Analytical Methods

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Determination of Enantiomeric Excess in Amine Derivatives with Molecular Self-Assemblies**

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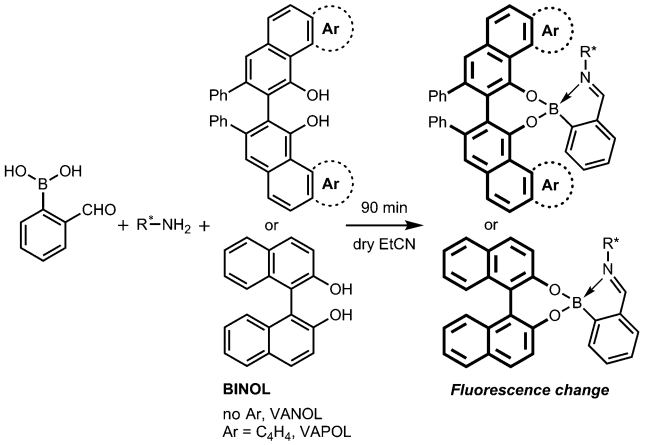
Abstract: *We report the first fluorescence-based assay for the rapid determination of the ee value of amines, amino alcohols, and amino acid esters. The method uses the self-assembly of 2-formylphenylboronic acid with a chiral diol and a chiral amine or derivatives (of unknown chirality) to produce two diastereomeric iminoboronates that differ in their fluorescence intensity and polarization. The approach allows for the accurate determination of the ee value of chiral amines with errors of just 1–2 %. We believe that this application of orthogonal dynamic covalent self-assembly in the determination of the enantioselectivity will lead to the development of high-throughput procedures for the determination of chirality.*

Chiral amines play pivotal roles in biology[1] and the pharmaceutical chemistry[2] and are also used as chiral auxiliaries.[3] Given its importance, the determination of the enantiomeric excess (*ee*) has become central to organic and medicinal chemistry and necessitates the development of methods for the fast and reliable determination of the *ee* value.[4]

One of the most common methods for the determination of enantiomeric purity is the measurement of optical rotation.[5] In addition, NMR spectroscopy,[6] GC, and HPLC are also useful techniques.[7] As an alternative, circular dichroism (CD) has been used and recently brought to a high-throughput level.[8] Alas, these methods require high concentrations of analytes, strict conditions, and—with few exceptions—are too slow for high throughput-screening (HTS).[9]

For the above reasons we have decided to develop a sensitive and HTS-amenable assay for chiral amines and their derivatives. We take advantage of a ternary system comprising an α-chiral primary amine, 2-formylphenylboronic acid (FPBA), and enantiomerically pure 1,1′-bi-2-naphthol (BINOL), which assemble rapidly and lead to characteristic ^{1}H NMR spectroscopic shifts for each enantiomer of the amine (Scheme 1).[10] This system has also been employed for determination of *ee* values by using electrochemical[11] or CD methods.[12] Our assumption is that the twist angle of the fluorescent BINOL ligand, is affected by the chirality of the analyte during the assembly, thereby resulting in detectable fluorescence changes (i.e. intensity changes and spectral shifts). We have selected several aromatic diol derivatives: BINOL, 3,3′-diphenyl-2,2′-bi-1-naphthol (VANOL), and 2,2′-diphenyl-(4-biphenanthrol) (VAPOL), as the reporter moieties for the analysis of chiral amines, amino alcohols, and amino acids. VANOL and VAPOL are more sterically encumbered ligands, and are thus more likely to increase the enantioselectivity during the formation of the resulting iminoboronate esters. Here, the fluorescence from the diastereomeric product mixture (*S* ligand, chiral amine with various *ee* values, achiral 2-formylphenylboronic acid, FPBA, Scheme 1) is read rapidly using a microplate reader.



Scheme 1. Three-component self-assembly reaction between 2-formylphenylboronic acid, a primary amine, and aromatic diol derivatives.

The reaction in Scheme 1 was studied using mass spectrometry (see the Supporting Information), fluorescence spectroscopy, and single-crystal X-ray diffraction analysis. Figure 1 shows the X-ray structures of two enantiomeric iminoboronate esters (*S*,*S* and *R*,*R*) of FPBA, α-methylbenzylamine (MBA), and VAPOL in a 1:1:1 stoichiometry. Figure 1 also shows the pseudotetrahedral geometry at the boron atom. Such a geometry provides rigidity, which is a factor both in governing the enantioselectivity of the system and in communicating any binding events to the fluorophore.

Next, a quantitative determination of chiral enrichment in amines, amino alcohols, and amino acids (in the form of their methyl esters) was obtained by fluorescence titrations. An example is shown in Figure 2. Here, the fluorescence of (*S*)-VANOL is quenched upon formation of the iminoboronate

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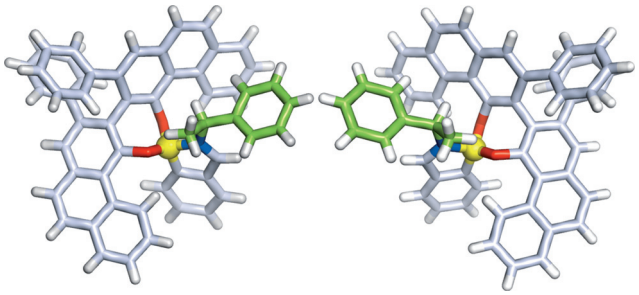

Figure 1. X-ray crystal structures of (*S*,*S*)- (left)and (*R*,*R*)-iminoboronate esters (right) self-assembled from FPBA, MBA, and VAPOL. C: gray (or green for MBA), B: yellow, N: blue, O: red, and H: white.

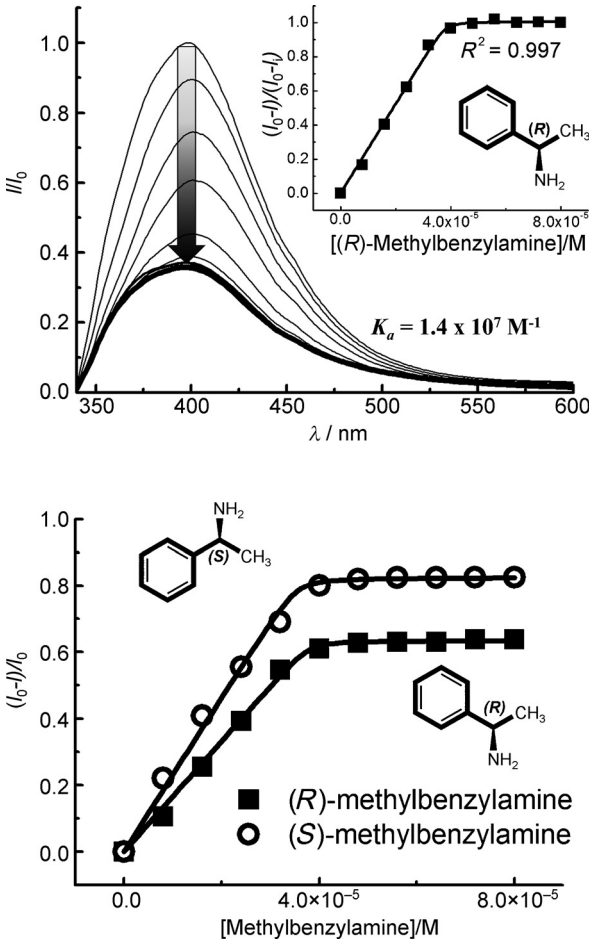

Figure 2. Top: Fluorescence spectra of (*S*)-VANOL (40 μM) with FPBA (40 μM) upon addition of (*R*)-methylbenzylamine in dry EtCN. λ_{ex} = 335 nm. [(*R*)-methylbenzylamine] = 0–80 μM. Bottom: The binding isotherms for (*S*)- and (*R*)-methylbenzylamine show the two enantiomers have distinctly different behavior.

ester. Figure 2 (top) shows the attenuation of the fluorescence upon addition of (*R*)-methylbenzylamine. Figure 2 (bottom) shows two isotherms corresponding to the fluorescence response to (*R*)- and (*S*)-methylbenzylamine. The quenching is likely due to photoinduced electron transfer from the nitrogen atom of the complexes.[13] A red-shift of the fluorescence arising from the formation of an oxazolidine boronate ester is observed in the presence of amino alcohols.[14] Notably, the changes in the fluorescence signals of the

Table 1: The apparent association constants (K_a, M^{-1})[a] obtained from fluorescence titration in dry EtCN.

Guest	(*S*)-BINOL K_a, [M^{-1}]	(*S*)-VANOL K_a, [M^{-1}]	(*S*)-VAPOL K_a, [M^{-1}]
(*S*)-methylbenzylamine (MBA)	7.0×10^6	2.3×10^7	3.1×10^6
(*R*)-methylbenzylamine	2.4×10^6	1.4×10^7	4.5×10^6
(*S*)-methylphenethylamine (MPA)	9.9×10^5	$>10^8$	1.9×10^6
(*R*)-methylphenethylamine	5.6×10^5	$>10^8$	1.3×10^6
(*S*)-2-aminobutane (AMB)	4.2×10^5	1.5×10^7	7.9×10^6
(*R*)-2-aminobutane	1.2×10^6	1.2×10^6	4.0×10^6
(*S*)-1-(2-naphthyl)ethylamine (NEA)	2.4×10^6	8.0×10^6	1.6×10^6
(*R*)-1-(2-naphthyl)ethylamine	2.2×10^6	3.6×10^6	2.1×10^6
(*S*)-phenylalanine (Phe)	4.1×10^3	3.3×10^4	2.0×10^4
(*R*)-phenylalanine	5.9×10^3	1.3×10^5	3.2×10^4
(*S*)-valine (Val)	5.4×10^3	7.4×10^4	2.6×10^4
(*R*)-valine	7.2×10^3	1.1×10^4	3.1×10^4
(*S*)-2-amino-3-phenyl-1-propanol (APP)	9.7×10^5	5.8×10^6	1.7×10^6
(*R*)-2-amino-3-phenyl-1-propanol	1.5×10^7	3.0×10^6	6.7×10^6

[a] The K_a values were calculated based on the change in fluorescence intensity upon addition of each guest. The errors in the curve fitting were < 20%.

diastereomers are different, which suggests that the determination of the enantiomeric excess can be monitored from changes in the fluorescence intensity and polarization.

Importantly, the two diastereomeric complexes (*S*)-VANOL + (*S*)-methylbenzylamine (+FPBA) versus (*S*)-VANOL + (*R*)-methylbenzylamine (+FPBA) show different fluorescence intensities, as well as different association isotherms and apparent association constants (K_a). On the other hand, a control experiment utilizing two enantiomeric mixtures (*S*)-BINOL + (*S*)-methylbenzylamine (+FPBA) versus (*R*)-BINOL + (*R*)-methylbenzylamine (+FPBA) showed identical isotherms.

Table 1 shows the apparent association constants (K_a, M^{-1}) for selected amines, amino alcohols, and amino acid esters. Importantly, the method is not dramatically sensitive to potential impurities such as water (up to 5% v/v, after which two phases separate), ethanol (tested up to 50% v/v), ethylene glycol, glucose, and citrate (all up to 4.0 mM in water, that is, 1 equiv).

The values in Table 1 suggest that the K_a values are different for enantiomeric amines, thus enabling the reliable detection of the *ee* value. The general order of association constants observed was amines ≈ amino alcohols > amino acid esters. These differences then contribute to the discrimination of analytes in an array-based assay.

The fluorescence data were recorded using a conventional plate reader and 384-well microplates (see the Supporting Information). Pattern recognition procedures[15] were then used to reveal the guest-specific trends in the overall response.

First, we performed a qualitative assay, run at a constant concentration of 40 μM and 1:1:1 stoichiometry of the amine, chiral auxiliary, and FPBA. The qualitative assay focused on the difference in fluorescence of the two enantiomeric amines. A large difference in the spectroscopic behavior of the enantiomers is manifested as a large distance between the

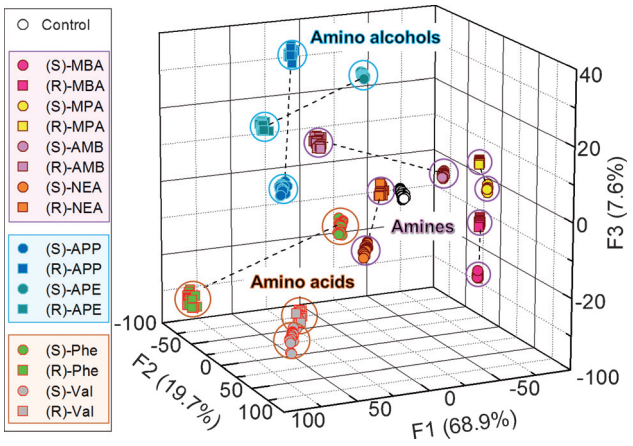

Figure 3. The qualitative linear discriminant analysis (LDA) of amine, amino alcohol, and amino acid enantiomers in EtCN by using a fluorescence assay show large distances between the clusters of enantiomer pairs, thus suggesting a high chance for the determination of the *ee* value (MBA: methylbenzylamine, MPA: methylphenethylamine, AMB: 2-aminobutane, NEA: 1-(2-naphthyl)ethylamine, APP: 2-amino-3-phenyl-1-propanol, APE: 2-amino-1-phenylethanol).

corresponding clusters in the linear discriminant analysis (LDA)[16] plot (Figure 3). LDA is a frequently used supervised pattern recognition method for reduction of dimensionality and classification of the multivariate data. LDA models the similarity by maximizing the distance between the classes and minimizing the distance between the trials within the clusters. A cross-validation procedure was performed to ascertain the level of correct classification of the observations within the clusters. From the score plot one can see that the responses could discriminate 16 analytes and a control with 100% correct classification of all 340 data points (corresponding to 16 analytes and a control). The assay recognized the amino derivatives and sorted them into three groups: amines, amino alcohols, and amino acids. Importantly, the obtained results reveal that the assay can discriminate different enantiomers of amines, amino alcohols, and amino acids and that the *S* isomers (circles) are well separated from the *R* isomers (squares).

The following semiquantitative assay (Figure 4) shows results of analyses of varying the *ee* value of four different amino derivatives: methylbenzylamine, 1-(2-naphthyl)ethylamine, 2-amino-3-phenyl-1-propanol, and phenylalanine. All four compounds were measured in 10 steps of different enantiopurity, from 0 to 100% *ee*. Once again, a 100% correct classification is observed. The linear trends in the individual series of the responses showed smooth progression from 0 to 100% *ee*, thereby suggest a high chance for successful linear regression analysis of the *ee* value.

For the quantitative % *ee* determination analysis of the guests we used a support vector machine (SVM) regression method, which is more suitable for modeling complex responses and nonlinear behavior of data.[17] Briefly, SVM is a supervised classification method that seeks to separate classes by mapping the input into an *n*-dimensional vector space using kernel functions. The SVM regression method constructs calibration models that serve to predict the *ee* values of unknown samples.

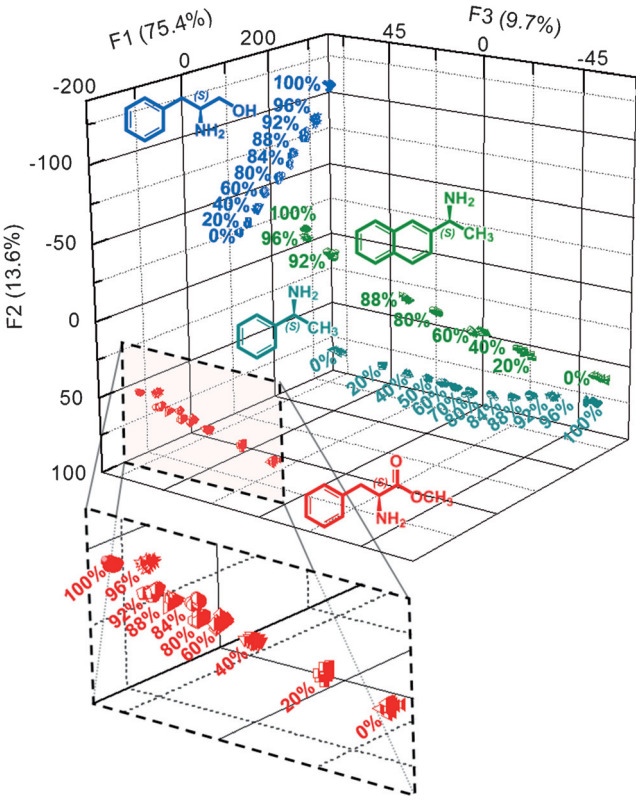

Figure 4. Results of LDA in EtCN for the semiquantitative assay of (*S*)-methylbenzylamine (cyan), (*S*)-1-(2-naphthyl)ethylamine (green), (*S*)-2-amino-3-pheyl-1-propanol (blue), (*S*)-phenylalanine (red).

The SVM regression allowed multiple points of enantiomeric excess to be predicted. We used 10 data points corresponding to various *ee* values to model the behavior of the data and two different % *ee* values to validate the model. The developed model was used to quantify two unknown samples (squares in Figure 5). The quantitative assay yielded

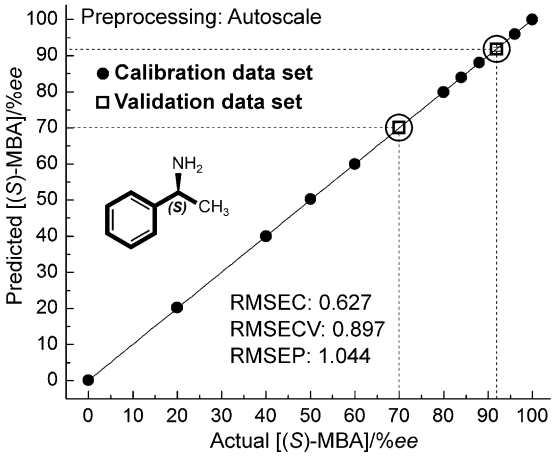

Figure 5. Results of the SVM regression for determination of the *ee* value of methylbenzylamine (MBA). The plot of actual versus predicted *ee* value shows high accuracy of the prediction for multiple *ee* values. The values of the root-mean-square errors (RMSEs) of calibration (C), cross-validation (CV), and prediction (P) (shown as squares) attest to the high quality of the model and prediction.

a very accurate % *ee* regression analysis of the amino derivatives, as shown by the root-mean-square errors (RMSEs). The RMSE of the prediction (RMSEP) revealed that the assay can predict the % *ee* value of each analyte with a maximum error of 1–2 % (Figure 5).

In summary, we have reported the first fluorescence-based assay for the determination of the enantiomeric excess of amines, amino alcohols, and amino acid esters. This method uses molecular self-assemblies based on boronate esterification. Fluorescence titrations showed highly variable substrate-dependent changes in fluorescence for various percentages of enantiomeric excess. The assay itself requires only a simple fluorescence plate reader and may be performed in a high-throughput fashion. Analysis of the % *ee* values with low errors was successfully achieved using support vector machines (SVMs). We believe that this application of orthogonal dynamic covalent self-assembly for the determination of *ee* values will lead to the development of high-throughput procedures for the determination of enantiomeric excess.

Experimental Section

Enantiopure aromatic diol derivative (BINOL, VANOL, or VAPOL; 1.1 equiv) and 2-formylphenylboronic acid (1 equiv) were dissolved in EtCN to form a 4 mM stock solution. Subsequently, amine (1 equiv) of known or unknown *ee* value was added and the reaction mixture incubated for 90 min to ensure complete formation of the diasteriomeric iminoboronate esters. Each reaction mixture was then diluted 100 times with pure propionitrile to a final concentration of 40 μM.

The diluted reaction mixtures were dispensed into the microplates and read with a microplate reader with the following sets of filters: Fluorescence intensity (FI): 300 nm excitation/380 nm emission (1st channel) and 320 nm excitation/370 nm emission (2nd channel), and fluorescence polarization (FP) at 330 nm excitation and 420 nm dual emission.

Keywords: chirality · fluorescence · self-assembly · sensors · supramolecular chemistry

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