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Simultaneous Determination of Both the Enantiomeric Composition and Concentration of a Chiral Substrate with One Fluorescent Sensor

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Supporting Information

ABSTRACT: A fluorescent sensor is discovered to exhibit high sensitivity at one emission wavelength and high enantioselectivity at another when treated with a chiral diamine. By using this fluorescent sensor, it is demonstrated for the first time that both the concentration and enantiomeric composition of a chiral substrate can be determined simultaneously with one fluorescence measurement.

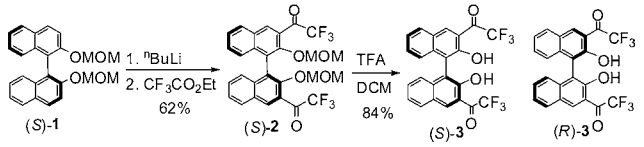
The potential application of enantioselective fluorescent sensors in rapid chiral assay has attracted significant research activity in this area. In recent years, a number of highly enantioselective fluorescent sensors have been developed for the recognition of chiral substrates such as carboxylic acids, amino acids, amines and amino alcohols.[1,2] These sensors can be used to determine the enantiomeric composition of a chiral substrate at a given concentration. Thus, an independent method to determine the concentration of the substrate is generally required. That is, two separate measurements are needed in order to determine both the concentration and the enantiomeric composition of a sample.[3]

In 2007, Anslyn reported the use of two UV absorption sensors (one chiral and one achiral with distinctively different absorptions) placed separately in a dual-chamber quartz cuvette to determine both the enantiomeric composition and concentration in one absorption measurement.[4] In 2010, we reported the use of a pseudoenantiomeric sensor pair in a fluorescent chiral assay.[5] A pseudoenantiomeric sensor pair contains a mixture of two sensors that have emissions at two different wavelengths (λ_1 and λ_2) with the opposite fluorescent responses to the two enantiomers of a chiral molecule. When this pseudoenantiomeric sensor pair is applied to a chiral assay, we have demonstrated that using the fluorescent intensity difference ($I_1 - I_2$) (I_1 = fluorescence intensity at λ_1, I_2 = fluorescence intensity at λ_2) can determine the enantiomeric composition of the substrate and using the fluorescence intensity sum ($I_1 + I_2$) can determine the concentration. That is, one fluorescent measurement could give both data with the use of the sensor mixture.

The above study leads us to propose another fluorescent method to determine both the concentration and enantiomeric composition of a chiral molecule: If a dual emission fluorescent sensor could exhibit a highly concentration-dependent emission at λ_1 and a highly enantioselective emission at λ_2, it might be possible to use the fluorescent responses of this sensor at the two emission wavelengths to determine both the concentration and the enantiomeric composition of a chiral molecule. Herein, we wish to report our discovery of the first example of such a system to simultaneously determine both the enantiomeric composition and the concentration of a chiral diamine with one fluorescent sensor.

We synthesized the 1,1′-bi-2-naphthol-based trifluoromethyl ketone molecule (*S*)-**3** and its enantiomer (*R*)-**3** as a potential fluorescent sensor for chiral amines according to Scheme 1.

Scheme 1. Preparation of Compounds (*S*)- and (*R*)-3



The use of trifluoromethyl ketone-based molecular sensors has been studied previously. In 1974, Herman reported the use of a trifluoromethyl aryl ketone for the selective electrochemical detection of carbonates.[6] It was later established that this selectivity is due to the nucleophilic addition of carbonates to the highly electrophilic trifluoromethyl ketone.[7] In 1991, Simon reported the use of the trifluoromethyl aryl ketone-based membranes as optical sensors for humidity and ethanol.[8] In these studies, the nucleophilic addition of water or ethanol to the trifluoromethyl carbonyl group disrupts the extended conjugation, leading to hypsochromic shifts of the absorption band. Further development of the trifluoromethyl ketone-based absorption and fluorescence sensors has been achieved in recent years for the recognition of many nucleophilic species such as alcohols, amines, and various anions.[9] In 2010, Anh also reported that a binaphthyl-based chiral trifluoromeththyl ketone could be used to distinguish the enantiomers of amino acids by using NMR spectroscopic methods.[10] In spite of these studies, however, no report has appeared on using the trifluoromethyl ketone-based molecules for enantioselective fluorescent recognition.

We studied the optical properties of (*S*)-**3**. The UV spectrum of (*S*)-**3** in methylene chloride displays absorptions at $\lambda_{max}(\varepsilon)$ = 228 (4.5×10^4), 263 (5.2×10^4), 319 (3.0×10^4) and 432 (4.8×10^3) nm (Figure 1a). When (*S*)-**3** (1.0×10^{-5} M in CH_2Cl_2) was treated with a chiral diamine *trans*-1,2-diaminocyclohexane (*R*,*R*)- or (*S*,*S*)-**4** (5.0×10^{-3} M), there were large absorption decreases at λ_{max} = 263, 319, and 432 nm, a large increase at

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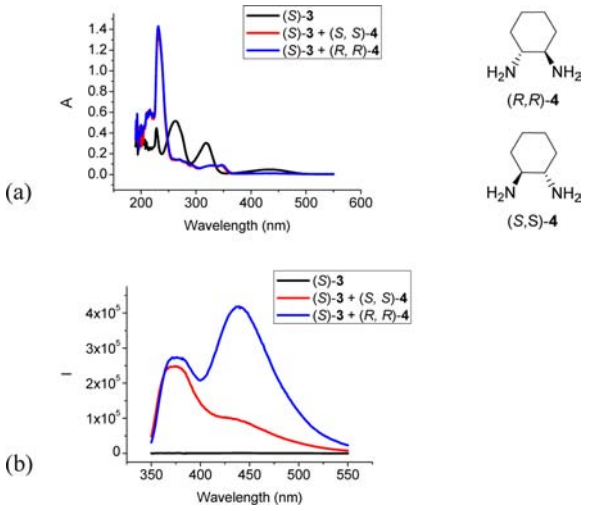

Figure 1. UV/vis absorption spectra (a) and fluorescence spectra (b) of (*S*)-**3** (1.0×10^{-5} M) with/without (*R*,*R*)- and (*S*,*S*)-**4** (5.0×10^{-3} M). (Solvent: CH_2Cl_2. λ_{exc} = 343 nm, slit = 2/2 nm.)

λ_{max} = 231 nm and a new absorption at λ_{max} = 345 nm, but no enantioselectivity was observed (Figure 1a). Unlike many 1,1′-binaphthyl molecules, (*S*)-**3** was found to be nonemissive at all in solution (Figure 1b). When its solution (1.0×10^{-5} M in CH_2Cl_2) was treated with (*R*,*R*)-**4** (5.0×10^{-3} M), a dramatic fluorescent enhancement was observed with dual emissions at 370 (λ_1) and 438 (λ_2) nm (Figure 1b). When (*S*)-**3** was treated with (*S*,*S*)-**4**, a similar large fluorescence enhancement at λ_1 was also observed, but the fluorescence enhancement at λ_2 was much smaller. Thus, (*S*)-**3** exhibits high sensitivity toward the chiral diamine at λ_1 and high enantioselectivity at λ_2. This molecule represents a rare example of an enantioselective fluorescent *enhancement* sensor for a chiral diamine.[11] We also studied the fluorescence response of (*R*)-**3**, the enantiomer of (*S*)-**3**, toward the chiral diamine. The expected mirror image responses were observed, which confirmed the observed chiral discrimination.

We have studied the effects of the concentration of the chiral diamine on the fluorescence responses of (*S*)-**3** at λ_1 and λ_2. Figure 2a plots the fluorescence intensity (I_1) of (*S*)-**3** at λ_1 versus the increasing concentration of (*R*,*R*)- and (*S*,*S*)-**4**. It shows that I_1 is strongly dependent on the concentration of the diamine but not significantly on its chiral configuration. Figure 2b plots the fluorescence intensity (I_2) of (*S*)-**3** at λ_2 versus the increasing concentration of (*R*,*R*)- and (*S*,*S*)-**4**, which shows high enantioselectivity. We further found that the fluorescence intensity ratio I_1/I_2 is independent of the concentration of the chiral diamine in the range of 5.0×10^{-4} to 5.0×10^{-3} M but is only dependent on the chiral configuration of the substrate. As shown in Figure 2c, the I_1/I_2 ratio for (*S*,*S*)-**4** remains constant at 2.60 and that for (*R*,*R*)-**4** at 0.67.

We have plotted I_1/I_2 of (*S*)-**3** (1.0×10^{-5} M in CH_2Cl_2) versus (*S*,*S*)-**4**% for the chiral diamine samples with varying enantiomeric composition and concentration (5.0×10^{-4} to 5.0×10^{-3} M) in Figure 3. This plot demonstrates that the enantiomeric purity of the chiral diamine can be determined by measuring the fluorescence responses of (*S*)-**3** at λ_1 and λ_2 without the need to know the concentration of the sample.

As described above, I_1 is strongly influenced by both (*S*,*S*)- and (*R*,*R*)-**4** (Figure 2a), and I_1/I_2 is only dependent on the enantiomeric composition (Figure 3). Figure 2a also shows that

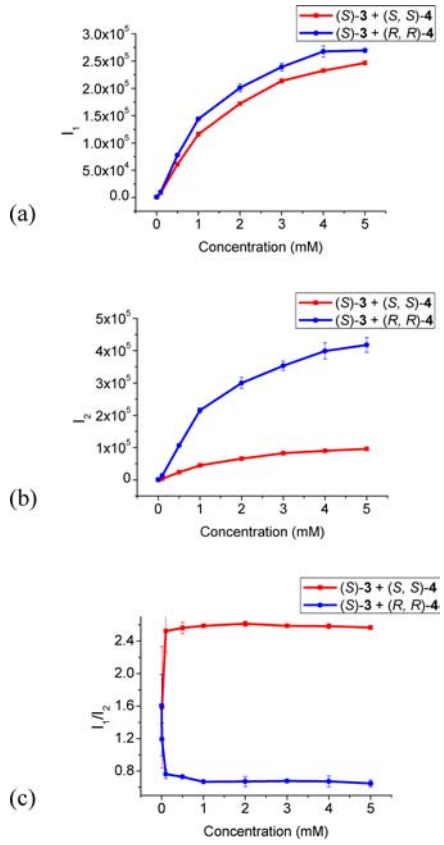

Figure 2. Plots of I_1 (a), I_2 (b), I_1/I_2 (c) for (*S*)-**3** (1.0×10^{-5} M) in the presence of varying concentrations of (*R*,*R*)- and (*S*,*S*)-**4**. (Fluorescence intensity I_1 at λ_1 = 370 nm and I_2 at λ_2 = 438 nm. Solvent: CH_2Cl_2. λ_{exc} = 343 nm, slit = 2/2 nm.)

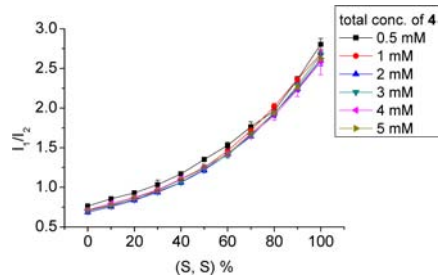

Figure 3. Plots of I_1/I_2 vs (*S*,*S*)-**4**% at various diamine concentrations (mM). (Solvent: CH_2Cl_2. λ_{exc} = 343 nm, slit = 2/2 nm.).

the chiral configuration of the diamine has a small effect on I_1. In order to more accurately determine the concentration of the substrate, we have plotted I_1 and I_1/I_2 of (*S*)-**3** against the diamine concentration of the samples containing varying compositions of (*S*,*S*)- and (*R*,*R*)-**4** in Figure 4. This plot takes into consideration the effects of the chiral configuration of the diamine. It demonstrates that the concentration of a chiral diamine sample can be determined by measuring the fluorescence responses I_1 and I_2 of the sensor (*S*)-**3**.

In the above experiments, when a given sample of the chiral diamine is treated with the fluorescent sensor (*S*)-**3**, one fluorescence measurement will give the fluorescence intensity I_1 and I_2. By using I_1/I_2, the enantiomeric composition of the sample can be determined from Figure 3. By using I_1 and I_1/I_2, the total concentration of the two enantiomers of the diamine can be determined from Figure 4. Therefore, both the

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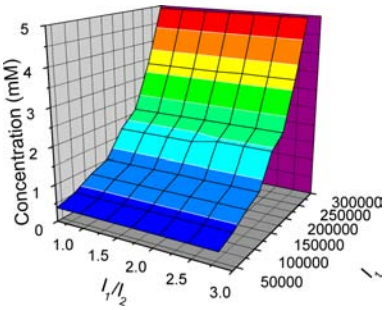


Figure 4. Plot of I_1, I_1/I_2 vs the total concentration of **4** with various enantiomeric composition.

concentration and the enantiomeric composition of a chiral molecule can be simultaneously determined by one fluorescence measurement with the use of only one fluorescent sensor.

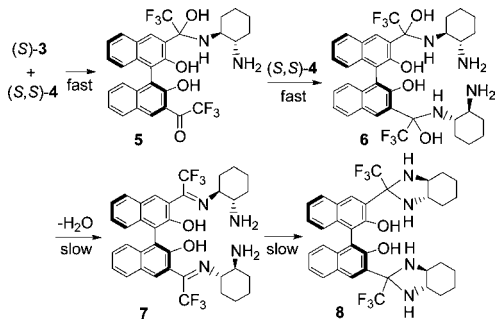
We have applied Figures 3 and 4 to analyze the ee's and concentrations of five test samples of the chiral diamine **4**. As the results summarized in Table 1 show, the values of (*S*,*S*)-**4**% and the sample concentrations from the fluorescent measurements had average errors of 10.6 and 8%, respectively.

Table 1. Determination of (*S*,*S*)-4% and Concentration of Test Samples by Using Figures 3 and 4.

sample	1	2	3	4	5
measured (*S*,*S*)-**4**%	99	80	55	26	96
actual (*S*,*S*)-**4**%	95	75	50	20	95
error	4%	7%	10%	30%	1%
measured concentration (mM)	0.6	0.9	1.6	2.9	3.4
actual concentration (mM)	0.6	1	1.8	2.5	3.5
error	0%	10%	11%	16%	3%

In order to gain further understanding on the interaction of (*S*)-**3** with the chiral diamine, we have conducted a ^{19}F NMR titration for the interaction of (*S*)-**3** with (*S*,*S*)-**4**. To an NMR tube containing (*S*)-**3** (0.4 mL, 5.0 mM) in $CDCl_3$, (*S*,*S*)-**4** was gradually added. After each addition, the solution was mixed well before its ^{19}F NMR spectrum was taken. The ^{19}F NMR spectrum of (*S*)-**3** gave a singlet at δ −70.06. With the addition of (*S*,*S*)-**4**, two new peaks at δ −69.94 and −83.83 started to appear with the same integration, while the signal of (*S*)-**3** at δ −70.06 was decreasing and then completely disappeared with the addition of 4.7 equiv of the diamine. This indicates the formation of the 1:1 adduct homosemiaminal **5** at this stage (Scheme 2).[9c,12a] After that, the signal at δ −69.94 started to decrease, while the signal at δ −83.83 was increasing until all the peaks were converted to the peak at δ −83.83 with the addition of 27 equiv of the diamine. This indicates the formation of the 2:1 adduct disemiaminal **6**.[9c,12a] Further addition of the diamine did not change the ^{19}F NMR spectra during the 2 h period. In the subsequent few days, slow appearance of new peaks at δ −72.09 and −80.64 was observed, which were then slowly converted to the peak at δ −80.64. The signal at δ −72.09 is attributed to the formation of **7**,[12b] and that at δ −80.64 is attributed to the formation of the aminal **8**.[12c] Compound **8** was also prepared from the reaction of (*S*)-**3** with (*S*,*S*)-**4** in the presence of molecular sieves at room temperature in 2 d. ^{19}F NMR titration of (*S*)-**3** with (*R*,*R*)-**4** exhibited similar responses.

Scheme 2. Proposed Mechanism for the Reaction of (*S*)-3 with the Chiral Diamine



The above NMR study has revealed that the addition of the chiral diamine to (*S*)-**3** led to a fast formation of the amine-ketone adducts **5** and **6**, but the formation of the condensation product **7** and the subsequent aminal product **8** was slow. Therefore, the observed large fluorescence enhancement of (*S*)-**3** in the presence of the chiral diamine can be attributed to the formation of **5** and **6**. We have examined the fluorescence spectra of (*S*)-**3** (1×10^{-5} M) when mixed with (*S*,*S*)-**4** or (*R*,*R*)-**4** (5×10^{-3} M) for over five hours, and found no significant change in both the shape and intensity.

In summary, we have discovered a fluorescent sensor that exhibits very different fluorescence responses at two emission wavelengths toward a chiral diamine, one with high sensitivity and one with high enantioselectivity. On the basis of this difference in fluorescence response, it has been demonstrated for the first time that both the concentration and enantiomeric composition of a chiral substrate can be determined simultaneously by one fluorescence measurement with the use of only one fluorescent sensor. This system should significantly simplify the application of the enantioselective fluorescent sensor.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Detailed synthesis and characterization data of compounds **2**, **3** and **8**. Additional spectroscopic data. This information is available free of charge via the Internet at http://pubs.acs.org/.

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Notes

The authors declare no competing financial interest.

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