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Rapid Optical Methods for Enantiomeric Excess Analysis: From Enantioselective Indicator Displacement Assays to Exciton-Coupled Circular Dichroism

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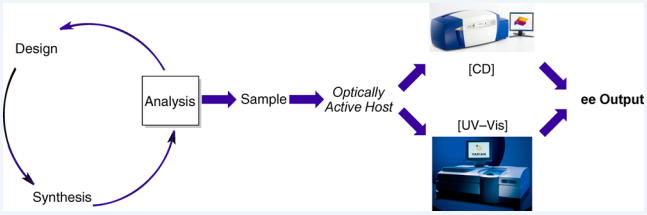
CONSPECTUS: The advent of high-throughput screening (HTS) for chiral catalysts has encouraged the development of fast methods for determining enantiomeric excess (ee). Traditionally, chromatographic methods such as chiral HPLC have been used for ee determination in HTS. These methods, however, are not optimal because of high duty cycle. Their long analysis time results in a bottleneck in the HTS process. A more ideal method for HTS that requires less analysis time such as chiroptical methods are thus of interest.



In this Account, we summarize our efforts to develop host−guest systems for ee determination. The first part includes our enantioselective indicator displacement assays (eIDAs), and the second part focuses on our circular dichroism based host−guest systems. Our first eIDA utilizes chiral boronic acid receptors, along with prescreened indicators, to determine ee for chiral α-hydroxyacids and vicinal diols with ±7% average error (AE). To further the practicality for this system, a HTS protocol was developed. Our second eIDA uses diamino chiral ligands and Cu^{II} as the receptor for the ee determination of α-amino acids. The system reported ±12% AE, and a HTS protocol was developed for this system.

Our first CD based host−guest system uses metal complexes composed of Cu^{I} or Pd^{II} with enantiopure 2,2′-diphenylphosphino-1,1′-binaphthyl (BINAP) as host to determine the ee of chiral vicinal diamines (±4% AE), primary amines (±17% AE), and cyclohexanones (±7% AE). Primary amines and cyclohexanones were derivatized to form chiral imines or chiral hydrazones to allow coordination with the metal complex. Upon coordination of chiral analytes, the metal-to-ligand (BINAP) charge transfer band was modulated, thus allowing the discrimination of chiral analytes. As an effort to improve the accuracy for chiral primary amine ee determination, a system with a host composed of *o*-formylphenyl boronic acid (FPBA) and enantiopure 1,1′-bi-2-naphthol (BINOL) was used to reduce the AE to ±5.8%. In the presence of amines, the FPBA−BINOL host forms an imine-coordinated boronic ester, thus affecting the CD signal of the boron complex. Another chiral primary amine ee determination system was developed with Fe^{II} and 3-hydroxy-2-pyridinecarbaldehyde. The chiral imines, formed by the pyridinecarbaldehyde and chiral amines, would coordinate to the Fe^{II} ion yielding exciton-coupled circular dichroism (ECCD) active metal complexes. This system was able to determine the ee of chiral amines with ±5% AE. Furthermore, this imine−Fe^{II} complex system also successfully determined the ee of α-chiral aldehydes with ±5% AE. Other ECCD based hosts were subsequently developed; one with bisquinolylpyridylamine and Cu^{II} for chiral carboxylates and amino acids and another multicomponent system with pyridine chromophores for chiral secondary alcohol ee determination. Both of the systems were able to determine ee of the chiral analytes with ±3% AE.

Overall, our group has developed ee determining host−guest systems that target various functionalities. To date, we are able to determine the ee of vicinal diols, α-hydroxyacids, vicinal diamines, cyclohexanones, amines, α-chiral aldehydes, carboxylates, amino acids, and secondary alcohols with ±7% or lower average error. Future development will involve improving the average error and employing the current systems to analyze real-life samples resulting from parallel syntheses.

1. INTRODUCTION

Enantiomers have different biological activities. This is best exemplified in the pharmaceutical industry, where the enantiomer of a bioactive drug could have unforeseeable detrimental effects on humans. Recent advances in asymmetric synthesis have provided a multitude of ways to efficiently obtain one preferred enantiomer over the other.[1] These asymmetric transformations are often carried out with either a chiral catalyst or chiral auxiliary.[2,3] One method of chiral catalyst and auxiliary discovery is high-throughput screening (HTS) where rapid quantification of product enantiomeric excess (ee) and yield is required to achieve high efficiency.[4,5] Currently, the use of chiral chromatographic methods is the most common strategy to measure ee values.[6−10] These methods are associated with high cost (solvent, column replacement) and low duty cycle (equilibration time) and therefore are not ideal for HTS. To achieve the speed required for true HTS, methods that avoid elution are desirable.

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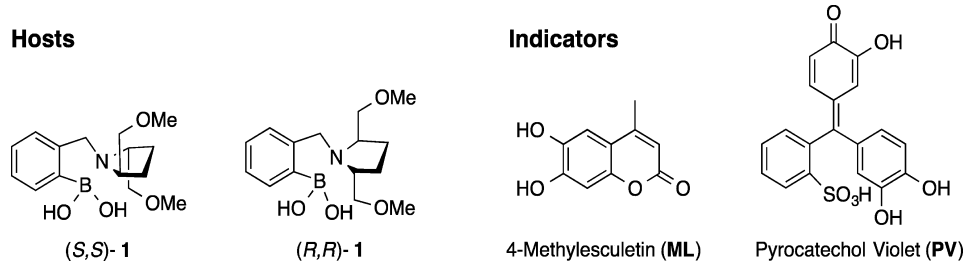

Figure 1. Examples of structures of the hosts and indicators used in the study.

Optical spectroscopy based ee determination is attractive due to the inherently short analysis time and lack of chromatographic separation. These methods, however, often require derivatization of the analyte, which adds additional steps to the screening process.[5] Hence, host–guest systems that selectively target the asymmetric transformation products are ideal. Recently, our group has developed optical spectroscopy based host–guest systems with the aim of rapid determination of enantiomeric excess. In these systems, ee values can be easily determined with reproducible calibration curves or appropriate patterning techniques; exact association constants for the two enantiomers are not required. This Account focuses solely on work from our group. For further reading about host–guest based ee determination, several reviews and primary literature are available.[11−13]

This Account starts with enantioselective indicator displacement assays (eIDAs) that target α-hydroxycarboxylates,[14−16] vicinal diols,[14−18] and α-amino acids.[19−22] This is followed by circular dichroism (CD) and exciton coupled circular dichroism (ECCD) techniques for diamines, amines, carboxylic acids, amino acids, secondary alcohol, cyclohexanones, and aldehydes.

2. ENANTIOSELECTIVE INDICATOR DISPLACEMENT ASSAY (EIDA) AS DETECTION METHOD

In an enantioselective indicator displacement assay (eIDA), the quantification of enantiomeric excess (ee) is based on displacement of an indicator by a chiral analyte.

2.1. eIDA for α-Hydroxycarboxylates and Vicinal Diols

One of our first eIDAs exploited the binding of boronic acids to α-hydroxyacids and vicinal diols.[14−16] The assay was developed with boronic acid receptors and catechol indicators. A representative example is shown in Figure 1. It was hypothesized that the presence of stereocenters neighboring the boron atom in the host would result in enantioselective association with chiral guests. In addition, *o*-aminomethyl functionality was used to aid the association equilibrium.[15] In the initial study with boronic acid receptor (*S*,*S*)-**1** and various α-hydroxycarboxylates, using PV as the colorimetric indicator, an average of ±15% error was observed for the determined ee values.

In an effort to enhance the assay's sensitivity and improve the accuracy in ee measurements, fluorescent indicators were investigated.[14] 4-Methylesculetin (ML), along with receptor (*S*,*S*)-**1**, was used to develop mathematical relationships that correlate optical signal to ee and concentration. It was found that iteratively fitting the curve of fluorescence intensity vs ee using Origin software gave the best results. Through this approach, the average error in ee was found to be ±7%. Further studies with a series of receptors, a series of indicators, and other guest substrates gave a protocol that could be used to optimize indicator selection, concentration of the indicator, and the chiral receptor concentration.[16]

The boronic acid and vicinal diol host–guest eIDA was further explored to extend the scope of detection and practicality.[17,18] In the reported study,[17] four pairs of *syn*-vicinal diol enantiomers were selected as guests along with three indicators (Figure 2).

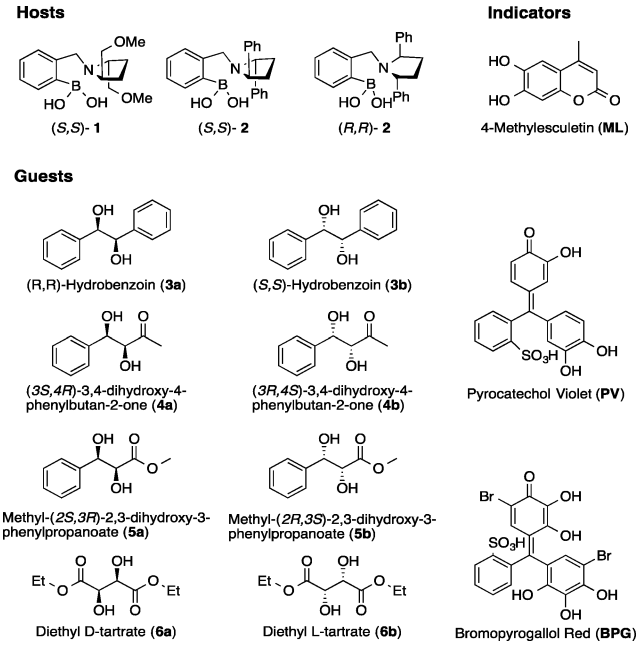

Figure 2. Hosts, guest enantiomers, and indicators used for developing HTS protocol.

Two additional chiral hosts, (*S*,*S*)-**2** and (*R*,*R*)-**2**, were introduced to the system along with the previously reported host (*S*,*S*)-**1** to enhance the enantioselectivity. Using 96-well plates and a UV–vis plate reader, absorbance data were collected at different wavelengths with each host–indicator pair (three for each pair, nine total). The wavelengths were selected on the basis of the largest absorbance changes. Principal component analysis (PCA) on the absorbance data showed excellent differentiation of both the identity of the diols and their enantiomers (Figure 3). Furthermore, the eIDA successfully differentiated samples with different guest concentration at various ee values on a PCA score plot (Figure 4). To demonstrate the predictive power of this system, artificial neural network (ANN) analysis with 14 absorbance inputs yielded an average absolute error of ±0.08 mM for sample concentration and ±7% for ee.

In an effort to perform HTS utilizing this eIDA procedure, a stepwise process for protocol development for concentration and ee determination was published.[18] The protocol described a five step process that includes optimization of the eIDA host and indicator concentration using UV–vis titrations, screening for the best host–indicator combination to discriminate the

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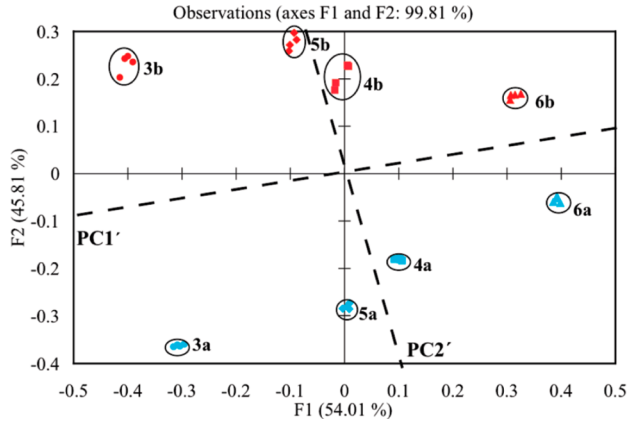

Figure 3. PCA plot of the diol enantiomers discriminated with (*S*,*S*)-**1**−PV, (*R*,*R*)-**2**−ML, and (*S*,*S*)-**2**−PV receptor−indicator pair. The study was conducted in 10 mM *p*-toluenesulfonic acid/Hunig's base buffer (pH 7.4) in 100% MeOH at 25 °C.

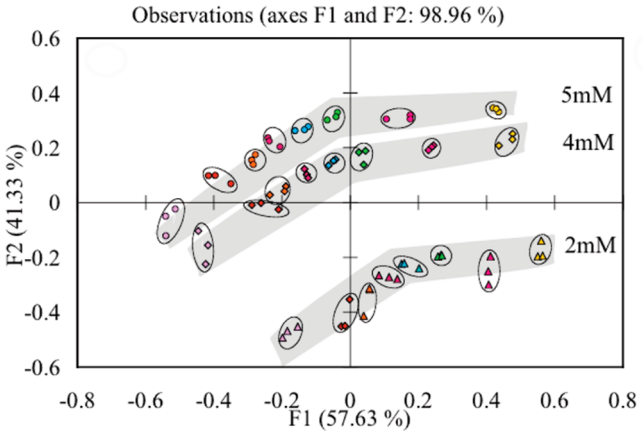

Figure 4. PCA plot of diol **5** with varying ee and at three different concentrations discriminated by (*S*,*S*)-**1**−BPG, (*R*,*R*)-**2**−ML, and (*S*,*S*)-**2**−PV receptor−indicator pair. The study was conducted in 10 mM *p*-toluenesulfonic acid/Hunig's base buffer (pH 7.4) in 100% MeOH at 25 °C.

enantiomers of interest, training of an ANN, analyzing unknown ee analytes, and, last, loading the absorbance results onto the trained ANN to determine ee and concentration. Except for the first step, which is only required to be done once per host−indicator pair, all the steps can be performed on a well-plate reader allowing for true HTS. The developed protocol was used to analyze samples of hydrobenzoin with unknown ee and an average error of ±0.17 mM in the range of 3−8 mM concentration and ±2.4% for ee. When tested with samples synthesized with established Sharpless asymmetric dihydroxylation reactions, the protocol was able to identify the best ligand as reported by literature.[17]

2.2. eIDA for α-Amino Acids

Another system of eIDAs was developed for ee determination of α-amino acids.[19−22] The system utilized Cu^{II} with chiral ligands **7** and **8** as hosts complexed to chromazurol S to form the receptor (Figure 5). Upon enantioselective coordination of chiral α-amino acids to form diastereomeric complexes, the selected colorimetric indicators were released, thus resulting in changes in absorbance. With X-ray crystallographic data, the enantioselectivity was postulated to have arisen from both the favored

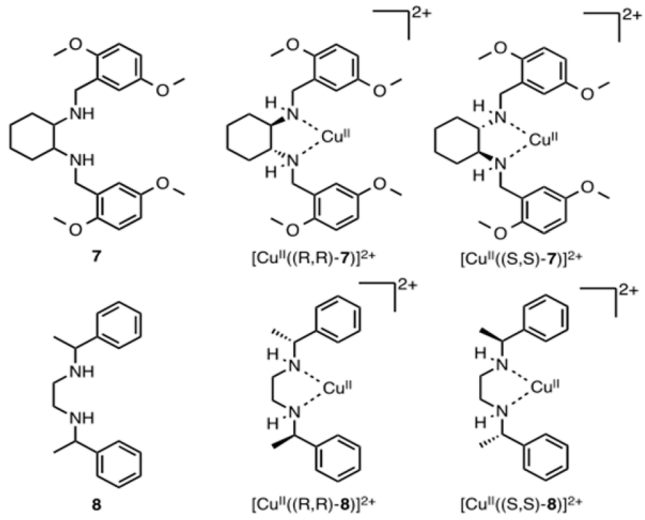

Figure 5. Structures of the chiral ligands (**7** and **8**) and the receptors formed with Cu^{II} and chiral ligand.

positioning of the dimethoxybenzylic rings on ligand **7** and the steric interactions of the phenyl groups on ligand **8** with the amino acid side chain. With this eIDA, 13 out of the 17 examined amino acids were enantioselectively discriminated. Further study with ee calibration curves showed around ±11.9% average error for ee determination. To further demonstrate the practicality, a high throughput screening protocol utilizing this eIDA was developed with 96-well plates and four α-amino acids. (Figure 6).[22]

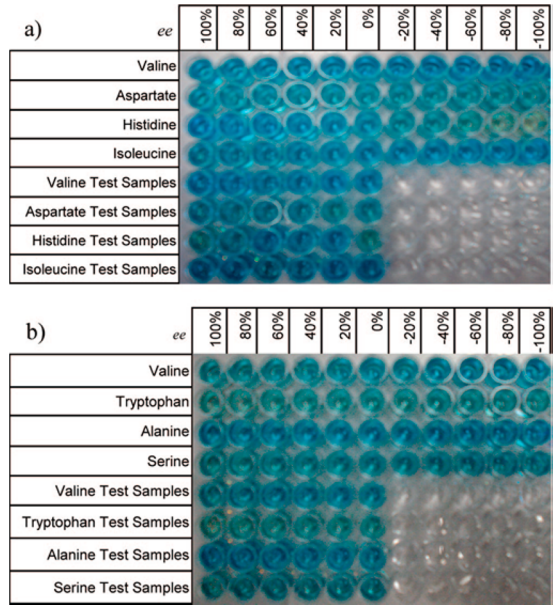

Figure 6. The 96-well plate used for making the ee calibration curves. Each plate had four rows of amino acid samples for making the calibration curve with six test samples on the bottom: (a)$[Cu^{II}((R,R)\text{-}7)]^{2+}$ was used as the receptor; (b) $[Cu^{II}((R,R)\text{-}8)]^{2+}$ was the receptor.

The average absolute error of ee determination for all four of the amino acids was ±9.7% using only receptor $[Cu^{II}((R,R)\text{-}7)]^{2+}$; receptor $[Cu^{II}((R,R)\text{-}8)]^{2+}$ had unsatisfactory results. ANN analysis was applied to the data collected in hopes to improve the eIDA's predictive power. With ANN, the average absolute error of ee was found to be ±10.0%. An asymmetrically synthesized α-amino acid with unknown ee was subjected to the eIDA.

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The determined ee, from the reported eIDA, was found to be in good agreement with values measured with chiral HPLC and a ^{1}H NMR chiral shift agent.

In our research, focus for ee determination switched from UV−vis and fluorescence based systems to circular dichroism (CD) based methods. One reason was the signal dependence on the concentration of chiral analyte. Further, with UV−vis based systems, anything that can absorb in the region of detection could interfere with the signal of interest. This fact, in the context of HTS for chiral catalysts, would mean additional purification steps, which are not ideal for the process. A second reason for the change was simplification in host design. The hosts for eIDAs were often not commercially available and required synthesis, whereas most of our CD based hosts are formed with commercially available compounds or ligands that can be made with simple synthetic procedures. For the reasons given here, we moved the focus of our work to various forms of CD spectroscopy.

3. CIRCULAR DICHROISM (CD) AS A DETECTION METHOD

Circular dichroism (CD) spectroscopy is an optical technique that is inherently sensitive to chirality, enables one to analyze and differentiate analytes in a chiral host−guest system, and is applicable to high-throughput screening (HTS). Most chiral building blocks do not display strong Cotton effects in CD spectroscopy. However, intense Cotton effects can be produced when metal complexes have MLCT bands or when identical chromophores have a helical twist, leading to exciton-coupled circular dichroism (ECCD). The sign of the Cotton effect gives valuable information for determining the absolute configuration of the chiral analyte, and the CD signals can be directly correlated to the ee of the sample. For our CD based sensors, titration studies are typically performed to find the saturation point and the binding stoichiometry of the host−guest interaction. The use of guest concentrations beyond the saturation point leads to concentration independent spectral responses.

In combination with statistical and chemometric techniques, such as linear discriminant analysis (LDA), artificial neural networks (ANNs), and principal component analysis (PCA), the CD spectral data can be further analyzed to find trends in the data as well as uncover characteristics of the data that best differentiate the chemo- and enantio-identity of the products. LDA is a technique to find linear combinations of features that maximize the separation between classes and minimize the separation within classes,[23,24] and PCA is a tool that can classify and identify variance in data.[25] These analysis techniques allow for highly accurate discrimination of chemoselectivity and enantioselectivity in sample reactions.

3.1. Metal-to-Ligand-Charge Transfer (MLCT) CD Assays

Our first CD-based assay for ee involved analysis of a metal complex that has CD-active metal-to-ligand-charge transfer (MLCT) bands.[26,27] The MLCT band in the visible region of the CD spectrum was of interest because most organic functional groups are CD-silent in this range, showing signals only in the 190−220 nm region. A simple inorganic coordination complex with *R*- or *S*-BINAP (2,2′-diphenylphosphino-1,1′-binaphthyl), a binaphthyl diphosphine ligand with axial chirality, was used as a host system to discriminate the chirality of diamines, primary amines, and cyclohexanones.[26−30] The axial chirality of BINAP arises from the limited rotation at room temperature of the bond linking the two sterically hindered naphthyl rings. The coordination complex with a transition metal, such as copper or palladium, introduces structural rigidity to the BINAP and its ligand derivatives.[28] Upon addition of chiral guest, the MLCT bands were modulated, allowing enantiomeric differentiation. BINAP was also chosen because both of its enantiomerically pure forms are commercially available. The guest molecules can be directly used with the metal complex when the number of host binding sites and the number of functional handles of the guest are the same. Otherwise, the guests need to be derivatized in order to bind to the metal complex.

3.1.1. Racemic and Chiral Metal Complexes as Hosts for Diamines. Chiral metal complexes that have CD active MLCT bands, such as $[Cu^{I}(BINAP)(MeCN)_2]PF_6$ (**9**) or $[Pd^{II}(BINAP)(MeCN)_2]PF_6$ (**10**), were used to differentiate enantiomers of chiral vicinal 1,2-diamines (Figure 7).[26] Both

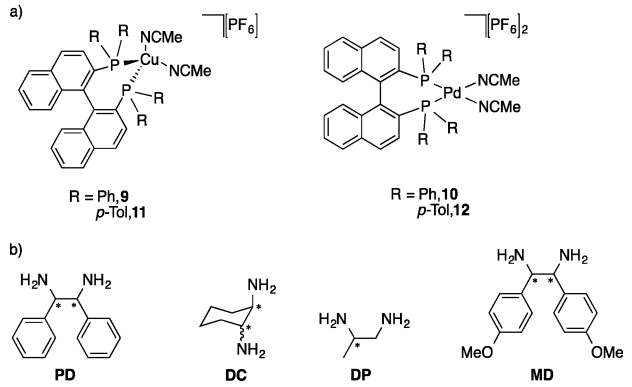

Figure 7. (a) Racemic metal complexes employed. (b) Diamines employed: 1,2-phenylethylenediamine (PD); 1,2-diaminocyclohexane (DC); 1,2-diaminopropane (DP); bis(4-methoxyphenyl)-1,2-diaminoethane (MD).

enantiomers of **9** have MLCT bands around 340 nm in the CD spectrum, giving opposite Cotton effects for the *R* and *S* copper-coordinated complexes (Figure 8). Because there are no CD

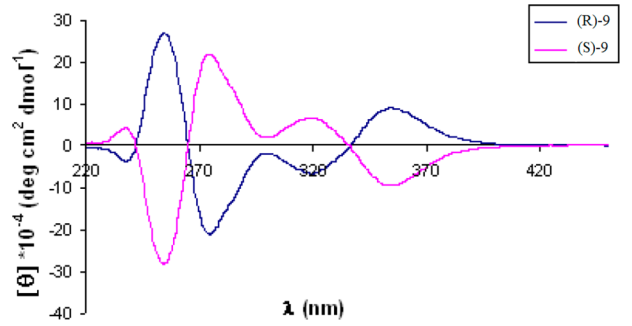

Figure 8. CD spectra of *R*-**9** [0.4 mM] and *S*-**9** [0.4 mM] between 220 and 450 nm.

signals above 300 nm for the diamine analytes tested or for the metal alone with diamines, the signals above 300 nm are indicative of the chemical identity, chirality, and concentration of the guests. Distinctive CD-active MLCT bands were observed for each diamine and its enantiomer when complexed with *R*- or *S*-**9**. Also, by comparing the intensity difference between CD signals of the complexes of diamine enantiomers with *R*-**9** or *S*-**9**, ee for diamines was evaluated with an average error of ±3.8%.

The LDA plot in Figure 9, generated from the CD data of all the receptors at chosen wavelengths, showed chemical

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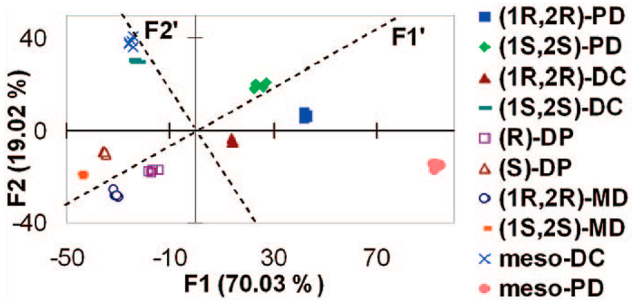


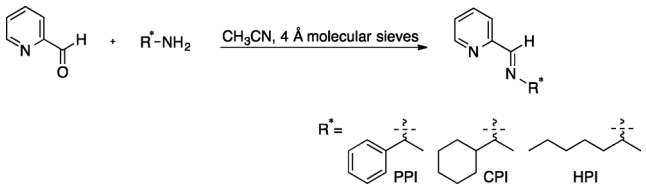
Figure 9. Response patterns for all the analytes using (*R*)-**9** receptor obtained by LDA.

identification and chiral discrimination of all of the diamine analytes, which was determined by their individual clustering through the four quadrants. When the same data was analyzed with multilayer perceptron (MLP) ANNs, ee and concentration ($[G]_t$) were determined with average errors of ±3.8% and ±18.6%, respectively.

The racemic mixtures of **9** and **10** were also employed to discriminate enantiomers of chiral diamines.[27] Of course, the racemic mixture does not show any CD signals alone, but upon binding with enantiopure vicinal 1,2-diamines, CD active MLCT bands are observed. The LDA plot successfully classified the diamines and their handedness.

3.1.2. Chiral Metal Complexes as a Host for α-Chiral Primary Amines. The same metal complex **9** was employed to discriminate α-chiral primary amines.[29] However, the addition of underivatized chiral amines to *R*-**9** does not show any signal modulation by CD spectroscopy. Therefore, a simple derivatization of chiral amines to form imines that can coordinate with *R*-**9** was necessary. Chiral imines were formed from the condensation of chiral amines with 2-pyridinecarboxaldehyde *in situ* (Scheme 1). The modulated MLCT signal, which is indicative of

Scheme 1. Derivatization of the Amines To Form the Corresponding Schiff Bases



the coordination of the imines with *R*-**9**, was observed in the CD spectrum (Figure 10). The CD signals were characteristic of each analyte, and the data was further analyzed with LDA and PCA. In the PCA plot, the F1 axis defines chirality with negative values for *R*-enantiomers and positive values for *S*-enantiomers, and the F2 axis defines concentration. The average error for ee was ±17%, which led us to develop a method that could give a more accurate enantiodiscrimination.[31]

3.1.3. Chiral Cu(I) Metal Complexes as Hosts for α-Chiral Cyclohexanones. Complex **9** was also applied to the enantiodiscrimination of α-chiral cyclohexanones.[30] In order to create bidentate ligands that produce a twist upon binding with *R*- or *S*-**9**, enantiomerically pure α-chiral cyclohexanones were derivatized with 1-methyl-1-(2-pyridyl) hydrazine to form hydrazones (Scheme 2). The nitrogen atoms in the pyridyl group and hydrazone moieties coordinate to a Cu^I, forming a metal complex. Upon addition of hydrazone to enantiomerically

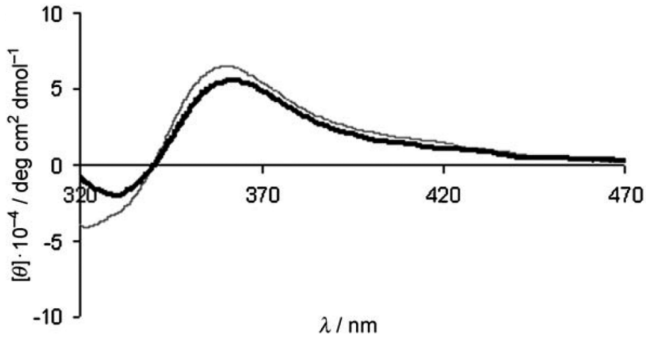
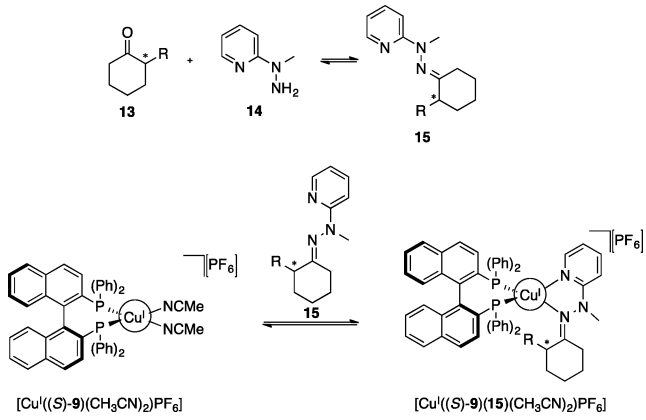


Figure 10. CD spectrum for (*R*)-**9** [0.4 mm] and the enantiomers of CPI [0.8 mm](thin line = (*R*)-CPL; thick line = (*S*)-CPL).

Scheme 2. Derivatization of *R*-Chiral Cyclohexanones (13) with 1-Methyl-1-(2-pyridyl) Hydrazine (14) To Produce a Bidentate Analyte (15), Followed by Complexation to (9)



pure **9**, diastereomers will be produced with different twist angles. Different twist angles between the naphthyl rings in BINAP were predicted based upon steric interactions between the phosphine ligand and the R group on the ketones. The degree of twist is reflected in the CD spectrum, allowing for discrimination between the two enantiomers. The *R*-enantiomers of hydrazones with *S*-**9**, compared with *S*-enantiomers, will cause a larger change in the twist, inducing a larger change in the CD signals from the original MLCT band of **9** (Figure 11). The enantiomeric host *R*-**9** produces a mirror image CD spectrum. Through the use of calibration curves, these studies allow ee determination of the chiral cyclohexanones to be performed with an absolute error of ±7%.

3.2. A Boronic Acid Receptor for Chiral Primary Amines

Another method for the analysis of chiral primary amines was created using an assembly from the Bull and James groups,[32,33] where enantiopure 1,1′-bi-2-naphthol (BINOL) assembles with *o*-formylphenyl boronic acid (FPBA) and α-chiral primary amines (Scheme 3). Enantiomerically pure BINOL and BINOL−FPBA mixture both have a CD signal that is modulated upon addition of *R* or *S* guest amines.[34] The three-component assembly forms an imine coordinated boronate ester, and it was hypothesized that the absolute configuration of the amine would modify the torsional angles of *S*- or *R*-BINOL to cause modulation in CD. However, molecular modeling experiments using Spartan showed no evidence of distortion of the dihedral angle of BINOL in the product assembly. The change in CD occurs from extending the chromophore ability of the chiral

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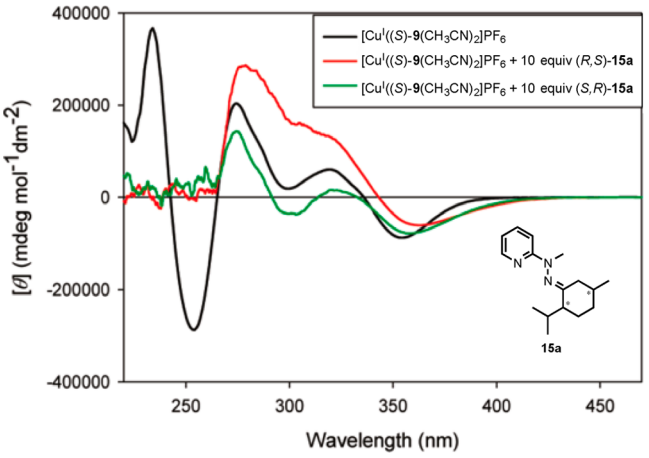
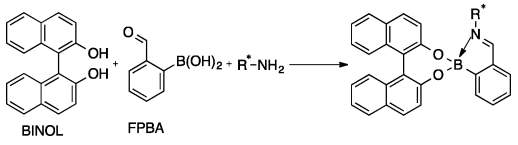


Figure 11. CD spectra of **9** (401 μM) in CH_3CN (black), *S*-**9** (401 μM) mixed with (*R*,*S*)-**6** (4 mM) (red), and *S*-**9** (401 μM) mixed with (*S*,*R*)-**6** (4 mM) (green). Themolar.

Scheme 3. Assembly Developed by the Bull and James Groups



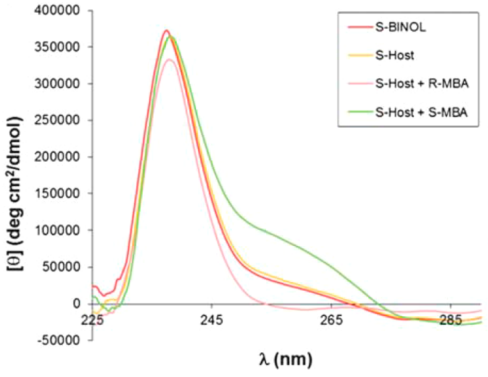


Figure 12. CD spectra of the assembly of methyl boronic acid (MBA) with (*S*)-BINOL–FPBA.

amine through the condensation with the aromatic boronic acid (Figure 12).

Various amine analytes were assembled with BINOL and analogues and with FPBA (Figure 13). The CD spectra of the resulting complexes showed differences in intensity and shape, making it possible to discriminate their identities and chirality. With all data obtained from varying hosts and guests, PCA and LDA plots were generated to classify the amines. The ee analysis done was highly accurate, giving an average absolute error of ±5.8% using the calibration curves generated.

3.3. Exciton-Coupled Circular Dichroism (ECCD)

One form of CD, namely, exciton-coupled circular dichroism (ECCD),[35] has been widely employed in chirality sensing for various analytes.[36−55] When a compound contains two or more chromophores that can be oriented in a helical fashion, ECCD signals are generated. This phenomenon leads to bisignate CD curves centered at the UV−vis absorption maximum. Enantiomers have mirror image CD spectra, and the sign of the Cotton effect is used to determine the absolute configuration of

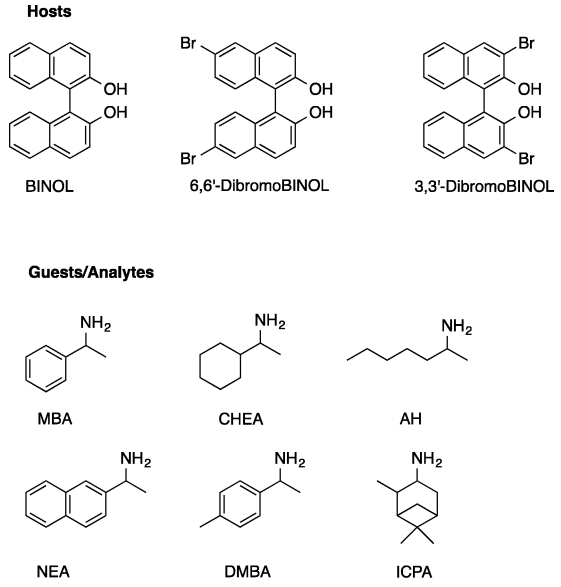
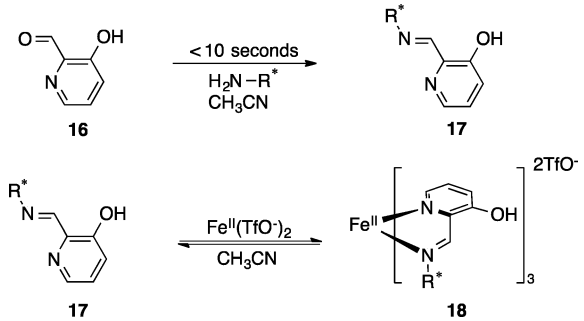


Figure 13. Structures of the compounds used as hosts and guests/analytes.

the analyte. Practically, chiral analytes must be derivatized with chromophores or need to be bound to receptors containing chromophores through supramolecular interactions.

3.3.1. In Situ Generated Fe^{II} Complexes as a Host for Chiral Amines. The method described in section 3.1.2 for chiral monoamines[29] suffers from several drawbacks. First, the derivatization of 2-pyridinecarboxaldehyde to form bidentate imines takes 2 h. Second, it gave a moderately high average error of ±17%. Lastly, the calibration curves are concentration dependent. In an effort to eliminate the need for the synthesis of a host and to create a simple and quick assay, our group has turned to self-assembly. In one example, Fe^{II} was used as a metal center to coordinate three equivalents of bidentate imines, which were created from the condensation between a chiral amine and aldehyde **16**.[31] In order to reduce derivatization time, 3-hydroxy-2-pyridinecarbaldehyde was allowed to react with the chiral primary amines to form chiral imines (Scheme 4). Followed by

Scheme 4. Aldehyde 16 Reacts Rapidly with an Amine To Form Imine 17, Followed by Complexation with Fe^{II} To Form 18



this in situ amine derivatization, Fe^{II} was added to form octahedral complexes that possess different helical twists. There are four possible stereoisomers for enantiomerically pure amines, and 24 possible stereoisomers for mixtures of *R* and *S* amines. These isomers result from helical isomerism (clockwise, counterclockwise), configurational isomerism (*fac* and *mer*), and *R* and *S* amines. However, this complexity does not interfere in ee

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determination and enantiomeric differentiation because the isomers interchange rapidly in equilibria. The three asymmetrically oriented ligands bonded to Fe^{II} generate ECCD signals, which correlate with the identity of the stereogenic center of the imines and the helicity of the complex (Figure 14). Imines with an *R* stereogenic center induce a counterclockwise twist and have a negative ECCD couplet and vice versa.

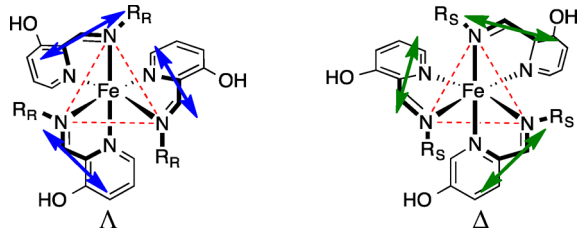


Figure 14. Helical arrangements of the transition dipoles that couple to give rise to the positive and negative ECCD couplets for the Δ-(*R*)- and Λ-(*S*)-*fac* isomers, respectively.

Amines with aromatic, cyclic, and acyclic functionality were differentiated by the CD signal intensity and shape (Figure 15). A concentration-independent calibration curve was generated to determine ee with low average error of ±5%.

3.3.2. Fe^{II} Complexes as a Host for α-Chiral Aldehydes. Adapting two protocols previously discussed,[29,31] an assay for chiral aldehydes was created.[56] Imines generated from compound **14** with various chiral aldehydes coordinate with metal upon the addition of Fe^{II} triflate (Scheme 4 and Figure 16). Compared with our previous approach,[29] this assay was advantageous because the derivatization of the amine to form an imine was reduced from 2 h to 30 min. The complexation of synthesized bidentate imines **19**–**21** with Fe^{II} led to large CD signals, which were used to determine ee of α-chiral aldehydes. The CD signal was used to discriminate absolute configuration of the α-chiral aldehydes as well as their ee values with an absolute average error of ±5%.

3.3.3. A Cu^{II} Complex as a Host for Chiral Carboxylates and α-Amino Acids. Our group has also exploited ECCD for the analysis of chiral carboxylates.[57] Achiral host [(BQPA)-$Cu^{II}(ClO_4)$], **22**, is easy to synthesize and has an empty coordination site for monodentate carboxylate binding (Scheme 5).

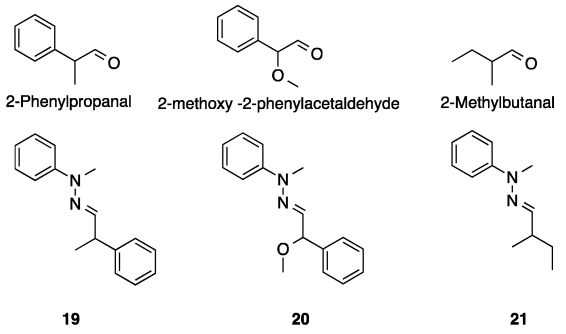
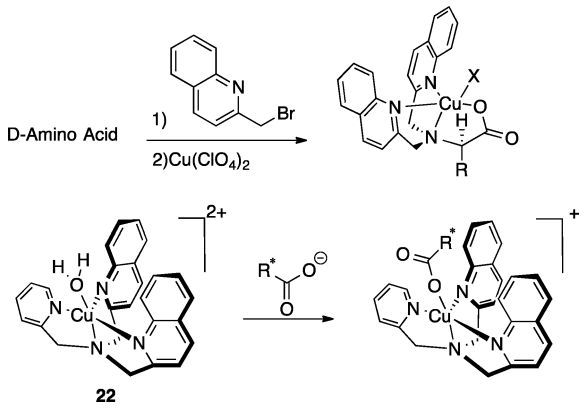


Figure 16. Structures of aldehyde studied and **19**, **20**, and **21** created by reaction with **15**.

Scheme 5. Protocol To Determine Absolute Configuration of Chiral Amino Acids (top) and Proposed Complex Formation between [(BQPA)$Cu^{II}(ClO_4)_2$] Host, 22, and Chiral Carboxylate Guest



Host complex **22** alone has two different helical isomers that exchange rapidly in equilibria resulting in no CD signal. However, binding of a chiral guest causes one twist to dominate and thereby generate the corresponding ECCD couplet (Figure 17). This method has the advantage of avoiding an analyte derivatization step. The guest forms a complex that has a minimum steric interaction with the groups on the stereocenter, thus dictating the helicity. *R*-Enantiomers gave negative CD couplets,

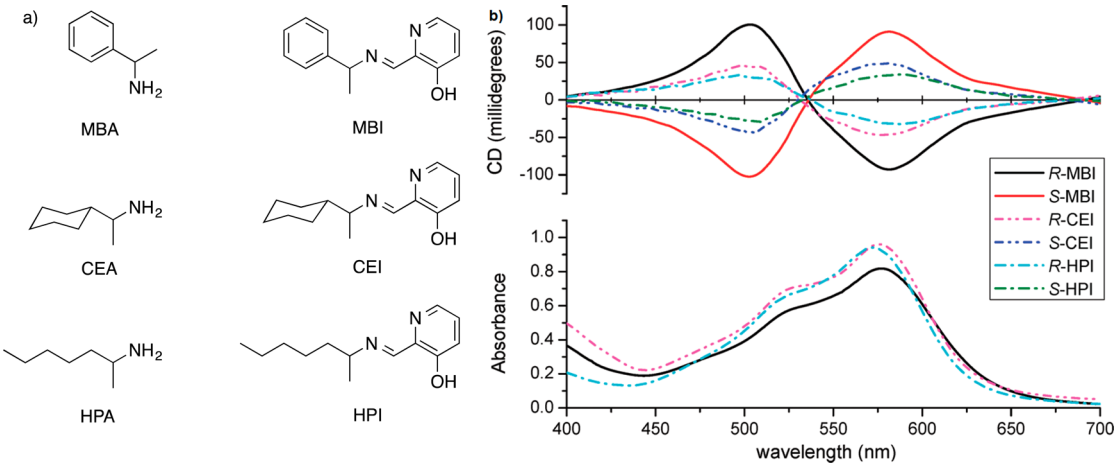


Figure 15. (a) Structures of (left) MBA, CEA, and HPA, the three amines studied, and (right) MBI, CEI, and HPI, the three imines formed after reaction of the amines with aldehyde **16**. (b) UV–vis and CD spectra of the MLCT bands for the three different imines studied, MBI (3 mM), CEI (6 mM), and HPI (7 mM), at 100% and −100% ee in acetonitrile with 1 mM Fe^{II} in a 0.1 cm quartz cell from 400 to 700 nm.

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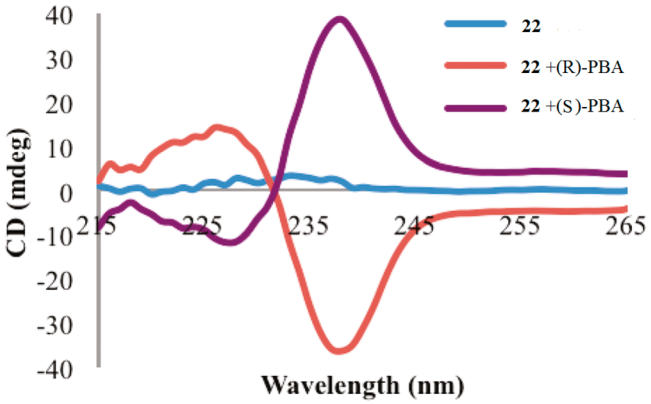


Figure 17. CD spectra of host **22** (0.5 mM) by itself and with each enantiomer of PBA (1.0 mM) in default buffer (75% MeCN/H_2O with 20 mM HEPES buffer at pH 7.4).

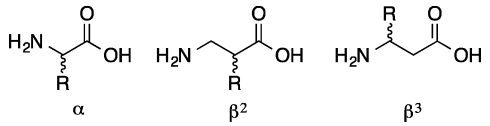


Figure 18. Types of amino acids.

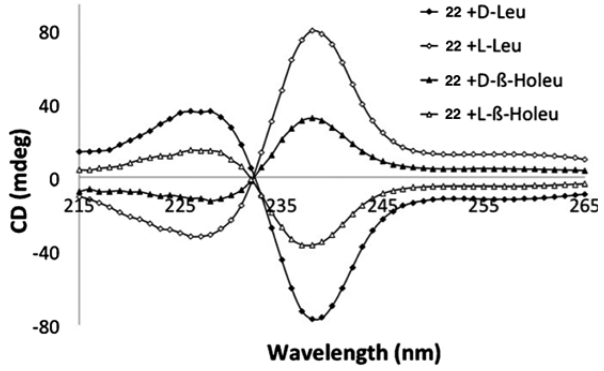
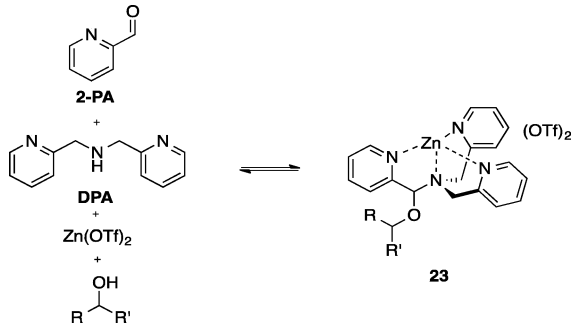


Figure 19. CD spectra for each indicated guest (1.0 mM) with host **22** (0.5 mM) in default buffer.

Scheme 6. Four-Component Reversible Covalent Assembly for Secondary Alcohol Binding



which are indicative of a P helical isomer with a counterclockwise twist and vice versa. The difference in steric size of the groups attached to the stereocenter is directly correlated to the magnitude of the CD signal. Calibration curves for the determination of ee of carboxylates were generated and gave an average absolute error of ±3%.

Amino acids contain carboxylate groups, and hence host **22** was also used as a sensor for *α*-amino acids and *β*-homoamino acids.[58] Boc-protected *α*-amino acids and Boc-protected *β*-amino acids follow the same operating principles as the previously studied carboxylates. For both *α*-amino acids and *β*-homoamino acids, D-isomers led to a P-type helix whereas L-isomers led to an M-type helix. However, due to the increase in the degrees of rotational freedom in *β*-homoamino acids, reduced CD signals were observed (Figures 18 and 19). *γ*-Amino acids were not suitable for this system.

3.3.4. A Zn^{II} Mediated Multicomponent Assembly as a Host for Chiral Secondary Alcohols. Our most recent ECCD-based sensor targets chiral secondary alcohols and was formed via a dynamic multicomponent assembly process.[59] Four components, 2-pyridinecarboxaldehyde, di(2-picolyl)amine, zinc^{II} triflate, and the chiral alcohol were mixed together, and reversible covalent bonding formed assembly **23** (Scheme 6). The assembly possesses a helical twist of the pyridines that depends on the handedness of the stereocenter at the hemiaminal ether carbon. The stereocenter, in turn, is dictated by the handedness of the alcohol. The helical twist of the tris(pyridine)

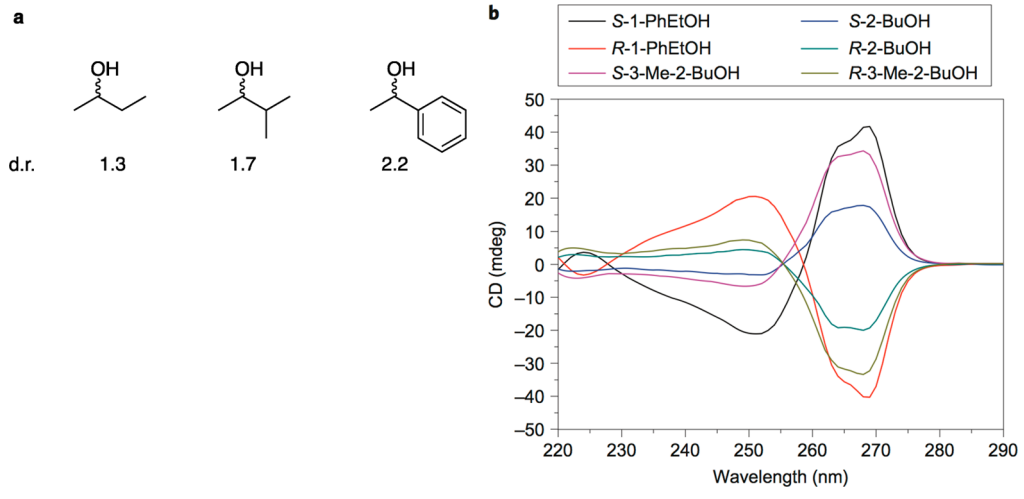


Figure 20. (a) Diastereomeric ratio values for assemblies with chiral mono-ols (*R* or *S*) obtained from ^{1}H NMR. (b) CD spectra of assembly derived from three alcohols (0.175 mM 2-PA, 0.525 mM mono-ol).

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complex induces a large Cotton effect in the CD spectrum resulting from ECCD. *R*-Alcohols lead to a preference of an *S*-stereocenter at the hemiaminal ether carbon, giving a preferential P twist of the tris(pyridine) ligand and a negative ECCD couplet, while the opposite is true for *S*-alcohols.[60] The sign of the Cotton effect is therefore indicative of the handedness of the alcohol stereocenter and the unique CD signals for each alcohol allow determination of alcohol identity. This system has successfully been used to quantify ee values of chiral secondary alcohols with a ±3% error.

The diastereomeric ratio (dr) of the assembly with chiral alcohols was linearly correlated with the magnitude of the CD signal (Figure 20).[61] Further, Charton steric parameters linearly correlate with the dr values and thereby also the ECCD intensity. From these correlations, the magnitude of CD values of various alcohols could be predicted with average absolute error of ±9.5%.

4. CONCLUSIONS AND OUTLOOK

The use of optical spectroscopy for the determination of enantiomeric excess values has the potential to revolutionize reaction and catalyst discovery. Our group, among others, has pioneered this effort with a focus on either colorimetric or circular dichroism approaches. Within our own group, a series of assays targeting several chiral functional groups have been created: diols, diamines, amines, alcohols, carboxylic acids, aldehydes, and ketones. In addition, a series of strategies for optical modulations have been implemented: indicator-displacement assays, CD-active MLCT excitation, and exciton-coupled circular dichroism. While the absolute errors in ee values are not yet as low as those traditionally associated with chiral HPLC analysis, advances in lowering the errors can be anticipated in the near future. The error in ee values arises primarily from variations in quantitative syringing and pipetting during sample generation. These variations can be reduced by adopting a fully automated protocol often seen in HTS. Even with errors of 5% to 10%, one can still quickly identify trends in data and find the "hits" within hundreds of samples. Although these errors seem relatively large compared with HPLC, chemists in big-pharma have routinely told our group that they are perfectly acceptable to HTS. Thus, true high-throughput screening is right around the corner. We end this Account ends with the question - "How will the process of discovery of catalysts and reactions for asymmetric induction change when chemists are able to measures hundreds of ee values in a hour or less?"

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The authors declare no competing financial interest.

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