An enantioselective fluorescence sensing assay for quantitative analysis of chiral carboxylic acids and amino acid derivatives†

Christian Wolf,* Shuanglong Liu and Brian C. Reinhardt

Received (in Cambridge, UK) 11th July 2006, Accepted 9th August 2006
First published as an Advance Article on the web 1st September 2006
DOI: 10.1039/b609880k

A chiral 1,8-diacridylnaphthalene-derived fluorosensor exhibiting a C_2-symmetric cleft designed for stereoselective interactions with hydrogen bond donors has been used for the determination of both concentration and enantiomeric composition of carboxylic acids and amino acid derivatives.

The prospect of high-throughput screening of asymmetric reactions has directed increasing attention to the development of enantioselective UV and fluorescence methods during recent years.[1] Fluorescence spectroscopy offers a variety of advantages over traditional chromatographic and NMR spectroscopic techniques such as different detection modes (fluorescence quenching, enhancement, and lifetime measurements), high sensitivity, low cost of instrumentation, waste reduction, and time-efficiency.[2] The potential of fluorescence sensing for the determination of the enantioselectivity of asymmetric reactions has been demonstrated with the titanium tartrate-catalyzed addition of trimethylsilyl cyanide to an immobilized aldehyde and with the enzymatic kinetic resolution of *trans*-1,2-diaminocyclohexane by Pu *et al.* and our group.[3] In both cases, fluorescence sensing was found to provide accurate ee's and proved advantageous over laborious and time-consuming chromatographic methods. Anslyn *et al.* reported a practical approach to enantioselective fluorosensing of bifunctional α-hydroxycarboxylates and diols based on indicator-displacement assays with chiral boronic acid receptors.[4] However, a method providing the enantiomeric composition and concentration for a wide range of mono- and multifunctional carboxylic acids and amino acid derivatives has been elusive to date.

Previous X-ray and NMR spectroscopic studies conducted in our laboratories have shown that 1,8-diquinolyl- and 1,8-diacridylnaphthalenes are highly congested, rigid structures that possess remarkable one-dimensional flexibility.[5] While the two cofacial heteroaryl rings perpendicular to the naphthalene framework show little splaying, the torsional angle, τ, can change over a range of 50°, in particular upon binding to a hydrogen bond donor. The usefulness of these fluorescent sensors for the enantioselective analysis of chiral compounds has been attributed to this one-dimensional conformational flexibility which facilitates the accommodation of substrates of varying size in the C_2-symmetric pocket, Fig. 1.[6] We wish to report the synthesis of axially chiral *anti*-1,8-bis(3′-*tert*-butyl-9′-acridyl)naphthalene **1** and its use as a practical fluorosensor for the determination of both enantiomeric excess and concentration of mono- and multifunctional carboxylic acids and amino acids.

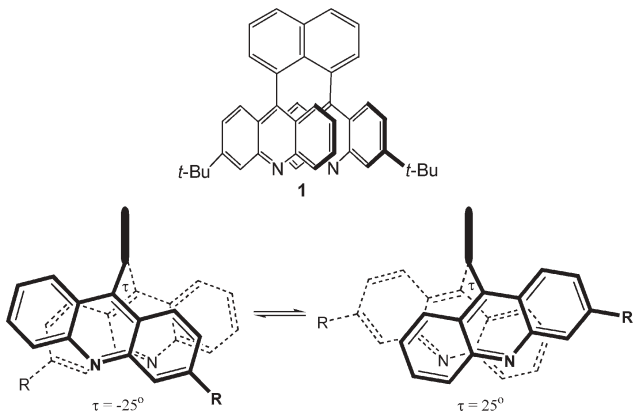
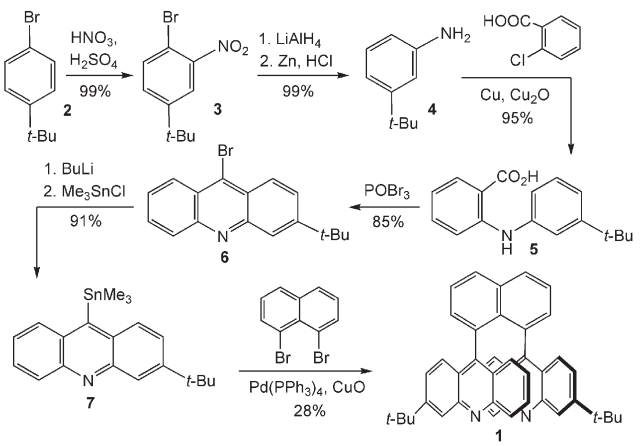


Fig. 1 Structure of fluorosensor **1** and one-dimensional conformational flexibility of 1,8-diacridylnaphthalenes.

The synthesis of **1** involved regioselective acridine ring construction from *N*-3-*tert*-butylphenylanthranilic acid **5** which was obtained from 4-*tert*-butylbromobenzene **2** in three steps.[7] Lithiation and subsequent stannylation of 9-bromo-3-*tert*-butylacridine **6** produced **7**, which was then employed in a Stille cross-coupling with 1,8-dibromonaphthalene to give 1,8-diacridylnaphthalene **1**, Scheme 1. Slow evaporation of a racemic mixture of **1** in isopropyl alcohol–hexanes (1 : 1) at room temperature produced a single crystal suitable for X-ray analysis, Fig. 2.‡ The



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

Department of Chemistry, Georgetown University, Washington, DC 20057, USA. E-mail: cw27@georgetown.edu; Fax: (+1) 202 687 6209; Tel: (+1) 202 687 3468

† Electronic supplementary information (ESI) available: Synthesis and characterization of **1**. Stern–Volmer and Benesi–Hildebrand plots and fluorosensing measurements of the concentration and enantiopurity of several samples. See DOI: 10.1039/b609880k

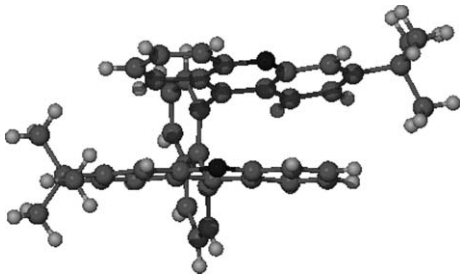


Fig. 2 Single crystal structure of **1**.

splaying and torsional angles between the acridyl rings of **1** were determined as 5.3° and 23.4°, respectively. Because of the inherent fluorescence and one-dimensional flexibility of 1,8-diacridylnaphthalenes in solution, this C_2-symmetric bidentate ligand was expected to effectively embed hydrogen bonding interactions with chiral carboxylic acids and amino acids into a highly stereoselective environment. We anticipated that the formation of diastereomeric adducts could be conveniently measured and quantified by fluorescence spectroscopy.

After the screening of several chiral HPLC columns, we found that the enantiomers of *anti*-**1** can be resolved on a Chiralpak AD column. Enantiopure **1** (excitation at 360 nm, emission maximum at 535 nm) and carboxylic acids and amino acids **8–19**, Fig. 3, were then employed in fluorescence titration experiments using acetonitrile as solvent. We were pleased to find that a concentration of 3.5×10^{-6} M of the fluorosensor **1** suffices to effectively differentiate between minute quantities of the enantiomers of all analytes studied.

For example, the (*R*)-enantiomers of α-halogenated carboxylic acids **8** and **9** showed little quenching whereas the fluorescence of (−)-**1** decreased dramatically even when the (*S*)-enantiomers were present at only millimolar concentrations, Fig. 4. Diacridylnaphthalene **1** has two potential binding sites and can undergo simultaneous hydrogen bonding with two substrates. Benesi–Hildebrand plots revealed that (−)-**1** forms stronger 1 : 2 complexes with (*S*)-**8** and (*S*)-**9** than with the corresponding (*R*)-enantiomers which explains the more pronounced fluorescence quenching observed with the levorotatory sensor. As expected, the (+)-sensor affords opposite enantioselectivity, Fig. 4. We were pleased to find that **1** also differentiates between the enantiomers of carboxylic acids and amino acids **10–19**, see ESI.† Since

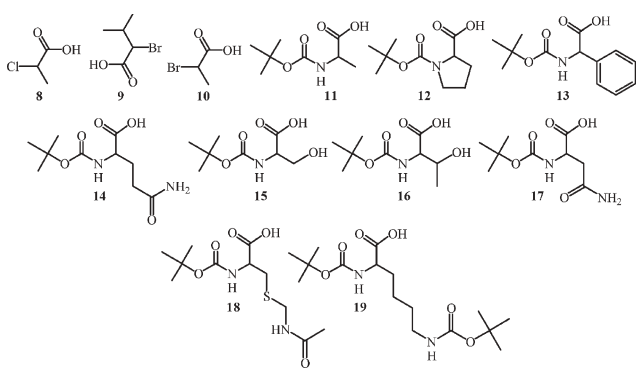


Fig. 3 Structures of chiral carboxylic acids and amino acids.

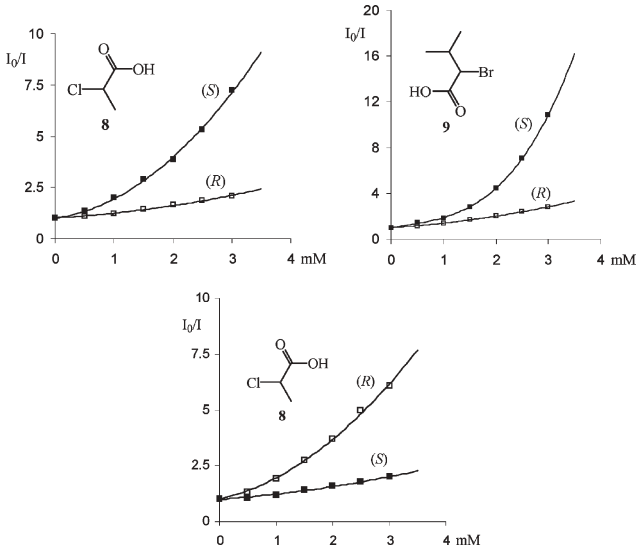


Fig. 4 Stern–Volmer plots showing enantioselective fluorescence quenching of (−)-**1** in the presence of **8** and **9** (top). Sensing of the enantiomers of **8** with (+)-**1** (bottom). Sensor concentration: 3.5×10^{-6} M. Excitation (emission) wavelength: 360 nm (535 nm).

diacridylnaphthalene **1** exhibits a strong fluorescent signal that effectively responds to chiral interactions with **8–19** at low concentration, it better exploits the inherent sensitivity of fluorescence spectroscopy than previously reported sensors and extends this technique to enantioselective analysis of minute sample amounts.

With this new efficient sensor in hand, we developed a fluorescence method that allows accurate measurements of both the total amount and the enantiomeric excess of a chiral compound. This was realized by the combination of two assays using racemic and enantiopure sensor **1**. First, the total concentration of a chiral analyte with unknown enantiomeric composition was determined by fluorescence sensing with racemic diacridylnaphthalene **1**. As expected, fluorescence titration of racemic **1** using either enantiomer of 2-chloropropionic acid **8** gave superimposable Stern–Volmer plots, see ESI.† We therefore envisioned that (±)-**1** could be used for quantitative non-stereoselective analysis of a chiral sample, while the enantiomeric excess could then be uncovered using the enantiopure sensor in a succeeding assay. In order to evaluate the accuracy and reproducibility of our sensing method, we prepared different samples of chiral acid **8** having concentrations of 1.5 and 3.0 mM, respectively, and enantiopurities varying from 5 to 95%. Through comparison of the averaged fluorescence response of racemic **1** with a calibration curve we calculated concentrations of **8** ranging from 1.47 to 1.51 and 2.97 to 3.06 mM, respectively. Having determined the individual sample concentrations, we were then able to uncover the enantiomeric composition based on fluorescence quenching experiments with (−)-**1**, Table 1.

In general, the results obtained for the six samples were within ±2% of the actual concentration of 2-chloropropionic acid **8** and within ±3% of the actual enantiopurity. For example, fluorescence analysis of sample C (1.50 mM, 95.0% (*S*)-**8**) gave a concentration of 1.51 mM and 96.8% (*S*)-**8**. Excellent

Table 1 Concentration and enantiomeric composition of six samples of acid **8** determined by fluorescence quenching with (−)-**1**

Sample	Actual concentration/ mM	Actual % (*S*)	Calculated concentration/ mM[a]	Calculated % (*S*)[a]
A	1.50	5.0	1.47	6.4
B	1.50	55.0	1.48	56.2
C	1.50	95.0	1.51	96.8
D	3.00	15.0	2.97	16.2
E	3.00	55.0	3.02	56.9
F	3.00	85.0	3.06	87.8

[a] Average of three fluorescence measurements at 535 nm.

results were also obtained with the other samples, including A and D which contained substantial quantities of the (*R*)-enantiomer. The data demonstrate the high reproducibility and accuracy of this method which is suitable for the screening of samples covering a wide range of enantiopurities and a high excess of either enantiomer.

In conclusion, we have prepared *anti*-1,8-bis(3′-*tert*-butyl-9′-acridyl)naphthalene **1** exhibiting a highly congested C_2-symmetric pocket for chiral recognition of carboxylic acids and amino acid derivatives and demonstrated its use for practical stereoselective fluorescence analysis. Because **1** affords a strongly fluorescent signal that effectively responds to enantioselective interactions with chiral acids, this new sensor is suitable for quantitative analysis of minute sample amounts. We have developed a simple method that utilizes diacridylnaphthalene **1** in two facile fluorescence sensing assays. We believe that this approach 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 simple assays that provide accurate values with high reproducibility; it eliminates the need for substrate derivatization; and it utilizes a cost-effective and sensitive technique (fluorescence spectroscopy) that minimizes solvent waste.

Funding from the NSF (CAREER Award, CHE-0347368) and the PRF administered by the ACS (PRF40897-G4) is gratefully acknowledged.

Notes and references

‡ Crystal structure data for **1**·*i*-PrOH: formula $C_{47}H_{43}N_2O$, M = 652.34, crystal dimensions: 0.1 × 0.1 × 0.1 mm, triclinic, space group $P\bar{1}$, a = 10.7101(14) Å, b = 11.3527(15) Å, c = 15.434(2) Å, α = 77.800(3)°, β = 87.817(3)°, γ = 76.455(3)°, V = 1783.1(4) Å^3, Z = 2, ρ_{calcd} = 1.134 g cm^{-3}, μ = 0.070 mm^{-1}, range of θ for data collection: 1.35 to 28.45°, T = 173(2) K, 8379 independent reflections (R_{int} = 0.1305), $R1$ = 0.0828, w$R2$ = 0.1815 with $I > 2\sigma(I)$, GooF = 0.907, $\Delta\rho_{max}$ = 0.379 e Å^{-3}, $\Delta\rho_{min}$ = −0.322 e Å^{-3}. The crystal contains a disordered molecule of isopropyl alcohol in the asymmetric unit. Single crystal X-ray diffractions were performed at −100 °C using a Siemens platform diffractometer with graphite monochromated Mo-Kα radiation (λ = 0.71073 Å). Data were integrated with the Siemens SAINT program[8a] and corrected for the effects of absorption using SADABS.[8b] The structures were solved by direct methods and refined with full-matrix least-square analysis using SHELX-97-2 software.[8c] Non-hydrogen atoms were refined with anisotropic displacement parameters and all hydrogen atoms were placed in calculated positions and refined with a riding model. CCDC 606070. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b609880k

1 (*a*) L. Pu, *Chem. Rev.*, 2004, **104**, 1687–1716; (*b*) L. Zhu and E. V. Anslyn, *J. Am. Chem. Soc.*, 2004, **126**, 3676–3677.

2 (*a*) T. D. James, K. R. A. S. Sandanayake and S. Shinkai, *Nature*, 1995, **374**, 345–347; (*b*) Y. Yan and M. L. Myrick, *Anal. Chem.*, 1999, **71**, 1958–1962; (*c*) G. Beer, K. Rurack and J. Daub, *Chem. Commun.*, 2001, 1138–1139; (*d*) M. Reetz and S. Sostmann, *Tetrahedron*, 2001, **57**, 2515–2520; (*e*) J. Lin, Q.-S. Hu, M.-H. Xu and L. Pu, *J. Am. Chem. Soc.*, 2002, **124**, 2088–2089; (*f*) J. Zhao, T. M. Fyles and T. D. James, *Angew. Chem., Int. Ed.*, 2004, **43**, 3461–3464; (*g*) Z.-B. Li, J. Lin and L. Pu, *Angew. Chem., Int. Ed.*, 2005, **44**, 1690–1693.

3 (*a*) Z.-B. Li, J. Lin, Y.-C. Qin and L. Pu, *Org. Lett.*, 2005, **7**, 3441–3444; (*b*) G. E. Tumambac and C. Wolf, *Org. Lett.*, 2005, **7**, 4045–4048.

4 L. Zhu, Z. Zhong and E. V. Anslyn, *J. Am. Chem. Soc.*, 2005, **127**, 4260–4269.

5 (*a*) C. Wolf and X. Mei, *J. Am. Chem. Soc.*, 2003, **125**, 10651–10658; (*b*) G. E. Tumambac and C. Wolf, *J. Org. Chem.*, 2004, **69**, 2048–2055; (*c*) X. Mei and C. Wolf, *J. Org. Chem.*, 2005, **70**, 2299–2305; (*d*) G. E. Tumambac and C. Wolf, *J. Org. Chem.*, 2005, **70**, 2930–2938.

6 (*a*) X. Mei and C. Wolf, *Chem. Commun.*, 2004, 2078–2079; (*b*) X. Mei and C. Wolf, *J. Am. Chem. Soc.*, 2004, **126**, 14736–14737; (*c*) X. Mei, R. M. Martin and C. Wolf, *J. Org. Chem.*, 2006, **71**, 2854–2861.

7 (*a*) X. Mei, A. T. August and C. Wolf, *J. Org. Chem.*, 2006, **71**, 142–149; (*b*) C. Wolf, S. Liu, X. Mei, A. T. August and M. D. Casimir, *J. Org. Chem.*, 2006, **71**, 3270–3273.

8 (*a*) G. M. Sheldrick, *SAINT*, Bruker AXS Inc., USA, 2003; (*b*) G. M. Sheldrick, *SADABS, Program for area detector adsorption correction*, Institute for Inorganic Chemistry, University of Göttingen, Germany, 1996; (*c*) G. M. Sheldrick, *SHELX-97-2*, University of Göttingen, Germany, 1997.