Supplementary Information

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

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1. Synthesis

General Methods: All reactions were carried out using commercially available reagents and solvents were used without further purification. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) measurements were conducted in $CDCl_3$ unless otherwise noted. Flash column chromatography was accomplished using silica gel (particle size 32-63 μm) or aluminum oxide (activated, basic, 150 mesh, 58 Å).

1-Bromo-4-*tert*-butyl-2-nitrobenzene (3). To 1-bromo-4-*tert*-butylbenzene (5.28 mL, 31.0 mmol) at 0 ^{o}C was added dropwise 9.6 mL of HNO_3/H_2SO_4 (1:1.4). The solution was allowed to warm to room temperature and stirred for 12 hours. The reaction mixture was quenched with ice and extracted with hexanes. The combined organic layers were dried over anhydrous magnesium sulfate, filtered and concentrated to yield 8.42 g (99% yield) of an orange oil. ^{1}H NMR δ: 1.34 (s, 9H), 7.44 (dd, J = 2.5 Hz, 8.5 Hz, 1H), 7.64 (d, J = 8.5 Hz, 1H), 7.83 (d, J = 2.5 Hz, 1H). ^{13}C NMR δ: 31.68, 34.87, 112.74, 112.77, 116.08, 129.28, 146.49, 152.78. EI-MS (70 eV): m/z = 257 (50, M^+); 242 (100, M^+ - Me). Anal. Calcd for $C_{10}H_{12}BrNO_2$: C: 46.53 H: 4.69 N: 5.43; Found: C: 46.44 H: 4.61 N: 5.46.

3-*tert*-Butylaniline (4). To a solution of $LiAlH_4$ in THF (148 mL, 1 M) at 0 ^{o}C was added dropwise 1-bromo-4-*tert*-butyl-2-nitrobenzene (8.4 g, 29.5 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 12 hours. The solution was then cooled to 0 ^{o}C and quenched with a mixture of THF/water (1:1, 25 mL). The mixture was filtered, rinsed with ether and the filtrate was concentrated to produce a light orange residue.[1] To a solution of the di-3-*tert*-butylphenyldiazene in EtOH (150 mL) was added granular Zn (5.8 g, 88.5 mmol), concentrated HCl until a pH ≈ 3 was obtained and the mixture was stirred at room temperature for 12 hours. The reaction mixture was quenched with aqueous Na_2CO_3 until the pH was neutral and EtOH was removed in *vacuo*. The remaining aqueous solution was extracted with ether and the combined organic layers were dried over anhydrous magnesium sulfate, filtered and concentrated to yield 4.50 g (99% yield) of an orange oil. For elemental analysis, aniline **4** was treated with hydrogen chloride (diethyl ether, 2.0 M) to give **4**-HCl. ^{1}H NMR δ: 1.31 (s, 9H), 6.53 (ddd, J = 1.0 Hz, 2.0 Hz, 7.8 Hz, 1H), 6.74 (dd, J = 2.0 Hz, 2.0 Hz, 1H), 6.82 (ddd, J = 1.0 Hz, 2.0 Hz, 7.8 Hz, 1H), 7.11 (dd, J = 7.8 Hz, 7.8 Hz, 1H). ^{13}C NMR δ (DMSO): 30.85, 34.45, 120.06, 120.42, 124.93, 129.32, 131.45, 152.41. EI-MS (70 eV): m/z = 149 (70, M^+); 134 (80, M^+ - Me). Anal. Calc. for $C_{10}H_{16}ClN$: C: 64.68 H: 8.68 N: 7.54, Found: C: 64.49 H: 8.60 N: 7.46.

2-(3-*tert*-Butylphenylamino)benzoic acid (5).[2] To a flask containing 2-chlorobenzoic acid (2.1 g, 13.4 mmol), Cu powder (76.6 mg, 1.21 mmol), Cu_2O (76.7 mg, 0.54 mmol) and K_2CO_3 (3.7 g, 26.8 mmol) was added 3-*tert*-butylaniline, **4**, (2.0 g, 13.4 mmol) dissolved in ethoxyethanol (4.3 mL). The reaction mixture was heated to reflux (135 ^oC) and stirred for 12 hours. The mixture was cooled to room temperature, diluted with aqueous Na_2CO_3, filtered through celite and acidified with concentrated HCl until a pH of approximately 4 was reached, which caused precipitation of crude **5**. The residue was separated from water, dissolved in methylene chloride, dried over anhydrous magnesium sulfate, and concentrated to yield 3.5 g (95% yield) of a white powder. Anthranilic acid **5** was isolated as the HCl salt by addition of hydrogen chloride (diethyl ether, 2.0 M) for elemental analysis. ^1H NMR δ: 1.47 (s, 9H), 7.56 (dd, J = 1.2 Hz, 8.2 Hz, 8.2 Hz, 1H), 7.68-7.81 (m, 2H), 8.14 (d, J = 2.1 Hz, 1H), 8.19 (dd, J = 0.9 Hz, 8.9 Hz, 1H), 8.31 (dd, J = 0.6 Hz, 9.2 Hz, 1H), 8.36 (m, 1H). ^{13}C NMR δ (DMSO): 31.07, 34.42, 112.29, 113.57, 117.16, 118.66, 118.78, 120.26, 129.07, 131.89, 134.19, 140.07, 147.29, 152.23, 169.96. Anal. Calc. for $C_{17}H_{20}ClNO_2$: C: 66.77 H: 6.59 N: 4.58, Found: C: 67.27 H: 7.16 N: 4.93.

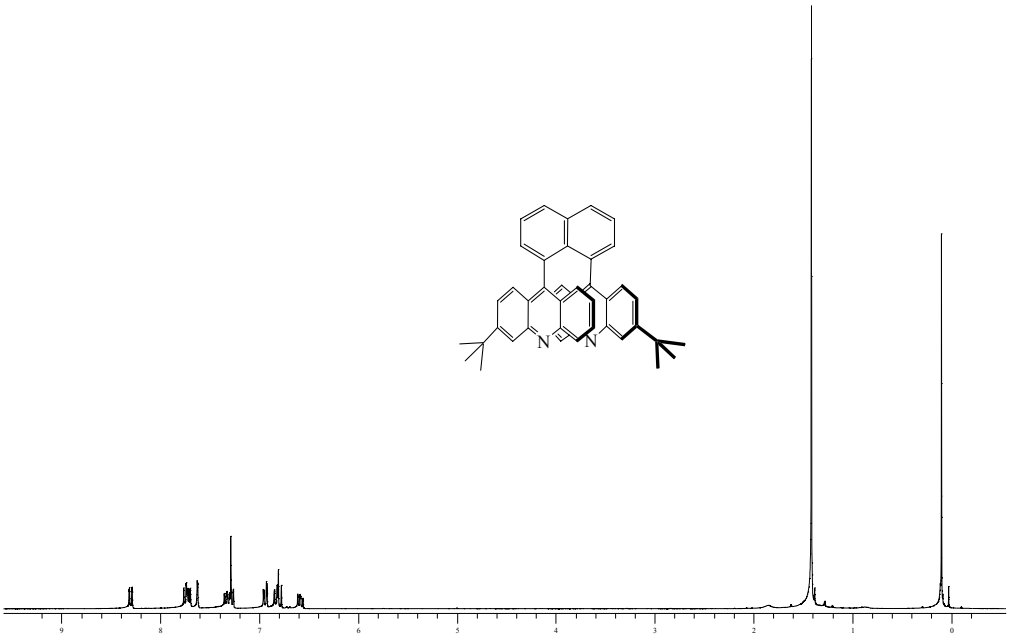
9-Bromo-3-*tert*-butylacridine (6).[3] To a flask containing 2-(3-*tert*-butylphenylamino)benzoic acid (3.78 g, 14 mmol) was added $POBr_3$ (24.9 g, 87 mmol). The solid mixture was heated to 120 ^oC and stirred for 2.5 hours. The reaction mixture was cooled to room temperature, poured on ice and extracted with dichloromethane. The combined organic layers were dried over anhydrous magnesium sulfate, filtered and concentrated to produce a crude oil, which was purified by column chromatography on basic aluminum oxide using hexanes/EtOAc/triethylamine (70:30:1) to yield 3.7 g (85%) of **6** as a yellow oil. ^1H NMR δ: 1.48 (s, 9H), 7.60 (ddd, J = 0.9 Hz, 6.6 Hz, 8.7 Hz, 1H), 7.74 (dd, J = 1.8 Hz, 9.0 Hz, 1H), 7.79 (ddd, J = 1.5 Hz, 6.6 Hz, 8.7 Hz, 1H), 8.13 (d, J = 2.1 Hz, 1H), 8.19 (m, 1H), 8.34 (d, J = 9.6 Hz, 1H), 8.39 (m, 1H). ^{13}C NMR δ: 31.43, 35.97, 124.90, 125.35, 126.64, 127.50, 127.64, 127.67, 128.11, 130.26, 131.02, 135.94, 149.66, 150.00, 154.51. EI-MS (70 eV): m/z = 313 (80, M^+); 298 (100, M^+ - Me); 270 (30, M^+ - Me - HCN); 234 (5, M^+ - Br). Anal. Calc. for $C_{17}H_{16}BrN$: C: 64.98 H: 5.13 N: 4.46, Found: C: 65.44 H: 5.18 N: 4.62.

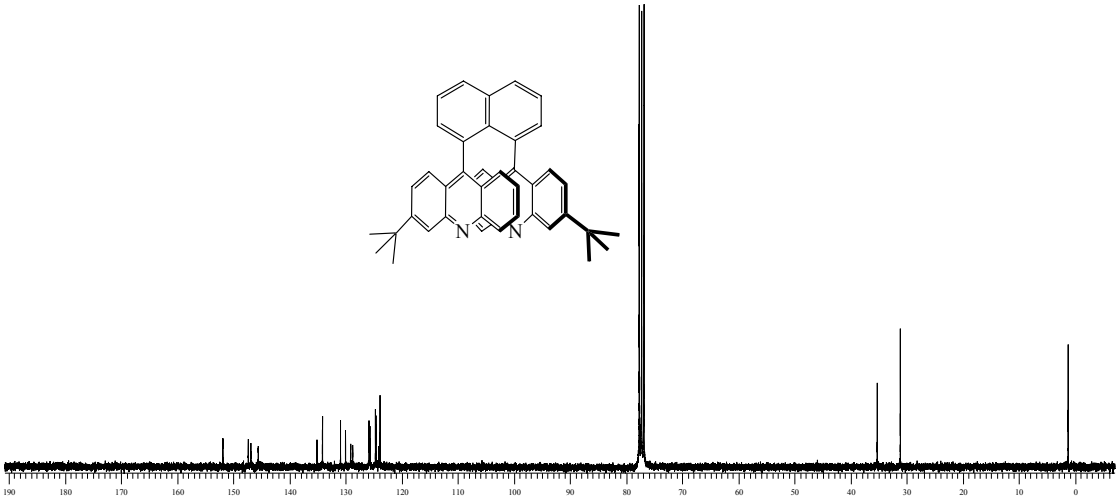
3-*tert*-Butyl-9-trimethylstannylacridine (7). To a flame-dried flask containing 9-bromo-3-*tert*-butylacridine (630 mg, 2.01 mmol) was added dropwise *n*-butyllithium (1.51 mL, 1.6 M in hexane) at -78 ^oC under nitrogen. After the lithium exchange was complete (~1 hour), a solution of trimethyltin chloride in THF (2.81 mL, 1.0 M) was added dropwise and the reaction mixture was allowed to warm to room temperature and stirred for another 5 hours. Then the reaction mixture was concentrated, dissolved in ethyl acetate and purified by column chromatography using hexanes/ethyl acetate/triethylamine (50:50:1) as the mobile phase to yield 726 mg (91%) of **7** as a colorless oil. The stannane is not very stable and was employed in the following step without any further purification. ^1H NMR δ: 0.67 (s, 9H), 1.47 (s, 9H), 7.50 (m, 1H), 7.65 (dd, J = 1.8 Hz, 9.0 Hz, 1H), 7.73 (dd, J = 7.2 Hz, 7.2 Hz, 1H), 8.08 (d, J = 10.5 Hz, 1H), 8.12 (dd, J = 0.6 Hz, 8.7 Hz, 1H), 8.17 (d, J = 2.1 Hz, 1H), 8.23 (dd, J = 0.6 Hz, 9.0 Hz, 1H). ^{13}C NMR δ: -4.02, 31.45, 35.86, 125.52, 125.58, 130.11, 130.22, 130.68, 130.88, 132.54, 133.85, 148.26, 148.45, 153.42.

1,8-bis(3'-*tert*-butyl-9'-acridyl)naphthalene (1). A mixture of 1,8-dibromonaphthlene (65 mg, 0.23 mmol), $Pd(PPh_3)_4$ (53 mg, 0.045 mmol) and CuO (36 mg, 0.45 mmol) was stirred at 140 ^oC in anhydrous DMF for 5 min. Then a solution of stannane **7** (360 mg, 0.90 mmol) in 2 mL DMF was added. After 16 h, the reaction mixture was cooled down to room temperature and dilute NH_3 was added. The mixture was extracted with dichloromethane and the combined organic layers were evaporated under vacuum. Purification by column chromatography with hexanes/ethyl acetate/triethylamine (60:40:1) afforded equimolar amounts of *syn*- and *anti*-**1** as pale yellow solids. (38 mg, 28%)

***Anti*-1**: ^1H (300 MHz, $CDCl_3$) δ = 1.32 (s, 18 H), 6.48 (ddd, *J* = 1.2 Hz, 6.6 Hz, 8.6 Hz, 2H), 6.68-6.75 (m, 4H), 6.83 (dd, *J* = 2.0 Hz, 9.0 Hz, 2H), 7.16-7.26 (m, 4H), 7.52 (d, *J* = 2.0 Hz, 2H), 7.60-7.66 (m, 4H), 8.20 (dd, *J* = 1.2 Hz, 8.6 Hz, 2H). ^{13}C (75 MHz, $CDCl_3$) δ = 29.7, 33.9, 122.4, 122.7, 123.2, 123.3, 124.2, 124.3, 124.4, 127.3, 127.7, 128.6, 129.5, 132.7, 133.7, 144.1, 145.5, 145.9, 150.4. Anal. Calcd for $C_{44}H_{38}N_2$: C, 88.85; H, 6.44; N, 4.71. Found: C, 88.49; H, 6.40; N, 4.73.

***Syn*-1**: ^1H (300 MHz, $CDCl_3$) δ = 1.28 (s, 18 H), 6.54-6.65 (m, 6H), 6.78 (dd, *J* =1.5 Hz, 9.3 Hz, 2H), 7.16 (d, *J* = 6.8 Hz, 2H), 7.32 (ddd, *J* = 1.2 Hz, 6.3 Hz, 8.3 Hz, 2H), 7.52 (d, *J* = 1.2 Hz, 2H), 7.59-7.66 (m, 4H), 8.19 (d, *J* = 8.3 Hz, 2H). ^{13}C (75 MHz, $CDCl_3$) δ = 29.8, 34.0, 122.3, 122.5, 123.1, 123.3, 123.7, 124.0, 124.2, 124.7, 127.6, 127.9, 128.5, 129.7, 133.0, 133.5, 133.6, 144.3, 145.8, 146.2, 150.6.

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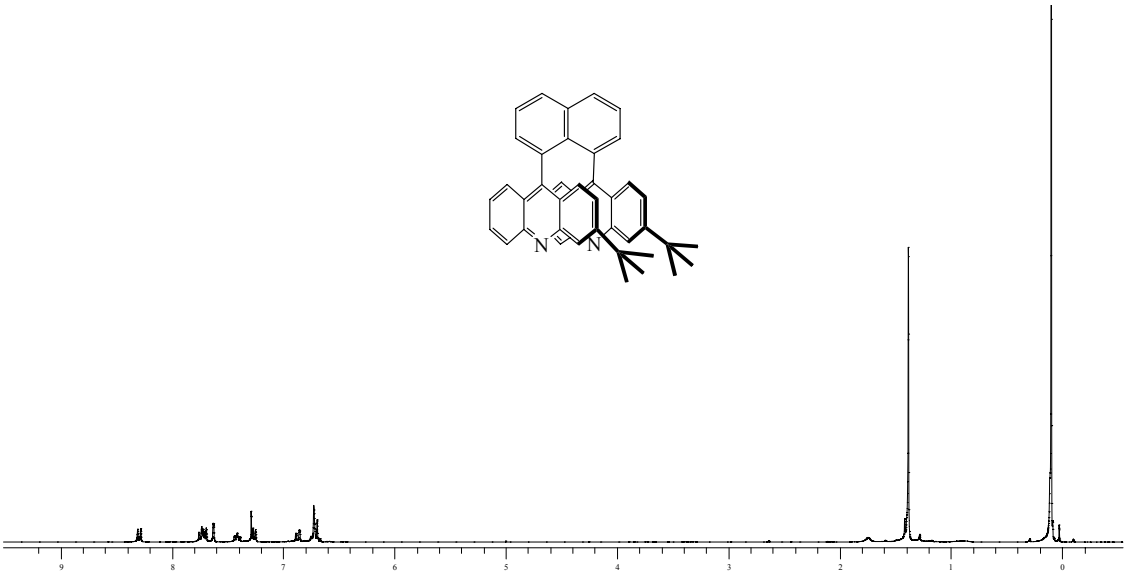
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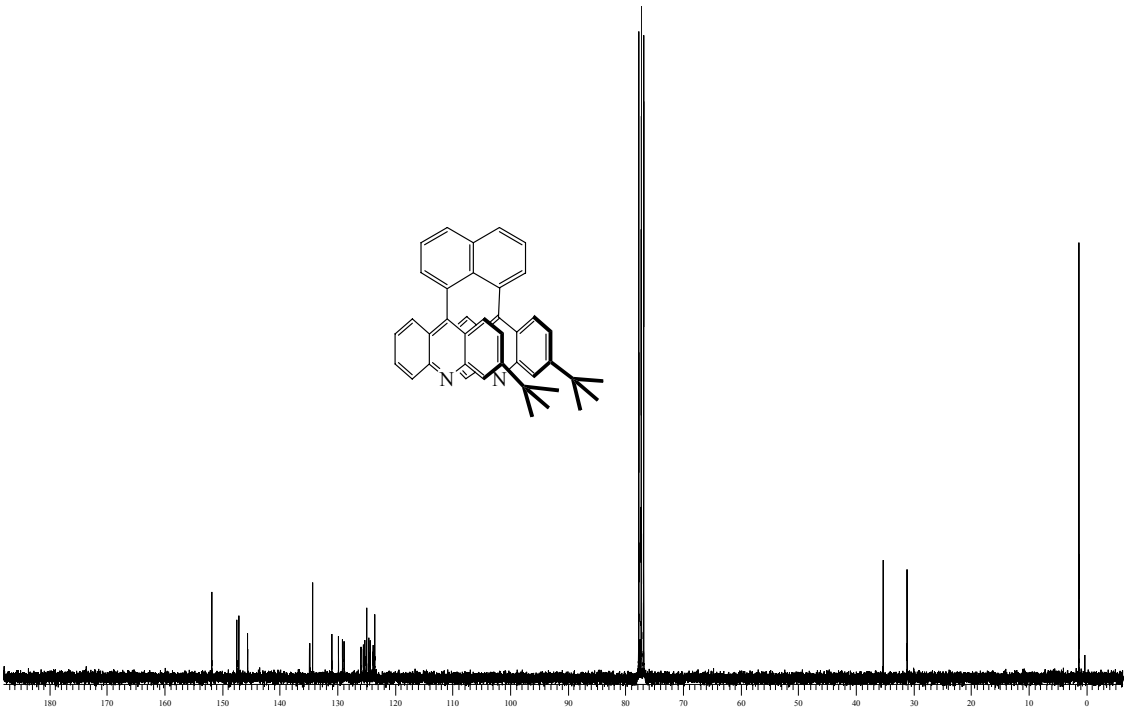


Figure 1.1. NMR spectra of the *syn*- and *anti*-diastereomers of 1,8-bis(3'-*tert*-butyl-9'-acridyl)naphthalene.

2. Single Crystal X-ray Analysis of 1

Single crystal X-ray diffractions of **1** were performed at -100 ^{o}C using a Siemens platform diffractometer with graphite monochromated Mo-Kα radiation (λ = 0.71073 Å). The structures were solved by direct methods and refined with full-matrix least-squares analysis using SHELX-97-2 software. Non-hydrogen atoms were refined with anisotropic displacement parameters and all hydrogen atoms were placed in calculated positions and refined with a riding model. Data were corrected for the affects of absorption using SADABS. Crystal data, collection parameters, refinement details, and key molecular parameters are shown in Table 2.1.

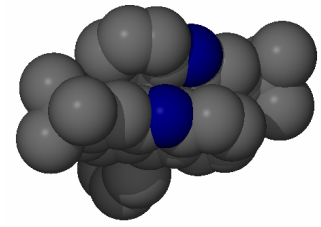


Figure 2.1. Space filling model of the crystal structure of **1**.

Table 2.1. Crystal data and structure refinement for **1**-*i*-PrOH.

Empirical formula	$C_{47}H_{43}N_2O$	
Formula weight	652.34	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	Triclinic	
Space group	P-1	
Unit cell dimensions	a = 10.7101(14) Å	α= 77.800(3)°.
	b = 11.3527(15) Å	β= 87.817(3)°.
	c = 15.434(2) Å	γ = 76.455(3)°.
Volume	1783.1(4) $Å^3$	
Z	2	
Density (calculated)	1.134 mg/m^3	
Absorption coefficient	0.070 mm^{-1}	
F(000)	648	
Crystal size	0.1 x 0.1 x 0.1 mm^3	
Theta range for data collection	1.35 to 28.45°.	
Index ranges	-14<=h<=14, -14<=k<=14, -20<=l<=20	
Reflections collected	16643	
Independent reflections	8379 [R(int) = 0.1305]	
Completeness to theta = 28.00°	96.0 %	
Absorption correction	Multi-scan	
Max. and min. transmission	0.9479 and 0.9233	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	8379 / 0 / 461	
Goodness-of-fit on F^2	0.907	
Final R indices [I>2sigma(I)]	R1 = 0.0828, wR2 = 0.1815	
R indices (all data)	R1 = 0.2799, wR2 = 0.2707	
Extinction coefficient	0.027(3)	
Largest diff. peak and hole	0.379 and -0.322 $e.Å^{-3}$	

3. UV and Fluorescence Measurements

All UV absorption and fluorescence emission spectra were collected under nitrogen using a 3.5 x 10^{-6} M solution of **1** in carefully degassed anhydrous acetonitrile under inert atmosphere. The quantum yield of **1** was determined as 0.11 using literature procedures and anthracene as the reference.[4]

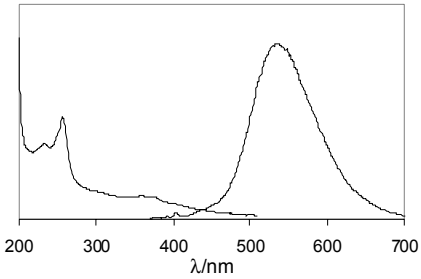
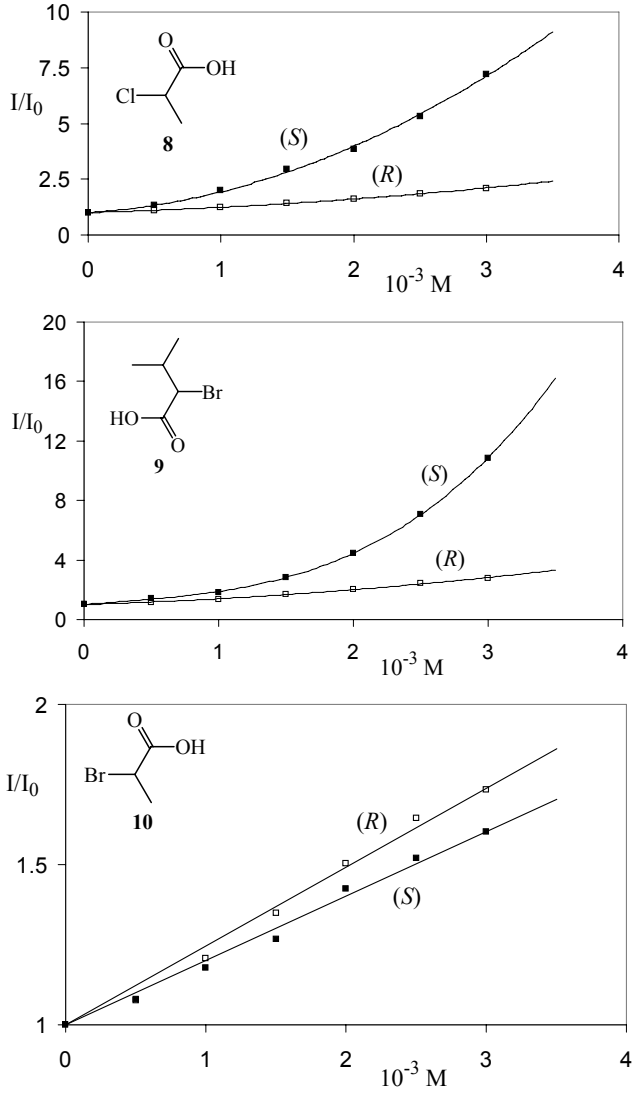
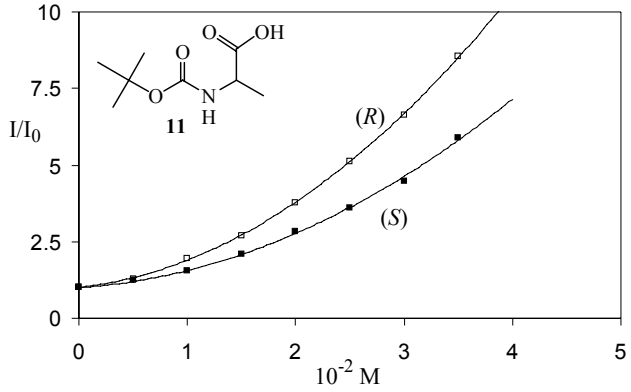


Figure 3.1. UV and fluorescence spectrum of **1** in acetonitrile. Excitation (emission) wavelength: 360 nm (535 nm).

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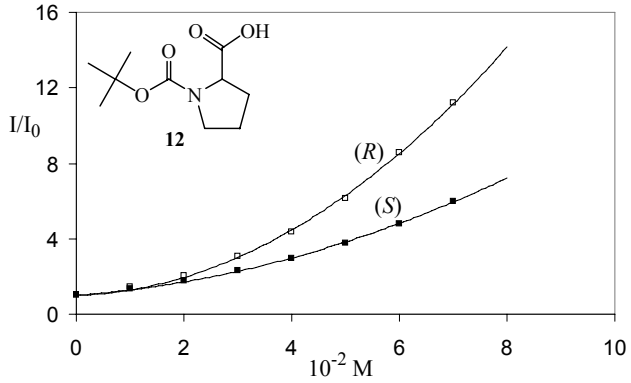
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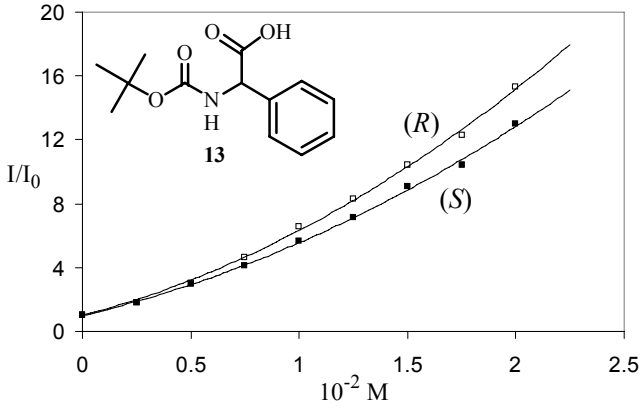
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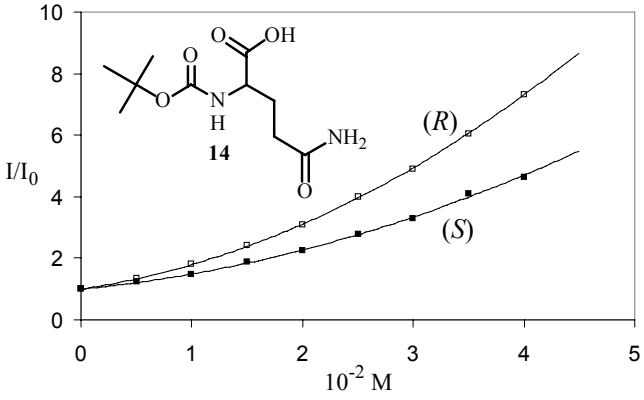
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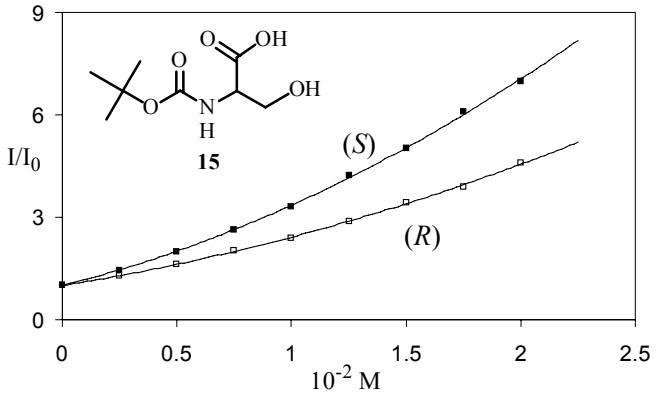
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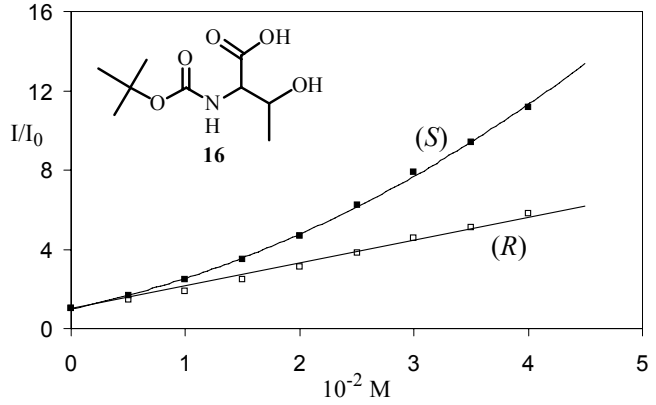
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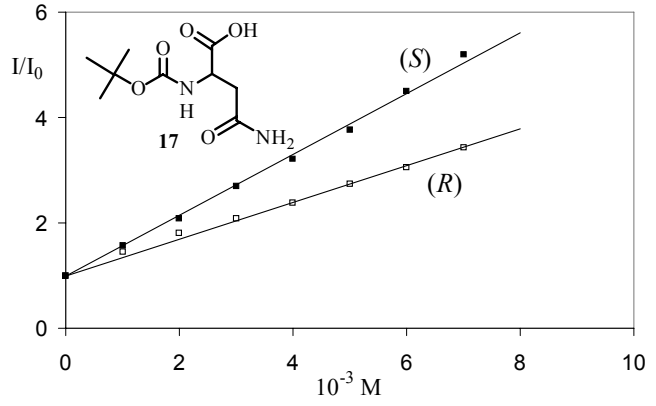
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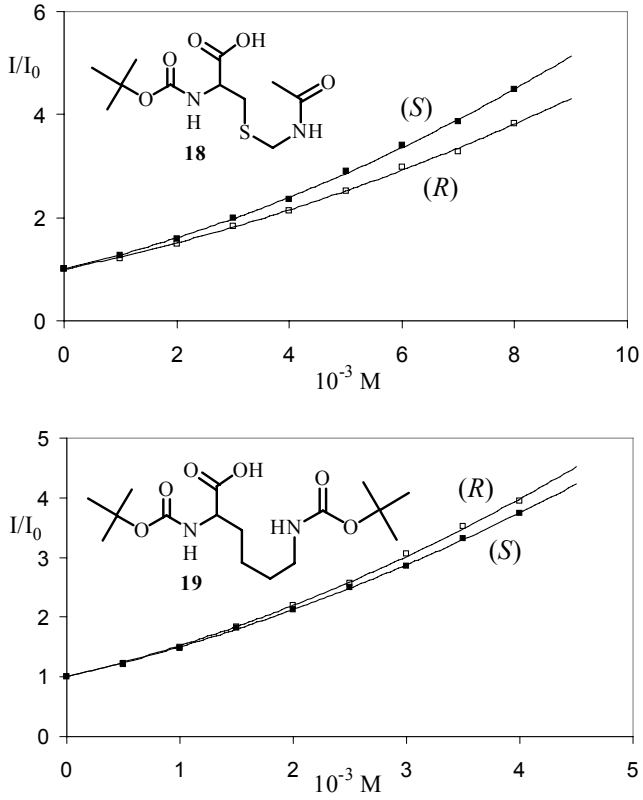

Figure 3.2. Stern-Volmer plots showing enantioselective fluorescence quenching of (-)-**1**. The concentration of the sensor was 3.5 x 10^{-6} M. Excitation (emission) wavelength: 360 nm (535 nm).

In order to verify that our observations are indeed a result of enantioselective recognition and not due to impurities that could have a strong quenching effect and be present in only one of the enantiomeric analytes, we determined the fluorescence response of (+)-**1** during titration with the enantiomers of **8**. As expected, the fluorescence of the dextrorotatory sensor was more effectively quenched by the (*R*)-enantiomer while the (*S*)-enantiomer showed little effect.

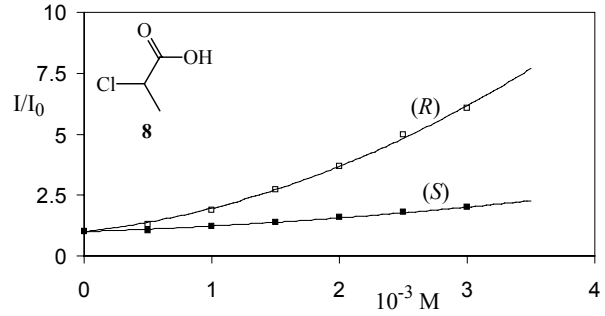

Figure 3.3. Stern-Volmer plot showing enantioselective fluorescence quenching of (+)-**1**. The concentration of the sensor was 3.5 x 10^{-6} M. Excitation (emission) wavelength: 360 nm (535 nm).

Because diacridylnaphthalene **1** has two potential binding sites, the quenching effects obtained with carboxylic acids **8** and **9** were analyzed using Benesi-Hildebrand equations derived for 1:2 complexation (equation 3.1.) in order to determine the association constants of the corresponding diastereomeric hydrogen adducts (Figure 3.4.):

$$\frac{I_0}{I\text{-}I_0} = \frac{b}{a\text{-}b}\left\{\frac{1}{K[\mathrm{M}]^2} + 1\right\} \quad (3.1.)$$

where I_0 is the inherent fluorescence intensity of (-)-**1**, I is the fluorescence intensity in the presence of an analyte, [M] is the analyte concentration, and K is the association constant, a and b are constants.

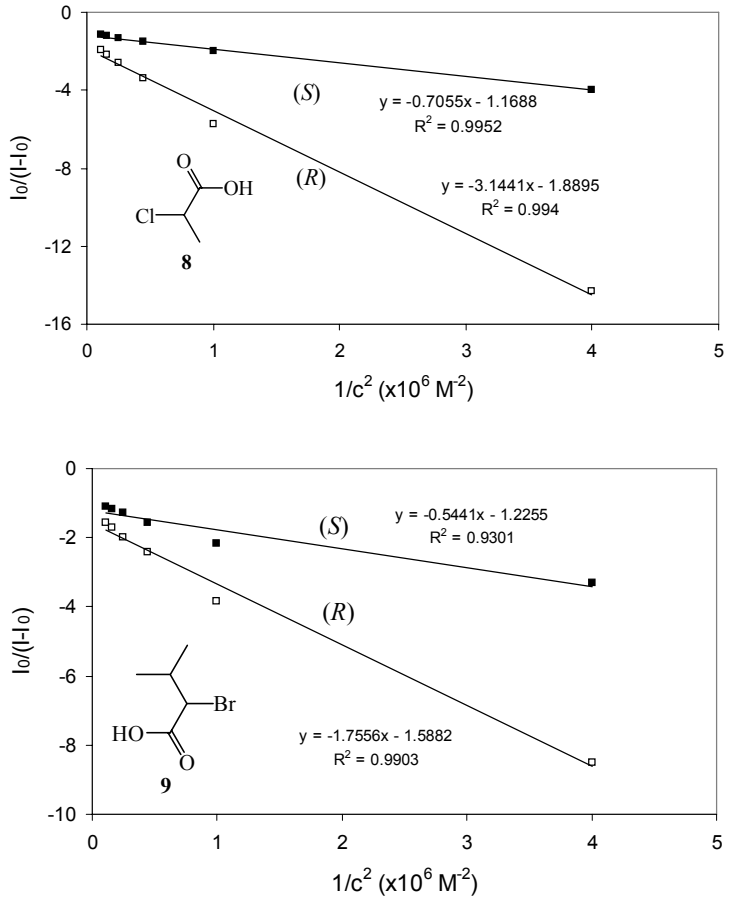

Figure 3.4. Benesi-Hildebrand plots obtained for the diastereomeric 1:2 adducts of (-)-**1**.

The association constants for the formation of (-)-**1**-[(*S*)-**8**]$_2$ and (-)-**1**-[(*R*)-**8**]$_2$ were determined as 1.7 x 10^6 M^{-2} and 6.9 x 10^5 M^{-2}, respectively. The association constants for the formation of (-)-**1**-[(*S*)-**9**]$_2$ and (-)-**1**-[(*R*)-**9**]$_2$ were calculated as 2.3 x 10^6 M^{-2} and 9.0 x 10^5 M^{-2}, respectively.

4. Quantitative Fluorescence Assays

Fluorescence titration of racemic **1** using either enantiomer of 2-chloropropionic acid **8** gave superimposable Stern-Volmer plots (Figure 4.1.). While the plots shown above prove enantioselective quenching of the enantiopure sensor, the plots in Figure 4.1. demonstrate non-stereoselective quenching when racemic **1** is used.

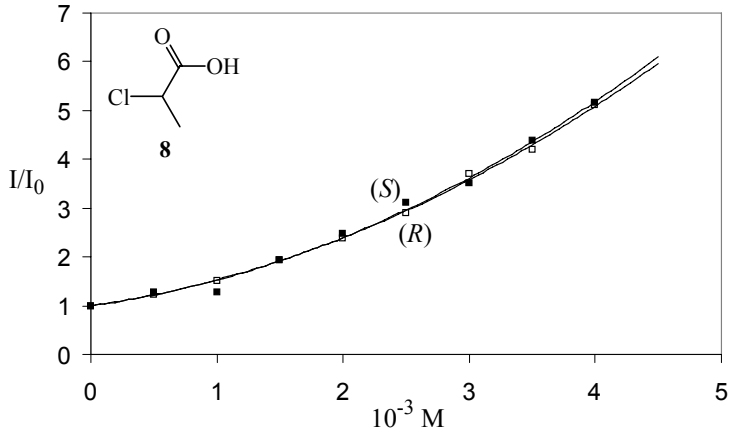

Figure 4.1. Fluorescence quenching of racemic **1** using either enantiopure (*R*)- or (*S*)-**8**. The concentration of the sensor was 3.5 x 10^{-6} M in ACN. Excitation (emission) wavelength: 360 nm (535 nm).

The non-stereoselective quenching observed with racemic **1** was then used to determine the amounts (concentrations) of different samples of chloropropionic acid **8** having concentrations of 1.5 x 10^{-3} and 3.0 x 10^{-3} M in acetonitrile, respectively, and different ee's (Table 4.1.). The analyte concentration was determined using the calibration curve shown in Figure 4.1. Each sample was measured three times. The fluoresecnce analysis of six samples gave very accurate and highly reproducible results within +/- 2% of the actual concentrations.

Table 4.1. Determination of the concentration of six samples of **8** using racemic fluorosensor **1**.

sample	actual conc. [mM]	actual (%) (*S*)	fluorescence intensity measured[a]	I_0/I	calculated conc. [mM]
A	1.50	5.0	145095	1.96999	1.47
B	1.50	55.0	144805	1.97393	1.48
C	1.50	95.0	142770	2.00207	1.51
D	3.00	15.0	81890	3.49048	2.97
E	3.00	55.0	80635	3.54480	3.02
F	3.00	85.0	79275	3.60561	3.06

[a]Average of three measurements at 535 nm.

Then, the enantiomeric excess of each sample was determined using a calibration curve obtained by fluorescence quenching of (-)-**1** (3.5 x 10^{-6} M in ACN) with chloropropionic acid **8** of varying ee (0 to 100%) at fixed concentrations of 1.5 x 10^{-3} and 3.0 x 10^{-3} M,

respectively (Figure 4.2.). The results are shown in Table 4.2. The fluoresecnce analysis of the six samples gave very accurate and highly reproducible results within +/- 3% of the actual percentage of (*S*)-**8**.

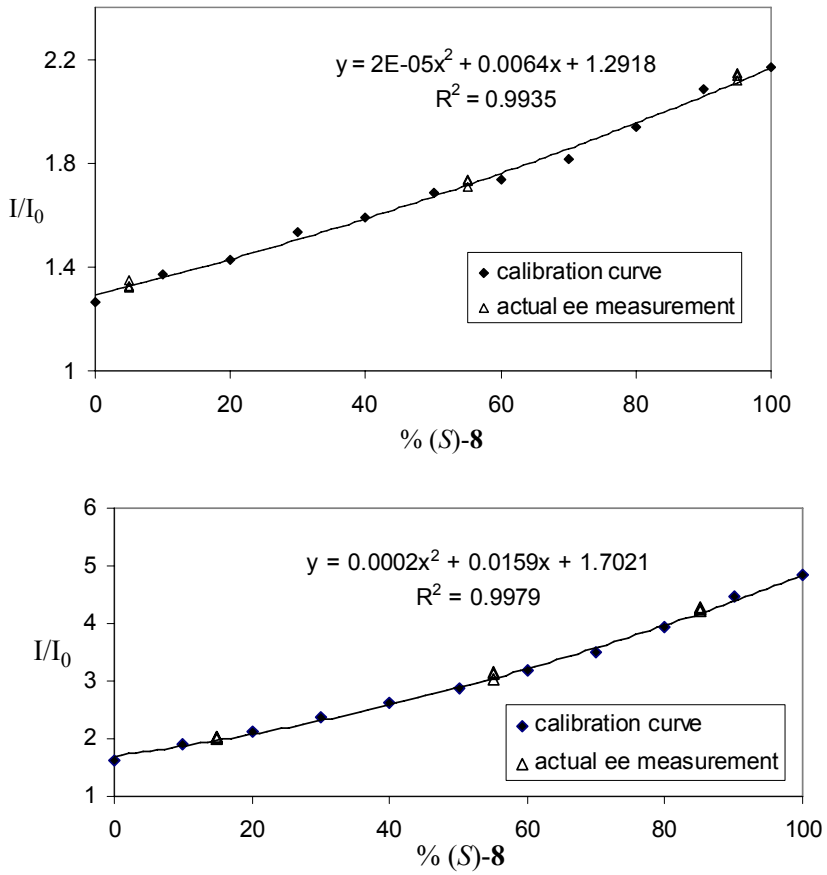

Figure 4.2. Enantioselective fluorescence quenching of (-)-**1** (3.5 x 10^{-6} M in ACN) as a function of the enantiomeric composition of **8**. The total concentration of **8** was 1.5 x 10^{-3} M (top) and 3.0 x 10^{-3} M (bottom) in ACN. Excitation (emission) wavelength: 360 nm (535 nm).

Table 4.2. Determination of the enantiomeric composition of six samples of **8** based on enantioselective fluorescence quenching of enantiopure (-)-**1**.

sample	actual conc. [mM]	actual (%) (*S*)	fluorescence intensity measured[a]	I_0/I	calculated (%) (*S*)
A	1.50	5.0	191823	1.33119	6.4
B	1.50	55.0	148020	1.72508	56.2
C	1.50	95.0	119620	2.13462	96.8
D	3.00	15.0	127717	2.02238	16.2
E	3.00	55.0	83280	3.10221	56.9
F	3.00	85.0	60698	4.25518	87.8

[a]Average of three measurements at 535 nm.

5. Chiral HPLC, Specific Rotation, and Circular Dichroism of 1

The enantiomers of **1** were separated on Chiralpak AD using hexane/EtOH (95:5) as the mobile phase.

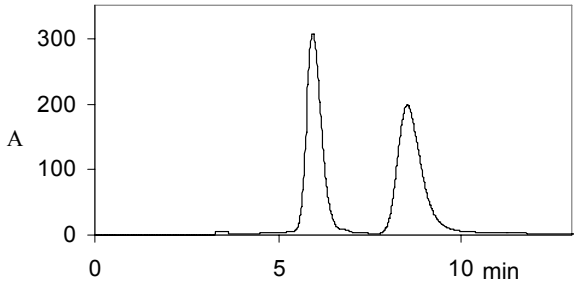

Figure 5.1. HPLC chromatogram using Chiralpak AD.
Diluent: hexanes/EtOH (75:25); mobile phase: hexanes/EtOH (95:5); flow rate = 1 mL/min; 25 ^{o}C; UV detection: 254 nm; injection volume: 50 µL; sample concentration: 1 mg/mL. Retention times: R_1 = 6.0 min, R_2 = 8.6 min.

Specific rotations of the enantiomers of diacridylnaphthalene **1** were determined by polarimetry as $[\alpha]^{589}_{25}$ = -276 (1st eluted enantiomer, c = 12.6 mg/100 ml in anhydrous ACN) and $[\alpha]^{589}_{25}$ = +288 (2nd eluted enantiomer, c = 12.0 mg/100 ml in anhydrous ACN).

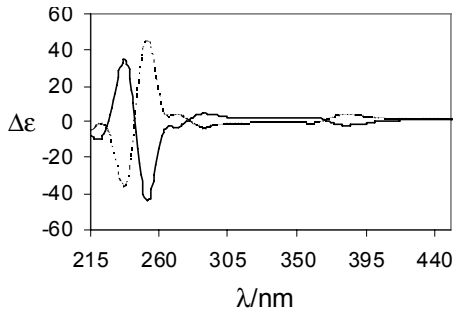

Figure 5.2. CD spectrum of the enantiomers of **1** (c = 7.0 x 10^{-5} mol/L) in acetonitrile.
Solid line: first eluent, (-)-**1**; dashed line: second eluent, (+)-**1**.

6. References and Notes

(1) This step affords di-3-*tert*-butylphenyldiazene which was confirmed by GC/MS.
(2) (a) Mei, X.; August, A. T.; Wolf, C. *J. Org. Chem.* **2006**, *71*, 142-149. (b) Wolf, C.; Liu, S.; Mei, X.; August, A. T.; Casimir, M. D. *J. Org. Chem.* **2006**, *71*, 3270-3273.
(3) Because of decomposition during chromatographic purification on silica gel, isolation of **6** requires flash chromatography on basic alumina.
(4) Jones II, G.; Jackson, W. R.; Choi, C.-Y. *J. Phys. Chem.* **1985**, *89*, 294-300.