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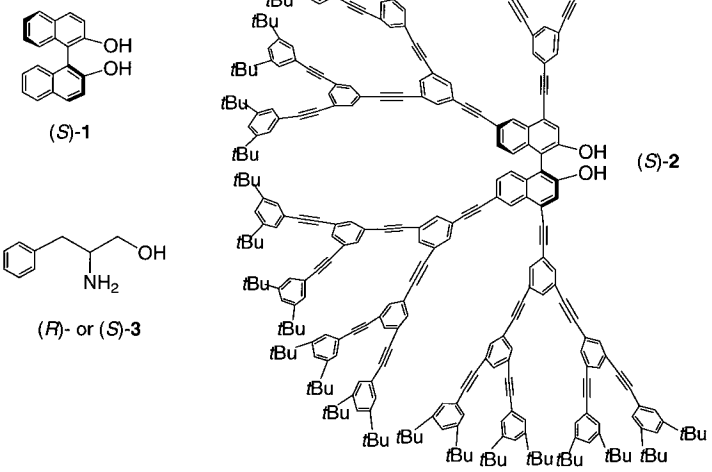
The First Dendrimer-Based Enantioselective Fluorescent Sensor for the Recognition of Chiral Amino Alcohols**

Vincent J. Pugh, Qiao-Sheng Hu, and Lin Pu*

The development of fluorescence-based molecular sensors has received broad attention in research in recent years.[1–3] The use of fluorescence spectroscopic methods for molecular recognition has many advantages: fluorescence provides many more signaling modes for substrate detection, such as quenching, fluorescence enhancement, excimers, exciplexes, and lifetimes, than electronic absorption. The high sensitivity of fluorescence techniques requires the use of only very small amounts of sensor molecules. Fluorescence spectrometers are also of low cost and widely available. Fluorescence sensors can be further applied to continuous monitoring and remote sensing by using optical fibers. A large number of fluorescent sensors have been designed for applications in the detection of metal ions, phosphates, and neutral molecules.[1–3] Recently, a fluorescent sensor has also been used in the combinatorial search for catalysts.[4a] If such fluorescent sensors could be made enantioselective, they would allow a rapid analysis of the enantiomeric composition of thousands of chiral molecules generated by the combinatorial synthesis. This process would greatly facilitate the combinatorial discovery of asymmetric catalysts or reagents since the current chromatographic analysis of enantiomers is inherently a slow process. An enzyme-catalyzed release of fluorophores has been used in the search for catalytic antibodies for the enantioselective hydrolysis of acetates.[4b] Chiral discrimination by luminescence has also been studied in the past two decades.[5–9] These studies involve a variety of luminescent materials including inorganic complexes,[5] organic molecules,[6–8] and enzymes.[9] Enantioselective responses have been observed when chiral luminophores are treated with chiral quenchers or enhancers. The relationship between the fluorescence properties of the sensors and the enantiomeric purity of the substrates have been established in a few cases.[5b, 6g, 7, 9b]

Recently, we carried out a program to incorporate chiral dendrimers[10] into enantioselective fluorescent sensors as a real-time technique to quantitatively or semi-quantitatively determine the enantiomeric composition of chiral molecules. Properly designed dendritic materials have been found to show efficient migration of energy from the dendrons or periphery groups to the more conjugated units or core, which has led to greatly enhanced fluorescence intensity.[11–17] The strong fluorescence signals of such dendrimers should be very useful in the development of fluorescent sensors. Based on the structure of chiral 1,1′-bi-2-naphthol ((*S*)-**1**)[18] and the den-



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drons of Xu and Moore,[11] we have synthesized optically active dendrimers such as (*S*)-**2**.[19] We have found that there is very efficient migration of energy from the phenylacetylene dendrons of (*S*)-**2** to the chiral binaphthyl core.[19] Thus, the dendritic arms of (*S*)-**2** serve as light-harvesting antenna. This result is similar to that observed by Xu and Moore in the optically inactive phenyleneacetylene dendrimer system.[11] Herein we report that dendrimer (*S*)-**2** can carry out enantioselective fluorescent sensing of chiral amino alcohols.

We have compared the fluorescence of dendrimer (*S*)-**2** with that of the parent molecule (*S*)-**1**, and have found that the fluorescence intensity of the dendrimer is dramatically increased over that of (*S*)-**1**. This enhancement occurs because of the increased number of absorbing units as well as from the efficient intramolecular migration of energy in (*S*)-**2**.[19] Dendrimer (*S*)-**2** emits at $\lambda_{em} = 422$ and 441 (sh) nm when excited at 310 nm. Figure 1 shows the fluorescence spectra of dendrimer (*S*)-**2** (excited at 310 nm, the maximum in its

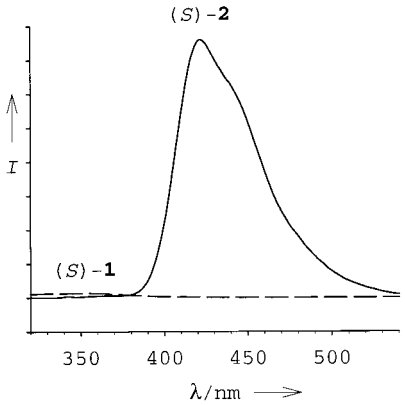

Figure 1. Comparison of the fluorescence spectrum of dendrimer (*S*)-**2** with that of (*S*)-**1** in benzene:hexane (20:80) (uncorrected).

excitation spectrum) and (*S*)-**1** (excited at 280 nm, the maximum in its excitation spectrum) at the same concentration (4.0×10^{-8} M) in a mixed solvent of benzene and hexane (20:80). Only when the concentration of (*S*)-**1** is increased over 170 times, does its fluorescence intensity become comparable with that of (*S*)-**2**. The greatly enhanced fluorescence of the dendrimer makes it potentially more useful as a fluorescent sensor than the weakly fluorescent 1,1′-bi-2-naphthol. (*S*)-**2** is a second generation dendrimer. Its fluorescence is also much stronger than that of the lower generation ones.[19]

We have studied the fluorescence of dendrimer (*S*)-**2** in the presence of chiral amino alcohols and amines, such as 2-amino-1-propanol, 3-methyl-1-butanol, 2-amino-3-phenyl-1-propanol, 2-amino-4-methyl-1-pentanol (leucinol), and *trans*-1,2-diaminocyclohexane. We found that of the compounds studied the enantiomers of 2-amino-3-phenyl-1-propanol (phenylalaninol, (*R*)- and (*S*)-**3**) quench the fluorescence of (*S*)-**2** very efficiently and also with a significantly different rate. Other chiral amino alcohols or amines also quench the fluorescence of (*S*)-**2** efficiently, but with smaller enantioselective responses. Figure 2 shows the Stern–Völmer

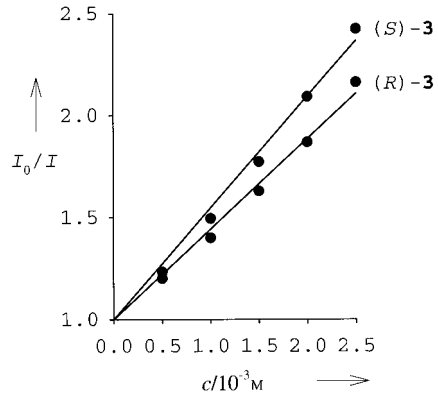

Figure 2. The Stern–Völmer plot of dendrimer (*S*)-**2** in the presence of (*R*)- and (*S*)-**3**.

quenching plot of (*S*)-**2** (4.0×10^{-8} M) in the presence of (*R*)- and (*S*)-**3** in a mixed solvent of benzene and hexane (20:80).[20] The amino alcohols were purchased from Aldrich with a purity of 98% for both (*R*)- and (*S*)-**3**. These two compounds were dissolved in methanol and filtered through silica gel, and then recrystallized from EtOAc:hexane (2.5:1). As shown in Figure 2, the fluorescence quenching of the chiral dendrimer by the amino alcohol is enantioselective. The Stern–Völmer constant is $556\,M^{-1} \pm 2\%$ (K_{sv}^{S}) in the presence of (*S*)-**3** and $473\,M^{-1} \pm 2\%$ (K_{sv}^{R}) in the presence of (*R*)-**3**. Thus, $K_{sv}^{S} - K_{sv}^{R} = 83\,M^{-1}$ and $K_{sv}^{S}/K_{sv}^{R} = 1.18$. The *S* enantiomer of **3** quenches the fluorescence of the chiral dendrimer more efficiently than the *R* enantiomer. The opposite enantiomer of the dendrimer shows the opposite trend in quenching efficiency for the amino alcohol enantiomers, which indicates there is a chirality-based luminescence-quenching selectivity.

In 1992, Iwanek and Mattay found certain enantioselectivity in the quenching of the luminescence of (*R*)- and (*S*)-**1** by optically active amines and amino alcohols.[6e] We have also studied the fluorescence of (*S*)-**1** in the presence of (*S*)- and (*R*)-**3** in the mixed solvent of benzene and hexane (20:80) and found the Stern–Völmer constants of 121 and $118\,M^{-1}$, respectively. Thus, $K_{sv}^{S} - K_{sv}^{R} = 3\,M^{-1}$ and $K_{sv}^{S}/K_{sv}^{R} = 1.03$. This indicates that dendrimer (*S*)-**2** not only provides much stronger fluorescence signals than (*S*)-**1** for observation, but also leads to higher enantioselectivity in chiral recognition. The much larger Stern–Völmer constants of the dendrimer demonstrate that the fluorescence of the dendrimer is much more sensitive to the amino alcohol quencher than that of the parent binaphthol.

The amine-induced fluorescence quenching of (*R*)- and (*S*)-**1** were attributed to both ground-state hydrogen bonding as well as excited-state deprotonation of individual binaphthol molecules.[6e] We found that the ground-state deprotonation of (*S*)-**1** in benzene:hexane (20:80) by NaOH was accompanied by a large red-shift (45 nm) in the lowest energy absorption in the UV/Vis spectrum. A similar large red-shift (50 nm) of the lowest energy UV/Vis absorption of dendrimer (*S*)-**2** was also observed in the presence of NaOH in the same mixed solvent. The deprotonated form showed very weak fluorescence. However, a red-shift of only 5 nm was observed for (*S*)-**2** in the presence of **3** (2.0×10^{-3} M) in benzene/hexane (20/80). This indicates a hydrogen-bonding interaction be-

tween (*S*)-**2** and **3** rather than proton transfer in the ground state. It was found that the lowest singlet-excited-state pK_a^* value for β-naphthol is 2.8, whereas the ground-state pK_a value is 9.5.[21] The acidity of β-naphthol is increased by over six orders of magnitude from the ground state to the excited state. A similarly small pK_a^* value is expected for (*S*)-**2** in its excited state. Thus, dendrimer (*S*)-**2** in the presence of **3** should be deprotonated in the excited state. Therefore, the possible pathways for the fluorescence quenching of (*S*)-**2** by **3** might include a radiationless decay of the hydrogen-bonded complex formed between (*S*)-**2** and **3** and the formation of a poorly emissive proton-transfer complex in the excited state.

We have also found that the hydroxyl groups of (*S*)-**2** are necessary for efficient quenching by amino alcohols as there was no significant quenching by (*S*)-**3** when the two hydroxyl groups of (*S*)-**2** were converted into two methoxy groups. The hydroxyl groups of (*S*)-**2** allow the fluorescence quenching to occur at the binaphthyl core, to where the light-harvesting dendrons funnel the energy.[11, 19] This effect increases the sensitivity of the dendrimer toward the amino-alcohol quencher and explains the much larger Stern–Völmer constants of dendrimer (*S*)-**2** than those of the small molecule (*S*)-**1**.

The enantioselective quenching of the fluorescence of (*S*)-**2** by (*R*)- and (*S*)-**3** has led us to study the effect of the enantiomeric purity of **3** on the value of the Stern–Völmer quenching constant of the dendrimer. When (*S*)-**2** (4.0×10^{-8} M) was treated with **3** (2.0×10^{-3} M) in which the ratio of the *R* and *S* enantiomers were varied a linear plot of the K_{sv} value versus the percentage of the *S* enantiomer in the *R* and *S* mixture of **3** was obtained (Figure 3). The fluorescence signal

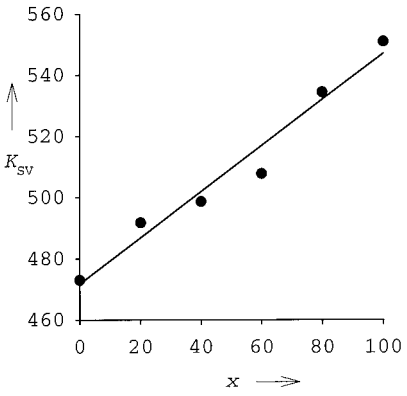

Figure 3. The relationship of the K_{sv} value of the dendrimer (*S*)-**2** with the enantiomeric composition of chiral amino alcohol **3**; *x*: percent of (*S*)-**3**.

of the dendrimer is quenched by about 48–53% at this concentration. As the percentage of the *S* enantiomer increases, there is a linear increase in the Stern–Völmer constant. This experiment demonstrates that the enantiomeric composition of a chiral molecule can be determined with the use of a fluorescent dendritic sensor. Thus, the K_{sv} value of the fluorescent dendrimer can be used to predict the enantiomeric excess (*ee*) of a chiral quencher.

In summary, we have discovered that there is a dramatic enhancement of fluorescence intensity from 1,1′-bi-2-naphthol to the corresponding dendrimer, as a result of the exponentially increased number of absorbing units and an efficient intramolecular migration of energy. The fluorescence of the chiral binaphthol-based dendrimer can be enantioselectively quenched by a chiral amino alcohol. A linear relationship has been established between the Stern–Völmer constant of the dendrimer and the enantiomeric composition of the chiral amino alcohol. Such enantioselective fluorescent sensors may allow a rapid determination of the enantiomeric composition of chiral molecules, and are potentially useful in the high-throughput combinatorial search for asymmetric catalysts and reagents. The strong fluorescence of the dendrimer provides intense signals for detection and allows the use of very small amounts of the chiral materials. In addition, the light-harvesting antennas of the dendrimer funnel energy to the center where it is quenched by the amino alcohol. This allows the dendrimer sensor to have much more sensitive fluorescence responses than the corresponding small molecule sensor. We are currently working on extending the observed enantioselective fluorescence response of this class of chiral dendrimers to other chiral organic substrates in order to develop sensors for practical applications. The influence of the dendrimer generation on the enantioselectivity and sensitivity will also be studied.

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Catalyst for DNA Ligation: Towards a Two-Stage Replication Cycle**

Jingdong Ye, Yahaloma Gat, and David G. Lynn*

The chemical methods available for the ligation of complementary oligomers along a DNA template are complex, requiring transition metal and imidazole catalysis of the condensation reaction. We were interested in simplifying the required conditions by converting the DNA template itself into a better catalyst, and report a modified backbone linkage which greatly simplifies the reaction. As related backbone linkages have been prepared by chemically reading the DNA sequence, this reaction completes a replication cycle, simplifying the ubiquitous three-stage replication cycle, the central dogma of biology,[1] to a two-stage chemical process.

Several laboratories have contributed to optimizing the reaction conditions for template-directed phosphodiester formation.[2] In the BrCN, imidazole, $NiCl_2$ cocktail, BrCN has a half-life of several minutes in the aqueous media. Premixing the cocktail generates *N*-cyanoimidazole, the reagent required to activate ligation.[2d,e] While more stable than BrCN under the reaction conditions, displaying a half-life of several hours, *N*-cyanoimidazole will chemoselectivity ligate a nick site along a DNA template. Many aromatic drugs are known to bind tightly within the narrow minor groove of DNA, particularly A/T tracts,[3] including netropsin, distamycin, Hoechst 33258, and the bis-amidine compounds benenil and pentamidine, and do so by displacing specific well-ordered H_2O molecules known to line this cavity.[4] It is therefore possible that *N*-cyanoimidazole associates with the nick site better than BrCN to facilitate the activation and/or the ligation steps in the condensation.

In an attempt to replicate this effect by template modification, five synthetic templates, T_{N-H}, T_{N-pr}, T_{N-bu}, T_{N-im}, and T_{N-ea}, were compared with the native DNA (T_P) under ligation conditions (Figure 1). Each template was prepared

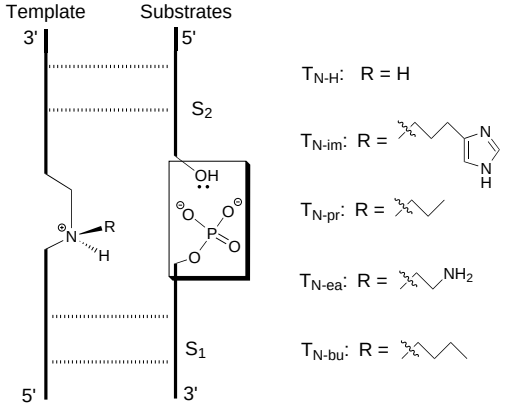

Figure 1. The ternary complex formed by template and complementary substrates, 8mer S_1 and 12mer S_2. The sequence of the template is HO-dCpCpGpTpTpCpGpTpTpTpTXTpCpTpGpTpCpTpCpG-OH, S_1 is HO-dApCpGpApApCpGpGp-OH, and S_2 is HO-dCpGpApGpApCp-ApGpApApApA-OH. X in the sequence represents the site of insertion of the various linkage structures. In Tp, X represents the native phosphodiester linkage of DNA.

with the appropriate synthetic amine thymidine dimer incorporated into standard solid-phase synthesis protocols.[5d] The 20mer templates were designed such that the complementary DNA substrates, S_1 and S_2, displayed high binding affinity with the template. Thermal melting analyses indeed established that the melting temperature for all template – substrate duplexes T/S_1 ($35 \pm 2\,^\circ C$) and T/S_2 ($45 \pm 2\,^\circ C$) were very similar and $> 10\,^\circ C$ above the reaction temperature. A 1:1:1 stoichiometry therefore generated significant and equivalent concentrations of the ternary complexes (T:S_1:S_2) for each reaction.

All modified templates and substrates were purified prior to reaction by reverse-phase chromotography, and purity was

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