

Rapid Enantiomeric Excess and Concentration Determination Using Simple Racemic Metal Complexes

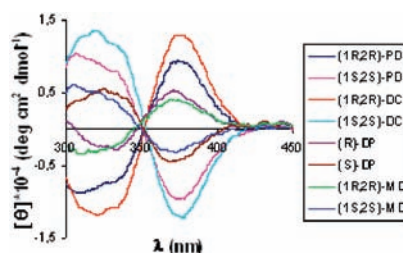
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ABSTRACT



Racemic-metal complexes were used to determine identity, enantiomeric excess, and concentration of chiral diamines using metal-to-ligand charge transfer bands in circular dichroism spectroscopy. It takes under just 2 min per sample to determine $[G]_t$ and %R with tolerable errors (19% and 4%, respectively). The simplicity of the achiral receptors employed confers to this technique great potential for high-throughput screening.

In recent years high-throughput screening (HTS) assays have been developed for the discovery of efficient catalysts for asymmetric reactions.¹ The classic methods employed for enantiomeric excess (ee) determination are HPLC or GC.² These methods are relatively laborious and slow. Moreover, usually these methods do not permit simultaneous concentration determination. Therefore, it is desirable to create standard protocols that accelerate the quantification of yield and enantiomeric excess (ee) of chiral products in a high-

throughput fashion.³ Toward these goals, the development of optical spectroscopic assays eases the instrumentation dependence and increases the speed of HTS assays.⁴

Progress in the development of indicator-displacement assays (IDA) have been made in recent years by our group,⁵ with either one receptor or an array of receptors, combined with pattern recognition protocols for the creation of fast

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optical techniques that quantitate both ee and concentration. Only a few other techniques have been shown to have this capacity.⁶

Recently, we reported an even simpler optical technique, which is based on changes in metal-to-ligand charge transfer (MLCT) bands in the circular dichroism (CD) spectra of an inorganic complex. This method allowed us to determine the identity, ee, and concentration of chiral diamines.⁷ We focused the previous study on the use of one chiral receptor (**1**) for the ultimate goal of differentiating achiral guests from chiral guests. Here we report a simplification, where we concentrate on the use of racemic receptors. The use of racemic commercial receptors have clear economic and synthetic advantages over using pure enantiomers.⁸ Herein we describe the use of a small library of racemic complexes and show they can be used to quantitate ee and concentration, and differentiate the analytes. We employ pattern recognition protocols to analyze not only the behavior of one selected analyte but also the fingerprint obtained for several analytes from the simultaneous combination of receptors.

CD spectroscopy has become a versatile tool for configuration assignment and the structural evaluation of molecules.⁹ Because CD active MLCT bands arise from achiral complexes upon chiral analyte binding,^{7,10} our protocols use simple inorganic complexes. In this study, our analytes are chiral vicinal 1,2-diamines (see Figure 1A).^{11,12}

For the creation of racemic metal complexes that have CD active MLCT bands when a chiral analyte is added, we turned our attention to Cu(I) and Pd(II) as metals, and *rac*-Binap (2,2'-bis(diphenylphosphino)-1,1'-dinaphthyl) and *rac*-*p*-Tol-Binap (2,2'-bis(di-*p*-tolyl-phosphino)-1,1'-dinaphthyl) as ligands. The compounds [Cu(P-P)(NCMe)₂][PF₆] (P-P = Binap **1**,⁷ Tol-Binap **3**¹³) and [Pd(P-P)(NCMe)₂][PF₆]₂ (P-P = Binap **2**, Tol-Binap **4**) were prepared (see Supporting Information). The racemic receptors **1–4** do not show any

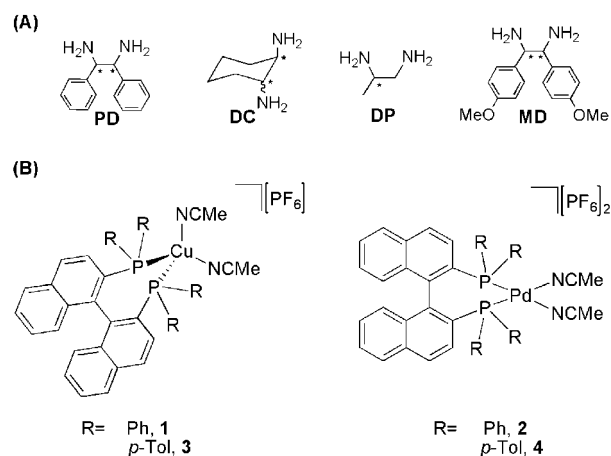


Figure 1. (A) Diamines employed: 1,2-phenylethylenediamine (**PD**), 1,2-diaminocyclohexane (**DC**), 1,2-diaminopropane (**DP**), bis(4-methoxyphenyl)-1,2-diaminoethane (**MD**). (B) Racemic-metal complexes employed.

CD signals, as expected. Also, there are no CD signals above 320 nm for the chiral diamine analytes or for copper or palladium alone with the diamines. Therefore, any signals that arise above this wavelength after the addition of the chiral guests to the receptors would be indicative of the chirality, identity, and concentration of the analyte.

We first focused on the identification of diamines. Therefore, we analyzed complexes **1–4** with 2 equiv of the enantiomers of each diamine. The CD showed that the MLCT bands of these complexes were characteristic to the analyte. Every diamine leads to a different CD spectrum, and the

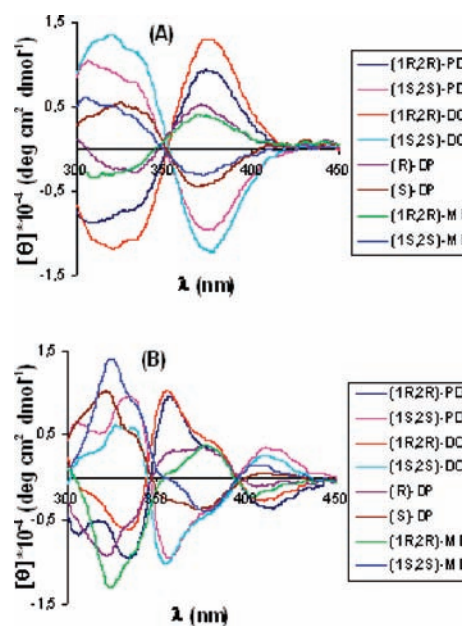


Figure 2. CD spectra of receptors [0.4 mM] with the guests [0.8 mM]: (A) (*rac*)-**1** and (B) (*rac*)-**2**.

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enantiomeric analytes gave identical and opposite spectra (see representative examples in Figure 2). These CD spectra allow for enantiomer identification based on the sign of the Cotton effect, as well as the identity of the diamines as judged by the intensity of the signals at selected wavelengths.

Therefore, to construct an identification pattern, the CD spectra of all four receptors at 0.4 mM were recorded at 0.8 mM concentration of each analyte. To ensure reproducibility each experiment was performed five times. The data were analyzed at four selected wavelengths (330, 345, 375, 395 nm) for **1** and **3** and at four different wavelengths (320, 335, 355, 410 nm) for **2** and **4** and subjected to linear discriminate analysis (LDA) to identify diagnostic patterns.¹⁴

The LDA plots for receptors **1**, **2**, **3**, and **4** show discrimination of all of the guests. As an example, Figure 3A shows the results from receptor **1**, revealing tight

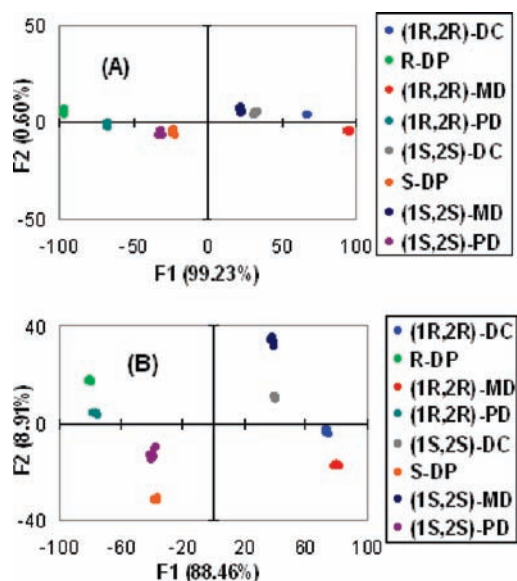


Figure 3. LDA plots obtained with the: (A) receptor **1**, (B) receptor **2**.

clustering of data relative to spatial separation of diamines and their enantiomers, but only along the F1 axis. This shows that one linear discriminate equation is enough to correlate the differences between ellipticities at the chosen wavelengths to chemical identity and chirality. The same result was found for receptor **3** (see Supporting Information). However, in the case of the palladium complexes **2** (Figure 3B) and **4** (Figure S4B, Supporting Information), the clusters are spread through the four quadrants of the LDA plot. This is indicative of the fact that the differences in ellipticities at the four chosen wavelengths for each individual analyte cannot be expressed by one simple linear equation, thereby revealing differential response of the CD curves to each analyte at the wavelengths analyzed. One can readily see this by comparing Figure 2A and B, where in A the CD spectra have few crossing points, whereas in B there is extensive crossing of the spectra.

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To test the ability of a combination of all of the receptors to discriminate the analytes, we created an LDA plot using the CD data of all of the receptors, employing the same wavelengths previously cited (see Figure 4). The LDA shows

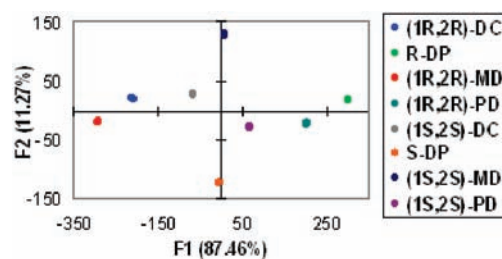


Figure 4. Linear discriminate analysis for a combination of all receptors.

the analytes clearly discriminated with the greatest cross-reactivity reflected in the axes. The precision of the LDA plots was examined using a jackknife analysis,¹⁵ and 100% accuracy in chemo- and enantioselectivity was established.

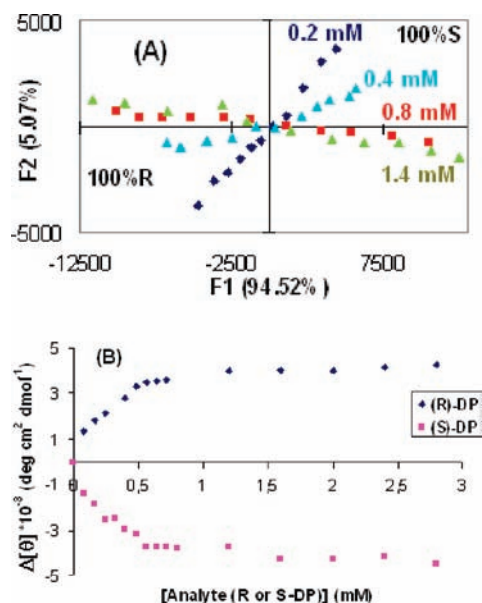


Figure 5. (A) Titration of receptor **2** [0.4 mM] with enantiomers of **DP** at 381 nm. (B) PCA plot of **DP** with four ee training sets at four different [G], values: 0.2, 0.4, 0.8, 1.4 mM.

After the determination of chirality and identity, we decided to investigate the capacity to determine both concentration and ee for a known analyte. We employed receptor **2** and **DP** combined with a robotically controlled 96-well plate CD reader (JASCO ASU-605). The wells were loaded with 0.4 mM of receptor **2** in acetonitrile and then charged with four concentrations of **DP** (0.2, 0.4, 0.8, and

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1.4 mM) with 11 ee values for each (1, 0.8, 0.6, 0.4, 0.2, 0, -0.2, -0.4, -0.6, -0.8, and -1). The same plate was loaded with five unknown samples created independent of the training set.

We analyzed the trainings sets using principle component analysis (PCA) (see Figure 5A).¹⁴ This plot shows that the F1 axis defines chirality with negative scores for the samples enriched in (*R*)-enantiomer and positive scores for the (*S*)-enantiomer. The F2 axis defines concentration. From 0.2 to 0.8 mM the differences in concentration are considerable; however, after 0.8 mM the differences relative to 1.4 mM are clearly smaller. These results correlate with the titrations, which show that after approximately 0.8 mM saturation is achieved (Figure 5B).

Table 1. Determination of Concentration and ee of DP Samples by ANN

	1	2	3	4	5
[G] _t (mM)	0.80	1.40	0.50	1.00	1.20
[G] _t (mM) (ANN)	0.92	0.94	0.66	0.97	1.36
% <i>R</i>	90	80	50	30	20
% <i>R</i> (ANN)	85	80	42	26	18

Furthermore, we analyzed the same data using multilayer perceptron (MLP) artificial neural network (ANN)¹⁶ on the basis of our previous experience^{5d,7,17} and as a means to test the accuracy of this protocol for ee and concentration

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determination. The architecture of MLP consists of an input, an output, and a hidden layer where the data is processed through a back-propagation algorithm. The input layer consists of 51 ellipticities from CD₃₂₀ to CD₄₂₀ at 2 nm intervals. The training set data resulted in a MLP-ANN with 12 hidden layers. The five unknowns were computed, and the values of [G]_t and %*R* had average errors of 18.6% and 3.8% (Table 1), which is clearly amenable to a first approximation in a HTS protocol.

In summary, we have described a technique based on a library of achiral receptors that fingerprints chemical identity, chirality, and concentration. The optical CD signatures can be interpreted by a variety of pattern recognition protocols depending on the goal of the analysis. A CD spectrometer interfaced with a robotic 96-well plate serves to generate data that trains an ANN and subsequently yields acceptable accuracy in the analysis of true unknowns. The technique allows for the determination of both concentration and ee values in under 2 min per sample. In this specific study we avoided chiral receptor synthesis and/or resolution, analyte derivatization, and pretreatment of the data, as well as the necessity of knowing the analyte concentration in advance. We are currently expanding the technique to a wide variety of chiral organic functional groups.

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Supporting Information Available: Experimental procedures and crystallographic data in CIF format. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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