Fluorescent Recognition

A Cyclohexyl-1,2-diamine-Derived Bis(binaphthyl) Macrocycle: Enhanced Sensitivity and Enantioselectivity in the Fluorescent Recognition of Mandelic Acid**

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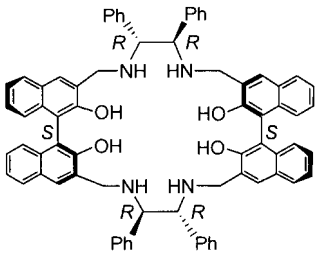
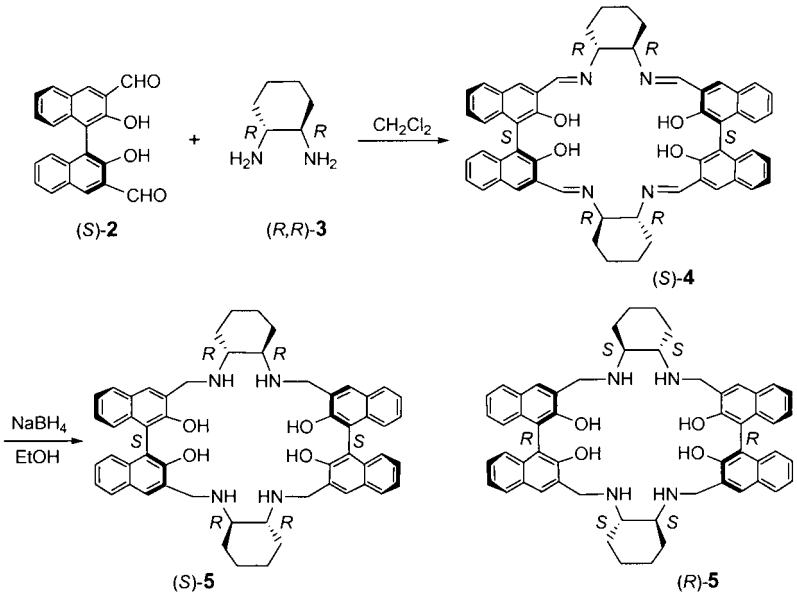


(*S*)-**1**

Separation-based techniques such as HPLC and GC equipped with chiral columns are the main analytical tools used today in both academy and industry for the determination of the enantiomeric composition of chiral compounds. With the development of the combinatorial chemistry technique, a large number of organic compounds including those with chirality can be synthesized very rapidly.[1] This presents a great challenge to analyze the chiral composition of these compounds because of the inherently slow separation techniques. A number of techniques such as mass spectrometry, electrophoresis, IR thermography, and UV absorption, circular dichroism, and fluorescence spectroscopies[2–6] are being developed for the fast assay of chiral compounds. We are particularly interested in the application of fluorescence spectroscopy in chiral recognition because it not only provides a real-time analysis but also offers high sensitivity and diverse sensing modes. With the use of a fluorescence microplate reader or a fluorescence imaging technique, hundreds of samples can be analyzed very quickly.

Application of fluorescence spectroscopy in chiral recognition has received growing attention in recent years.[4–6] Among these studies, significant work has been conducted with 1,1′-binaphthyl molecules.[5,6] This is because the unique chiral and aromatic structure of the 1,1′-binaphthyl unit could provide both excellent chiral recognition capability and interesting fluorescence signals. For example, we recently found that the macrocyclic compound (*S*)-**1**, with two 1,1′-binaphthyl and two 1,2-diphenylethylenediamine units, is capable of enantioselective fluorescent recognition.[6c,d] This compound shows emissions both from the monomer as well as an excimer. The enantioselective fluorescent response of (*S*)-**1** in the presence of mandelic acid was high for the emission of the excimer, but much smaller for that of the monomer. Herein we report a modified bis(binaphthyl) macrocycle that exhibits an extremely high enantioselective fluorescent response in the emission from the monomer in the presence of mandelic acid.

Similar to the reaction reported by Brunner and Schiessling,[7] a remarkable four-component condensation of (*S*)-**2** and (*R*,*R*)-**3** occurred at room temperature in CH_2Cl_2 to form the chiral macrocyclic Schiff base (*S*)-**4** (Scheme 1). Reduction of (*S*)-**4** with $NaBH_4$ in ethanol gave (*S*)-**5**. Although compound (*S*)-**5** is structurally similar to (*S*)-**1**, purification of (*S*)-**5** proved to be much more difficult. There was always approximately 5% impurity persistent in (*S*)-**5** when purified either by column chromatography or by recrystallization. Finally, we found that (*S*)-**5** formed a gel-like material in water when its solution in CH_2Cl_2 was treated with aqueous HCl. After the organic phase was removed, neutralization ($NaHCO_3$) and extraction (CH_2Cl_2) of the aqueous phase, followed by removal of the solvent led to analytically pure (*S*)-**5**.[8] The specific optical rotation of (*S*)-**5** was $[\alpha]_D = -86.3$ ($c = 0.22$, C_6H_6). The ^1H NMR spectrum of (*S*)-**5** shows two signals at $\delta = 4.27$ and 4.43 ppm (AB, 8H, $J = 14.4$ Hz) for the diastereotopic methylene protons at the 3,3′-positions of the



Scheme 1. Synthesis of the bis(binaphthyl) macrocycle (*S*)-**5**.

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binaphthyl units. Compound (*R*)-**5**, the enantiomer of (*S*)-**5**, was synthesized similarly starting from (*R*)-**2** and (*S,S*)-**3**.

Although the concentration of (*S*)-**5** had little effect on the positions of the peaks and the shapes of the absorption spectra, a large concentration dependence was observed for the fluorescence spectra of the macrocycle. As the concentration increased from 1.0×10^{-6} to 1.0×10^{-4} M in benzene, the emission intensity of the macrocycle (*S*)-**5** at the long-wavelength band ($\lambda = 435$ nm) increased greatly (Figure 1).

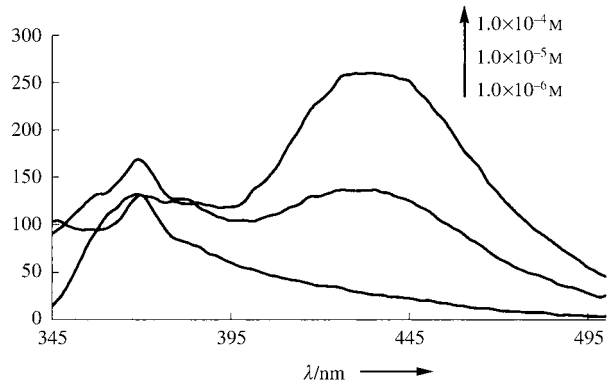


Figure 1. Concentration effect on the fluorescence spectra of (*S*)-**5** in benzene ($\lambda_{ex} = 332$ nm, ex/em slits = 3.5/6.5 nm).

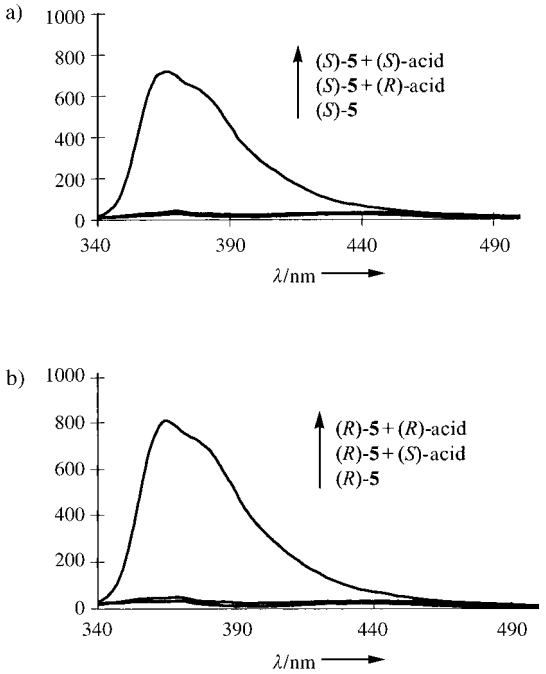


Figure 2. Fluorescence emission spectra of a) (*S*)-**5** and b) (*R*)-**5** in the absence/presence of (*R*)- and (*S*)-mandelic acid ($\lambda_{ex} = 332$ nm, ex/em slits = 3.5/3.5 nm).

At 1.0×10^{-4} M, the long-wavelength emission dominated. The change at the short-wavelength emission band ($\lambda = 370$ nm) with respect to the concentration was much smaller. According to our previous study on the macrocycle (*S*)-**1**,[6d] we attribute the long-wavelength band of (*S*)-**5** to emission from its excimer and the short-wavelength band to emission from the monomer.

The interaction of the macrocycle (*S*)-**5** with the enantiomers of mandelic acid was studied. The UV/Vis spectrum of the macrocycle showed only a slight decrease in the absorbance intensity when treated with mandelic acid, but no changes in the shape or position of the peak and almost no difference between the effects of (*R*)- and (*S*)-mandelic acid were observed. In contrast, a dramatic difference was observed for the fluorescence responses of the macrocycle towards (*R*)- and (*S*)-mandelic acid.[9] As shown in Figure 2a, (*R*)-mandelic acid (5.0×10^{-4} M) had almost no effect on the fluorescence of (*S*)-**5** (1.0×10^{-5} M in benzene containing 0.05% 1,2-dimethoxyethane (DME)). However, under the same conditions, (*S*)-mandelic acid caused an increase in the fluorescence intensity of monomeric (*S*)-**5** by over 20-fold.

To ascertain that the observed large difference in the fluorescence responses of (*S*)-**5** toward (*R*)- and (*S*)-mandelic acid is the result of inherent chiral recognition, we studied the interaction of (*R*)-**5**, the enantiomer of (*S*)-**5**, with (*R*)- and (*S*)-mandelic acid. Figure 2b presents the fluorescence emission spectra of (*R*)-**5** (1.0×10^{-5} M in benzene/0.05% DME) in both the presence and absence of (*R*)- and (*S*)-mandelic acid (5.0×10^{-4} M). Whereas (*S*)-mandelic acid caused little change in the fluorescence of (*R*)-**5**, (*R*)-mandelic acid greatly enhanced its fluorescence emission. Thus, there is a mirror-image relationship in the results of Figure 2a and Figure 2b. This demonstrates that the fluorescence interaction of the macrocycle with mandelic acid is indeed highly enantioselective.

The fluorescence enhancement (I/I_0) of (*S*)-**5** as a function of the concentrations of (*R*)- and (*S*)-mandelic acid is plotted in Figure 3a. As the concentration of the acid increased, the

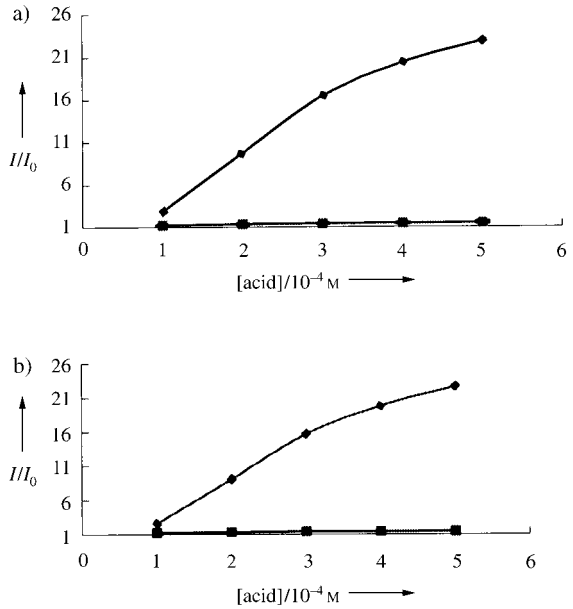


Figure 3. Fluorescence enhancement of a) (*S*)-**5** and b) (*R*)-**5** (1.0×10^{-5} M in benzene/0.05% DME) versus concentrations of (*R*)- and (*S*)-mandelic acid ($\lambda_{ex} = 332$ nm). a) The average of four experiments are presented; av (*S*):◆; av (*R*):■. b) (*R*) acid:◆; (*S*) acid:■.

(*S*) enantiomer greatly enhanced the fluorescence of (*S*)-**5**, but the *R* enantiomer did not. At a concentration of acid of 5.0×10^{-4} M, the enantiomeric fluorescence difference ratio, $ef=(I_S-I_0)/(I_R-I_0)$, was as high as 46. Figure 3b shows the fluorescence enhancement of (*R*)-**5** versus the concentration of (*R*)- and (*S*)-mandelic acid. The fluorescence response shown in Figure 3b mirrors that shown in Figure 3a.

The fluorescence enhancement of (*S*)-**5** reached a maximum as the concentration of (*S*)-mandelic acid increased to around 7.0×10^{-4} M. A further increase in the concentration of (*S*)-mandelic acid led to a small decrease in the fluorescence intensity. Over the concentration range 1.0×10^{-4} to 2.0×10^{-3} M, (*R*)-mandelic acid caused little change on the fluorescence of (*S*)-**5**.

The effect of the enantiomeric purity of mandelic acid on the fluorescence of (*R*)-**5** was also studied. Figure 4 curve a shows the fluorescence enhancement of (*R*)-**5** (1.0×10^{-5} M in benzene/0.05% DME) in the presence of mandelic acid (5.0×10^{-4} M) at various compositions of the *R* and *S* enantiomers. Figure 4 curve b shows the fluorescence enhancement of (*R*)-**5** (1.0×10^{-5} M in benzene/0.05% DME) when treated with the optically pure (*R*)-mandelic acid at concentrations that correspond to those of (*R*)-mandelic acid in the enantiomeric mixture. Thus, at the same amount of the *R* enantiomer, the optically pure (*R*)-mandelic acid caused a greater enhancement in fluorescence emission than the mixture containing both the *R* and *S* enantiomers. That is, even though (*S*)-mandelic acid could not increase the fluorescence intensity of the sensor (*R*)-**5**, it might be still in competition with (*R*)-mandelic acid in binding with the sensor which reduced the effective concentration of (*R*)-mandelic acid. Apparently, the binding of (*R*)-mandelic acid with (*R*)-**5** was much stronger than that of (*S*)-mandelic acid, and the racemic mixture of mandelic acid still led to a large fluorescence enhancement. For samples containing less than 20% (*R*)-mandelic acid, the fluorescent enhancement of (*R*)-**5** was small because of the large amount of (*S*)-mandelic acid (>80%). However, this sample could be analyzed by using the enantiomeric sensor (*S*)-**5** and should show a large fluorescence enhancement. Thus, by using both enantiomers of the sensor and by measuring the difference between their fluorescence responses toward the substrate under the same conditions will allow the determination of any enantiomeric composition of the chiral acid.

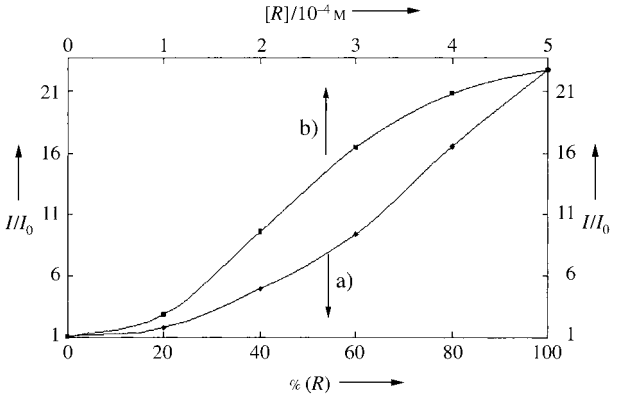
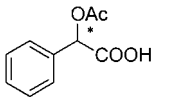


Figure 4. Fluorescence enhancement of (*R*)-**5** in the presence of samples containing either the optically pure (*R*)-mandelic acid or various enantiomeric compositions (% (*R*) values of the total acid concentration: 5×10^{-4} M).

We also studied the interaction of (*S*)-**5** with O-acetyl mandelic acid. Under the same conditions employed with mandelic acid, almost no enhancement in the fluorescence emission was observed with either (*R*)- or (*S*)-*O*-acetyl mandelic acid. This demonstrates that both the α-hydroxy group and its chiral configuration are very important for the binding of the acid with the macrocyclic receptor. Under similar conditions, (*S*)-**5** showed various degrees of chiral recognition toward other chiral acids such as $PhCH(NHCO_2CH_2Ph)CO_2H$ ($ef=6.7$), $PhCH_2CH(NHCO_2CH_2Ph)CO_2H$ ($ef=2.1$), and $PhCH_2CH(OH)CO_2H$ ($ef=3.0$).



O-acetyl mandelic acid

We previously reported that the highest enantioselectivity of (*S*)-**1** ($ef\approx12$) in the fluorescent recognition of mandelic acid was observed with the emission of excimeric (*S*)-**1** which required high concentrations of both (*S*)-**1** (1.0×10^{-4} M) and the acid (2.0×10^{-2} M).[6c,d] At the emission of the (*S*)-**1** monomer, the enantioselectivity was lower with $ef=3.2$. With the newly synthesized sensor (*S*)-**5**, we have observed the highly enantioselective fluorescent recognition of mandelic acid (*S*)-**5** with $ef\approx46$. Furthermore, as this high enantioselectivity is observed at the emission of the (*S*)-**5** monomer, it decreases the working concentrations of the sensor and mandelic acid by one and two orders of magnitude, respectively. Even at the greatly decreased concentrations of both the sensor and substrate, the fluorescence enhancement of (*S*)-**5** in the presence of (*S*)-mandelic acid can be over 10-times greater than that of (*S*)-**1** in the presence of (*S*)-mandelic acid. Therefore, (*S*)-**5** is a sensor of greatly increased enantioselectivity and sensitivity over (*S*)-**1**.

An NMR spectroscopic study on the interaction of the bis(binaphthyl) macrocycle with mandelic acid was also conducted. We found that a 1:1 mixture of (*S*)-**5** and (*S*)-mandelic acid dissolved in a solvent system of [D_6]acetone (4%) in [D_6]benzene caused a large upfield shift ($\Delta\delta=1.0$–1.1 ppm) for the signal of the α proton of (*S*)-mandelic acid; that is, from $\delta=5.20$ to $\delta=4.1$–4.2 ppm. However, under the same conditions, the chirality-mismatched mixture of (*S*)-**5** and (*R*)-mandelic acid led to only a small upfield shift ($\Delta\delta=0.25$ ppm) for the α proton of (*R*)-mandelic acid. This proton only showed an upfield shift of 0.02 ppm when mandelic acid was treated with dibenzylamine. These observations suggest that in the macrocycle–mandelic acid complex, (*S*)-mandelic acid is probably located much deeper inside the chiral cavity of (*S*)-**5** than (*R*)-mandelic acid which significantly shields the α proton of (*S*)-mandelic acid by the aromatic rings of the macrocycle. This could be the origin of the dramatic difference in the fluorescence responses of (*S*)-**5** toward the two enantiomers of mandelic acid. The ^{1}H NMR spectroscopic study also indicates that (*S*)-**5** probably binds more than one

equivalent of (*S*)-mandelic acid. Further studies on the structure of the sensor–substrate complex are in progress in our laboratory.

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[8] Preparation and characterization of (*S*)- and (*R*)-**5**: Aldehyde (*S*)-**2** (274 mg, 0.80 mmol) and cyclohexane-1,2-diamine ((*R*,*R*)-**3**, 93 mg, 0.80 mmol) were dissolved in dry CH_2Cl_2 under N_2, and the mixture was stirred at RT for 2 days. After evaporation of the solvent, the resulting Schiff base (*S*)-**4** was purified by passing through a short column of silica gel. Compound (*S*)-**4** was then combined with $NaBH_4$ (88 mg, 2.32 mmol) and ethanol (25 mL), which was degassed with nitrogen, and heated at reflux for 4 h. After removal of the solvent, the residue was dissolved in CH_2Cl_2 (10 mL) and aqueous HCl (0.4 N, 30 mL) was added. The organic layer was separated from the gel-like aqueous layer and discarded. The aqueous layer was neutralized with $NaHCO_3$ and then extracted with CH_2Cl_2 (3×20 mL). Removal of the solvent gave pure (*S*)-**5** as a white solid (170 mg, 55 %). m.p. $> 230\,^{\circ}C$ (dec.); $[\alpha]_D = -86.30$ ($c = 0.22$, C_6H_6); ^1H NMR ([D$_6$]acetone, 300 MHz): $\delta = 0.86$–1.16 (m, 8H), 1.48–1.60 (m, 4H), 2.01–2.18 (m, 4H), 2.18–2.32 (m, 4H), 4.27 and 4.43 (AB, 8H, $J = 14.4$ Hz), 7.08–7.28 (m, 12H), 7.67(s, 4H), 7.76–7.83 ppm (m, 4H); ^{13}C NMR ([D$_6$]acetone, 75 MHz): $\delta = 24.86$, 32.34, 51.58, 61.55, 117.36, 122.65, 124.82, 125.46, 126.94, 127.35, 127.92, 128.54, 134.45, 154.82 ppm; HRMS (MALDI): *m*/*z* calcd for $C_{56}H_{57}N_4O_4$ [MH^+]: 849.4374; found: 849.4382; Elemental analysis (%) calcd for $C_{56}H_{56}N_4O_4$: C 79.22, H 6.65, N 6.60; found: C 79.18, H 6.77, N 6.45.

[9] Preparation of samples for fluorescence measurements: The enantiomers of mandelic acid were purchased from Aldrich and recrystallized from methanol. All of the solvents were HPLC grade. The stock solutions of the sensors in benzene were freshly prepared for each measurement. For the fluorescence enhancement study, a solution of the sensor was mixed with a solution of mandelic acid at RT in a 5-mL volumetric flask, and the mixture was diluted to the desired concentration. The resulting solution was allowed to stand at RT for 2–4 h before measurement of the fluorescence emission.

