DOI: 10.1002/chem.200902650

A Facile Circular Dichroism Protocol for Rapid Determination of Enantiomeric Excess and Concentration of Chiral Primary Amines

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Abstract: A protocol for the rapid determination of the absolute configuration and enantiomeric excess (*ee*) of α-chiral primary amines with potential applications in asymmetric reaction discovery has been developed. The protocol requires derivatization of α-chiral primary amines through condensation with pyridine carboxaldehyde to quantitatively yield the corresponding imine. The Cu^{I} complex with 2,2′-bis (diphenylphosphino)-1,1′-dinaphthyl (BINAP–Cu^{I}) with the imine yields a metal-to-ligand charge-transfer (MLCT) band in the visible region of the circular dichroism (CD) spectrum upon binding. Diastereomeric host–guest complexes give CD signals of the same signs but different amplitudes, allowing for differentiation of enantiomers. Processing the primary optical data from the CD spectrum with linear discriminant analysis (LDA) allows for the determination of the absolute configuration and identification of the amines, and processing with a supervised multilayer perceptron artificial neural network (MLP-ANN) allows for the simultaneous determination of the *ee* and concentration. The primary optical data necessary to determine the *ee* of unknown samples is obtained in two minutes per sample. To demonstrate the utility of the protocol in asymmetric reaction discovery, the *ee* values and concentrations for an asymmetric metal-catalyzed reaction are determined. The potential of the application of this protocol in high-throughput screening (HTS) of *ee* is discussed.

Keywords: amines · artificial neural network · circular dichroism · enantiomeric excess · high-throughput screening

Introduction

Asymmetric catalysis is a commonly employed method for the synthesis of enantiomerically enriched compounds.[1] Traditionally, asymmetric catalysts are developed from a combination of intuition, knowledge of the reaction mechanism, molecular modeling, and trial and error, which are time-consuming processes. Combining traditional techniques with those used in the screening of large libraries of potential asymmetric catalysts could potentially accelerate the discovery process.[2] However, this requires the rapid determination of enantiomeric excess (*ee*), which is difficult with chromatographic techniques such as HPLC and GC.[3] A variety of methods are currently being developed to overcome this problem.[4]

For instance, optical techniques for the determination of *ee* have emerged as a powerful approach to high-throughput screening (HTS) because they are fast, simple, inexpensive, and easily adaptable to determine concentration.[5] For example, our group and others have exploited the use of optical techniques based on enantioselective indicator-displacement assays (*e*IDAs).[6]

Recently, we reported a simple protocol based on the combination of circular dichroism (CD) spectroscopy[7] and pattern-recognition-based techniques.[8] The protocol is based on monitoring changes in the metal-to-ligand charge-transfer (MLCT) bands of chiral and racemic metal complexes upon the addition of chiral diamine analytes. This technique allows for rapid screening of *ee* and the determination of concentration.

α-Chiral primary amines are important building blocks in chemical and pharmaceutical transformations.[9] Several methods for the asymmetric synthesis of chiral amines are under development.[10] Rapid screening of asymmetric reactions might hasten the discovery of an efficient route to these versatile building blocks. Thus, a method for the rapid screening of *ee* for α-chiral primary amines is desirable.[11]

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.200902650.

Herein, we describe a protocol for the rapid screening of *ee* of α-chiral primary amines. The protocol utilitizes the receptors (*R*)- and (*S*)-[Cu^{I}(BINAP)(NCMe)$_2$]PF$_6$ (**1**), in which BINAP is 2,2′-bis (diphenylphosphino)-1,1′-dinaphthyl (See Figure 1 a). Both (*R*)- and (*S*)-**1** complexes show

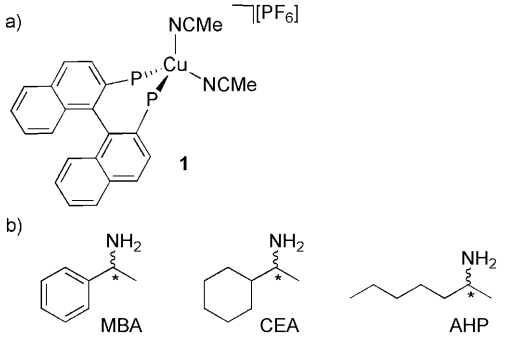


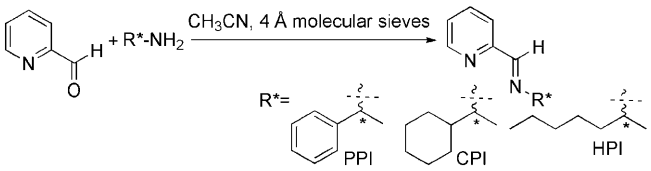
Figure 1. a) (*R*)- and (*S*)-**1** complexes employed as receptors; b) Chiral primary amines employed as analytes: a-methyl-benzylamine (MBA), 1-cyclohexyl-ethylamine (CEA), 2-aminoheptane (AHP).

MLCT bands around 340 nm in the CD spectra, and the changes in these MLCT bands after the addition of enantiomerically enriched chiral guests give a method for enantiodiscrimination and concentration determination of the chiral monoamines (shown in Figure 1 b). To test the practicality of the protocol, a sample from an asymmetric reaction was prepared. The *ee* of the sample was determined by the CD protocol and was compared with values obtained from the well-accepted ^{1}H NMR protocol developed by James and co-workers,[11a,b] showing that the CD protocol allows for rapid screening with comparable accuracy to standard iterative protocols.

Results and Discussion

Design: For the protocol to be of use in rapid screening, a system in which the analyte (chiral amine) does not have to be derivatized is preferable. Derivatization is time consuming and frequently requires purification before and after derivatization. Additionally, either a commercially available receptor or one that required only a one–two step synthesis was sought because this would increase its usefulness to the general chemistry community. Unfortunately, there was no signal modulation through addition of an underivatized chiral amine to (*R*)-**1**. Thus, we developed a method for derivatization that is fast and does not require purification. This minimizes the amount of time required for the derivatization step and still allows the protocol to be amenable to rapid *ee* determination.

Detection scheme: As previously mentioned, signal modulation of the receptor was not possible upon the addition of underivatized chiral amines. To overcome this problem, the chiral amine was condensed with 2-pyridine carboxaldehyde to form the corresponding chiral Schiff base in situ (Scheme 1). The overall reaction was fully complete in under two hours and resulted in quantitative formation of the imine. The imines were directly used without purification.[12]



Scheme 1. Derivatization of the amines to form the corresponding Schiff bases.

The addition of the chiral imines to a solution of receptor (*R*)-**1** modulated the signal in the CD spectrum spanning from approximately 320 nm to 470 nm (Figure 2). No CD

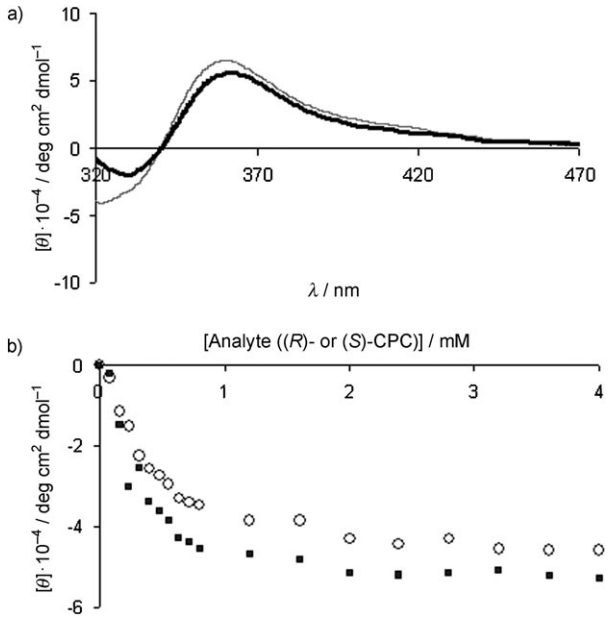


Figure 2. a) CD spectrum for (*R*)-**1**[0.4 mM] and the enantiomers of CPI [0.8 mM] (——=(*R*)-CPL, ——=(*S*)-CPL);(b) Titration of (*R*)-**1**[0.4 mM] with (*R*)- and (*S*)-CPI in acetonitrile at 354 nm. (○=(*R*)-CPL, ●=(*S*)-CPL).

signals above 320 nm were observed for the chiral imines, for copper alone with the imines, or for free BINAP. Thus, the signal is the result of a MLCT in the coordination complex of the imines with (*R*)-**1**.

Identification of amine and enantiodiscrimination: The absolute configuration and the identity of the amines can be determined by analyzing the primary optical data through linear discriminant analysis (LDA).[13] Titration of (*R*)-**1** with the imines showed that saturation was reached at two equivalents (see Figure 2 b). For this system, saturation gives

the maximum signal-to-noise ratio, and is thus best suited for data collection and analysis; hence, all data was collected at two equivalents. Each experiment was repeated five times to ensure reproducibility. The ellipticities were recorded at four different wavelengths (350, 355, 360, and 380 nm) and analyzed with LDA. The selected wavelengths were chosen around the positive maximum presented in the Cotton effect to try to obtain all of the possible information from the spectra. The use of more wavelengths does not give any improvement to the resulting LDA plot. The analytes showed good separation in the LDA plot (Figure 3). Positive

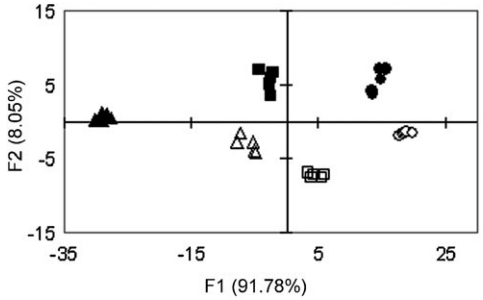


Figure 3. LDA plot of receptor (*R*)-**1**[0.4 mM] with all the analytes [0.8 mM] (■ = (*R*)-CPI, ● = (*R*)-HPI, ▲ = (*R*)-PPI, □ = (*S*)-CPI, ○ = (*S*)-HPI, △ = (*S*)-PPI).

scores along F2 were found for the *R* amines and negative scores for the *S* amines. As expected, the opposite behavior is observed for receptor (*S*)-**1** (see the Supporting Information). Jackknife analysis led to 100% identification and enantioselective discrimination of the chiral primary amines.[14] Thus, this method permits the identification and determination of handedness of chiral primary amines.

Quantitative determination of *ee* and concentration: The next goal was the quantitative determination of *ee* and concentration for the chiral guest CEA by using the CD data and an MLP-ANN.[15] All optical data was collected by using a CD spectrometer and a robotically controlled 96-well plate interface (ASU-605-JACSO, UK).[16]

Because a supervised MLP-ANN was used, a training set was necessary. The training set consisted of the ellipticities from $[\theta]_{340}$ to $[\theta]_{400}$ at 1 nm intervals for 0.4 mM of receptor (*R*)-**1** in acetonitrile, charged with three different concentrations (0.2 mM, 0.8 mM, and 1.4 mM) of CPI (the imine derivative of CEA), along with 11 *ee* values for each concentration (1, 0.8, 0.6, 0.4, 0.2, 0, −0.2, −0.4, −0.6, −0.8, and −1), giving 33 solutions as the training set.

First, this training set was analyzed by using principle component analysis (PCA)[13] to demonstrate that simultaneous *ee* and concentration determination is feasible (Figure 4). A slightly rotated axis shows concentration along PC1′ and *ee* along PC2′. This PCA plot was performed to display the ANN training data simply in two dimensional space, and not to show reproducibility with multiple replicates. The three strips parallel to PC2′ span the *ee* values

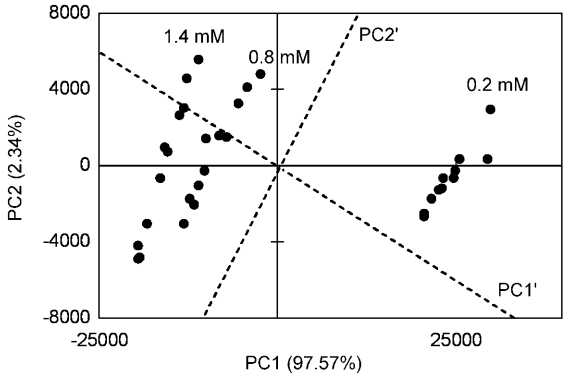


Figure 4. Two-dimensional PCA plot of CPI with three *ee* trainings sets at three different $[G]_t$ values: 0.2 mM, 0.8 mM and 1.4 mM.

from −1 to 1 in the eleven values listed above. The relative weights of the PC1 and PC2 axes clearly show that this system is much more responsive to concentration than *ee* values. Yet, as described below, the errors found for the *ee* values are still acceptable.

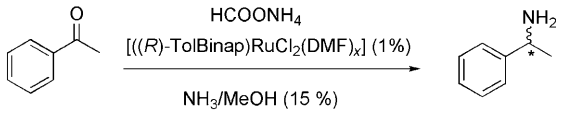
A MLP-ANN was trained with the ellipticities found for the 33 samples of varying concentrations and *ee* values. The number of hidden layers was varied until the MLP-ANN could accurately generate the outputs from the optical inputs. An MLP-ANN with 11 hidden layers gave the best results

To assess the generality of the function developed by the MLP-ANN, the ellipticities from $[\theta]_{340}$ to $[\theta]_{400}$ for six unknown samples, independent of the training set, were collected. These ellipticities were used as inputs in the MLP-ANN, and its ability to generate the correct *ee* values and concentrations was tested. The MLP-ANN calculates the concentration $[G]_t$ with an average error of 14.7% and calculates the *ee* with an average error of 11.7%. The results are summarized in Table 1.

Table 1. Determination of concentration and % *ee* of CEA test samples by using ANN.

Samples	1	2	3	4	5	6
$[G]_t$ [mM]	0.80	1.40	1.00	0.50	0.30	1.20
$[G]_t$ (ANN) [mM]	0.77	1.47	1.13	0.24	0.07	1.36
% *ee*	80	60	−40	0	−20	−60
% *ee* (ANN)	94	78	−48	8	10	−72

Analyzing an asymmetric reaction: The applicability of this technique in the screening of asymmetric reactions was demonstrated. To show the generality of the method, a different amine than CEA was analyzed. Asymmetric synthesis of α-methylbenzylamine (MBA) was done by reacting acetophenone and ammonium formate in the presence of a chiral ruthenium catalyst according to a literature procedure (see Scheme 2).[10b] The crude sample was identified as α-methylbenzylamine by ^{1}H NMR and ^{13}C NMR spectroscopy. The enantiopurity of the sample was first determined by the NMR protocol used by James and co-workers,[11a,b] giving a



Scheme 2. Asymmetric reaction used to synthesize a sample of MBA of unknown *ee*.

value of 72% of the *R* enantiomer (44% *ee*, see the Supporting Information).

Next, the *ee* was also determined by using the protocol reported here. A training set was loaded onto a 96-well plate consisting of PPI (the imine derivative of MBA) at three different concentrations (0.2, 0.8, and 1.4 mM) and eleven *ee* values at each concentration (1, 0.8, 0.6, 0.4, 0.2, 0, −0.2, −0.4, −0.6, −0.8, and −1). An MLP-ANN was trained by using the ellipticities from $[\theta]_{340}$ to $[\theta]_{400}$ at 2 nm intervals as inputs. A network containing twenty hidden layers gave the best results.

The imine (PPI) was synthesized in situ by direct addition of 2-pyridine carboxaldehyde to the crude reaction mixture (Scheme 2). Without purification, the imine was loaded onto the same 96-well plate that was used for the training set. There were three sets at two different concentrations (0.7 and 1.1 mM). The samples were run in triplicate at two different concentrations to demonstrate both that this protocol works at different concentrations, and that even with impurities from the reaction mixture, the average errors would be comparable to those previously obtained by using prepared solutions of pure imines.

The *ee* and concentration values calculated by using the MLP-ANN are summarized in Table 2. The average value

Table 2. Determination of % *ee* and $[G]_t$ of MBA for unknown samples from the asymmetric reaction.

Samples	1_1	1_2	1_3	2_1	2_2	2_3
% ee (ANN)	60	62	62	52	62	68
$[G]_t$ (ANN) [mM]	0.84	0.86	0.86	1.23	1.21	1.21
$[G]_t$ [mM]	0.70	0.70	0.70	1.10	1.10	1.10

obtained was 61% *ee*. Assuming that the James procedure yields the real value for the *ee*, the average error for *ee* is 17%, which is higher than but comparable to that of 11.7%, calculated by using solutions of the pure imine CPI. With respect to concentration, the average error for the asymmetric reaction is 13.5%, which is also similar for the error of 14.7% that was calculated by using solutions of a pure imine. The error found represents the accuracy of our method, which we note consistently gives a low *ee* value compared with the accepted method. In contrast, the precision, which is represented by a standard deviation of the *ee* values, is much better (5.2%).

Applications in rapid and high-throughput screening of *ee*: The term rapid screening is used throughout this paper instead of HTS. This is because true HTS requires the screening of a few thousand samples per day[1], which is not possible with this protocol. However, the primary optical data in this protocol is collected in only two minutes per sample. More traditional techniques such as chiral HPLC takes 10–20 minutes per sample.[1] Thus, this protocol outperforms traditional techniques and can reasonably be described as a rapid-screening method. Additionally, HPLC and GC methods usually require purification of the sample prior to analysis. Whereas the reported protocol requires a two hour derivatization, it can be done in situ and in parallel in 96-well plates. Thus, for the large sample sizes this protocol is designed for, the two hour time period is a relatively short amount of time when compared with the screening time.

The speed at which the optical data is collected could be improved allowing for HTS. Previously, our group has demonstrated that a 96-well plate reader can collect optical data for 84 samples in two minutes.[6i] However, in the case of the CD spectrometer, the commercially available automated plate interface uses a pump to inject samples from a 96-well plate into a cuvette. Injection of the samples is the most time-consuming part of the process, with the collection of the primary optical data occupying a relatively small portion of the time. Thus, with improvements to the automated plate interface, the protocol could approach sample sizes comparable to those previously reported for UV/Vis spectroscopy, which would allow for true high-throughput screening.

Conclusion

Our previous *e*IDA and CD methods analyzed chiral bidentate analytes, namely, diols, amino acids, and diamines. As described above, the monodentate primary amines did not show an optical response, whereas conversion to bidentate imines gave enantioselective discrimination. Although the scope here was the determination of *ee* and concentration of chiral monoamines, the approach of conversion to bidentate chelating ligands may prove to be a general strategy for analysis of simple functional groups, allowing the extension of this protocol to many functional groups by using a single-step in situ derivatization.

In summary, a new protocol that allows for the rapid and simultaneous determination of *ee* and concentration of chiral primary amines has been developed. Furthermore, the *ee* of a sample from an asymmetric reaction was determined by using this CD technique and displayed close agreement with a literature protocol. The speed, accuracy, and simplicity of this method, as well as the possibility of simultaneous concentration determination, make this method clearly amenable to rapid screening. Additionally, improvements to the automated plate reader on the CD spectrometer may allow for this protocol to be applied in true HTS. We are currently employing this technique in the analysis of a wide variety of asymmetric reactions and other organic functional groups.

Experimental Section

Preparation of the imines: A stoichiometric amount of the corresponding chiral amines was added to a stirred solution of 2-pyridine carboxaldehyde (9.5 mL, 0.1 mmol) in acetonitrile (2 mL) with 4 Å molecular sieves. After stirring for 2 h at room temperature, the solvent was removed in vacuo yielding the corresponding crude imines as pale-yellow oils. These imines were directly used in the analysis.

(*R*)- and (*S*)-*N*-(1-Phenylethyl)-2-pyridylmethanimine, (*R*)- and (*S*)-PPI: Yield: 19.3 mg, 92%; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.64$–8.63 (m, 1H), 8.48 (s, 1H), 8.06–8.04 (m, 1H), 7.85–7.81 (m, 1H), 7.49–7.26 (m, 6H), 4.77 (q, $J = 6.7$Hz, 1H), 1.56 ppm (d, $J = 6.6$Hz, 3H).

(*R*)- and (*S*)-*N*-(1-Cyclohexylethyl)-2-pyridylmethanimine, (*R*)- and (*S*)-CPI: Yield: 19.7 mg, 91%; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.62$ (d, $J =$ 4.8Hz, 1H), 8.3 (s, 1H), 7.98–7.96 (m, 1H), 7.84–7.79 (m, 1H), 7.40–7.37 (m, 1H), 3.18–3.15 (m, 1H), 1.84–1.68 (m, 4H), 1.48–1.46 (m, 2H), 1.32–1.24 (m, 2H), 1.21 (d, $J = 6.5$Hz, 3H), 1.19–0.96 ppm (m, 2H).

(*R*)- and (*S*)-*N*-(2-hepthyl)-2-pyridylmethanimine, (*R*)- and (*S*)-HPI: Yield: 19.2 mg, 94%; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.63$–8.62 (m, 1H), 8.34 (s, 1H), 7.97–7.95 (m, 1H), 7.83–7.82 (m, 1H), 7.39–7.37 (m, 1H), 3.42 (m, 1H), 1.58–1.57 (m, 2H), 1.29–1.22 (m, 9H), 1.11–1.10 ppm (m, 3H).

James procedure: In a dry vial with 4 Å molecular sieves 2-formylphenylboronic acid (14.9 mg, 0.1 mmol) and (*S*)-1,1′-bi-2-naphthol (28.6 mg, 0.1 mmol) were dissolved in $CDCl_3$ (1 mL). α-Methylbenzylamine (12.7 mL, 0.1 mmol) obtained from the asymmetric reaction was added onto the solution (see Scheme S1 in the Supporting Information). The mixture was stirred for 5 min and the corresponding ^{1}H NMR of the resulting solution is shown in Figure S2 in the Supporting Information.

Linear discriminant analysis (LDA): The XLSTAT program was used to carry out the LDA studies. LDA studies allow for differentiation and classification of the analytes. The generalization error of this classification method was measured by using Jackknife analysis.

Principle component analysis (PCA): XLSTAT was also used for PCA analysis. PCA allows multivariate data to be represented in lower-dimensional space. The first two principal components are invoked to visualize the objects in two-dimensional space. The first principal component (PC1) is directed along the maximum variance. The second principal component (PC2) is orthogonal to PC1 and carries the second maximum extent of variance.

Artificial neural network (ANN): The ANN analyses were carried out by employing the program Statistica Neural Networks 8.0.[16] The Statistica Neural Networks program has an embedded intelligent problem solver (IPS) function, which was requested to search for MLP consisting of networks three layers. During learning, output values from the ANN are compared to the true values and the coupling weights are adjusted to give the best network. The hidden activation chosen was hyperbolic tangent and the output activation was identity. In the case of the training set, data corresponding to MBA at 0.2 mM, the ellipticites values at 10, 30 and 50% of *R* were discarded.

Acknowledgements

This work was supported by the National Institutes of Health, GM77437 and the Welch Foundation.

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Received: September 25, 2009
Published online: November 27, 2009

