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Pseudoenantiomeric Fluorescent Sensors in a Chiral Assay

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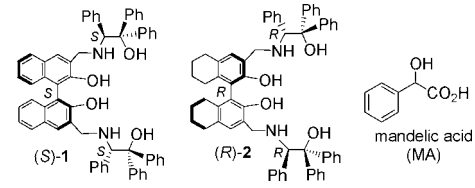
Abstract: Two enantioselective fluorescent sensors, namely, the 1,1′-binaphthol (BINOL)-amino alcohol (*S*)-**1** and the H_8BINOL-amino alcohol (*R*)-**2**, have been prepared as a pseudoenantiomeric pair. These two compounds have the opposite chiral configuration at both the axially chiral biaryl centers and the amino alcohol units. In methylene chloride solution, (*R*)-mandelic acid greatly enhances the emission of (*S*)-**1** at $\lambda_1 = 374$ nm and (*S*)-mandelic acid greatly enhances the emission of (*R*)-**2** at $\lambda_2 = 330$ nm. A 1:1 mixture of (*S*)-**1** and (*R*)-**2** was used to interact with mandelic acid at a variety of concentrations with various enantiomeric compositions. It was found that both the concentration of mandelic acid and its enantiomeric composition can be directly determined by measuring the sum and difference of the fluorescence intensities at λ_1 and λ_2.

In recent years, there has been growing interest in developing enantioselective fluorescent sensors because of their potential application as a rapid analytical tool in chiral assays.[1,2] A number of highly enantioselective fluorescent sensors for the recognition of chiral molecules such as carboxylic acids, amines, alcohols, amino alcohols, and amino acid derivatives have been reported.[1−3] These sensors can be used to determine the enantiomeric composition of a chiral substrate at a given concentration. Because the fluorescence of a chiral sensor is strongly influenced by both the concentration and the enantiomeric composition of the substrate, these two parameters need to be determined separately.[1m] It would be highly advantageous if both the concentration and the enantiomeric composition of the substrate could be determined simultaneously by one fluorescence measurement. This would greatly simplify the analysis of the reaction products generated from high-throughput screening experiments.

We propose the development of a novel strategy for simultaneously measuring both the concentration and the enantiomeric composition of a chiral substrate by using pseudoenantiomeric fluorescent sensor pairs. An enantiomeric fluorescent sensor pair is a racemic mixture that cannot be used for the desired chiral recognition. In contrast, the two sensors in a pseudoenantiomeric fluorescent sensor pair, (*S*)-A and (*R*)-B, have opposite enantioselectivities and emit at two distinctively different wavelengths, λ_A and λ_B. When a mixture of the pseudoenantiomeric sensor pair (*S*)-A and (*R*)-B is treated with an enantiomeric mixture of a chiral substrate, we assume that one enantiomer of the substrate should enhance the fluorescence of (*S*)-A at λ_A, giving a fluorescence intensity I_A, and the other enantiomer of the substrate should enhance the fluorescence of (*R*)-B at λ_B, giving a fluorescence intensity I_B. It is proposed that the difference in the fluorescence intensities, $I_A - I_B$, can be used to determine the concentration difference of the two enantiomers of the substrate and the sum of the fluorescence intensities, $I_A + I_B$, can be used to determine the total concentration of the two enantiomers. That is, both the enantiomeric composition of the substrate and its concentration could be determined by one fluorescence measurement with the use of the pseudoenantiomeric sensor pair. Herein, we demonstrate for the first time that such a pseudoenantiomeric fluorescent sensor pair can accomplish the desired chiral assay.

Chart 1



We conceived the use of the 1,1′-binaphthol (BINOL)-amino alcohol (*S*)-**1** and its analogue (*R*)-**2** (Chart 1) as a pseudoenantiomeric sensor pair. These two compounds have the opposite chiral configuration at both the axially chiral biaryl centers and the amino alcohol units. They are expected to exhibit emission at different wavelengths because of the much reduced conjugation in (*R*)-**2** relative to (*S*)-**1**.

Recently, we reported (*S*)-**1** as a generally enantioselective fluorescent sensor for α-hydroxycarboxylic acids in benzene solution.[3] Because of the reduced conjugation of (*R*)-**2** relative to (*S*)-**1**, benzene interferes with the fluorescence spectrum of (*R*)-**2** and is not a suitable solvent for this pseudoenantiomeric pair. We therefore examined the fluorescence response of (*S*)-**1** toward mandelic acid (MA) (Chart 1) in CH_2Cl_2. Even though CH_2Cl_2 is a much more polar solvent, highly enantioselective fluorescent responses were still observed. As shown in Figure 1a, (*R*)-MA greatly enhances the fluorescence of (*S*)-**1** at $\lambda_1 = 374$ nm, whereas (*S*)-MA causes only a very small fluorescence enhancement. It was found that the intensity ratio I_R/I_0 was 11.4 and that the enantioselective fluorescence enhancement ratio [ef $= (I_R - I_0)/(I_S - I_0)$] was 26.0. Figure 1b shows the fluorescence responses of (*S*)-**1** at various concentrations of (*R*)- and (*S*)-MA.

Compound (*R*)-**2** was obtained by using the partially hydrogenated BINOL (*R*)-H_8BINOL as the starting material. We studied

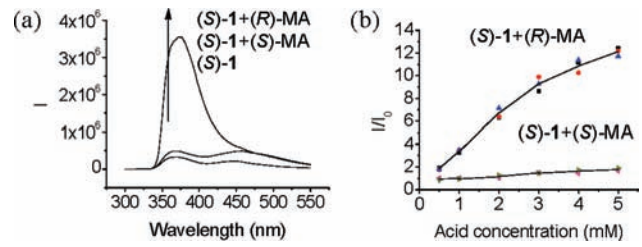


Figure 1. (a) Fluorescence spectra of (*S*)-**1** (1.0×10^{-4} M in CH_2Cl_2) with or without (*R*)- or (*S*)-MA (4.0×10^{-3} M). (b) Three independent measurements of the fluorescence enhancement of (*S*)-**1** (1.0×10^{-4} M in CH_2Cl_2) at $\lambda_1 = 374$ nm at various MA concentrations. ($\lambda_{exc} = 290$ nm, slit widths = 4.0/4.0 nm.)

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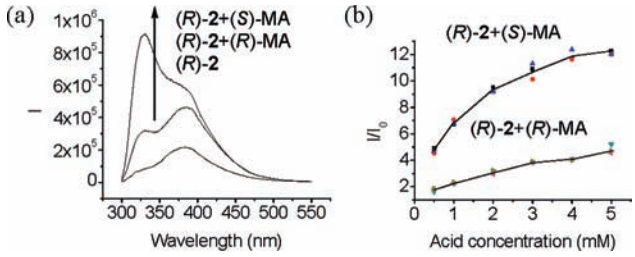

Figure 2. (a) Fluorescence spectra of (*R*)-**2** (1.0×0^{-4} M in CH_2Cl_2) with or without (*R*)- or (*S*)-MA (4.0×10^{-3} M). (b) Three independent measurements of the fluorescence enhancement of (*R*)-**2** (1.0×10^{-4} M in CH_2Cl_2) at $\lambda_2 = 330$ nm at various MA concentrations. ($\lambda_{exc} = 290$ nm, slit widths = 4.0/4.0 nm.)

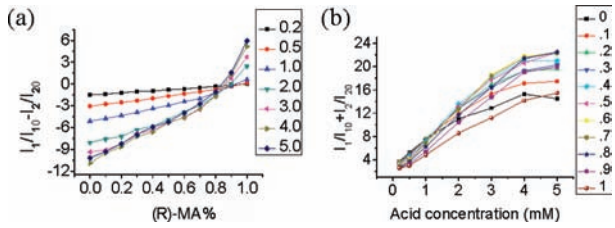

Figure 3. (a) Plots of $I_1/I_{10} - I_2/I_{20}$ vs (*R*)-MA% at various MA concentrations (mM). (b) Plots of $I_1/I_{10} + I_2/I_{20}$ vs MA concentration at various values of (*R*)-MA%. ($\lambda_{exc} = 290$ nm, slit widths = 4.0/4.0 nm.)

the fluorescence response of (*R*)-**2** toward (*R*)- and (*S*)-MA in CH_2Cl_2. As shown in Figure 2a, (*S*)-MA greatly enhances the fluorescence of (*R*)-**2** at $\lambda_2 = 330$ nm but (*R*)-MA causes a much smaller fluorescence enhancement. It was found that $I_S/I_0 = 11.7$ and ef = 3.6. Figure 2b shows the fluorescence responses of (*S*)-**1** at various concentrations of (*R*)- and (*S*)-MA.

The distinctively different fluorescence response wavelengths of (*S*)-**1** and (*R*)-**2** and their good and opposite enantioselectivities in the recognition of MA encouraged us to study the use of this pseudoenantiomeric sensor pair to interact with MA. A 1:1 mixture of (*S*)-**1** and (*R*)-**2** in CH_2Cl_2 in which each sensor's concentration was 1.0×10^{-4} M was prepared. This sensor pair solution was treated with MA at various enantiomeric compositions and total concentrations. The fluorescence intensity at $\lambda_1 = 374$ nm is labeled as I_{10} without MA and I_1 with MA, and the fluorescence intensity at $\lambda_2 = 330$ nm is labeled as I_{20} without MA and I_2 with MA. Figure 3a presents plots of the difference between the fluorescence intensity ratios at λ_1 and λ_2 ($I_1/I_{10} - I_2/I_{20}$) versus the enantiomeric purity of MA [(*R*)-MA%] as the total acid concentration was varied from 2.0×10^{-4} to 5.0×10^{-3} M. The data show that when pure (*R*)-MA was used [(*R*)-MA% = 1], $I_1/I_{10} > I_2/I_{20}$ and that when pure (*S*)-MA was used [(*R*)-MA% = 0], $I_1/I_{10} < I_2/I_{20}$. As (*R*)-MA% increased, $I_1/I_{10} - I_2/I_{20}$ changed from the negative region to the positive region. At the higher acid concentrations, the fluorescence intensity differences were greater, and as the acid concentration decreased, the fluorescence intensity difference decreased. Figure 3b displays plots of the sum of the fluorescence intensities at λ_1 and λ_2 ($I_1/I_{10} + I_2/I_{20}$) versus the total acid concentration for various values of (*R*)-MA%. These data show that as the acid concentration increased, $I_1/I_{10} + I_2/I_{20}$ increased. This increase was approaching a plateau point as the acid concentration was greater than 4 mM.

On the basis of Figure 3a,b, we plotted $I_1/I_{10} - I_2/I_{20}$ and $I_1/I_{10} + I_2/I_{20}$ against the MA concentration and (*R*)-MA%, respectively, in Figure 4. In Figure 4a, both 3D and 2D graphs are used to show the relationship of $I_1/I_{10} - I_2/I_{20}$ and $I_1/I_{10} + I_2/I_{20}$ to the MA concentration, and the data are color-coded according to the MA concentration. These graphs show that in the concentration range

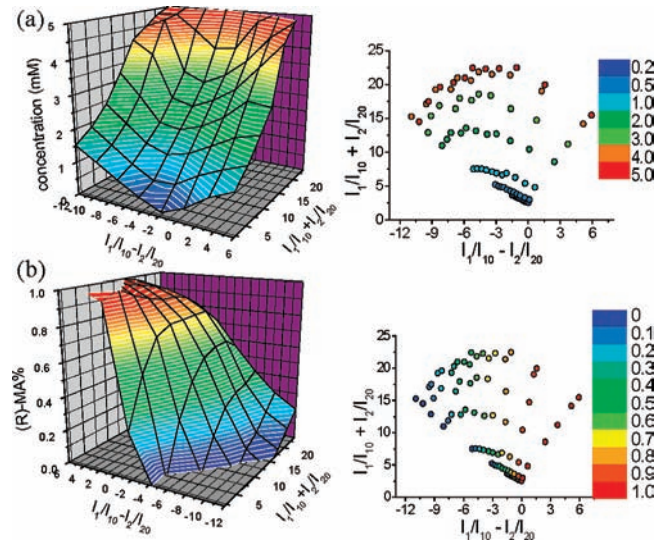

Figure 4. 3D and 2D plots of $I_1/I_{10} - I_2/I_{20}$ and $I_1/I_{10} + I_2/I_{20}$ with (a) the MA concentration (mM) and (b) (*R*)-MA%.

0.5−4 mM, $I_1/I_{10} - I_2/I_{20}$ and $I_1/I_{10} + I_2/I_{20}$ can be used to determine the total concentration of MA. The points start to overlap outside this concentration range. In Figure 4b, both 3D and 2D graphs are used to show the relationship of $I_1/I_{10} - I_2/I_{20}$ and $I_1/I_{10} + I_2/I_{20}$ to (*R*)-MA%, and the data are color-coded according to (*R*)-MA%. When $I_1/I_{10} + I_2/I_{20} > 5$, that is, the when the concentration of MA is >0.5 mM according to Figure 4a, the enantiomeric purity can be determined by using $I_1/I_{10} - I_2/I_{20}$ and $I_1/I_{10} + I_2/I_{20}$. Therefore, Figure 4a,b allow the direct determination of both the concentration and the enantiomeric composition of MA by one fluorescence intensity measurement of the sensor−substrate sample.

In conclusion, we have demonstrated that the pseudoenantiomeric molecular pair (*S*)-**1** and (*R*)-**2** are highly enantioselective fluorescent sensors toward MA with distinctively different emission response wavelengths. Each molecule in this sensor pair recognizes a different enantiomer of MA. When a 1:1 mixture of (*S*)-**1** and (*R*)-**2** is used to interact with the chiral acid, the sum and the difference of the fluorescence intensity ratios at the two emission wavelengths obtained in one fluorescence measurement can be used to directly determine both the concentration and the enantiomeric composition of the chiral substrate. This new strategy is potentially useful for the analysis of the chiral substrates generated from high-throughput catalyst or reaction screening experiments, which are expected to produce a great number of samples with varying concentrations and enantiomeric compositions.

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Supporting Information Available: Detailed synthesis and characterization data for (*R*)-**2** and fluorescence spectra for the interaction of (*S*)-**1** and (*R*)-**2** with MA. This material is available free of charge via the Internet at http://pubs.acs.org.

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