for rapid analysis; although one does not typically think of MS as a method that can distinguish the enantiomers of an analyte.[6] Indeed, both enantiomers of an analyte will afford identical mass spectra under the same experimental conditions. This problem can be overcome by the addition of another chiral compound (chiral selector), followed by the generation of charged diastereomeric complexes in the gas phase by an appropriate ionization method.

The methods that have demonstrated chiral recognition by MS can be divided into two main groups, ones which rely on the formation of mass-labelled, covalent diastereomers, and ones where non-covalent diastereomers are formed by the admixture of analyte and the appropriate chiral selector(s). The use of mass-labelled chiral derivatizing reagents requires an additional derivatization step where kinetic resolution between the chiral analyte and a mixture of mass-labelled chiral derivatizing reagents must be observed in order for the relative amounts of the derivatized products to be related back to the enantiomeric composition of the analyte.[7–9]

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The methods which rely on non-covalent complexation between analyte and selector(s) reported to this point can be sub-divided into two groups, ones that use a single-stage mass spectrometric measurement, and ones that use tandem mass spectrometry (MS/MS). The single-stage methods typically rely on relative peak heights of intermolecular complexes in the mass spectrum. This method has had limited application, being applied mainly to chiral crown ether–chiral ammonium ion type complexes.[10–14] The tandem mass spectrometric measurements typically 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 of these tandem measurements has mainly been applied to cyclodextrin–analyte complexes.[15–17] The rate at which the analyte exchanges for an achiral reagent gas in the cyclodextrin–analyte complex is used as a metric for determining the stereochemical composition of the analyte. For the other tandem experiments, higher order complexes are mass-selected and allowed to undergo CID, while the observed relative branching ratios are related to the enantiomeric composition by the kinetic method.[18–26]

Herein we report a new approach to the development of enantiomer assays by MS. This initial report demonstrates chiral recognition by MS through the adaptation of chromatographically derived chiral recognition systems. Solutions of soluble analogues of Pirkle-type chiral stationary phases (CSP),[27,28] when mixed with an analyte whose enantiomers are known to be resolved on the corresponding CSP, are shown to afford selector–analyte complexes in the mass spectrum. When pseudo-enantiomeric selectors are used, where each enantiomer is mass-labelled at a site remote to its requisite interaction sites, the relative intensities of the selector–analyte complexes in the mass spectrum are dependent on the enantiomeric composition of the analyte.

## 2. Results and discussion

The initial selectors and analytes chosen for the mass spectral studies were based on soluble analogues of CSPs **1**–**3** (Chart 1).[29–33] The enantiomers of soluble analogues of CSP **1** are readily resolved on CSPs **2** and **3**. The enantiomers of analyte **4**, a soluble analogue of CSP **1**, afforded chromatographic separation factors of 3.41 and 3.74 on CSPs **2** and **3**, respectively (using 10% 2-propanol/90% hexanes at 2 mL/min as the mobile phase). In both cases, the analyte enantiomer that is more retained has the same stereochemical designation as the chiral selector [i.e., (*S*)-**4** is more retained on (*S*)-CSP **2**, and (*R*)-**4** is more retained on (*R*)-CSP **3**]. In addition, the enantiomers of soluble analogues of CSPs **2** and **3** are resolved on CSP **1**. For example, it is reported that the enantiomers of the *n*-propyl amide of dinitrobenzoyl(DNB)-leucine **5** [X = $NH(CH_2)_2CH_3$] afforded a separation factor of 26.6 on CSP **1**.[31] Owing to the mutual chiral recognition displayed between analyte **4** and DNB-amino acid derivatives, such as **5** and **6**, these were chosen as the initial analyte–selector combinations to develop the mass spectral enantiomer analysis.

A 1:1 mixture of the butyl amide of (*S*)-DNB-leucine **7** and the pentyl amide of (*R*)-DNB-leucine **8** was prepared and used as the pseudo-enantiomeric chiral selectors. It was expected that the length of the amide chain would not significantly affect the sense or the extent of the selectivity of the chiral selectors. Electrospray ionization (ESI) was performed on solutions of chiral selectors **7** and **8**, and analyte **4** (Scheme 1) (see Experimental for MS details). Over the course of screening a number of selector–analyte solutions, it was discovered that substantial selector–analyte complexes were observed in the mass spectrum with the addition of lithium chloride (the $Li^+$ adducts). Figure 1 shows the mass spectrum of a solution of the chiral selectors with racemic-**4** and lithium chloride. This spectrum was obtained by infusing the sample into the spectrometer at a rate of 5 μL/min with a syringe pump.

The monomeric ions were observed at *m*/*z* 309 (**4** + $Li^+$), 387 (**7** + $Li^+$) and 401 (**8** + $Li^+$). The lithiated homodimeric ions were observed at *m*/*z* 611 ($\mathbf{4}_2$ + $Li^+$), 767 ($\mathbf{7}_2$ + $Li^+$) and 795 ($\mathbf{8}_2$ + $Li^+$), while the hetero-dimers were observed at *m*/*z* 689 (**4** + **7** + $Li^+$), 703 (**4** + **8** + $Li^+$) and 781 (**7** + **8** + $Li^+$).

Solutions were then prepared where the enantiomeric composition of **4** was varied, and the ESI-MS spectrum recorded. It was observed that the relative intensity of the selector–analyte complex ions varied with enantio-

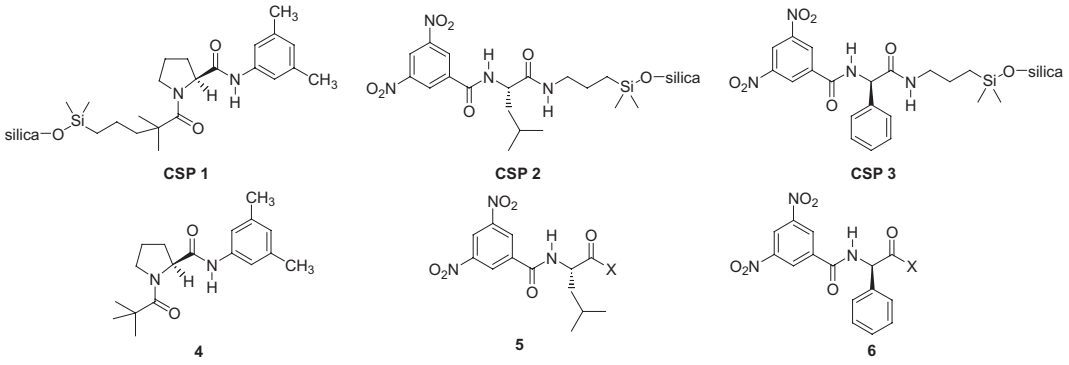
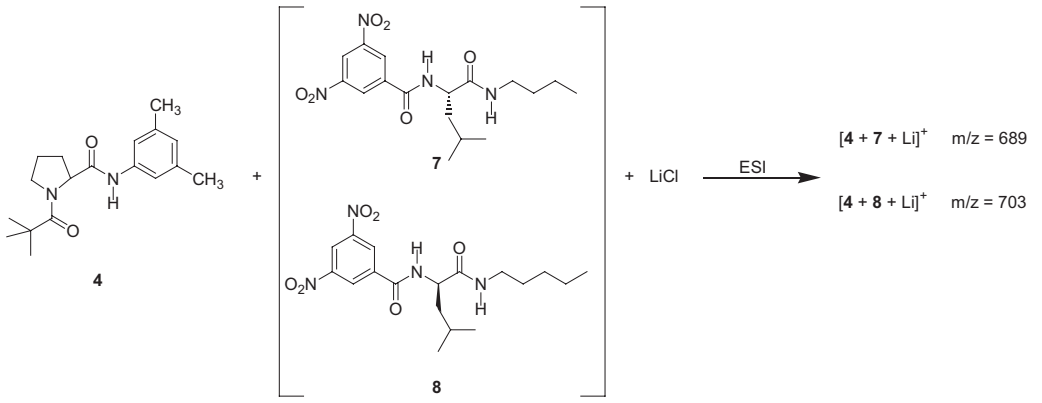



**Chart 1.**




**Scheme 1.**

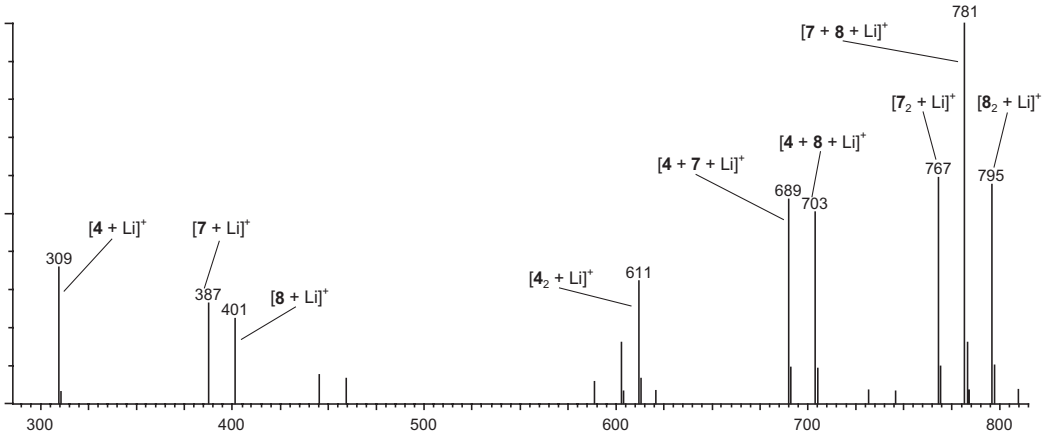



**Figure 1.** Mass spectrum of a solution of pseudo-enantiomeric selectors **7** and **8** (2.5 mM) and analyte **4** (0.25 mM) with added lithium chloride (25 mM) in methanol/water/acetone (1:1:2).

meric composition of analyte **4**. Figure 2 shows the relevant portion of the mass spectrum for solutions highly enriched in both enantiomers of analyte **4**, as well as the racemate. As can be seen from the data, the relative intensities of the selector–analyte peaks are different, and the sense of chiral recognition is what would be predicted from the chromatographic data. When the sample is enriched in the (*S*)-enantiomer of analyte **4**, the larger peak in the mass spectrum is the complex between the (*S*)-selector and the analyte, while when enriched with the (*R*)-analyte, the complex with the (*R*)-selector is larger. The other peaks in the spectrum remained relatively invariant with enantiomeric compositions of the sample.

It was expected that the relative intensities of the selector–analyte peaks in the mass spectrum should readily be related to the enantiomeric composition of the sample. If a selectivity factor, $\alpha$, is defined as the relative affinity of an analyte enantiomer for the two pseudo-enantiomeric chiral selectors, then the relative peak intensities observed in the mass spectrum should equal this value for the spectrum obtained using the pure analyte enantiomer (assuming that the ionization efficiencies for the two complexes are equal and that the concentrations of the chiral selectors are equal). The relative peak intensities for the opposite analyte enantiomer would

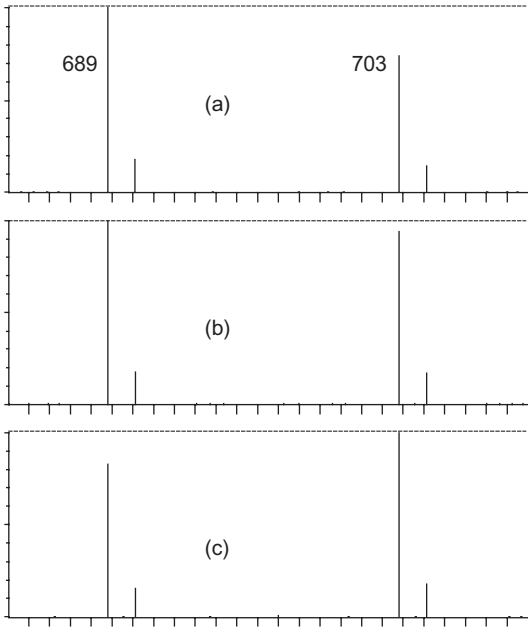



**Figure 2.** Partial mass spectra of pseudo-enantiomeric selectors (*S*)-**7** and (*R*)-**8** (2.5 mM) and analyte **4** (0.25 mM) with added lithium chloride (25 mM) in methanol/water/acetone (1:1:2). Spectrum: (a) 9.1% (*R*)-**4**, 90.9% (*S*)-**4**; (b) racemic **4**; (c) 89.8% (*R*)-**4**, 10.2% (*S*)-**4**.

then be equal to the reciprocal of this selectivity factor (given that the pseudo-enantiomeric selectors behave as enantiomers), and the ratio of peak intensities for the racemate should be unity. These three points [i.e. (mole fraction (*R*)-analyte enantiomer, relative peak intensity): $(0, \alpha^{-1})$, $(0.5, \alpha^{0})$, $(1, \alpha^{1})$] define a linear semi-log relationship between the relative peak intensities of the complexes and the enantiomeric composition of the sample, where the slope equals twice the natural log of the selectivity factor ($2 \ln \alpha$). Any differences in ionization efficiencies of the complexes, or concentrations of selectors would manifest itself in the intercept of the semi-log plot.

A plot of the natural log of the ratio of the intensities of the peaks at *m*/*z* 703 and 689 versus the mole fraction of the (*R*)-**4** in the sample (as determined by chiral HPLC) for 11 different enantiomeric compositions is presented in Figure 3. The plot indeed affords a straight line with a correlation coefficient of 0.997. Given the linearity of this plot, this provides a simple method for determining the enantiomeric composition of the analyte, provided that care is taken to ensure that the ratio of pseudo-enantiomeric chiral selectors remains the same. This was accomplished by using a stock solution of the chiral selectors. An aliquot of the chiral selector stock solution can then be added to the required amount of analyte in order to prepare the solution for mass spectral analysis. One then merely needs to acquire the mass spectrum, determine the ratio of peaks at *m*/*z* 703 and 689, and use the calibration curve to determine the enantiomeric composition of the analyte.

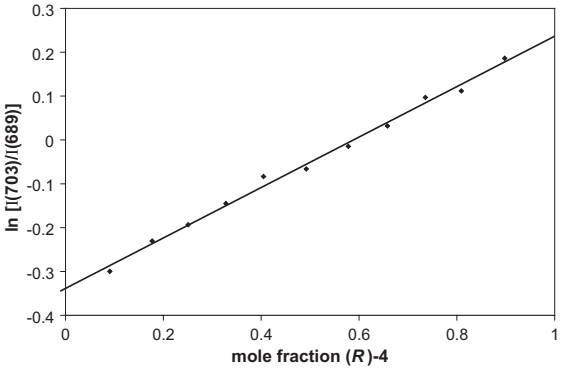



**Figure 3.** Plot of the natural log of the ratio of peaks at *m*/*z* 703 and 689 in the ESI-MS versus the mole fraction of (*R*)-**4** in the solution, using pseudo-enantiomeric chiral selectors **7** and **8** (slope = 0.575, intercept = −0.339, $r^2 = 0.997$).

These data were further analyzed by using the leave-one-out cross validation technique. In this method, one data point is removed from the analysis and the remaining data are fit to a calibration line, which is then used to determine the enantiomeric composition of the sample that was not used as part of the calibration. The results of this analysis for all 11 samples are shown in Table 1. As can be seen from Table 1, the enantiomeric composition determinations by MS are in good agreement with the values obtained by chiral chromatography. The average absolute difference between these measurements is 2.2 with a standard deviation of 1.2.

**Table 1.** Enantiomeric composition of analyte **4** by mass spectrometry using chiral selectors **7** and **8** compared to the enantiomeric composition as measured by chiral chromatography

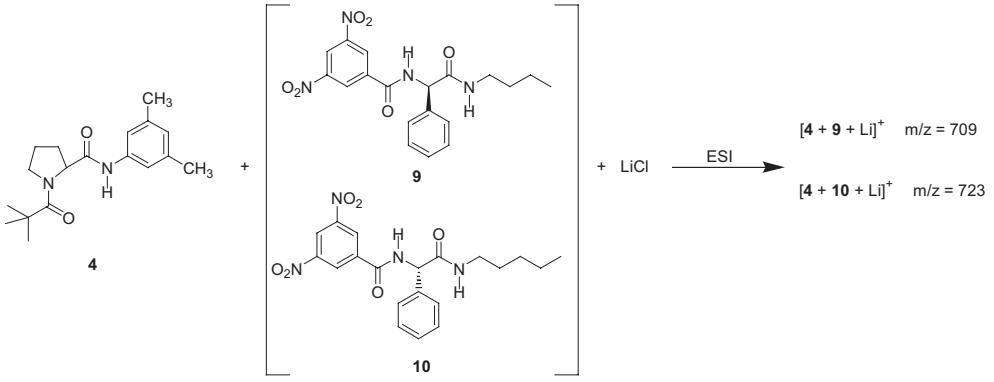
| % (*R*)-**4** by HPLC | % (*R*)-**4** by MS | Difference |
|---|---|---|
| 89.8 | 92.0 | 2.2 |
| 80.9 | 77.5 | −3.4 |
| 73.6 | 76.2 | 2.6 |
| 65.8 | 64.2 | −1.6 |
| 57.8 | 56.2 | −1.6 |
| 49.2 | 47.2 | −2.0 |
| 40.5 | 44.8 | 4.3 |
| 32.8 | 33.8 | 1.0 |
| 25.1 | 25.3 | 0.2 |
| 17.7 | 19.1 | 1.4 |
| 9.1 | 5.6 | −3.5 |

Nine replicate samples were analyzed at various enantiomeric compositions of (*R*)-**4** (as determined by HPLC) and applied to the calibration curve. Determination of the ratio of the intensities of the peaks at *m*/*z* 703 and 689 for samples with a composition of 89.8%, 49.2% and 9.1% (*R*)-**4** yields values of 90.6 ± 5%, 48.6 ± 5% and 7.5 ± 5%, respectively, at the 95% confidence limit.

Similarly, analogues of DNB-phenylglycine (CSP 3) were prepared, and the ability of a pseudo-enantiomeric mixture of chiral selectors of this type was used to determine whether chiral recognition would be observed with analyte **4** (Scheme 2). The ESI-mass spectrum of a solution of the butyl amide of (*R*)-DNB-phenylglycine **9** and the pentyl amide of (*S*)-DNB-phenylglycine **10**, with added lithium chloride, afforded the monomeric and dimeric species as before with the DNB-leucine analogues.

The dimeric ions between each pseudo-enantiomer of DNB-phenylglycine and analyte **4** were observed to vary regularly with the enantiomeric composition of analyte **4** (Fig. 4). As the amount of (*R*)-**4** was increased in the sample, the relative intensity of the peak at *m*/*z* 709 increased (with respect to the peak at *m*/*z* 723). Likewise, as the amount of (*S*)-**4** in the sample increased, the relative intensity of the peak at *m*/*z* 723 increased (with respect to the peak at *m*/*z* 709). This clearly demonstrates that the sense of chiral recognition in these samples is the same as is observed chromatographically. The (*R*)-selector has a higher affinity for (*R*)-**4**, therefore when the sample is enriched in (*R*)-**4**, the intensity of the (*R*)-**9** + (*R*)-**4** ion (*m*/*z* 709) in the mass spectrum will be increased. Likewise, an increase in (*S*)-**4** would increase the intensity of the (*S*)-**10** + (*S*)-**4** ion (*m*/*z* 723).

A plot of the natural log of the intensities of the selector–analyte complex ions in the mass spectrum versus the enantiomeric composition of analyte **4** afforded a straight line with a correlation coefficient of 0.998




**Scheme 2.**

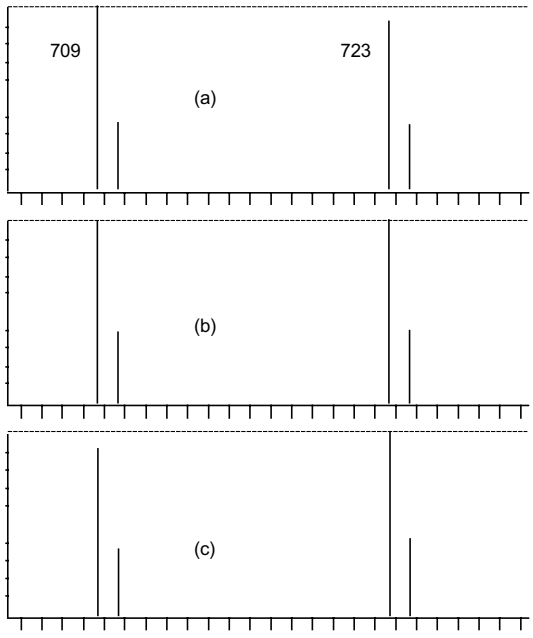



**Figure 4.** Partial mass spectra of pseudo-enantiomeric selectors (*R*)-**9** and (*S*)-**10** (1.0 mM) and analyte **4** (0.10 mM) with added lithium chloride (10 mM) in methanol/water/tetrahydrofuran (2:1:2). Spectrum: (a) 89.8% (*R*)-**4**, 10.2% (*S*)-**4**; (b) racemic **4**; (c) 8.9% (*R*)-**4**, 91.1% (*S*)-**4**.

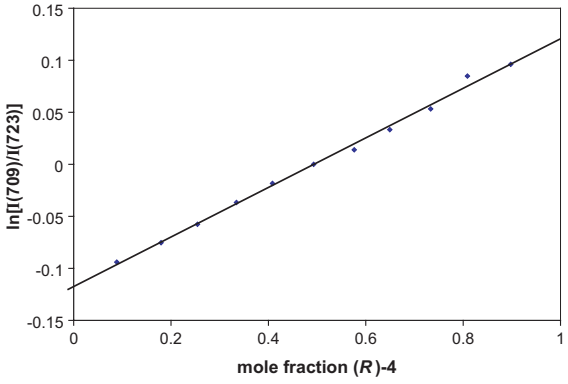



**Figure 5.** Plot of the natural log of the ratio of peaks at *m*/*z* 709 and 723 in the ESI-MS versus the mole fraction of (*R*)-**4** in the solution, using pseudo-enantiomeric chiral selectors **9** and **10** (slope = 0.238, intercept = −0.117, $r^2 = 0.998$).

(Fig. 5). As before, this demonstrates the potential use of this type of pseudo-enantiomeric chiral selectors for enantiomeric composition determinations by MS.

These data were also analyzed using the leave-one-out cross validation techniques. The results of this analysis for all 11 samples are presented in Table 2. As can be seen from Table 2, the enantiomeric composition determinations by MS are in good agreement with the values obtained by chiral chromatography. The average absolute difference between these measurements is 1.4 with a standard deviation of 1.6.

These initial findings clearly demonstrate that chiral recognition is observed in the ESI-mass spectrum using pseudo-enantiomeric chiral selectors. The ratio of peaks corresponding to the selector–analyte complexes in the mass spectrum at *m*/*z* 689 and 703 using chiral selectors **7** and **8** with analyte (*S*)-**4** is 0.71, and with analyte (*R*)-**4** is 1.27. The overall selectivity, given by the ratio of peak ratios for pure (*R*)-**4** and for pure (*S*)-**4** is 1.78. Likewise, for pseudo-enantiomeric chiral selectors **9** and **10**, the ratio of peaks corresponding to the selector–analyte complexes in the mass spectrum at *m*/*z* 709 and 723 for analyte (*S*)-**4** is 1.13, and for analyte (*R*)-**4** is 0.89, giving a selectivity (ratio of ratios) of 1.27. The observed selectivity of the DNB-leucine derived chiral selectors is higher than the selectivity observed for the DNB-phenylglycine derived chiral selectors, as demonstrated by

**Table 2.** Enantiomeric composition of analyte **4** by mass spectrometry using chiral selectors **9** and **10** compared to the enantiomeric composition as measured by chiral chromatography.

| % (*R*)-**4** by HPLC | % (*R*)-**4** by MS | Difference |
|---|---|---|
| 89.8 | 89.6 | −0.2 |
| 80.9 | 86.3 | 5.4 |
| 73.3 | 71.3 | −2.0 |
| 64.9 | 63.1 | −1.8 |
| 57.6 | 54.9 | −2.7 |
| 49.3 | 49.3 | 0.0 |
| 40.8 | 41.7 | 0.9 |
| 33.4 | 34.0 | 0.6 |
| 25.4 | 25.1 | −0.3 |
| 17.9 | 17.6 | −0.3 |
| 8.9 | 10.3 | 1.4 |

the relative peak ratios afforded for both enantiomers of analyte **4**, which is consistent with the relative slopes of the calibration curves shown in Figures 3 and 5, even though the chromatographic separation factors for the enantiomers of analyte **4** are very similar on both CSPs **2** and **3**. This can likely be attributed to a number of factors, each of which will be thoroughly investigated in subsequent studies. First, the concentrations of the chiral selectors were different (**7**/**8** 2.5 mM; **9**/**10** 1.0 mM), although the ratio of selector to analyte was maintained for each (10:1). Additionally, the solvent used for each system was different, mainly for solubility reasons. Also, it is unclear at this point as to the role that $Li^+$ plays in affecting chiral recognition between chiral selector and analyte, although $Li^+$ is definitely required for efficient ionization. The primary interactions between these chiral selectors and analyte **4** which are responsible for chiral recognition, as has been demonstrated through a number of studies,[29–31] are (1) a π-stacking interaction between the π-acidic dinitro aromatic ring of the selector and the π-basic dimethylanilide ring of the analyte; (2) a hydrogen bond between the DNB amide proton of the selector and the pivaloyl carbonyl of the analyte; and (3) a hydrogen bond between the C-terminal carbonyl of the selector and the anilide proton of the analyte. Given that the chromatographic separation factors are larger than the selectivities observed by ESI-MS, it is likely that the presence of $Li^+$ in solution may actually interfere with some of these requisite interactions, particularly the hydrogen bonding interactions. Further experiments that compare the selectivity of the selector–analyte complex in solution versus what is observed by ESI-MS are needed to determine the effect of $Li^+$ on chiral recognition. Additionally, the design, synthesis and evaluation of chiral selectors that incorporate functional groups specifically for ionization remote from the requisite chiral interaction sites are currently underway.

## 3. Conclusion

The adaptation of two chromatographically derived chiral recognition systems to mass spectrometric chiral recognition has been demonstrated. Given that mass spectrometry is well suited for rapid analysis, this method has the potential to allow high throughput enantiomeric composition determinations. Such a high throughput method would be an invaluable tool for the evaluation of potential chiral catalysts that were prepared combinatorially, and for the discovery of new chiral selectors, particularly for the screening of combinatorial libraries of potential chiral selectors. It is envisioned that the high throughput screening of combinatorial libraries of potential chiral selectors could quickly be effected for any analyte of interest. Discovery of high selectivity chiral selectors can then not only be used for mass spectrometric enantiomeric composition determinations by this method, but also be adapted to provide optimized chiral stationary phases for preparative enantiomer separations.[34–38] Further work in this area will be directed towards developing high throughput enantiomer analyses, and applying these to the applications discussed herein.

## 4. Experimental

### 4.1. Materials

The chiral stationary phases, CSP **2** and CSP **3**, analyte **4** and chiral selectors **7**, **8**, **9** and **10**, were prepared by literature methods.[29–33] All solvents were HPLC grade and used without further purification.

### 4.2. Mass spectrometry

Eleven stock solutions of analyte **4** were prepared by mixing differing amounts of (*R*)-**4** and (*S*)-**4** to afford the desired enantiomeric composition. The enantiomeric composition of each stock solution was also measured by HPLC (vide infra). Stock solutions of pseudo-enantiomeric selectors **7** and **8**, selectors **9** and **10**, and lithium chloride were also prepared. Metered amounts of stock solutions of the chiral selectors, the analyte and lithium chloride were combined and diluted to afford a final concentration of 2.5 mM for chiral selectors **7** and **8**, 0.25 mM for analyte **4** and 25 mM for lithium chloride in methanol/water/acetone (1:1:2). Likewise, solutions with a final concentration of 1.0 mM for chiral selectors **9** and **10**, 0.1 mM for analyte **4** and 10 mM for lithium chloride in methanol/water/tetrahydrofuran (2:1:2) were prepared.

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 infused with a syringe pump into the ESI source at a rate of 5 μL/min for the solutions with selectors **7** and **8**, and 8 μL/min for the solutions with selectors **9** and **10**. Spectrometer conditions were as follows: capillary voltage, 3.5 kV; cone voltage, 15 V; extractor voltage, 1.0 V; RF lens, 0.5 V; source temperature, 80 °C; desolvation temperature, 350 °C; cone gas flow, 70 L/h; desolvation gas flow, 757 L/h. For each experiment, data were collected for approximately 2 min, each full scan requiring ~0.7 s, with all the scans averaged to afford the final spectrum.

### 4.3. HPLC

The analyte solutions were assayed on (*S*)-CSP **2** and (*R*)-CSP **3**. HPLC conditions are as follows: mobile phase, 10% 2-propanol/90% hexanes; flow rate, 2 mL/min; column dimensions, 4.6× 250 mm. Elution orders were determined by injection of (*R*)-**4** and (*S*)-**4**, which were synthesized beginning with D-proline and L-proline, respectively. Chromatography of **4** on (*S*)-CSP **2**: $k_1 = 6.03$; $\alpha = 3.41$; more retained enantiomer = (*S*). Chromatography of **4** on (*R*)-CSP **3**: $k_1 = 2.52$; $\alpha = 3.74$; more retained enantiomer = (*R*).

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