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Rapid Optical Determination of Enantiomeric Excess, Diastereomeric Excess, and Total Concentration Using Dynamic-Covalent Assemblies. A Demonstration Using 2-Aminocyclohexanol and Chemometrics.

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ABSTRACT: Optical analysis of reaction parameters such as enantiomeric excess (*ee)*, diastereomeric excess (*de)*, and yield are becoming increasingly useful as assays for differing functional groups become available. These assays typically exploit reversible covalent or non-covalent assemblies that impart optical signals, commonly circular dichroism (CD), that are indicative of the stereochemistry and *ee* at a stereocenter proximal to the functional group of interest. Very few assays have been reported that determine *ee* and *de* when two stereocenters are present, and none have targeted two different functional groups that are vicinal and lack chromophores entirely. Using a CD assay that targets chiral secondary alcohols, a separate CD assay for chiral primary amines, a UV-Vis assay for *de*, and a fluorescence assay for concentration, we demonstrate a work-flow for speciation of the enantiomers and diastereomers of 2-aminocyclohexanol as a test-bed analyte. Due to the fact the functional groups are vicinal, we found that the *ee* determination at the two stereocenters is influenced by the adjacent center, and this led us to implement a chemometric patterning approach, resulting in a 4% absolute error in full speciation of the four stereoisomers. The procedure presented herein would allow for the total speciation of around 96 reactions in 27 minutes using a high-throughput experimentation routine. While 2-aminocyclohexanol is used to demonstrate the methods, the general workflow should be amenable to analysis of other stereoisomers when two stereocenters are present.

Introduction

Over the last few decades asymmetric reaction discovery has been fueled by the advent of high-throughput experimentation (HTE).[1-2] Many thousands of experimental conditions can be explored in a parallel fashion to identify the most efficient asymmetric transformations. However, a significant bottleneck is encountered with reaction analysis, due to the sheer number of reactions that can be conducted.[3-4]

Chiral chromatography is the most common method for characterizing the enantiomeric excess (*ee*) of the product of an asymmetric transformation. Recent developments in ultrafast chiral separations for high-throughput *ee* determination have made significant strides, with average analysis times on the 5 – 20 minute timescale; however, in some cases, sub-minute separation times were achieved.[5-7] Despite these remarkable advances, the serial nature of chromatography is not readily amenable to high-throughput screening (HTS) protocols. Parallelization of chromatographic techniques has been accomplished but requires specialized instrumentation and software.[8-9] In an effort to overcome this bottleneck, optical methods have been developed for asymmetric reaction discovery.[10-14] In contrast to chromatographic techniques, optical analyses can be accomplished in microwell plates, allowing for simple, fast, and cost-effective implementation in HTE protocols. To date, there have been numerous reports of optical methods such as UV-Vis,[15-18] fluorescence,[19-23] and circular dichroism (CD)[24-28] spectroscopy being successfully employed to determine the enantioselectivity of a given asymmetric transformation, as well as reaction yield.

While optical approaches to *ee* determination have had extensive success,[29] few reports of optical assays for both enantiomers and diastereomers have been reported.[23, 30] Diastereomers are non-mirror image stereoisomers, and are possible whenever there are two or more stereocenters. Of course, the stereocenters can be far apart or proximal. In an asymmetric transformation that simultaneously sets more than one stereocenter, the stereocenters are commonly proximal, and in particular, vicinal.[31-33] In such a scenario, the *ee* is typically determined within

each diastereomeric set, e.g. *cis*- and *trans*- (or *ethryo* and *threo*) via chiral chromatography. Likewise, the most common technique to measure diastereomeric ratio (*dr*) is HPLC, but NMR and other spectroscopic methods can be used.[34-36] These methods are again serial, and represent a bottleneck for rapid method development. Finally, while reaction yield is best defined by "isolated yield", in HTE procedures it would be useful to have rapid optical methods to monitor percent conversion *in situ*, and such techniques have been created.[37-39]

In one report that has addressed the stereochemical determination of both *ee* and *dr*, Pu and coworkers utilized a pair of enantiomeric fluorescent sensors (**1** and **2**) that were both enantioselective and diastereoselective towards the four stereoisomers of *N*-carbobenzyloxy-threonine (**3**, Figure 1A).[30] It was demonstrated that the fluorescence responses of the enantiomeric sensors at two emission wavelengths could be used to differentiate the four stereoisomers. Based on this observation, the authors stated that this system could be used to determine the relative concentration of each isomer in a mixture of four stereoisomers, but total speciation of the stereoisomers was not reported.

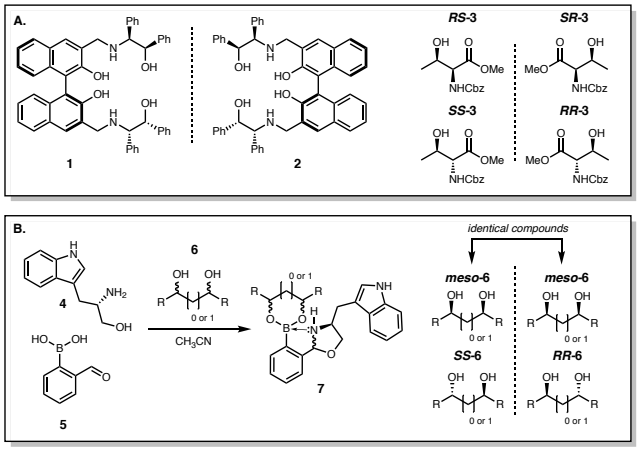


Figure 1. A. A pair of enantiomeric BINOL sensors for differentiating the four stereoisomers of *N*-carboxybenzoyl threonine methyl ester. B. A dynamic covalent multi-component assembly for the differentiation of stereoisomeric diols, where there are only three possible stereoisomers due to the internal mirror plane present in the *RS*/*SR* stereoisomer.

In another approach, Anzenbacher et al. utilized a dynamic covalent assembly of enantiopure tryptophanol (**4**) and *o*-formyl phenyl boronic (**5**) acid for enantio- and diastereoselective fluorosensing of symmetrical chiral 1,2- or 1,3-diols (**6**) (Figure 1B).[23] The stereochemistry at both -OH stereocenters imparts an effect on the fluorescence readout of complex **7**, allowing for differentiation of three stereoisomers, (*RR*, *SS*, and *meso*). It was demonstrated that an artificial neural network (ANN) analysis could be used to determine the *ee* of the stereoisomeric mixture and the total concentration of diol, but there was a caveat that only 5% of the *meso* form could be tolerated. Thus, diastereomeric excesses (*de*) were not determined and full stereoisomeric speciation was not demonstrated. No group has yet reported using optical methods to perform a full speciation of stereoisomers when two stereocenters of differing chemical functionality are present. Achieving this was our fundamental goal, and we chose an amino-alcohol as a test-bed analyte.

Vicinal aminoalcohols are privileged scaffolds in organic synthesis both in terms of their role in chirality transfer processes and pharmacologically active natural products.[40] As such, the synthesis of vicinal aminoalcohols remains a major thoroughfare of inquiry in the development of asymmetric methodologies. Several approaches have been demonstrated, including enantio- and diastereoselective reductive coupling of imino compounds with carbonyl compounds,[41] diastereoselective addition of nucleophiles to enantiopure α-aminocarbonyl compounds[42], diastereoselective oxidations of allyl amines[43], and enantioselective ring opening of epoxides.[44] In most cases, the absolute and relative configuration of synthetic vicinal aminoalcohols were determined for the *N*-derivatized substrates via chiral chromatography using racemic or enantiopure standards with chromatographic methods ranging from 12 to 25 minutes per run; however, it is noteworthy to mention that these approaches were not applicable to all vicinal aminoalcohols synthesized. For some substrates, the stereochemistry was determined via crystallographic methods where derivatization at the amine or alcohol stereocenter was often required to yield crystals suitable for crystallographic analysis.

With this in mind, we set out to create optical methods to determine *ee*, *de*, and reaction yield, and chose 2-aminocyclohexanol (**8**) as a model substrate. Not only are the stereocenters vicinal, but they contain different functionality, thus presenting the challenge of using two separate assays. Further, the structures do not possess chromophores, allowing the trivial characterization of *ee* values via CD spectropolarimetry on pure, underivatized samples. Here, we report on the utilization of dynamic covalent chemistries for the differentiation of the four stereoisomers of 2-aminocyclohexanol, and their concentrations (Figure 2). We discuss the complications encountered in doing such an analysis, and explore solutions to the complexities by using chemometric methods.

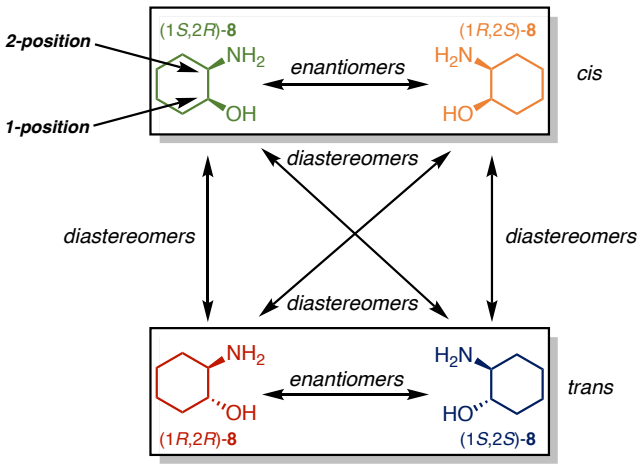


Figure 2. The four possible stereoisomers of 2-aminocyclohexanol (**8**), where the 1-position is the alcohol stereocenter and the 2-position is the amine stereocenter.

Results and Discussion

I. Design Criteria and Strategy

Optical assays for *ee* function by a chiral analyte interacting with a molecular sensor, inducing a spectroscopic readout that is dependent on the absolute configuration of the stereocenter being targeted. The current optical protocols for *ee* determination are designed for common functional groups, usually with a single α- (sometimes β- or γ-) stereocenter. Except for vibrational circular dichroism (VCD),[45] optical methods for determining *ee* have focused primarily on molecules with only one stereocenter, and hence, exist only as enantiomers. A problem arises when diastereomers exist. Because the optical methods assign *R* or *S* to individual stereocenters independently, the assignment of *R* or *S* is within both diastereomers. For example, an assay focused on stereocenter 1 would potentially report a *1S,2R* stereoisomer the same as *1S,2S*. Thus, we derived a mathematical relationship between the *R* and *S* configurations of two individual stereocenters with the *ee* of each set of diastereomers.[46] As described in the previous report, one requires an assay for *de* (or *dr*) in order to perform a complete speciation of all 4 stereoisomers when two stereocenters are present (such as in Figure 2). When the optical signal for each individual stereocenter is not influenced by the additional stereocenter, an *ee* value for each stereocenter, and a *de* value for the four stereoisomers, allows for complete speciation of the four stereoisomers. Complete speciation allows one to calculate *ee* within each diastereomeric set.

Our group has reported chirality sensing systems for mono-amines[28, 47-48] and secondary mono-ols[49-54] via CD spectroscopy, and we anticipated the tandem use of these systems would be ideally suited for stereoisomeric differentiation of the 2-aminocyclohexanol isomers.

The chirality sensing system for primary-amines relies on the *in situ* generation of iron (II) complexes with exciton coupled circular dichroism (ECCD) active absorption bands in the UV region as the result of coupling between pyridyl chromophores (200-400 nm) and CD active absorption bands due to intense metal to ligand charge transfer (MLCT) bands in the visible region (400-700 nm). Imine formation between a chiral amine and 3-hydroxy-2-pyridinecarboxaldehyde (**9**) followed by 3:1 ligand complexation to an iron (II) center forms a stereoisomeric mixture of octahedral complexes (**12**) that possess helical chirality (Figure 3). These isomers are the result of helical isomerism (Λ- and Δ-), configurational isomerism (*fac*- and *mer*-) and the stereochemistry of the mono-amines (*R*- and *S*-). This results in bisignate CD curves in the visible region due to (MLCT). The observed CD signal correlates to the helicity of the octahedral iron (II) complex and the stereochemistry of the chiral amine. Concentration independent calibration curves are generated that correlate CD intensity to *ee* with an average error of ±5%. Importantly, this assay requires no synthesis; both 3-hydroxy-2-pyridinecarboxaldehyde and iron (II) triflate are commercially available reagents, making it a facile method for the *ee* determination of chiral primary-amines.

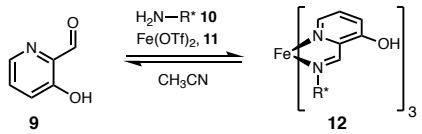


Figure 3. *in situ* generated octahedral iron (II) complexes for the *ee* determination of chiral primary primary-amines.

An ECCD sensing system based on a dynamic multicomponent assembly process targets chiral secondary alcohols (Figure 4). Iminium formation between di-(2-picolyl)amine (**13**) and 2-pyridinecarboxaldehyde (**14**) is followed by alcohol incorporation to yield a tren-like ligand (**19**). Complexation of the tris(pyridine) ligand to a zinc (II) center results in an ECCD-active trigonal bipyramidal complex as a result of the coupling between the pyridyl chromophores. The arrangement of the pyridinyl chromophores about the zinc (II) center is dependent on the stereochemistry of the hemiaminal ether stereocenter that is formed upon incorporation of the mono-ol into the tris(pyridine) ligand, whose stereochemistry in turn is dictated by the stereogenicity of the alcohol analyte. Thus, the sign of the Cotton effect is indicative of the stereochemistry of the chiral analyte. Concentration independent calibration curves are generated that correlate CD intensity to *ee* to give extrapolated *ee* values with an average error of ±3%. As with the assay for chiral amines, all reagents are commercially available making this a simple method for *ee* determination.

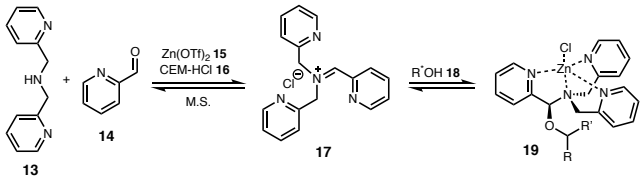
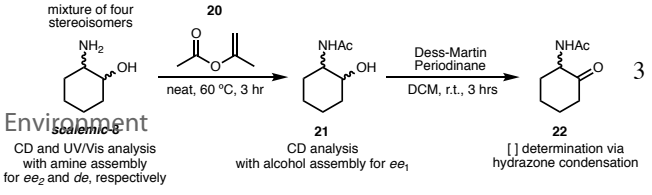


Figure 4. Dynamic covalent multi-component assembly for the *ee* determination of chiral secondary alcohols, where CEM-HCl is 4-(2-chloroethyl)morpholine hydrochloride and M.S. is 4Å molecular sieves.

Our strategy for characterizing a stereoisomeric mixture of 2-aminocyclohexanol (**8**) is shown in Scheme 1. We hypothesized the total concentration of **8** could be determined via hydrazone formation of an oxidized form of the analyte and fluorescence spectroscopy (Scheme 1). With the concentration of the analyte known, both the amine and alcohol sensing systems discussed above (Figures 3 and 4, respectively) could be conducted at concentrations above saturation to ensure that the CD signals indicative of *ee* were concentration independent. The amine assay given in Figure 3 would also allow for the differentiation of the *cis*- and *trans*-1,2-aminoalcohols via UV-Vis spectroscopy.

Scheme 1. Workflow for Total Stereoisomeric Speciation



II. Stereoisomeric Speciation

Concentration Determination

As shown in Scheme 1, the total concentration of the four stereoisomers of **8** was determined with a simple three-step procedure that can be done in parallel reactions in plates: 1) *in situ* acetylation of the amino group with isopropenyl acetate (**20**), 2) *in situ* Dess-Martin oxidation of the resulting amidoalcohol to the corresponding amidoketone (**22**), and 3) addition of fluorescent 7-(diethylamino)coumarin-3-carbohydrazide (Figure S21). These transformations proceed quantitatively and require no chromatographic purifications. We found the oxidation to the ketone was advantageous for an additional reason besides yield determination. The oxidation removes diastereomeric differences, such that a mixture of four diastereomers become only enantiomers, which have the same emission.[55] The products can be transferred from plates for future *ee* and/or *de* determination in parallel, via simple extractions, and concentration under centrifugal evaporation. In this workflow, it is necessary to first determine the concentration of the analyte in order to run the *ee* and *de* assays at the concentrations necessary for assembly formation.

Alcohol *ee* Determination

In order to monitor the stereochemistry at the 1-position (alcohol), it was necessary for the amine moiety to be protected. This was accomplished by the quantitative acetylation procedure introduced above, releasing only an equivalent of acetone that is innocent in our assemblies (Scheme 1).[56] All four stereoisomers of **21** were efficiently incorporated into the alcohol assembly and characteristic Cotton effects were observed at 270 nm (Figure 5). The *cis*-diastereomers gave significantly higher CD signals ($|CD|_{270\ nm}$ = 20 mdeg) compared to *trans* ($|CD|_{270\ nm}$ = 7 mdeg).

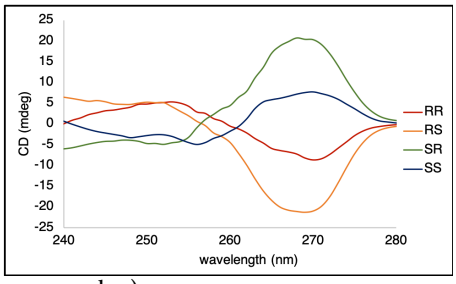


Figure 5. ECCD signals for the four stereoisomers *N*-(2-hydroxycyclohexyl)acetamide (**21**) with dipicolylamine, pyridine-2-carboxaldehyde, 4-(2-chloroethyl)morpholine hydrochloride, and $Zn(OTf)_2$. The ECCD spectra were recorded in CH_3CN at 25 °C (1.75 mM pyridine-2-carboxaldehyde, 8.75 mM **21**, 1 mm cell).

The difference in the observed CD intensity at 270 nm for the diastereomers can be attributed to two possibilities. The stereochemistry at the 2-position could have an effect on the orientation of the pyridine rings in the tripodal zinc complex, or the diastereomers of **8** could be incorporated to different extents. In previous studies, we've determined that five equivalents of alcohol relative to 2-pyridinecarboxaldehyde are needed to ensure efficient incorporation into the tris(pyridine) ligand. Thus, using five equivalents of either diastereomer of **21**, we found that the yield of the hemiaminal ether zinc complex (**19**) as determined via NMR integrals (Figures S1- S7) showed no significant differences in analyte incorporation. Hence, it was concluded that the difference in CD intensity at 270 nm was due to the adjacent stereocenter influencing the arrangement of the pyridine chromophores of zinc complex **19**.

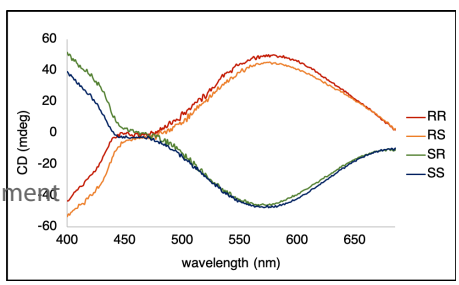
However, with the use of five equivalents of alcohol, there is still the possibility that one diastereomer of **21** may preferentially be incorporated into the assembly (Scheme S2). If this is the case, the observed CD signal will instead reflect the preferred diastereomer of the analyte, and any resulting *ee* determinations will be incorrect. To verify a statistical distribution of incorporated alcohol, 1:1 diastereomeric mixtures were made with 5 equivalents of alcohol e.g. 2.5 eq: 2.5 eq (*1R,2R*):(*1R,2S*) and (*1S,2S*):(*1S,2R*) and subjected to the reaction conditions for alcohol assembly formation (Figure 4). As CD is a function of absorbance, it can be treated analogously as UV-Vis spectra, which is simply additive. Thus, the CD of a solution with multiple components is the sum of the CD signals of the individual components. In our case, it was observed that the observed CD signal for the 1:1 diastereomeric mixtures were indeed additive. This was further confirmed by analyzing 1:1 diastereomeric mixtures that were made up from two enantiopure solutions, giving the same CD signals (Figure S12).

Amine *ee* Determination

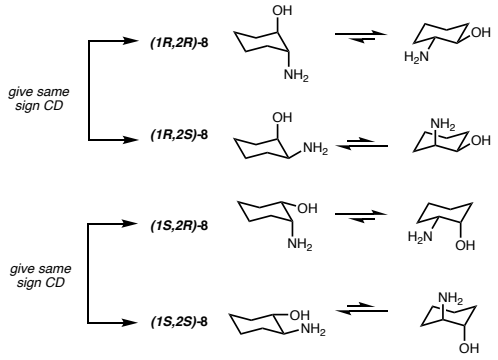
We found that the stereochemistry of the 2-position (amine) and the relative stereochemistry to the 1-position (alcohol) could be studied directly without prior derivatization of 2-aminocyclohexanol. Imine formation between 2-aminocyclohexanol and 3-hydroxypyridine-2-carboxaldehyde and subsequent complexation to an iron (II) center (Figure 3) gave intense MLCT bands with characteristic Cotton effects (Figure 6). The *cis*- and *trans*-**8** gave CD signals with similar intensities ($|CD|_{600\ nm}$ = 45 mdeg), albeit the (*1S,2S*) and (*1S,2R*) stereoisomers gave essentially the same CD spectra from 450 - 700 nm (see overlap of *RR* and *RS* in Figure 6; this coincidental overlap of CD spectra is not expected to be general and is not required for stereoisomeric differentiation). Thus, the arrangement of the pyridine chromophores around the iron center is primarily due to the configuration at the 1-position.

Figure 6. CD traces for four stereoisomers of 2-aminocyclohexanol with 3-hydroxy-pyridine-2-carboxaldehyde and $Fe(OTf)_2$. The CD spectra were recorded in CH_3CN at 25 °C (0.5 mM $Fe(OTf)_2$, 1.5 mM **8**, 1 cm cell).

In general, for **12**, the imine ligands exist in the *E*- con-



figuration and dock on the iron center with the bulkiest substituent oriented away from the iron (II) center.[47] In the context of 2-aminocylohexanol, there is the added complexity that each stereoisomer can exist in two different chair flips (Figure 7). For the *trans*- diastereomeric set, the alcohol and amine functional groups can either both be axially or equatorially oriented; whereas, in the *cis*-diastereomeric set, if one functional group is oriented axially, the other is necessarily oriented equatorially. Taking into consideration *A*-values (*A* for -NH_2 ranges from 1.23-1.7 and *A* for -OH ranges from 0.6-1.04), the most stable chair for each stereoisomer is the conformer that has the amine group in the equatorial position and thus, the imine in the assembly of Figure 3 is similarly anticipated to be equatorial.[57] Therefore, the alcohol is oriented axially in *cis*- and equatorially in the *trans*-, and this would give rise to a difference in the projection of the alcohol toward the pyridine rings in the octahedral Fe(II) complexes, and thereby affecting the arrangement of the pyridyl rings around the Fe(II) center. Thus, the observed

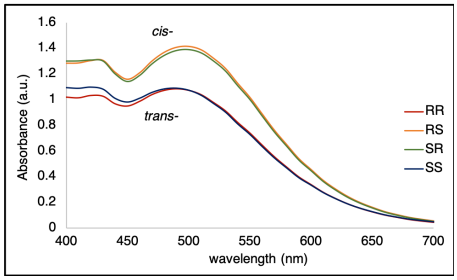


CD signals are strongly affected by the stereochemistry at the alcohol stereocenter.

Figure 7. Chair-flip equilibria for the four stereoisomers of 2-aminocyclohexanol.

Aminoalcohol *de* Determination

We anticipated the *cis*- and *trans*- diastereomers would be differentiated with the same octahedral iron complex via UV-Vis spectroscopy. As anticipated, we observed a distinct difference in absorbance values between the *cis*- and *trans*- diastereomers of **12** (Figure 8). This difference in absorbance is likely due to the different orientation of



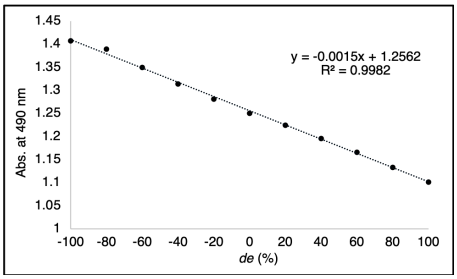
ligands around the iron (II) between the *cis*- and *trans*- diastereomeric sets.[58]

Figure 8. UV-Vis spectra for the four stereoisomers of 2-aminocyclohexanol with 3-hydroxy-pyridine-2-carboxaldehyde and $Fe(OTf)_2$. The UV-Vis spectra were recorded in CH_3CN at 25 °C (0.5 mM $Fe(OTf)_2$, 1.5 mM **8**, 1 cm cell).

A series of stereoisomeric solutions were made where the diastereomeric excesses (Eq. 1) were varied and monitored via UV-Vis spectroscopy; a linear correlation between *de* and absorbance was observed (Figure 9). As expected, no change in absorbance was seen when the *ee* of the solution was varied (Figure S19).

$$de = \left(\frac{trans - cis}{trans + cis}\right) X\ 100\%\ (Eq.\ 1)$$

The CD signals associated with the amine assembly are concentration independent if the iron (II) center is fully saturated with chiral imine ligands i.e. the CD signal will reflect the *ee* of the chiral amine (Figure 2). For most substrates, a 3:3:1 ratio of chiral amine, 3-hydroxy-2-pyridinecarboxaldehdye, iron triflate is suitable for complete assembly formation. The stereochemical complexity of the octahedral iron (II) complexes with a four component stereoisomeric mixture led us to perform experiments similar to those discussed above for the 1-position and the alcohol assembly, where we checked if the analytes were statistically incorporated into the assemblies or if one diastereomer was preferred (Figure S20). A statistical distribution was verified and the stereoisomeric complexity associated with the octahedral iron complexes was



determined to not affect chiroptical analysis as the complexes rapidly interconvert in equilibria.

Figure 9. Calibration curve for determining *de* of a solution of 2-aminocyclohexanol.

Comined *ee* and *de* Analysis

Having demonstrated enantiomeric differentiation of the four stereoisomers could be accomplished via CD spectroscopy of **12**/**19** and the relative configuration i.e. diastereomeric differentiation via UV-Vis spectroscopy of **12**, we turned our attention to determining the percent composition (speciation) for mixtures of the four stereoisomers. In a recent paper by our group, a mathematical relationship for the total speciation of a four component stereoisomeric mixture using *ee* and *de* values was described.[46] When discussing the success of an asymmetric transformation that forges two stereocenters, the enantioselectivity is typically characterized within the diastereomeric sets of enantiomers i.e. the *ee* is separately determined for the *cis*- and *trans*- isomers (Eqs. 2 and 3).

$$ee_{trans} = \frac{(1R,2R) - (1S,2S)}{(1R,2R) + (1S,2S)}\ X\ 100\%\ (Eq.\ 2)$$

$$ee_{cis} = \frac{(1R,2S) - (1S,2R)}{(1R,2S) + (1S,2R)}\ X\ 100\%\ (Eq.\ 3)$$

But, because our assays determine *ee* values at individual stereocenters, we defined the *ee*'s to be suited to our methods (Eqs. 4 and 5) while the traditional equation for diastereomeric excess remains unchanged (Eq. 6).

$$ee_1 = \frac{(1R,2R) + (1R,2S) - (1S,2R) - (1S,2S)}{(1R,2R) + (1R,2S) + (1S,2R) + (1S,2S)} X\ 100\%\ (Eq.4)$$

$$ee_2 = \frac{(1R,2R) + (1S,2R) - (1R,2S) - (1S,2S)}{(1R,2R) + (1R,2S) + (1S,2R) + (1S,2S)} X\ 100\%\ (Eq.5)$$

$$de = \frac{(1R,2R) + (1S,2S) - (1R,2S) - (1S,2R)}{(1R,2R) + (1R,2S) + (1S,2R) + (1S,2S)} X\ 100\%\ (Eq.6)$$

With these definitions, we aimed to individually home in on the absolute configuration at either the 1- or 2-position without considering the stereochemistry of the adjacent stereocenter. Rearrangement of the three equations above and substitution gave us equations for determining the percent composition of the four stereoisomers based on two *ee* values and a *de* value (Eqs. 7- 10).

$$\%\ (1R,2R) = \frac{1}{4}(ee_1 + ee_2 + de) + 25\ (\text{Eq. } 7)$$

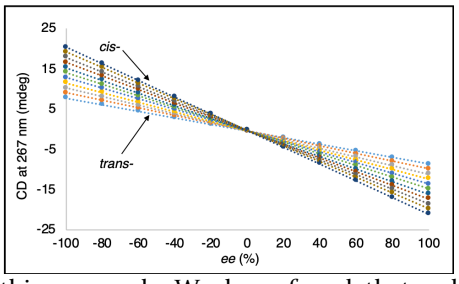
$$\%\ (1R,2S) = \frac{ee_1}{2} + 50 - \%\ (1R,2R)\ (Eq.8)$$

$$\%\ (1S,2R) = \frac{ee_2}{2} + 50 - \%\ (1R,2R)\ (Eq.9)$$

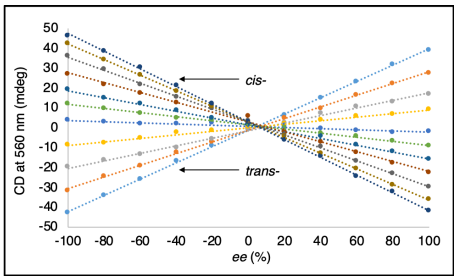
$$\%\ (1S,2S) = \frac{de}{2} + 50 - \%\ (1R,2R)\ (Eq.10)$$

For the above equations to give accurate percent compositions, the optical measurements should be unaffected by the adjacent stereocenter. For example, when monitoring the 1-position, (*1R,2R*) and (*1R,2S*) alcohols should give the same CD signal at 267 nm. But as discussed above, the adjacent stereocenter is imparting an effect on the spectroscopic signal. This can be clearly seen by considering the different intensities in CD magnitudes at the λ_{max} of both the alcohol and amine assemblies (Figures 10 and 11). These two plots show dramatic differences in the slopes of the ee vs CD signal for both the alcohol and amine assemblies, and in fact the amine assembly slope switches from negative values for *trans* to positive values for *cis*.

This difference between stereoisomers that possess the same stereochemistry at the stereocenter being investigated results in the inability to accurately determine the *ee* at that stereocenter. For example, considering a hypothetical stereoisomeric mixture of unknown composition of 2-aminocyclohexanol, one might observe a CD intensity of 10 mdeg at the λ_{max} of the alcohol assembly (Figure 10). Looking at Figure 10, a CD intensity of 10 mdeg corresponds to several different diastereomeric mixtures. At first glance, one might expect knowing the *de* of the mixture would allow for one to choose the line corresponding to the *de* in Figures 10 and 11 and determine ee_1 and ee_2; however, we have found that the mathematics do not



permit this approach. We have found that only a very special circumstance can one use this approach, and this is discussed extensively in reference 46. As a result, we were presented with the additional challenge of differentiating the stereochemistry of the adjacent stereocenter in order to accurately determine the *ee* values for the per-



cent composition calculations using Eqs. 7 - 10. Thus, we turned to chemometrics as a means to pattern the optical responses.

Figure 10. ECCD intensity of four component stereoisomeric mixtures of assembly **19** derived from the four stereoisomers of **21** plotted versus ee_1 values. Each line corresponds to a single *de* value (-100, -80, -60, -40, -20, 0, 20, 40, 60, 80, 100) and 11 ee_1 values (-100, -80, -60, -40, -20, 0, 20, 40, 60, 80, 100).

Figure 11. CD intensity of four component stereoisomeric mixtures of assembly **12** derived from the four stereoisomers of **8** plotted versus ee_2 values. Each line corresponds to a single *de* value (-100, -80, -60, -40, -20, 0, 20, 40, 60, 80, 100) and 11 ee_2 values (Left to right -100, -80, -60, -40, -20, 0, 20, 40, 60, 80, 100).

Chemometric Analysis

With the CD intensities for both the alcohol and amine assemblies, and the UV-Vis spectrum for the amine assembly as inputs, we employed a chemometric analysis (machine learning) to determine *ee* values at both stereocenters. We began with a training set of 70 samples and a test set of 10 samples and recorded the CD and absorbance spectra (Figures S22-S27). A visual representation of the space spanned by the training and test sets is shown in Figure 12. Note that we used values toward the high ends of *ee* and *de* for training, as these are the values that would be of most interest in a real screening scenario.

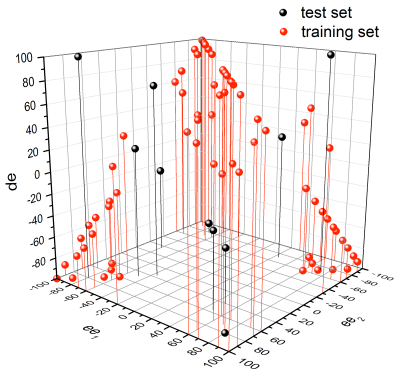
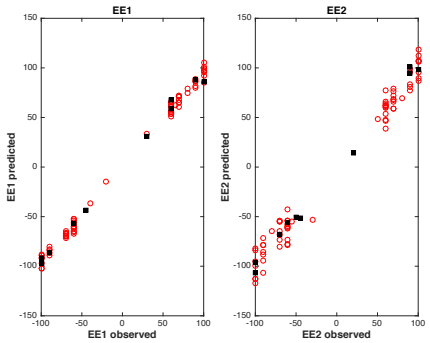


Figure 12. 3-D Map of ee_1, ee_2 and *de* space spanned by training and test set, where the x-axis is ee_1, y-axis is ee_2 and the *z*-axis is *de*.

The three sets of measurements collected, i.e., the CD intensities for **19** ($\mathbf{X}_{CD,\ alcohol}$) and for **12** ($\mathbf{X}_{CD,\ amine}$), and the UV-Vis spectra of **12** ($\mathbf{X}_{UV\text{-}Vis,\ amine}$) were used as predictors to build a linear regression model for the quantification of ee_1 and ee_2. In this framework, in order to take advantage of the multi-block nature of the data set, a novel multi-block regression method called sequential and orthogonalized covariance selection (SO-CovSel)[59] was applied to find the best relationship between the measured variables (i.e. the spectral intensities) and the quantities to be predicted (ee_1 and ee_2). This method (which is explained in greater detail in Section V of the Supporting Information) couples the search for the optimal regression parameter, leading to the most accurate predictions, with a highly effective variable selection strategy which, leading to parsimonious models (i.e., models based only on a small number of predictors), are more robust, more easily interpretable and also, more suitable to be implemented in sensors. In the present study, the CD and UV-Vis data measured on the calibration samples were used to build the regression model, i.e., to select the best subset of variables (spectral intensities) to be used as predictors and to estimate their associated regression coefficients for the prediction of the responses (the values of ee_1 and ee_2). The optimal model (which was selected based on a cross-validation procedure with 7 cancelation groups) was the one built using 11 variables from the alcohol CD block (the spectral intensities at 240, 249, 252, 254, 256, 259, 262, 266, 269, 271 and 273 nm) and only one (ellipticity at 560 nm) and one (absorbance at 500 nm) for the amine CD and amine UV-Vis blocks, respectively. These variables clearly would not have been chosen if one had simply assumed the λ_{max} values would be optimal for variable selection. When applied to the training set, this model resulted in absolute error of ±5.4% and ±10.7%, for the prediction of ee_1 and ee_2, respectively (Figure 13), and, in the validation stage, even better results were obtained on the test set (Table 1), ±6% for ee_1 and ±5% for ee_2, respectively.



With the ee_1 and ee_2 values predicted using SO-CovSel (Table S1) and *de* values determined directly from the absorbance at 490 nm of the octahedral iron complexes, Equations 7 – 10 were applied for total speciation and the percent composition of each stereoisomer (Table 1), resulting in an averaged absolute error of only ± 4%. Finally, these percent compositions were expressed as the commonly used parameters of *ee* within each diastereomeric set (i.e. ee_{cis} and ee_{trans}, Table 2) with similarly low absolute errors (± 3%).

Figure 13. Plots of known *ee* ($EE_{\#,observed}$) versus *ee* predicted using SO-CovSel regression method, where the red data points are the training set and the black data points are the test set.

Table 1. Total Stereoisomeric Speciation with *ee* Values Predicted Using SO-CovSel and a *de* Value from Absorbance Data[a]

RR			***SS***		
calc.	actual	abs. error	calc.	actual	abs. error
0.73	0	0.7	97.0	100	-2.9
4.64	0	4.6	4.37	0	4.4
91.1	95	-3.9	-1.26	0	-1.3
-4.07	0	-4.1	-7.90	0	-7.9
4.22	5.26	-1.0	8.10	5.26	2.8
13.0	10	3.0	68.0	65	3.0
38.5	35	3.5	3.47	5	-1.5
58.2	60	-1.8	5.43	0	5.4
31.2	20	11.3	30.6	20	10.6
0.06	0	0.1	67.8	65	2.8
RS			***SR***		
calc.	actual	abs. error	calc.	actual	abs. error
-3.90	0	-3.9	6.08	0	6.1
-2.78	0	-2.8	93.8	100	-6.2

8.16	5	3.2	2.02	0	2.0
102	95	6.7	10.3	5	5.3
20.2	21.05	-0.9	67.5	68.42	-0.9
11.6	15	-3.4	7.42	10	-2.6
62.1	60	2.1	-4.13	0	-4.1
-1.11	0	-1.1	37.5	40	-2.5
-9.19	0	-9.2	47.3	60	-12.7
15.9	15	0.9	16.34	20	-3.7

[a]All values are given in percentages.

Table 2. Calculated *ee* Values Within Each Diastereomeric Set[a]

ee_{cis}			ee_{trans}		
calc.	actual	abs. error	calc.	actual	abs. error
-9.98	0	-9.98	-96.4	-100	3.64
-96.6	-100	3.45	0.27	0	0.27
6.14	5	1.14	92.4	95	-2.65
91.4	90	1.39	3.82	0	3.82
-47.3	-47.4	0.09	-3.89	0	-3.89
4.13	5	-0.87	-54.9	-55	0.06
66.3	60	6.28	35.0	30	5.04
-38.6	-40	1.44	52.8	60	-7.21
-56.5	-60	3.50	0.62	0	0.62
-0.40	-5	4.60	-67.7	-65	-2.70

[a]All values are given in percentages.

Conclusion

Rapid optical assays for the determination of reaction parameters in parallel synthesis routines targeted to optimizing *ee* and *de* values are becoming increasingly necessary as automation methods permeate reaction discovery. To explore methods for both *ee* and *de* analysis (as well as reaction yield) we turned our first study to a very challenging analyte: 2-aminocyclohexanol. Not only is there no chromophore in this analyte, but the stereocenters are vicinal, which led to the optical signal being influenced by both the targeted functional group and the neighboring functional group. Thus, a simple mathematical approach to achieve complete speciation was not possible, but instead, a patterning technique (chemometrics) was implemented. This resulted in only a 4% absolute error on determining the percentages of each of the four stereoisomers in random mixtures. Further, optical analysis of 96 reactions can be accomplished in 27 minutes: 5 minutes for concentration determination via a single wavelength measurement, 5 minutes for simultaneous ee_2/*de* determination via a single wavelength measurement, and 17 minutes for ee_1 determination via 12 wavelength measurements. Because 2-aminocyclohexanol was simply chosen to be a challenging analyte for which to develop our methods, we are now turning towards using this general work-flow for screening reactions that simultaneously set two stereocenters in a single step with varying degrees of enantio- and diastereoselectivities, such as the reduction of diketide thioesters via ketoreductases.[31]

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Detailed synthetic protocols, optical analyses, training set data, test set data and predicted *ee* values usng SO-CovSel along are included in the Supporting Information, as well as Supporting Text, Supporting Figures (S1-S27) and a Supporting Table (S1). (PDF)

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