Supplemental Information

Enantioselective Analysis of an Asymmetric Reaction using Fluorescence Sensing

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1. Synthetic procedures.

All commercially available reagents and solvents were used without further purification. 1,8-Dibromonaphthalene was prepared from 1,8-diaminonaphthalene as described in the literature.[1] All reactions were carried out under nitrogen atmosphere and under anhydrous conditions. Organostannanes are highly toxic and should only be used in a vented hood and when wearing eye and skin protection. Products were purified by flash chromatography on SiO_2 (particle size 0.032-0.063 mm). GC-MS was performed on a Fison Instruments MD800 mass spectrometer equipped with a gas chromatograph using a 15 m DB-1 column. Atmospheric pressure chemical ionization (APCI) mass spectra were collected on a YMC-Pack CN column (4.6 x 250 mm) using an HPLC/MSD system equipped with electrospray and atmospheric pressure chemical ionization MS detection and hexanes/EtOH = 9:1 as the mobile phase. NMR spectra were obtained at 300 MHz (^{1}H NMR) and 75 MHz (^{13}C NMR) using $CDCl_3$ as the solvent. Chemical shifts are reported in ppm relative to TMS. Elemental analysis data were collected using a Perkin Elmer 2400 CHN.

4-Chloro-2-isopropylquinoline (5)

A solution of 0.7 M isopropyllithium in *n*-pentane (73.4 mL, 51.4 mmol) was added under nitrogen to a solution of 4-chloroquinoline, **4**, (7.0 g, 42.8 mmol) in 130 mL of anhydrous THF at –78 ^{o}C. After 1 h, the reaction was quenched with 10% NH_4OH, allowed to come to room temperature and extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and the solvents were removed in vacuo. The residue was dissolved in 50 mL of acetone and an aqueous solution of cerium (IV) ammonium nitrate (65.3 g, 120 mmol, 100 mL H_2O) was added. Acetone was removed after the dark red solution had turned to light yellow. The mixture was extracted with CH_2Cl_2, dried over $MgSO_4$ and the solvents were removed under reduced pressure. Flash chromatography on silica gel (75:25:1 hexanes: CH_2Cl_2: triethylamine) of the crude residue gave **7** (5.0 g, 57%) as a yellow oil.

^{1}H NMR: δ = 1.39 (d, *J* = 6.9 Hz, 6H), 3.23 (sept, *J* = 6.9 Hz, 1H), 7.43 (s, 1H), 7.58 (ddd, *J* = 1.4 Hz, 7.0 Hz, 8.2 Hz, 1H), 7.73 (ddd, *J* = 1.4 Hz, 7.0 Hz, 8.4 Hz, 1H), 8.06 (ddd, *J* = 0.5 Hz, 1.4 Hz, 8.4 Hz, 1H), 8.18 (ddd, *J* = 0.5 Hz, 1.4 Hz, 8.2 Hz, 1H). ^{13}C NMR: δ = 22.5, 37.3, 119.3, 123.8, 125.0, 126.5, 129.2, 130.1, 142.5, 148.5, 167.5. EI-MS (70 ev): m/z (%) = 205 (30, M^+), 190 (100, M^+- Me), 162 (15, M^+- *i*-Pr), 127 (17, M^+- *i*-Pr - Cl). Anal. Calc. for $C_{12}H_{12}ClN$: C, 70.07; H, 5.88; N, 6.81. Found: C, 69.96; H, 5.75; N, 6.64.

4-Iodo-2-isopropylquinoline (6)

4-Chloro-2-isopropylquinoline, **5**, (4.5 g, 22.0 mmol) was converted to its hydrochloride salt by adding 4 M HCl in 1,4-dioxane (6.1 mL, 24.2 mmol). After 15 minutes, the solvent was removed and the precipitate dried under reduced pressure. It was then suspended with dry NaI (16.5 g, 67.5 mmol) in anhydrous acetonitrile and the mixture was refluxed for 24 h. After cooling to room temperature, an aqueous solution containing 10% K_2CO_3 and 5% $NaHSO_3$ was added.

After the mixture was extracted with CH_2Cl_2, the combined organic layers were dried over $MgSO_4$ and the solvents were evaporated under reduced pressure. Purification by flash chromatography (100:10:1 hexanes: ethyl acetate: triethylamine) yielded **6** (5.5 g, 84%) as a yellow oil.

^{1}H NMR: δ = 1.39 (d, *J* = 6.9 Hz, 6H), 3.19 (sept, *J* = 6.9 Hz, 1H), 7.55 (ddd, *J* = 1.4 Hz, 6.9 Hz, 8.3 Hz, 1H), 7.70 (ddd, *J* = 1.4 Hz, 6.9 Hz, 8.3 Hz, 1H), 7.95-7.98 (m, 2H). ^{13}C NMR: δ = 22.5, 36.8, 112.2, 127.1, 128.8, 129.5, 130.1, 130.8, 130.9, 131.3, 147.4, 167.3. EI-MS (70 eV): 297 (21, M^+), 282 (60, M^+- Me), 170 (3, M^+- I), 155 (20, M^+- I - Me), 127 (18, M^+- I - *i*-Pr). Anal. Calc. for $C_{12}H_{12}IN$: C, 48.51; H, 4.07; N, 4.71. Found: C, 48.91; H, 3.91; N, 4.64.

2-Isopropyl-4-trimethylstannylquinoline (7)

4-Iodo-2-isopropylquinoline, **6**, (5.2 g, 17.5 mmol) was dissolved in 70 mL of anhydrous ether and cooled to -78 ^{o}C under nitrogen. A solution of *n*-BuLi in hexanes (13.0 mL, 1.6 M in hexanes) was added dropwise and the mixture was stirred for 30 minutes. To this solution, Me_3SnCl (26.0 mL, 1.0 M in hexanes) was added at once. The resulting mixture was allowed to come to room temperature, stirred for 5 h, quenched with 10% NH_4OH and extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. Purification by flash chromatography (150:10:1 hexanes: ethyl acetate: triethylamine) afforded **7** (4.2 g, 72%) as a viscous yellow oil.

^{1}H NMR: δ = 0.48 (s, 9H), 1.41 (d, *J* = 6.9 Hz, 6H), 3.23 (sept, *J* = 6.9 Hz, 1H), 7.46 (s, 1H), 7.48 (ddd, *J* = 1.4 Hz, 6.9 Hz, 1H), 7.66 (ddd, *J* = 1.4 Hz, 6.9 Hz, 8.4 Hz, 1H), 7.71 (dd, *J* = 1.4 Hz, 8.0 Hz, 1H), 8.06 (dd, *J* = 1.4 Hz, 8.4 Hz, 1H). ^{13}C NMR: δ = -8.4, 22.7, 37.3, 125.3, 127.7, 128.6, 129.3, 130.0, 132.2, 147.0, 153.9, 165.0. EI-MS (70 ev): m/z (%) = 335 (24, M^+), 320 (100, M^+- Me), 305(9, M^+- 2Me), 290 (31, M^+- Me), 275 (3, M^+- 4Me), 170 (32, M^+- Me_3Sn),

155 (11, M^+- Me - Me_3Sn). Anal. Calc. for $C_{15}H_{21}NSn$: C, 53.93; H, 6.34; N, 4.19. Found: C, 53.67; H, 6.70; N, 4.19.

1,8-Bis(2-isopropyl-4-quinolyl)naphthalene (9)

A solution of 1,8-dibromonaphthalene, **8**, (0.90 g, 3.1 mmol), $Pd(PPh_3)_4$ (368 mg, 10 mol %) and CuO (0.90 g, 11.7 mmol) in 30 mL of anhydrous DMF was stirred at 100 ^oC under N_2. After 5 minutes, 2-isopropyl-4-trimethylstannylquinoline, **7**, (4.0 g, 12.0 mmol) in 20 mL of DMF was added. The reaction was stirred at 100 ^oC for 13 h, cooled to room temperature and quenched with 10% NH_4OH. The aqueous layer was extracted with diethyl ether. The combined organic layers were washed with H_2O, dried over $MgSO_4$ and concentrated under vacuum. Purification by chromatography on silica gel (100:20:1 hexanes: ethyl acetate: triethylamine) gave **9** (585 mg, 1.3 mmol, 41%) as yellow crystals, mp 214-218^oC.

^1H NMR: δ = 0.80 (d, *J* = 6.9 Hz, 6H), 0.98 (d, *J* = 6.9 Hz, 6H), 2.34 (sept, *J* = 6.9 Hz, 2H), 6.34 (s, 2H), 7.18 (dd, *J* = 1.1 Hz, 8.2 Hz, 2H), 7.23-7.29 (m, 4H), 7.54 (ddd, *J* = 1.4 Hz, 6.7 Hz, 8.4 Hz, 2H), 7.62 (dd, *J* = 7.1 Hz, 8.2 Hz, 2H), 7.87 (d, *J* = 8.5 Hz, 2H), 8.13 (dd, *J* = 1.4 Hz, 8.4 Hz, 2H). ^{13}C NMR: δ = 21.1, 22.6, 36.1, 119.9, 125.1, 125.3, 125.4, 126.6, 128.3, 129.3, 129.5, 130.4, 130.9, 134.3, 135.7, 146.7, 147.9, 165.4. EI-MS: m/z (%) = 466 (100, M^+), 451 (94, M^+-Me), 436 (5, M^+- 2Me), 423 (23, M^+- *i*-Pr), 253 (5, M^+-isopropylquinolyl). LC-APCI-MS: m/z (%) =467 [100, $(M + H)^+$]. Anal. Calc. for $C_{34}H_{30}N_2$: C, 86.70; H, 7.68; N, 5.62. Found: C, 86.27; H, 7.23; N, 5.87.

1,8-Bis(2-isopropyl-4-quinolyl)naphthalene *N,N'*-dioxide 10

To a solution of 1,8-bis(2-isopropyl-4-quinolyl)naphthalene, **9**, (100mg, 0.2 mmol) in diethyl ether (10.0 mL) at 0 ^oC was added dropwise a solution of *m*-CPBA (170 mg, 1.0 mmol) in THF. After stirring for 30 minutes, the reaction mixture was allowed to come to room temperature and

stirred for 16 h. The solvent was removed, followed by neutralization with an aqueous solution of K_2CO_3. The aqueous layer was then extracted with CH_2Cl_2. After drying of the combined methylene chloride extracts over $MgSO_4$ and removal of the solvent, the crude residue was purified by flash chromatography (30:1 hexanes:ethanol) to give **10** (80 mg, 75%) as white crystals.

^{1}H NMR**:** δ = 0.67 (d, *J* = 6.9 Hz, 6H), 0.94 (d, *J* = 6.9 Hz, 6H), 3.49 (sept, *J* = 6.9 Hz, 2H), 6.42 (s, 2H), 7.26 (m, 6H), 7.63 (m, 4H), 8.16 (d, *J* = 8.0 Hz, 2H), 8.66 (d, *J* = 8.8 Hz, 2H). ^{13}C NMR: δ = 19.2, 19.8, 28.2, 119.4, 120.8, 126.1, 126.4, 127.8, 128.9, 129.8, 130.4, 131.3, 132.1, 134.5, 135.1, 137.5, 140.9, 152.2. LC-APCI-MS: 499 [(M+ H)$^+$]**.** Anal. Calcd for $C_{24}H_{30}N_2O_2$**:** C, 81.90; H, 6.06; N, 5.62; Found**:** C, 82.39; H, 6.43; N, 5.37.

Derivatization of 1,2-diaminocyclohexane 1 for HPLC analysis

To a stirred solution of diamine **1** (250mg, 2.23 mmol) in CH_2Cl_2 (25 mL) was added triethylamine (1.4 g, 13.4 mmol) at RT. The solution was cooled to -10 ^{o}C and *m*-toluoyl chloride (0.72 g, 4.7 mmol) was added. The reaction was allowed to warm to RT over 7h and then quenched with brine and extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo The crude residue was purified on silica using 1:1 hexanes:EtOAc to give *N,N'*-bis(*m*-toluoyl)diamide **12** (560 mg, 72%) as a white solid. Pure **12** was dissolved in 1:1 Hexanes:EtOH (c = 10 mg/mL) and analyzed by chiral HPLC on Chiralpak AD column using 80:20 Hexanes:EtOH as the mobile phase.

^{1}H NMR: δ = 1.26-1.45 (m, 4H), 1.71-1.78 (m, 2H), 2.10-2.20 (m, 2H), 2.36 (s, 6H), 3.94-3.42 (m, 2H), 7.14 (br s, 2H), 7.25-7.29 (m, 4H), 7.56-7.62 (m, 4H). ^{13}C NMR: δ = 21.6, 25.0, 32.5, 54.8, 124.3, 128.1, 128.6, 132.4, 134.4, 138.5, 168.7.

The enantiomers of **12** were separated by chiral HPLC using a Chiralpak AD column. The diluent was 1:1 hexanes:EtOH and the mobile phase was 80:20 hexanes:EtOH. The chromatographic enantioselectivity factor, α, was determined as 1.61.

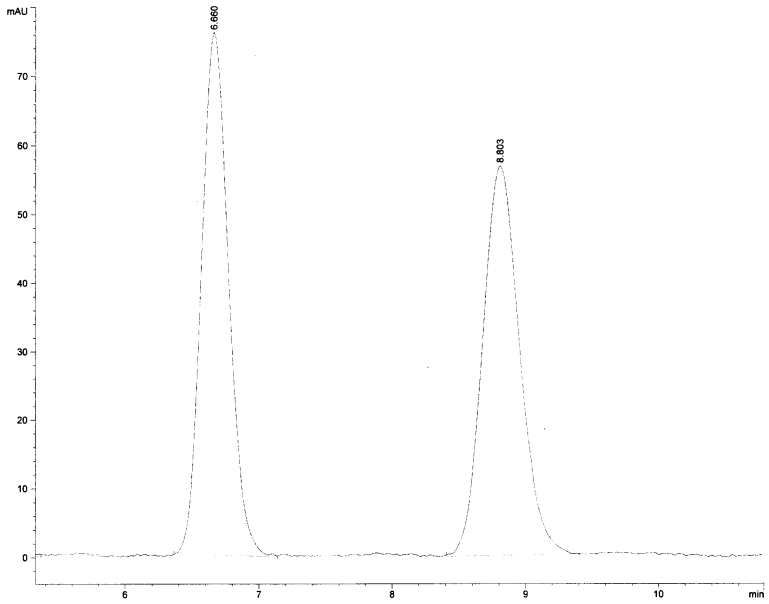

Figure 1. Enantioseparation of **12** on Chiralpak AD.

Enzymatic kinetic resolution

To a solution of racemic *trans*-1,2-diaminocyclohexane, **1**, (400 mg, 3.5 mmol) and dimethyl malonate, **2**, (460 mg, 3.5 mmol) in 1,4-dioxane (25 mL) was added lipase acrylic resin from *Candida Antarctica* (216 mg) under nitrogen. The reaction mixture was stirred at room temperature. Aliquots from the reaction mixture were taken after 1.5, 2.5, 3.5, 5 and 7 hours. For each aliquot portion, the enzyme was separated by filtration and washed with methylene chloride. HCl in ether (2M) was then added to the organic solution until precipitation of white solids was complete. The precipitate was filtered and dried under reduced pressure. (*R*,*R*)-Bisamidoester **3** was obtained as an off-white solid (530 mg, 48%).

^{1}H NMR: δ = 1.31-1.39 (m, 4H), 1.73-1.82 (m, 2H), 1.97-2.15 (m, 2H), 3.31 (s, 4H), 3.72-3.81 (m, 8H), 7.01-7.17 (br s, 2H). ^{13}C NMR: δ = 24.8, 32.2, 41.8, 52.7, 53.8, 166.5, 169.3.

For fluorescence analysis of the kinetic resolution, diamine **1** and monoamidoester **11** were isolated from the reaction mixture via precipitation with 2 N hydrogen chloride in diethyl ether and subsequent basic extraction. Because **1** is known to easily undergo oxidation the dried residue was immediately employed in fluorescence measurements. Relative amounts of **1** and **11** formed at 1.5, 2.5, 3.5, 5 and 7 hours were known from previous screening experiments, which enabled us to prepare samples containing 0.01 M of **1** for accurate fluorescence analysis using a calibration curve, vide infra.

2. HPLC enantioseparation of 10.

Chiral HPLC was carried out on an HP 1050 equipped with an autosampler and DAD detector using a Chiralpak AD column (250 mm x 4.6 mm, 5 μm). The samples were dissolved in hexanes:EtOH = 1:1 and separated into enantiomers using hexanes:EtOH = 40:60 as the mobile. The levorotatory enantiomer was eluted first and the HPLC enantioselectivity factor, α, was determined as 1.52. Preparative separations were performed by repetitive injections of 150 μL of **10** dissolved in the same diluent at a concentration of approximately 10 mg/mL.

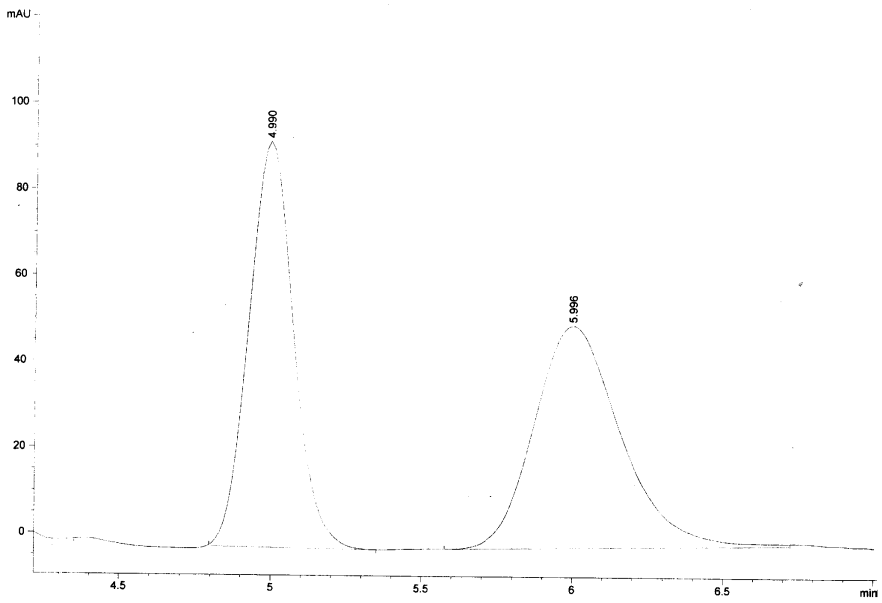

Figure 2. Enantioseparation of **10** on Chiralpak AD.

3. Circular dichroism and specific rotation of 10.

Semi-preparative HPLC enantioseparation of **10** on a Chiralpak AD column using 40:60 hexanes:ethanol as the mobile phase allowed determination of the CD spectra and specific rotations of the enantiomers. CD Spectra of 1,8-bis(2-isopropyl-4-quinolyl)naphthalene *N*,*N*'-dioxide **10** were obtained at a concentration of 3.0 x 10^{-5} M in 95:5 hexanes/ethanol using a JASCO J-710 circular dichroism chiroptical spectrometer. Specific rotations were determined on a Rudolph Instruments Digipol 781 polarimeter.

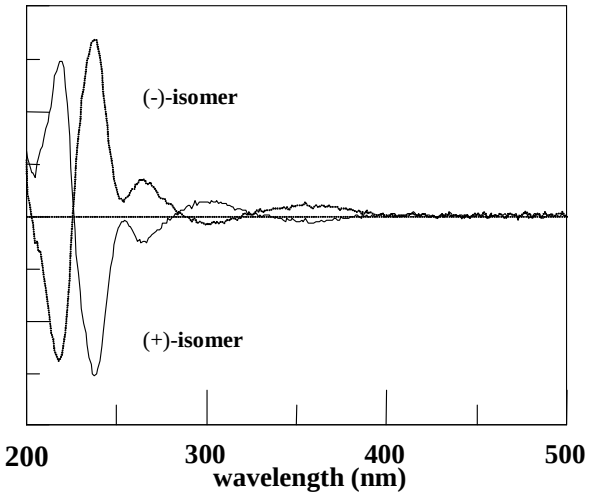

Figure 3. CD spectra of the enantiomers of **10**.

$[\alpha]^{24}_{546}$ = -116.0 (1st eluted enantiomer on Chiralpak AD, c = 30mg/ 100 mL CH_2Cl_2)

$[\alpha]^{26}_{546}$ = + 98.6 (2nd eluted enantiomer on Chiralpak AD, c = 30 mg/100 mL CH_2Cl_2)

4. Fluorescence measurements.

Fluorescence experiments were conducted using a Fluoromax-2 from Instruments S.A. Inc. All emission spectra were collected under nitrogen using a 3.5 10^{-5} M solution of **10** in carefully degassed chloroform under inert atmosphere. Excitation wavelength was 380 nm and emission wavelength was 430 nm.

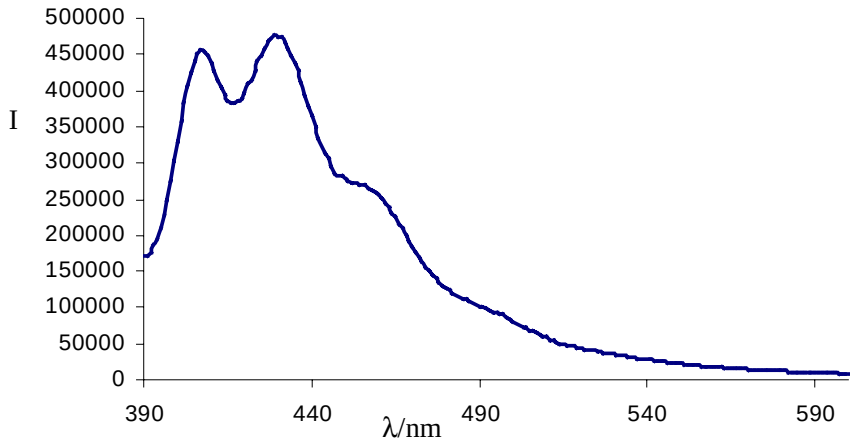

Figure 4. Fluorescence spectrum of **10**. The concentration of *N*,*N*'-dioxide **10** was 3.0 x 10^{-5} M in $CHCl_3$. The excitation wavelength was 380 nm.

Enantioselective fluorescence enhancement was also observed with (+)-**10** in the presence of (*R*,*R*)- and (*S*,*S*)-**1** in chloroform. The corresponding Stern-Volmer plot shows that (*S*,*S*)-**1** enhance fluorescence of (+)-**10** while (*R*,*R*)-**1** has little effect.

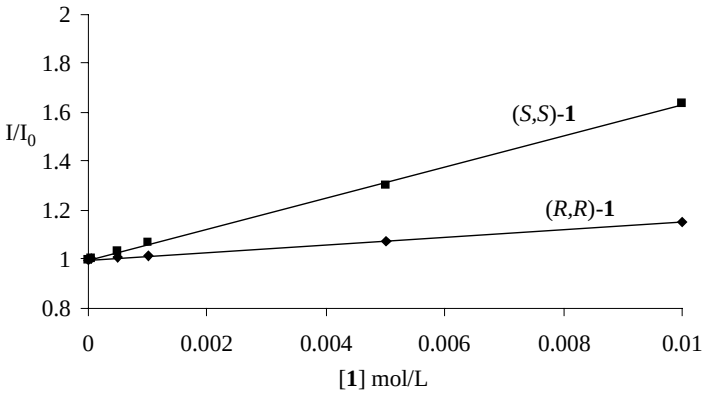

Figure 5. Stern-Volmer plot of 3.5 10^{-5} M (+)-**10** in the presence of (*R*,*R*)- and (*S*,*S*)-**1** in chloroform. Excitation wavelength was 380 nm; emission wavelength was 430 nm.

In order to evaluate the accuracy and reproducibility of the sensing method nine samples containing **1** in different enantiopurity were prepared and analyzed by the fluorescence sensing with **10**. Three sample sets containing 18, 57, and 96% of (*R*,*R*)-**1** in chloroform were prepared and the fluorescence response of **10** in the presence of the analyte mixture was measured to calculate the enantiomeric composition using a calibration curve.

Table 1. Fluorescence quenching of **10** in the presence of **1** measured for calibration.

% (*R*,*R*)-**1**	I	I_0/I
0	909584	1.053789
10	913757	1.058623
20	922694	1.068977
30	963778	1.116575
40	977564	1.132546
50	998009	1.156232
60	1046452	1.212356
70	1076001	1.246589
80	1115505	1.292356
90	1175926	1.362356
100	1265061	1.465623

I_0 was 863156.

Table 2. Determination of the actual enantiopurity of nine accurately weighed-in samples of **1** by enantioselective fluorosensing using the calibration curve.

% (*R*,*R*)	I	I_0/I	calculated % (*R*,*R*)	averaged % (*R*,*R*)	STD (%)
18	923789	1.070246	21		
18	922632	1.068905	20	20	0.12
18	921545	1.067646	20		
57	1037568	1.202063	59		
57	1040125	1.205026	60	60	0.18
57	1041102	1.206157	60		
96	1245689	1.443179	98		
96	1242356	1.439318	97	98	0.31
96	1250010	1.448185	98		

The concentration of *N*,*N*'-dioxide **10** was 3.0×10^{-5} M in $CHCl_3$. The concentration of **1** was 0.01 M. Excitation wavelength was 380 nm; emission wavelength was 430 nm.

Table 3. Enantiomeric excess of **1** obtained by enzymatic kinetic resolution using enantioselective fluorosensing and HPLC analysis.

time (h)	I	I_0/I	%ee (*S*,*S*)-**1** by fluorescence	%ee (*S*,*S*)-**1** by HPLC
	974623	1.129139	22	
1.5	976254	1.131028	20	24
	971956	1.126049	26	
	950232	1.100881	44	
2.5	951523	1.102377	44	44
	952256	1.103226	44	
	941235	1.090457	50	
3.5	941256	1.090482	50	56
	941562	1.090519	50	
	939562	1.088500	54	
5.0	938465	1.087447	55	64
	934321	1.082447	57	
	921989	1.068160	62	
7.0	920232	1.066125	64	68
	924965	1.071608	60	

Fluorescence: The concentration of *N*,*N'*-dioxide **10** was 3.0 x 10^{-5} M in $CHCl_3$. The concentration of **1** was 0.01 M. Excitation wavelength was 380 nm; emission wavelength was 430 nm. HPLC conditions: The analyte was dissolved in 1:1 hexanes:EtOH and separated into enantiomers using a Chiralpak AD column and 80:20 hexanes:EtOH as the mobile phase. UV detection at 254 nm.

5. References

(1) Seyferth, D.; Vick, S. C. *J. Organomet. Chem.* **1977**, 141, 178-187.