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Enantioselective Fluorescence Sensing of Chiral α-Amino Alcohols

Shuanglong Liu, John Paul C. Pestano, and Christian Wolf*

Department of Chemistry, Georgetown University, Washington, D.C. 20057

cw27@georgetown.edu

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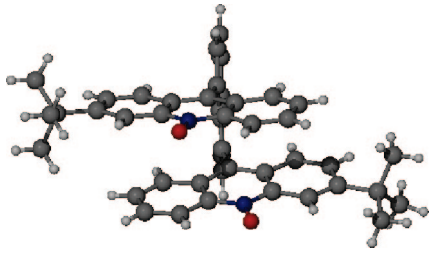


1,8-Bis(3-*tert*-butyl-9-acridyl)naphthalene *N*,*N*′-dioxide, **1**, has been synthesized in five steps from 3-*tert*-butylaniline and 2-chlorobenzoic acid in 29% overall yield. This C_2-symmetric ligand forms a highly fluorescent scandium complex that can be used for enantioselective sensing of chiral amino alcohols. A fluorescence ligand displacement assay that allows accurate measurements of both the total amount *and* the enantiomeric excess of several amino alcohols at micromolar concentrations is reported.

The high demand for time-efficient, accurate, and sensitive enantioselective analysis[1] provides a compelling rationale for developing chiral UV and fluorescence sensors that can be used to determine both the enantiomeric purity and overall concentration of chiral compounds.[2] We have introduced 1,8-diheteroarylnaphthalenes and *N*,*N*′-dioxide derivatives thereof that are designed to (a) closely embed hydrogen bonding interactions with chiral molecules into a highly stereoselective environment and (b) to utilize fluorescence and UV spectroscopy to quantitatively measure chiral recognition.[3] A thorough understanding of the stereodynamics and three-dimensional structure of pyridyl, quinolyl, and acridyl derivatives has been obtained based on crystallography, NMR spectroscopic analysis, and racemization studies (Figure 1).[4] While the two cofacial heteroaryl rings in 1,8-diheteroarylnaphthalenes remain perpendicular to the naphthalene framework and show little splaying, the torsion angle can change over a range of 50°, in particular upon binding to a guest molecule. The suitability of these chemosensors to enantioselective recognition of amino acids, carboxylic acids and other chiral hydrogen bond donors has been attributed to this one-dimensional flexibility, which facilitates accommodation of substrates of varying size in the chiral pocket.

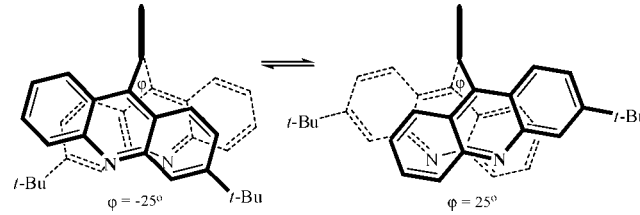
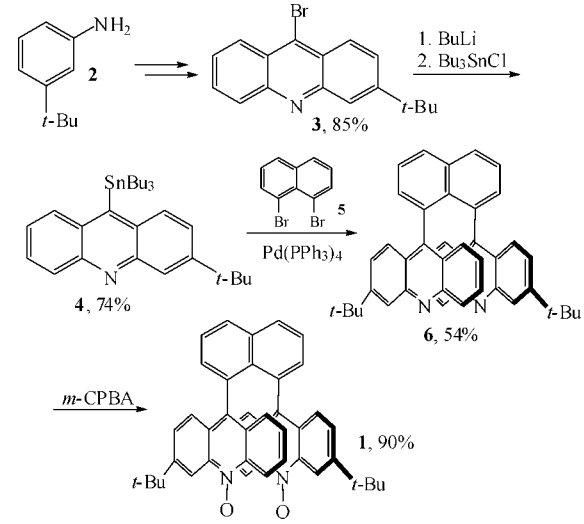


FIGURE 1. One-dimensional flexibility of a 1,8-diacridylnaphthalene.

SCHEME 1. Synthesis of 1,8-Bis(3-*tert*-butyl-9-acridyl)naphthalene *N*,*N*′-Dioxide, 1



To extend the application spectrum of currently known enantioselective sensors, we decided to synthesize 1,8-bis(3-

(1) (a) Reetz, M. T.; Becker, M. H.; Kuhling, K. M.; Holzwarth, A. *Angew. Chem., Int. Ed.* **1998**, *37*, 2647–2650. (b) Ding, K.; Shii, A.; Mikami, K. *Angew. Chem., Int. Ed.* **1999**, *38*, 497–501. (c) Guo, J.; Wu, J.; Siuzdak, G.; Finn, M. G. *Angew. Chem., Int. Ed.* **1999**, *38*, 1755–1758. (d) Reetz, M. T.; Becker, M. H.; Klein, H.-W.; Stockigt, D. *Angew. Chem., Int. Ed.* **1999**, *38*, 1758–1761. (e) Reetz, M. T.; Kuhling; Deege, A.; Hinrichs, H.; Belder, D. *Angew. Chem., Int. Ed.* **2000**, *39*, 3891–3893. (f) Abato, P.; Seto, C. T. *J. Am. Chem. Soc.* **2001**, *123*, 9206–9207. (g) Evans, M. A.; Morken, J. P. *J. Am. Chem. Soc.* **2002**, *124*, 9020–9021. (h) Taran, F.; Gauchet, C.; Mohar, B.; Meunier, S.; Valleix, A.; Renard, P. Y.; Creminon, C.; Grassi, J.; Wagner, A.; Mioskowski, C. *Angew. Chem., Int. Ed.* **2002**, *41*, 124–127. (i) Matsushita, M.; Yoshida, K.; Yamamoto, N.; Wirsching, P.; Lerner, R. A.; Janda, K. D. *Angew. Chem., Int. Ed.* **2003**, *42*, 5984–5987. (j) Markert, C.; Pfaltz, A. *Angew. Chem., Int. Ed.* **2004**, *43*, 2498–2500.

(2) (a) Pu, L. *Chem. Rev.* **2004**, *104*, 1687–1716. (b) Pagliari, S.; Corradini, R.; Galaverna, G.; Sforza, S.; Dossena, A.; Montalti, M.; Prodi, L.; Zaccheroni, N.; Marchelli, R. *Chem. Eur. J.* **2004**, *10*, 2749–2758. (c) Zhao, J.; Fyles, T. M.; James, T. D. *Angew. Chem., Int. Ed.* **2004**, *43*, 3461–3464. (d) Zhu, L.; Anslyn, E. V. *J. Am. Chem. Soc.* **2004**, *126*, 3676–3677. (e) Li, Z.-B.; Lin, J.; Qin, Y.-C.; Pu, L. *Org. Lett.* **2005**, *7*, 3441–3444. (f) Li, Z.-B.; Lin, J.; Pu, L. *Angew. Chem., Int. Ed.* **2005**, *44*, 1690–1693. (g) Zhu, L.; Zhong, Z.; Anslyn, E. V. *J. Am. Chem. Soc.* **2005**, *127*, 4260–4269. (h) Folmer-Andersen, J. F.; Lynch, V. M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2005**, *127*, 7986–7987. (i) Mei, X.; Wolf, C. *J. Am. Chem. Soc.* **2006**, *128*, 13326–13327.

(3) (a) Tumambac, G. E.; Mei, X.; Wolf, C. *Eur. J. Org. Chem.* **2004**, 3850–3856. (b) Mei, X.; Wolf, C. *J. Am. Chem. Soc.* **2004**, *126*, 14736–14737. (c) Mei, X.; Wolf, C. *Chem. Commun.* **2004**, 2078–2079. (d) Tumambac, G. E.; Wolf, C. *Org. Lett.* **2005**, *7*, 4045–4048. (e) Wolf, C.; Liu, S.; Reinhardt, B. C. *Chem. Commun.* **2006**, 4242–4244. (f) Mei, X.; Wolf, C. *Tetrahedron Lett.* **2006**, *47*, 7901–7904.

(4) (a) Tumambac, G. E.; Wolf, C. *J. Org. Chem.* **2004**, *69*, 2048–2055. (b) Tumambac, G. E.; Wolf, C. *J. Org. Chem.* **2005**, *70*, 2930–2938. (c) Mei, X.; Wolf, C. *J. Org. Chem.* **2005**, *70*, 2299–2305. (d) Mei, X.; Martin, R. M.; Wolf, C. *J. Org. Chem.* **2006**, *71*, 2854–2861. (e) Wolf, C. Ed. *Dynamic Stereochemistry of Chiral Compounds*; RSC Publishing: London, UK, 2008; pp 84−109.

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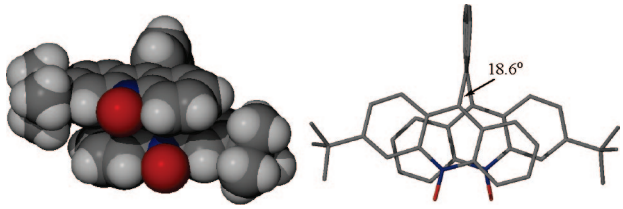

FIGURE 2. Single-crystal structure of **1**.

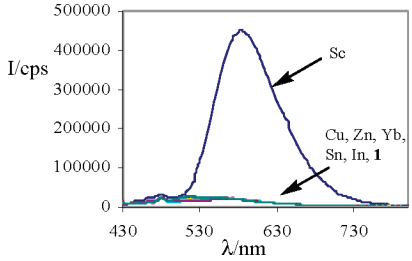

FIGURE 3. Fluorescence of *N*,*N*′-dioxide **1** in the presence of different metal ions. The concentration of **1** and M(OTf)$_x$ was 2.3 × 10^{-5} and 1.15 x10^{-5} M in anhydrous acetonitrile, respectively.

tert-butyl-9-acridyl)naphthalene *N*,*N*′-dioxide, **1**, and to explore its use for fluorescence sensing of chiral amino alcohols. Following methods previously reported from our laboratories,[5] we converted commercially available 3-*tert*-butylaniline, **2**, to acridyl bromide **3**, which was then treated with butyllithium and tributylstannyl chloride to furnish acridyltributylstannane **4**. Stille coupling of **4** and 1,8-dibromonaphthalene, **5**, using Pd(PPh$_3$)$_4$ as catalyst, gave 1,8-diacridylnaphthalene **6** in 54% yield (Scheme 1). We observed that stannane **4** produces significantly higher yields than its trimethylstannyl analogue, which undergoes extensive methyl transfer during the second transmetalation step, thus generating a 1-acridyl-8-methylnaphthalene derivative. Apparently, transmetalation of an *n*-butyl group from **4** to the palladium center does not occur under the reaction conditions used and sterically crowded **6** can be obtained in 54% yield.[6] The corresponding *N*,*N*′-dioxide **1** was obtained by oxidation with *m*-CPBA.

Careful solvent evaporation of a solution of *N*,*N*′-dioxide **1** in a 1:1 mixture of dichloromethane and ethanol afforded a triclinic single crystal belonging to the *P*1 space group. Crystallographic analysis showed that the acridyl rings are twisted about 18.6° but remain almost perfectly cofacial with a splaying angle of 5.3° (Figure 2). This bidentate ligand forms a C_2-symmetric cleft with two accessible *N*-oxide groups and we expected that **1** would form strong metal complexes.

We found that the fluorescence of **1** is dramatically increased by stoichiometric amounts of Sc(OTf)$_3$ but this was not the case in the presence of other metal ions, such as Cu(OTf)$_2$, Zn(OTf)$_2$, Yb(OTf)$_3$, Sn(OTf)$_2$, and In(OTf)$_3$ (Figure 3). Ligand **1** thus serves as a selective off/on sensor for scandium(III) ions. Fluorescence and UV titration experiments including Job plot analysis revealed that *N*,*N*′-dioxide **1** forms a strong Sc[(±)-**1**]$_2$ complex (p*K* 4.9). We then realized that the fluorescence emission of the scandium *N*,*N*′-dioxide complex at 588 nm

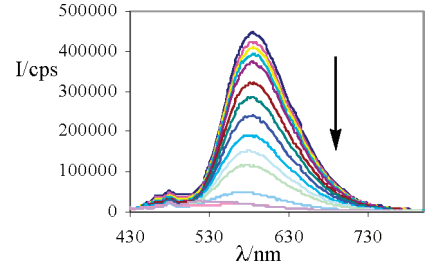

FIGURE 4. Fluorescence change of Sc[(±)-**1**]$_2$ upon addition of (*S*)-alaninol. The concentration of **1** and Sc(OTf)$_x$ was 2.3 × 10^{-5} and 1.15 × 10^{-5} M in anhydrous acetonitrile, respectively. The amino alcohol was added in increments of 1.15 × 10^{-5} M.

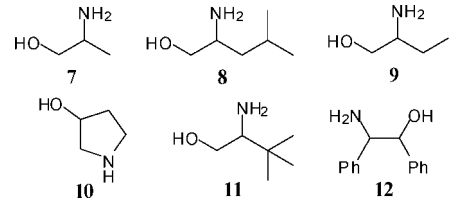

FIGURE 5. Amino alcohols employed in enantioselective sensing studies.

disappeared upon addition of amino alcohols such as alaninol, **7**, indicating the replacement of **1** by **7** (Figure 4).

A previously reported UV−vis sensing method developed in our laboratories[2i] and the results shown in Figure 4 encouraged us to explore the feasibility of an enantioselective fluorescence assay in which the *N*,*N*′-dioxide ligands would be subsequently replaced from an *enantiopure* scandium complex. Accordingly, titration of a strongly fluorescent solution of Sc[(+)-**1**]$_2$ with alaninol or another amino alcohol would result in the replacement of the first *N*,*N*′-dioxide ligand from the metal center by (*R*)- or (*S*)-alaninol and generate diastereomeric Sc[(+)-**1**(*R*)-**7**] and Sc[(+)-**1**(*S*)-**7**] complexes. This first exchange step would then be followed by another ligand substitution and produce fluorescence-silent alaninol-derived scandium complexes and free ligand (+)-**1**. Because the ligand exchange proceeds via diastereomeric scandium complex intermediates, one enantiomer of alaninol was expected to be more effective in displacing (+)-**1** than the other. As a result, the fluorescence signal of the *N*,*N*′-dioxide-derived scandium complex would be switched off enantioselectively, and this could be exploited for sensing purposes.

After screening several chiral HPLC columns, we found that the enantiomers of *anti*-**1** can be separated on an (*R*,*R*)-Whelk-O 1 column. Amino alcohols **7**−**12** were then employed in competitive binding experiments, using Sc[(+)-**1**]$_2$ or Sc[(−)-**1**]$_2$ as sensor (Figure 5). Fluorescence titration experiments showed that the sensor can effectively differentiate between the enantiomers of the amino alcohols tested, even at micromolar concentrations (see the Supporting Information). The excellent enantioselectivity in the case of alaninol is particularly remarkable (Figures 6 and 7). Adjusting the concentration of (*S*)-**7** to approximately 5.0 × 10^{-5} M results in almost quantitative quenching of the emission maximum of Sc[(+)-**1**]$_2$ (1.15 × 10^{-5} M), which is due to effective replacement of (+)-**1** ligands from the scandium center. At the same analyte concentration, the (*R*)-enantiomer of **7** shows little effect on the fluorescence of Sc[(+)-**1**]$_2$. To verify that the measured difference in fluorescence quenching originates from enantioselective ligand displacement

(5) (a) Wolf, C.; Liu, S.; Mei, X.; August, A. T.; Casimir, M. D. *J. Org. Chem.* **2006**, *71*, 3270–3273. (b) Mei, X.; August, A. T.; Wolf, C. *J. Org. Chem.* **2006**, *71*, 142–149. (c) Liu, S.; Pestano, J. P. C.; Wolf, C. *Synthesis* **2007**, 3519–3527.

(6) 1,8-Diacridylnaphthalene **6** is formed in an anti/syn ratio of 55:45.

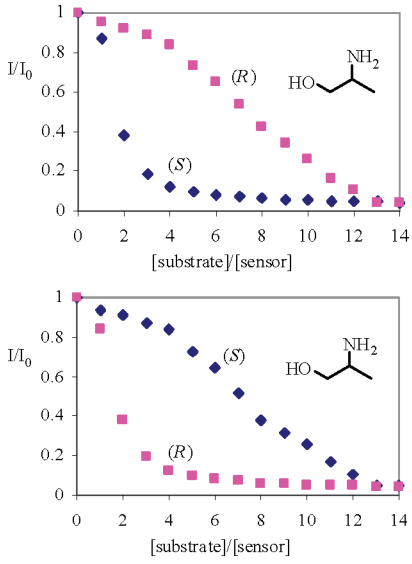

FIGURE 6. Enantioselective sensing of the enantiomers of alaninol **7**, using Sc[(+)-**1**]$_2$ (left) and Sc[(−)-**1**]$_2$ (right). Titrations were conducted by using acetonitrile as solvent and the concentration of **1** and Sc(OTf)$_3$ was 2.3 × 10^{-5} and 1.15 x10^{-5} M, respectively. The emission (excitation) wavelength was 588 (420) nm.

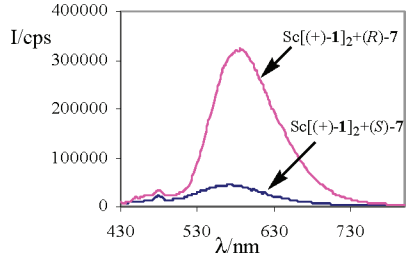

FIGURE 7. Fluorescence emission spectra of Sc[(+)-**1**]$_2$ (1.15 × 10^{-5} M) in the presence of (*R*)- and (*S*)-alaninol (5.75 × 10^{-5} M).

and is not due to impurities, Sc[(−)-**1**]$_2$ was employed in the same experiment. In this case, more effective ligand displacement was observed with (*R*)-alaninol than with the (*S*)-enantiomer.

We then developed a practical fluorescence method based on enantioselective stepwise displacement of **1** from Sc(*N*,*N*′-dioxide **1**)$_2$ for quantitative analysis of both the concentration and the ee of amino alcohols **7**−**12**. Nine samples containing alaninol at different concentrations and in varying enantiomeric composition were prepared. First, the quenching of the fluorescence intensity of *racemic* Sc(*N*,*N*′-dioxide **1**)$_2$ was measured and compared to a calibration curve (see the Supporting Information).[7] This gave very accurate results of the amount of **7** in each sample. For example, the calculated total concentrations of three samples (G, H, I) were 6.85 × 10^{-5}, 6.83 × 10^{-5}, and 6.97 × 10^{-5} M, which is in excellent agreement with the actual value of 6.90 × 10^{-5} M. Because the sensor was employed in its racemic form, the results obtained are independent of the enantiomeric composition of **7** (Table 1).

For quantitative determination of the enantiomeric composition of the same alaninol samples, three plots correlating the ee of **7** at 2.3, 4.6, and 6.9 × 10^{-5} M and the fluorescence intensity of *enantiopure*Sc[(+)-**1**]$_2$ were obtained (see the Supporting Information). With these calibration plots in hand, we were able to determine the ee of the 9 samples based on enantioselective fluorescence quenching. Again, results proved to be accurate and reproducible. For example, the calculated ee values of three samples having a concentration of 2.30 × 10^{-5} M were 6.6%, 51.1%, and 93.5%, which corresponds well with the actual values of 5%, 55%, and 95%, respectively (Table 2).

(7) It should be noted that the use of racemic **1** can result in the formation of mixtures of homo- and heterochiral scandium complexes.

TABLE 1. Determination of the Concentration of Nine Samples of Alaninol with Use of Racemic Sc(*N*,*N*′-dioxide 1)$_2$

sample	actual concn (10^{-5} M)	ee	calcd concn (10^{-5} M)
A	2.30	5	2.27
B	2.30	55	2.25
C	2.30	95	2.22
D	4.60	5	4.63
E	4.60	55	4.58
F	4.60	95	4.52
G	6.90	5	6.85
H	6.90	55	6.83
I	6.90	95	6.97

TABLE 2. Determination of the Enantiomeric Compositions of Nine Samples of Alaninol with Use of Sc[(+)-1]$_2$ in Acetonitrile

actual concn (10^{-5} M)	actual ee	I/I_0	calcd ee
2.3	5	0.880	6.6
2.3	55	0.634	51.1
2.3	95	0.340	93.5
4.6	5	0.796	7.0
4.6	55	0.415	58.0
4.6	95	0.116	98.0
6.9	5	0.626	3.4
6.9	55	0.314	59.4
6.9	95	0.139	91.0

In summary, 1,8-diacridylnaphthalene *N*,*N*′-dioxide **1** forms a highly fluorescent scandium complex that can be used for enantioselective recognition of chiral amino alcohols. We have developed a fluorescence ligand displacement assay that allows accurate measurements of both the total amount *and* the enantiomeric excess of several amino alcohols at micromolar concentrations. This sensing method combines several attractive features: it allows determination of both concentration and ee by the use of one sensor (in its racemic and enantiopure form), it depends on two simple assays suitable to automation and high-throughput screening, it generates accurate values with high reproducibility, it does not require cumbersome substrate derivatization, and it utilizes cost-effective and sensitive fluorescence spectroscopy that requires only very small sample amounts and thus minimizes solvent waste.

Experimental Section

Synthesis of 1,8-Bis(3-*tert*-butyl-9-acridyl)naphthalene *N*,*N*′-dioxide (1). To a solution of *anti*-**6** (23 mg, 0.039 mmol) in anhydrous CH_2Cl_2 was added a solution of *m*-CPBA (14 mg, 0.078 mmol) in 2 mL of CH_2Cl_2. The mixture was stirred at room temperature for 15 h. Then, the mixture was washed with 2 N NaOH. The organic layers were combined and the solvents were removed under reduced pressure. The residue was purified by flash chromatography on silica gel with 10% MeOH in CH_2Cl_2 as mobile phase to afford **1** as a red solid (21 mg, 90%). ^{1}H (300 MHz, $CDCl_3$) δ 1.41 (s, 18 H), 6.59 (ddd, J = 1.0, 6.6 Hz, 7.6 Hz, 2H), 6.76 (d, J = 9.3 Hz, 2H), 6.83 (d, J = 8.4 Hz, 2H), 7.03 (dd, J = 1.0, 9.0 Hz, 2H), 7.31 (dd, J = 1.0, 6.8 Hz, 2H), 7.38 (ddd, J = 1.2, 6.6, 7.8 Hz, 2H), 7.73 (dd, J = 7.1, 8.3 Hz, 2H), 8.29 (dd, J = 1.2, 8.3 Hz, 2H), 8.41 (d, J = 2.0 Hz, 2H), 8.50 (d, J = 9.0 Hz, 2H). ^{13}C (75 MHz, $CDCl_3$) δ 31.6, 36.2, 115.4, 120.2, 125.9, 126.1, 126.2,

126.4, 126.5, 126.8, 126.9, 130.1, 130.9, 132.3, 133.5, 134.7, 135.0, 135.7, 138.2, 138.5, 154.2. Anal. Calcd for $C_{44}H_{38}N_2O_2$: C, 84.31; H, 6.11; N, 4.47. Found: C, 84.05; H, 6.32; N, 4.35.

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Supporting Information Available: All synthetic procedures, full product characterization, and details of chromatographic, crystallographic, and spectroscopic measurements. This material is available free of charge via the Internet at http://pubs.acs.org.

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