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Enantioselective Analysis of an Asymmetric Reaction Using a Chiral Fluorosensor†

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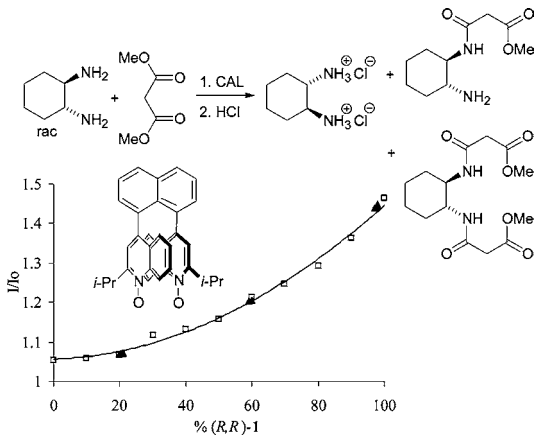
ABSTRACT



An axially chiral 1,8-dipyridylnaphthalene *N,N′*-dioxide has been employed in enantioselective fluorescence analysis of the enzymatic kinetic resolution of *trans*-1,2-diaminocyclohexane. The procedure eliminates cumbersome purification and derivatization steps required by traditional methods. The results demonstrate the potential of fluorescence spectroscopy using suitable chiral chemosensors for real-time analysis of the enantiomeric composition of chiral compounds and for high-throughput screening of asymmetric reactions.

Many biologically active compounds are chiral, and the majority of today's top-selling pharmaceuticals are single enantiomers. The ever-increasing demand for chiral chemicals has led to significant progress in asymmetric synthesis and development of combinatorial methods for rapid preparation of large libraries of compounds for comprehensive SAR screening. However, enantioselective analysis of asymmetric reactions usually involves time-consuming chromatographic techniques such as GC and HPLC on chiral stationary phases or NMR spectroscopic measurements of purified samples in the presence of chiral shift reagents. Since currently employed methods are often the rate-limiting factor during high-throughput screening efforts of asymmetric reactions, the development of fast stereoselective assays has received increasing attention. Recent advances in the development of enantioselective high-throughput analysis include ingenious methods based on mass spectrometry,[1] colorimetry,[2] UV absorption,[3] fluorescence spectroscopy,[4] IR thermography,[5] circular dichroism,[6] NMR spectroscopy,[7] cap-

† Dedicated to the memory of Professor Wilfried König who fundamentally contributed to enantioselective analysis and separation.

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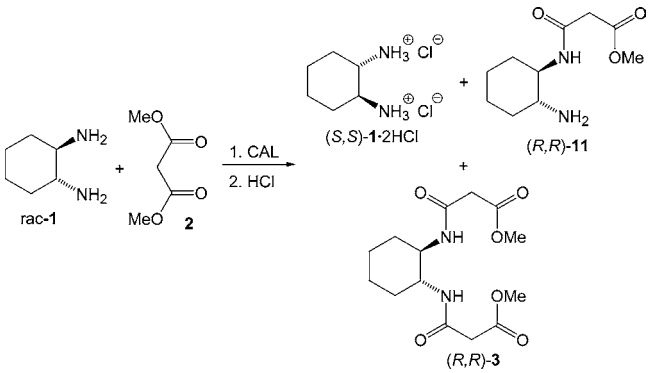
illary electrophoresis,[8] competitive immunoassay,[9] enzymatic assay,[10] and indicator-displacement assay.[11]

Enantioselective sensing based on fluorescence spectroscopy provides an attractive alternative because of its inherently high sensitivity, simple operation, waste reduction, time efficiency, and the possibility of performing real-time analysis. To date, a few enantioselective fluorescence sensors derived from BINOL[12] and selectively substituted 1,8-diacridylnaphthalenes[13] have been reported. However, these sensors have only been employed in chiral recognition studies of isolated samples, and none has been applied to enantioselective analysis of asymmetric reactions. We wish to report an enantioselective fluorescence assay that simplifies screening of the stereochemical course of the enzymatic kinetic resolution of *trans*-1,2-diaminocyclohexane, **1**, via acylation with dimethyl malonate, **2**, in the presence of a suitable lipase.

Gotor and co-workers reported that *Candida antarctica* lipase (CAL)-catalyzed aminolysis of racemic 1,2-diamine **1** using dimethyl malonate as the acyl donor in 1,4-dioxane provides enantiopure (*R*,*R*)-bisamidoester **3**, while enantiomerically enriched unreacted (*S*,*S*)-**1** could be isolated as its air-stable ammonium salt after treatment with anhydrous HCl, Scheme 1.[14] We anticipated that screening of the subsequent enantioselective enrichment of (*S*,*S*)-**1** during kinetic resolution with CAL would reveal important limitations and drawbacks of traditional enantioselective analysis. Determination of the enantiomeric purity of aliphatic analytes such as **1** by HPLC on a chiral stationary phase is generally complicated because of the absence of a chromophore that would allow facile UV detection. Similarly, enantioselective GC analysis would require a cumbersome derivatization step to increase the inherently low volatility of diamine **1**. Attempts to utilize chiral solvating agents such as BINOL, binaphthyl-2,2′-diyl hydrogen phosphate, and camphersulfonic acid or lanthanide shift reagents such as Eu(hfc)$_3$ for NMR spectroscopic analysis of the enantiomers of **1** proved unsuccessful as a result of low resolution of diastereotopic

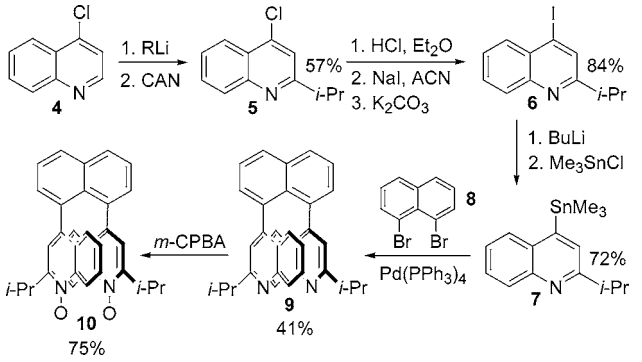
Scheme 1. Enzymatic Kinetic Resolution of Racemic 1,2-Diamine **1** Using *Candida antarctica* Lipase



signals. Accordingly, a literature survey of enantiomeric analyses of **1** provided few methods, all requiring derivatization prior to HPLC separation.[14]

We decided to develop a rapid fluorosensing method for enantiomeric analysis of the enzymatic kinetic resolution of racemic 1,2-diamine **1** that would favorably compare with traditional methods and avoid time-consuming derivatization steps. Starting from 4-chloroquinoline, **4**, we were able to prepare 1,8-bis(2-isopropyl-4-quinolyl)naphthalene *N*,*N*′-dioxide, **10**, in five steps, Scheme 2. Alkylation of **4** with

Scheme 2. Synthesis of 1,8-Bis(2-isopropyl-4-quinolyl)naphthalene *N*,*N*′-Dioxide, **10**



isopropyllithium and treatment with CAN gave **5** with 57% yield, which was converted to iodide **6** prior to lithiation and subsequent stannylation to yield 2-isoproyl-4-trimethylstannylquinoline **7** in 72%. Stille cross-coupling of **7** and 1,8-dibromonaphthalene, **8**, provided 41% of 1,8-bis(2-isopropyl-4-quinolyl)naphthalene, **9**, which was oxidized with *m*-CPBA to afford **10** with 75% yield. The enantiomers of **10** were separated by HPLC using a Chiralpak AD column.

We found that C_2-symmetric 1,8-diquinolylnaphthalene *N*,*N*′-dioxide **10** is suitable for chiral recognition of the enantiomers of diamine **1**. Fluorescence titration experiments showed that addition of the enantiomers of **1** to a solution of (−)-**10** in chloroform results in enantioselective changes

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of the fluorescence response of **10**. Stern−Volmer plotting demonstrates that (*R*,*R*)-**1** significantly enhances fluorescence of the sensor, whereas the (*S*,*S*)-enantiomer has little effect, Figure 1. The enantioselectivity factor, $\alpha = K^{RR}_{SV}/K^{SS}_{SV}$, was

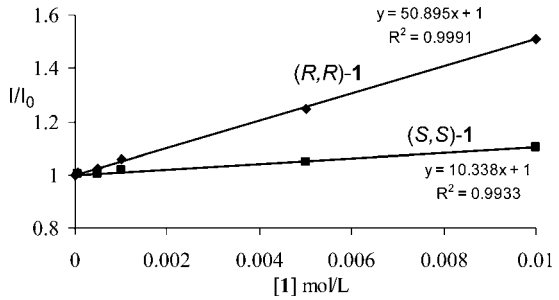

Figure 1. Stern−Volmer plot of 3.5 10^{-5} M (−)-**10** at optimized optical density in the presence of (*R*,*R*)- and (*S*,*S*)-**1** in chloroform. Excitation wavelength was 380 nm; emission wavelength was 430 nm.

calculated as 4.9. Importantly, no change in the fluorescence intensity of **10** was observed upon addition of monoamidoester, **11**, and bisamidoester **3**. In analogy to enantioselective fluorosensing of amines and amino acids observed with a C_2-symmetric diacridylnaphthalene *N*,*N*′-dioxide derivative, chiral recognition of the enantiomers of **1** by enantiopure diquinolylnaphthalene *N*,*N*′-dioxide **10** is attributed to formation of diastereomeric hydrogen bond adducts.[13a] On the basis of the high stereoselectivity and substrate-specificity, we expected that the sensor could be used to differentiate between the enantiomers of **1** even in the presence of other analytes such as **11**, i.e., without tedious purification and derivatization steps.

To prove the accuracy and reproducibility of our sensing method we prepared nine samples containing 18%, 57%, and 96% of (*R*,*R*)-**1** and determined the enantiomeric composition based on the fluorescence enhancement of (−)-**10**. Averaging the fluorescence measurements of the individually prepared samples we calculated 20%, 60%, and 98% enantiopurity using a calibration curve. The results are within ±3% of the actual enantiopurity of the samples and demonstrate high precision and accuracy of enantioselective fluorosensing with **10**, Figure 2.

We then applied our fluorosensing method to the enzymatic kinetic resolution of **1**. Aliquots from the reaction mixture were taken after 1.5, 2.5, 3.5, 5, and 7 h and analyzed by fluorosensing using *N*,*N*′-dioxide **10**. Diamine **1** and monoamidoester **11** were isolated through precipitation with 2 N hydrogen chloride in diethyl ether and subsequent basic extraction. Since relative amounts of **1** and **11** formed at these time intervals were known from previous screening experiments, we were able to prepare samples containing 0.01 M of **1** in anhydrous chloroform for accurate fluorescence analysis using a calibration curve. For comparison of the enantiopurity of **1** determined by fluorescence sensing, we

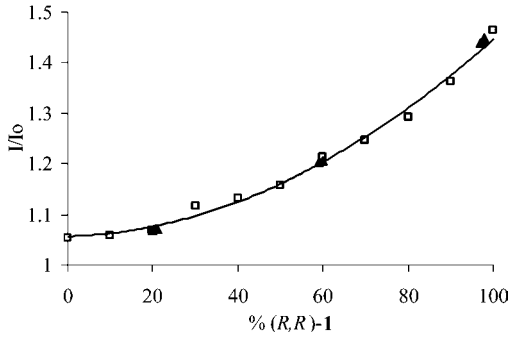

Figure 2. Calibration curve (□) and determination of the enantiomeric purity of nine accurately weighed-in samples (▲) having 18%, 57%, and 96% of (*R*,*R*)-**1**. Total concentration of **1** was 0.01 M. The concentration of (−)-**10** was 3.5 10^{-5} M.

used *m*-toluoyl chloride to prepare an UV-active diamide suitable for HPLC/UV analysis. Screening of a range of chiral HPLC columns revealed that *N*,*N*′-bis(*m*-toluoyl)-diamide **12** can be separated into enantiomers on Chiralpak AD. The enantiomeric composition of the individual samples of **1** obtained by both methods are shown in Table 1. As

Table 1. Enantiomeric Excess of **1** Obtained by Enzymatic Kinetic Resolution Using Enantioselective Fluorosensing and HPLC Analysis

time (h)	% ee (*S*,*S*-**1**) by HPLC	I/I_0 [a]	% ee (*S*,*S*-**1**) by fluorescence sensing[a]
1.5	24	1.1287	24
2.5	44	1.1022	44
3.5	56	1.0905	50
5.0	64	1.0861	56
7.0	68	1.0686	62

[a] Averaged value of three measurements.

expected, the fluorescence enhancement of (−)-**10**, I/I_0, diminished as the relative amount of (*R*,*R*)-**1** decreased as a result of enantioselective conversion to (*R*,*R*)-bisamidoester **3**. We were pleased to find that the results obtained by HPLC of derivative **12** and direct fluorescence analysis are in very good agreement. After 1.5 h, lipase-catalyzed enantioselective conversion of (*R*,*R*)-diamine **1** to (*R*,*R*)-bisamidoester **3** increased the enantiomeric excess of unreacted **1** to 24% of the (*S*,*S*)-enantiomer. Further screening of the reaction showed the continuing increase in the enantiopurity of (*S*,*S*)-**1** to 62−68% ee after 7 h according to HPLC and fluorescence analysis. The ability of **10** to differentiate between the enantiomers of **1** in the presence of other substrates including monoamide **11** thus greatly facilitates fast enantioselective analysis.

In conclusion, we have developed a C_2-symmetric fluorosensor that can be employed in direct enantioselective analysis of the enzymatic kinetic resolution of *trans*-1,2-diaminocyclohexane, eliminating cumbersome purification

and derivatization steps required by traditional methods. The results demonstrate the potential of fluorescence spectroscopy using suitable chiral chemosensors for real-time analysis of the enantiomeric composition of chiral compounds and for high-throughput screening of asymmetric reactions.

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Supporting Information Available: Synthetic procedures, fluorescence measurements, HPLC enantioseparation of **10** and **12**, and CD and specific rotation data of **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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