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Enantioselective Sensing of Chiral Carboxylic Acids

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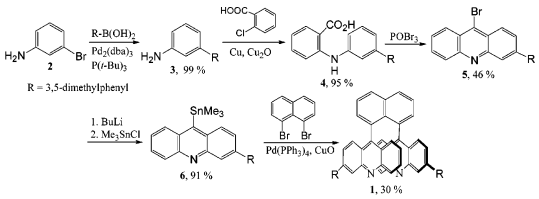
Chiral carboxylic acids and amino acids play an important role in natural product synthesis and drug development.[1] The development of time-efficient and cost-effective stereoselective assays is essential for (a) the verification of the enantiopurity and stereochemical integrity, i.e., stability to racemization, and (b) the screening of asymmetric reactions that afford chiral carboxylic acids and amino acids. Stereoselective luminescent sensing based on indicator-displacement assays has recently been reported to provide a promising entry to high-throughput screening (HTS) of chiral reactions.[2] Enantioselective sensing based on fluorescence spectroscopy offers a variety of advantages over NMR spectroscopy with chiral shift reagents and chiral chromatography, such as different detection modes (fluorescence quenching, enhancement, and lifetime), high sensitivity, low cost of instrumentation, waste reduction, time efficiency, and the possibility of performing real-time analysis. Because of the high sensitivity inherent to fluorescence spectroscopy, only a very small amount of the sensor is required, which makes this technique a cost-effective and practicable alternative. To date, only few enantioselective fluorescence sensors,[3] including chiral macrocycles,[4] dendrimers,[5] and oligomers,[6] have been reported. Enantioselectivity in energy-transfer reactions between a variety of chiral quencher molecules and photoexcited chiral Lanthanide chelates has also been observed by time-resolved or steady-state circularly polarized luminescence measurements.[7]

We have recently developed a synthetic route to highly congested C_2-symmetric diacridylnaphthalenes that do not show any sign of syn/anti isomerization, even at elevated temperature.[8] Herein, we report the preparation of 1,8-bis(3,3′-(3,5-dimethylphenyl)-9,9′-diacridyl)naphthalene (**1**) for enantioselective sensing of a broad variety of chiral carboxylic acids. The structure of this C_2-symmetric sensor is designed to (a) embed interactions with chiral acids into a highly stereoselective environment and (b) utilize fluorescence spectroscopy to monitor stereoselective recognition.

Suzuki coupling of commercially available 3-bromoaniline (**2**) and 3,5-dimethylboronic acid afforded 3-(3,5-dimethylphenyl)-aniline (**3**) in high yields (Scheme 1). Treatment of **3** with 2-chlorobenzoic acid gave *N*-3-(3,5-dimethylphenyl)anthranilic acid (**4**), which was converted to 9-bromo-3-(3,5-dimethylphenyl)acridine (**5**) using phosphorous oxybromide. Lithiation of **5** at −78 °C followed by stannylation with trimethylstannyl chloride resulted in the formation of 3-(3,5-dimethylphenyl)-9-trimethylstannylacridine (**6**) in high yields. Employing stannane **6** and 1,8-dibromonaphthalene in a CuO-promoted Stille coupling yielded 1,8-bis(3,3′-(3,5-dimethylphenyl)-9,9′-diacridyl)naphthalene (**1**).

Scheme 1. Synthesis of 1,8-Diacridylnaphthalene **1**



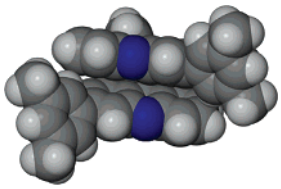
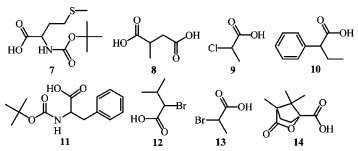


Figure 1. Single-crystal structure of **1**. View at the antiparallel acridyl rings (front) attached to the peri positions of naphthalene (rear). The *N*−*N* distance was determined as 4.38 Å, and the splaying angle between the acridyl rings is 11.6°.

Chart 1. Carboxylic Acids Tested



We were able to grow a monoclinic single crystal of **1** belonging to the *C*2/*c* space group through slow evaporation of a dichloromethane/acetonitrile solution (1:1 v/v) to reveal the three-dimensional structure of the sensor by X-ray analysis (Figure 1). The bidentate ligand exhibits two antiparallel acridyl moieties that form a C_2-symmetric cleft for stereoselective recognition of chiral carboxylic acids measurable by fluorescence spectroscopy.

Fluorescence studies of **1** in acetonitrile revealed an emission maximum at 550 nm. Despite its congested structure, diacridylnaphthalene **1** has a fluorescence quantum yield of 0.17 and a fluorescence lifetime of 5.46 ns. We were pleased to find that the anti isomers of **1** can be separated into enantiomers by HPLC on a WhelkO-1 column and decided to employ chiral analytes **7**−**14** in fluorescence titration experiments (Chart 1).

Titration studies using (+)-**1** at 2.6×10^{-6} M in acetonitrile (excitation at 360 nm, emission maximum at 550 nm) showed that the sensor can differentiate between the enantiomers of all carboxylic acids employed in this study. Fluorescence quenching was observed with enantioselectivities, α, up to 4.5 in the case of camphanic acid (**14**). Since diacridylnaphthalene (+)-**1** exhibits two potential binding sites, the fluorescence quenching was analyzed using the Stern−Volmer equations derived for 1:1 (1) and 1:2 (2) complexes to determine the enantioselectivity and binding mode of the corresponding diastereomeric complexes:

$$I_0/I = 1 + K_{SV}[Q] \quad (1)$$

$$I_0/I = 1 + K_{SV}[Q]^2 \quad (2)$$

where I_0 is the inherent fluorescence intensity of (+)-**1**, I is the fluorescence intensity in the presence of the quencher, Q, and K_{SV} is the Stern−Volmer constant.

We found that acids **7**−**10** form an equimolar complex with (+)-**1**, whereas two equivalents of **11**−**14** coordinate simultaneously to the diacridylnaphthalene sensor. For example, (+)-**1** was found to differentiate between (*R*)- and (*S*)-*N*-*t*-Boc-methionine (**7**), with

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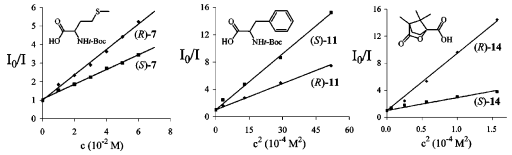

Figure 2. Linear Stern−Volmer plots for the enantioselective fluorescence quenching of (+)-**1** in the presence of carboxylic acids **7**, **11**, and **14**. The concentration of (+)-**1** in acetonitrile was 2.6×10^{-6} M. Excitation (emission) wavelength, 360 nm (550 nm).

Table 1. Enantioselective Fluorescence Quenching of (+)-**1** in the Presence of Carboxylic Acids **7**−**14**

analyte	ratio **1**/analyte	α	$K_{(+)-1-(R)-\text{analyte}}$[a]	$K_{(+)-1-(S)-\text{analyte}}$[a]
7	1:1	1.7 (*R*/*S*)	88.5 M^{-1}	56.5 M^{-1}
8	1:1	1.3 (*S*/*R*)	610.0 M^{-1}	840.0 M^{-1}
9	1:1	2.2 (*S*/*R*)	75.6 M^{-1}	241.3 M^{-1}
10	1:1	1.1 (*S*/*R*)	18.4 M^{-1}	20.0 M^{-1}
11	1:2	2.1 (*S*/*R*)	2100.0 M^{-2}	4900.0 M^{-2}
12	1:2	2.2 (*R*/*S*)	16000.0 M^{-2}	7100.0 M^{-2}
13	1:2	1.3 (*R*/*S*)	5300.0 M^{-2}	4700.0 M^{-2}
14	1:2	4.5 (*R*/*S*)	63000.0 M^{-2}	36000.0 M^{-2}

[a] Obtained using the Benesi−Hildebrand equation for 1:1 complexes (**7**−**10**) and 1:2 complexes (**11**−**14**).

an enantioselectivity factor, $\alpha = K^R_{SV}/K^S_{SV}$, of 1.7 based on a linear Stern−Volmer plot for the corresponding diastereomeric 1:1 complexes (Figure 2). By contrast, (+)-**1** forms a 1:2 complex with *N*-*t*-Boc-phenylalanine (**11**), with an enantioselectivity factor K^S_{SV}/K^R_{SV} of 2.1. The fluorosensor also differentiates between the enantiomers of methylsuccinic acid (**8**), 2-chloropropionic acid (**9**), 2-phenylbutyric acid (**10**), 2-bromo-3-methylbutyric acid (**12**), and 2-bromopropionic acid (**13**) (Table 1). To evaluate the accuracy and reproducibility of our sensing method, we prepared nine samples containing 15, 55, and 95% of (*R*)-**14** and determined the enantiomeric composition on the basis of the fluorescence quenching of **1**. Averaging the fluorescence measurements of the individually prepared samples, we calculated 16, 58, and 96% enantiopurity using a calibration curve. The results are within ±3% of the actual enantiopurity of the samples and thus demonstrate the high reproducibility and accuracy of this method.

1,1′-Binaphthyl-derived fluorosensors have been designed for chiral recognition of mandelic acid and its derivatives.[3e,5d,9] However, to the best of our knowledge, diacridylnaphthalene **1** is the first enantioselective fluorosensor applicable to a broad variety of carboxylic acids, including amino acids, aliphatic acids, arylalkanoic acids, and halogenated carboxylic acids. The observed change of the fluorescence intensity of excited (+)-**1** in the presence of carboxylic acids **7**−**14** is probably a result of static quenching through nonradiative relaxation of diastereomeric acid−base adducts.[10] The equilibrium binding constants of the diastereomeric complexes were obtained using the Benesi−Hildebrand equation for 1:1 complexes (**7**−**10**) and 1:2 complexes (**11**−**14**) (Table 1).[4d,11] The binding constants vary significantly and indicate that coordination between the sensor and the carboxylic acids may be attributed either to O−H···N hydrogen bonds or to charge-assisted carboxylate−acridinium $O^-\cdots H-N^+$ hydrogen bonding involving proton transfer in some cases.[12]

Achiral fluorosensors have been used for HTS of combinatorial libraries,[13] but only a few examples of enantioselective analysis with chiral fluoroprobes have been reported to date.[14] Since our results show that 1,8-diacridylnaphthalene **1** is a fluorosensor with a broad application spectrum, it may be employed in HTS efforts based on indicator-displacement assays to evaluate the asymmetric synthesis of chiral carboxylic acids, including non-steroidal anti-inflammatory drugs such as naproxen or ibuprofen.[2] However, it should be noticed that practical hurdles including solvent effects and interactions with chiral catalysts and reagents still have to be overcome before this methodology can become a widely useful tool for HTS.

In conclusion, we have developed a C_2-symmetric sensor that undergoes stereoselective interactions with a variety of chiral carboxylic acids, resulting in fluorescence quenching. The high sensitivity inherent to fluorescence spectroscopy, combined with the considerable stereoselectivity and broad application spectrum of this chemosensor, provides the potential for real-time analysis of the enantiomeric composition of chiral carboxylic acids. Further studies of the chiral recognition mechanism and the usefulness of 1,8-diacridylnaphthalenes for enantioselective fluorosensing of different classes of chiral compounds are currently underway in our laboratories.

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Supporting Information Available: Experimental procedures and full characterization of **1**, Stern−Volmer plots for all fluorescence titration experiments and actual measurements of the enantiopurity of different samples of **14** (PDF, CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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