

DIOS-MSEED: A Chip-Based Method for Measurement of Enantiomeric Excess by Kinetic Resolution/Mass Spectrometry

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Abstract. The determination of enantiomeric excess by kinetic resolution mass spectrometry has been implemented with the Desorption/Ionization On Silicon (DIOS) MS technique. Measurements can thereby be made much more rapidly than was previously possible, bringing this general methodology for screening asymmetric catalysts closer to true high-throughput status.

The measurement of enantiomeric excess (ee) of large numbers of samples is crucial to the discovery and/or refinement of asymmetric reactions by combinatorial means.^{1,2} The current state of the art encompasses only two methods for parallel analysis of many reaction mixtures at the same time: Capillary electrophoresis³ and array detection of dye-labeled samples by in situ kinetic resolution.⁴ The analysis of samples one at a time is presently most rapidly accomplished by HPLC with circular dichroism detection.⁵

In spite of the development of such methods, most combinatorial investigations of asymmetric catalysts or enantioselective organic reactions still rely upon the use of chiral stationary phases in HPLC and GC, which set the standard for accuracy, precision, and generality.^{6,7} These methods are often slow, requiring from 5 to 30 minutes or more per sample per instrument. Furthermore, a substantial amount of optimization of variables (i.e., elution solvents, gradients, flow rate) is often required to analyze new structures, and occasionally one finds organic structures that are not handled well by the standard commercially-available columns. The parallel or "high-throughput" methods thus far developed suffer from an opposite set of difficulties, being in general

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restricted to only a few types of samples, often requiring labeling with a chromophore.

We have focused on mass spectrometry (MS) as the basic technology on which to build a general method for the rapid determination of enantiomeric excess.^{8,9} MS provides the outstanding advantages of doing away with the need for a chromophoric tag and, often, the need for sample purification, since all other peaks except those for the masses of interest may be ignored. We report here the application of our Mass Spectrometry Enantiomeric Excess Determination (MSEED) method to the chip-based Desorption/Ionization On Silicon (DIOS) technique,¹⁰ giving rise to an improved method for general measurement of the enantiomeric content of organic compounds by mass spectrometry. Our approach relies upon the covalent derivatization of the analyte of interest with a mass-tagged chiral reagent prior to MS analysis, such that a very modest level of kinetic resolution is obtained. Such a step may be applied to an enormous range of structural types. Many other investigators, starting with Fales and Wright in 1977,¹¹ have also explored the detection of chirality by mass spectrometry, engineering the necessary diastereomeric interactions in different ways.^{12–21}

MSEED has thus far been implemented on electro-

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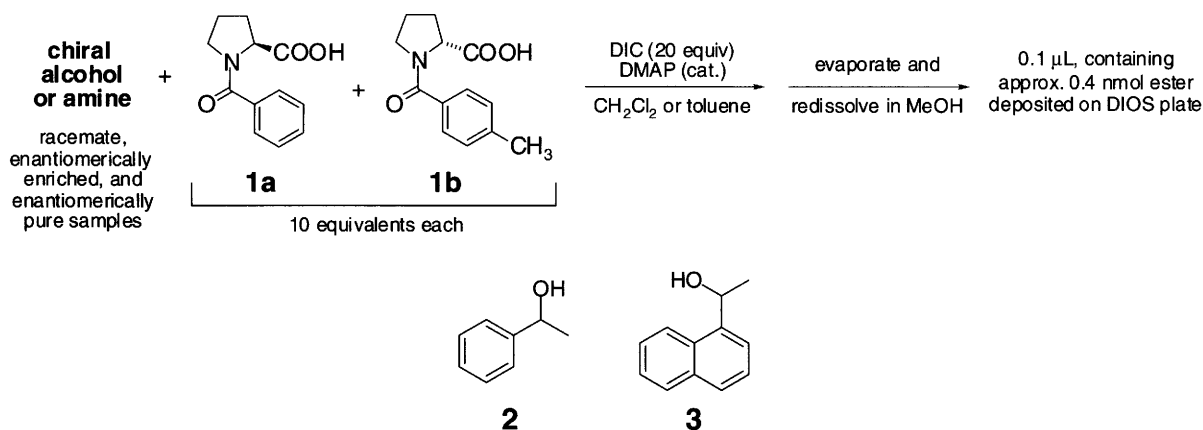
spray ionization (ESI) mass spectrometers, usually accessed by direct injection on an HPLC-MS instrument, bypassing the HPLC. Approximately 70 s is required for each injection to allow the material to clear the flow path of the instrument, and we typically average 3 injections for each sample to obtain the necessary data. To insure low background and no carryover from previous samples, the MS system is injected with solvent blanks. Taking into consideration the blank injections, the average rate of ee determination using electrospray MS is approximately 4 min per sample. Although it is conceivable to perform these analyses with an array of electrospray ionization sources,²² the practical limitation of this approach is approximately one sample every 30 s.

DIOS uses commercially available MALDI (Matrix-Assisted Laser Desorption/Ionization) instrumentation. The primary difference between DIOS and MALDI is that the former utilizes a porous silicon wafer on which the sample is deposited and eliminates the need for "matrix" material; therefore, DIOS can also detect small molecules (<700 Da) without matrix interference.^{23,24} The DIOS sample plates can be manufactured to include photopatterned images of the porous silicon, providing an array of active positions on the wafer; our standard ~6 cm² DIOS chip contains 100 such spots. Samples up to 1 µL in volume can be deposited at each position. After solvent evaporation, the entire chip can be scanned by rastering the laser with computer-controlled data acquisition, resulting in an analysis rate of 4–6 s per position. This system has been implemented for drug screening;²⁵ here we report its use in the determination of enantiomeric excess of an array of samples.

The MSEED technique for alcohols and amines relies upon the acylation of the compound of interest with

a mass-tagged mixture of acids, such that a modest level of kinetic resolution is achieved (Scheme 1).^{8,9} With calibration obtained from racemic and enantiopure samples of the substrate, the enantiomeric excess may be determined by measuring the relative amounts of the two esters or amides produced in the process. The method was applied to alcohols **2** and **3**, each prepared in 90%, 70%, 50%, and 20% enantiomeric excess, using mass-tagged acids **1a/1b** and a standard carbodiimide coupling method, as previously reported.⁸

To probe whether the DIOS technique allows for relative quantitation to a sufficient level of precision, we subjected the resulting reaction mixtures to ESI analysis in our normal fashion,⁸ and to DIOS analysis by the deposition of 0.5 µL samples in methanol to a porous silicon plate, followed by evaporation and DIOS-MS.^{26,27} ESI results were identical, within experimental error, to those previously reported;⁸ the figures obtained by DIOS-MS, shown in Table 1, reveal an equivalent outcome. Figure 1 shows a representative DIOS spectrum of a crude acylation reaction mixture. The presence of many peaks, reflecting the composition of the sample mixture, highlights an important virtue of the MSEED technique: No purification is necessary, since the masses of interest can usually be picked out (as shown in the inset) and quantified with sufficient precision regardless of other species that may be present. For DIOS analysis, between 20 and 128 laser pulses are averaged to obtain a spectrum, requiring 4–26 s. The results shown in Table 1 and Fig. 1 were obtained by averaging five such spectra for each sample, although we have subsequently found that one spectrum per sample provides almost the same level of precision. Throughout the data acquisition, the laser moves slightly over the sample spot, constantly exposing fresh



Scheme 1

Table 1. DIOS-MS analysis of samples of alcohols **2** and **3**, derivatized with mass tags **1a/1b**

actual % ee	20	50	70	90
measured ee for 2	24 ± 3	46 ± 5	72 ± 6	99 ± 9
measured ee for 3	20 ± 5	49 ± 6	71 ± 4	97 ± 9

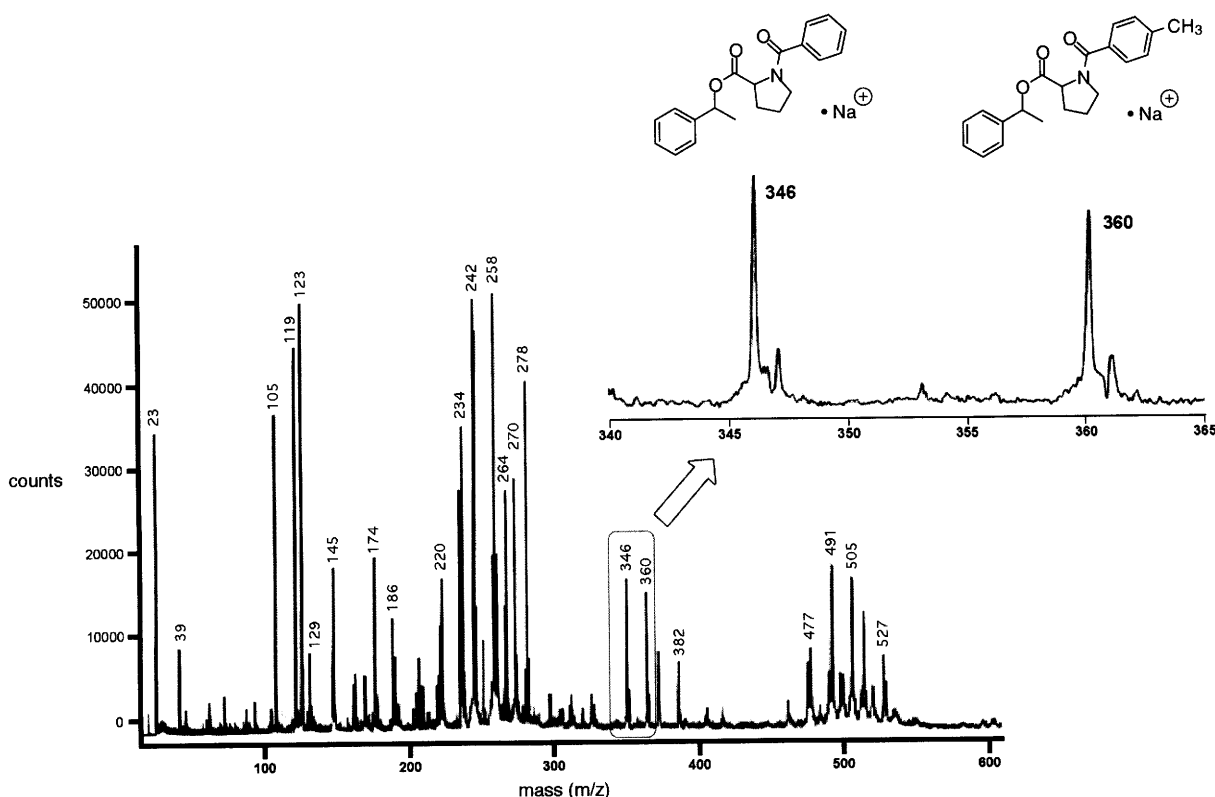


Fig. 1. DIOS-MS spectrum of the reaction of **2** with **1a/1b**. The magnified portion of the spectrum (*inset, upper right*) shows the two peaks of interest.

material to the mass spectrometer. The most severe limitation on the speed of DIOS analysis is presently imposed by the data acquisition rate, which will be increased to better than one spectrum per second with improvements in spectrometer hardware and software.

The DIOS technique is more tolerant than electrospray ionization of buffer components, impurities, and other potential contaminants, and gives equivalent resolution and sensitivity. Its automated nature and potentially rapid rate of data acquisition make it the best vehicle for implementation of the MSEED protocol for the analysis of enantiomeric composition, and for the high-throughput analysis of large numbers of com-

pounds in general. We are presently employing these methods for the combinatorial screening of asymmetric catalysts.

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 - (26) The laser desorption/ionization measurements were performed in a PerSeptive Biosystems (Framingham, MA) Voyager STR time-of-flight reflectron mass spectrometer with delayed extraction. The DIOS chips were attached to the MALDI target plates using conductive carbon tape. Samples were irradiated with a nitrogen laser operated at 337 nm, 5 Hz and attenuated with a neutral density filter. Ions produced by laser desorption were energetically stabilized during a delayed extraction period of 150 ns and then accelerated through the linear time-of-flight mass analyzer by a 20-kV potential pulse.
 - (27) DIOS chips were prepared by electrochemical etching of N-type <100> silicon wafers (0.005–0.02 W-cm resistivity) in a 25% HF/EtOH solution under white light illumination for 1 min at a constant current density of 4 mA/cm². A detailed description of the etching procedure is given in reference 23. The resulting porous silicon surfaces were oxidized by ozone and then treated with 5% aqueous HF. Photopatterning of the DIOS chips makes possible the deposition and analysis of many different samples on the same plate. Irradiation through a simple mask during etching allows for effective photopatterning of DIOS-active spots, since the etching rate on n-type silicon is strongly dependent on light intensity. In order to create patterns on the DIOS surface, the light from a fiber optic light source was passed through a printed mask and two achromatic lenses and then focused on the silicon surface. This simple procedure reproducibly produces sharply defined porous silicon spots.