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In Situ Evaluation of Kinetic Resolution Catalysts for Nitroaldol by Rationally Designed Fluorescence Probe

Takuya Matsumoto,[†,∥] Yasuteru Urano,[†] Yoshinori Takahashi,[‡,∥] Yusuke Mori,[‡,§,∥] Takuya Terai,[†,∥] and Tetsuo Nagano*[,†,∥]

[†]Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

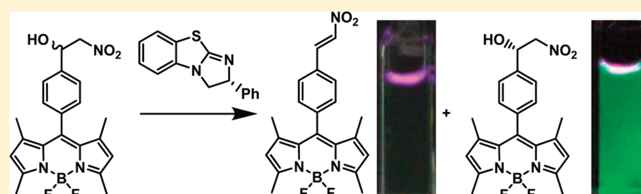
[‡]Graduate School of Engineering, Osaka University, Suita, Osaka 565-0871, Japan

[§]SOSHO, Inc., Osaka 541-0053, Japan

[∥]CREST, JST, Kawaguchi, Saitama 332-0012, Japan

S Supporting Information

ABSTRACT: Development of effective chemical catalysts is a key concern in organic chemistry. Therefore, convenient screening systems for chemical catalysts are required, and although some fluorescence-based HTS systems have been developed, little attempt has been made to apply them to asymmetric catalysts. Therefore, we tried to develop a chiral fluorescence probe which can evaluate the reactivity and enantioselectivity of asymmetric catalysts. We focused on kinetic resolution catalysts as a target of our novel fluorescence probe, employing β-elimination following acylation of nitroaldol. Once the hydroxyl group of nitroaldol is acylated, β-elimination occurs immediately, affording nitro olefin. Therefore, we designed and synthesized a fluorescence probe with an asymmetric nitroaldol moiety. Its fluorescence intensity decreases dramatically upon β-elimination, so the fluorescence decrease is an indicator of the reaction yield. Thus, the enantioselectivity of kinetic resolution catalysts can be assessed simply by measuring the fluorescence intensities of the reaction mixtures of the two enantiomers; it is not necessary to purify the product. This fluorescence probe revealed that benzotetramisole is a superior catalyst for kinetic resolution of nitroaldol. Furthermore, we established an HTS system for asymmetric catalysts, using a fluorescence probe and benzotetramisole. To our knowledge, this is the first fluorescence-based HTS system for asymmetric catalysts.



INTRODUCTION

Fluorescence probes are powerful tools in many fields of chemistry and biology, and have recently been introduced for high-throughput screening (HTS) of chemical catalysts. The creation of effective chemical catalysts is a central topic in organic chemistry, and although some have been designed on the basis of reaction mechanism, most have been developed through trial and error. Nowadays, combinatorial chemistry is used to obtain large libraries of candidate catalysts, but the yield and enantiomeric excess (ee) of the products are still determined by using high-performance liquid chromatography (HPLC) and gas chromatography (GC), which represent a bottleneck due to the relatively long analysis time. Optimizing the reaction conditions, i.e., solvent, concentration, temperature, time, etc., is also time-consuming. Therefore, a convenient HTS system for evaluation of the activities and enantioselectivities of many candidate catalysts in parallel would be very useful.[1−3]

HTS is already indispensable for discovering candidate drugs and for optimizing lead structures in pharmaceutical chemistry. Fluorescence detection is most commonly used in HTS systems, because its sensitivity is especially high, and in the case of screening for catalysts, it can be used to quantify the chemical yields in situ without purification of products. Several groups have attempted to develop HTS for chemical catalysts by means of MS,[4,5] NMR,[6] CD,[7−10] thermography,[11] fluorescence,[12−29] and so on. Among these techniques, fluorescence-based HTS seems the most useful, because fluorescence is easily measured, even if the solution contains impurities, and purification is unnecessary. But, most conventional fluorescence HTS systems focus on determining not enantioselectivity, but reaction yield. Therefore, to develop fluorescence-based HTS system for asymmetric catalysts is both important and challenging. For this reason, we tried to develop a novel chiral fluorescence probe whose fluorescence is controlled by asymmetric chemical reaction.

As a target of HTS we focused on the Henry reaction, which is a fundamental reaction for carbon−carbon bond formation and yields nitroaldol. Nitroaldol is a very important compound in pharmaceutical and synthetic chemistry because it is easily transformed into amino alcohol, nitro ketone, nitro olefin, and so on.[30−37] Nitroaldol often contains an asymmetric carbon, so we tried to develop an HTS system for kinetic resolution catalysts of nitroaldol. Here we report a rationally designed chiral

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fluorescence probe suitable for an HTS system aimed at kinetic resolution catalysts of nitroaldol, using BODIPY as a fluorophore.

RESULTS AND DISCUSSION

Development of HTS System for Kinetic Resolution Catalysts. BODIPY has been used not only as a fluorescent tag, but also as a core of fluorescence probes, because of its high fluorescence quantum yield with excitation in the visible range, and its relatively strong resistance to photobleaching. Furthermore, the fluorescence is not influenced by solvents and is relatively stable against chemical reaction.[38−40] These features are all favorable for application to HTS of chemical catalysts. Our group has designed and developed many BODIPY fluorescence probes based on photoinduced electron transfer (PET).[41−44] PET is a comprehensively reported mechanism for excited-state quenching, in which an electron is transferred from an electron donor moiety to an electron acceptor moiety (Figure 1a). If the electron acceptor has a low LUMO energy level, PET occurs and the fluorescence of the electron donor is quenched (Figure 1c).[45,46] A fluorescence probe controlled by PET would have the advantage that structural change at a moiety other than the fluorophore can lead to a fluorescence increase, and rational molecular design would allow us to maximize the fluorescence change caused by the reaction. We therefore employed BODIPY and PET to create a novel fluorescence probe which is strongly fluorescent before reaction (Figure 1b), but almost nonfluorescent (Figure 1c) after β-elimination, as a tool for an HTS system to find kinetic resolution catalysts of nitroaldol.

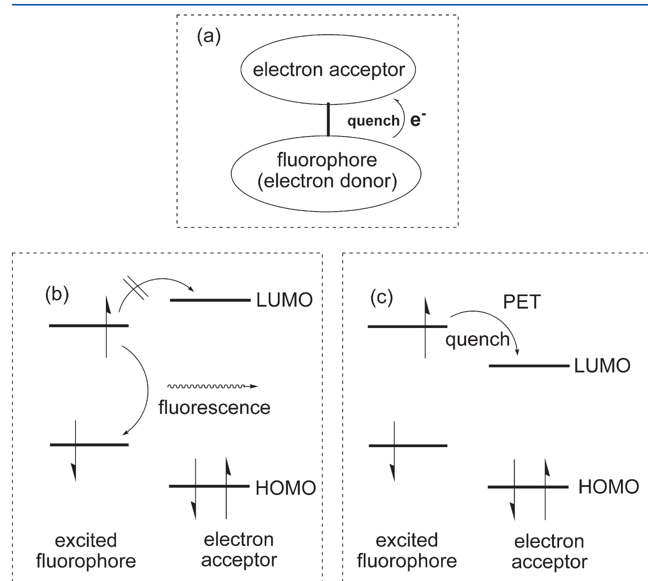


Figure 1. Schematic representation of PET strategy to control fluorescence: (a) switch "off" model in which PET occurs, resulting no fluorescence; (b), (c) schematic representation of relationship between LUMO energy level and PET; (b) when the electron acceptor has a high LUMO energy level, PET does not occur and fluorescence is not quenched; (c) when the electron acceptor has a low LUMO energy level, PET occurs and fluorescence is quenched.

As shown in Scheme 1, once the hydroxyl group of a nitroaldol is acylated, β-elimination occurs immediately, and nitro olefin is produced. Nitro olefin is an electron acceptor for the PET process, and quenches the fluorescence. If β-elimination occurs, we thought that the progress of the acylation could be evaluated simply by measuring the fluorescence decrease of the reaction mixture. The difference between the changes of fluorescence intensities of the two enantiomers reflects the enantioselectivity of a kinetic resolution catalyst.

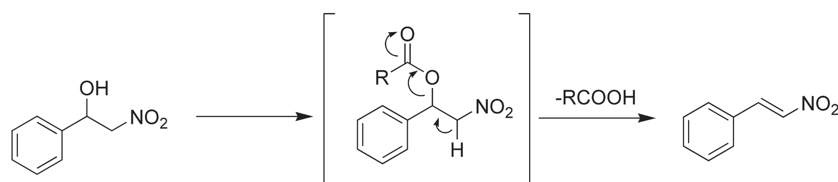
To test our hypothesis, these compounds were synthesized and their fluorescence properties were measured. Synthesis was performed according to Scheme 2. Nitroaldol BODIPY (**6**) was synthesized in 6 steps. When the hydroxyl group of **6** was acylated, nitro olefin BODIPY (**8**) was produced immediately, as we had expected. Next, the fluorescence spectra and properties of **6** and **8** were examined. The fluorescence and absorbance spectra are illustrated in Figure 2. As shown in Figure 2, the absorbance spectrum was not changed after elimination, but the fluorescence was dramatically decreased. Thus, nitro olefin appeared to act as an electron acceptor for PET, and the fluorescence of BODIPY was quenched. The decrease of fluorescence quantum yield (Φ_{fl}) also indicated that nitro olefin could quench the fluorescence of BODIPY; Φ_{fl} of **8** is extremely low (see Supporting Information).

The fluorescence change upon elimination was thus as expected, and **6** was found to be a superior fluorescence probe, which was expected to be suitable for use in an HTS system for kinetic resolution catalysts.

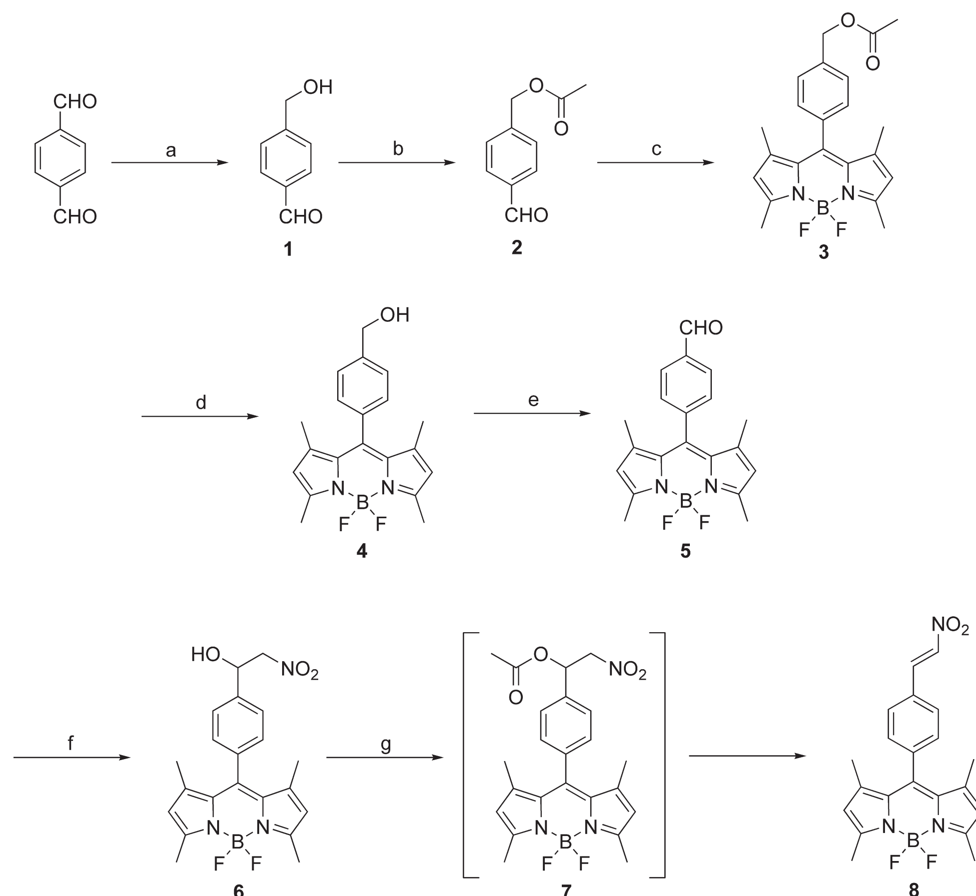
The strategy for an HTS system for kinetic resolution catalysts is illustrated in Figure 3. For example, if a candidate compound could catalyze acylation of (R)-**6** but could not catalyze acylation of (S)-**6**, the fluorescence intensity due to (R)-**6** would decrease but that due to (S)-**6** would not. So, the difference of fluorescence intensity reflects the enantioselectivity of the catalyst.

Before developing an HTS system for kinetic resolution catalysts, we performed optical resolution of racemic **6** by means of HPLC with a CHIRALPAK IA (DAICEL) column and 2-propanol/n-hexane = 1/9 as the eluent (see the Supporting Information). The separated enantiomers of **6** were iodinated according to Scheme 3 because a heavy atom was needed for accurate assignment of the absolute configuration. Iodinated (R)-**9** was recrystallized from CH_2Cl_2/n-hexane under stirring with a rotary shaker[47,48] at the rotation rate of 50 rpm for X-ray crystallography. The ORTEP plot of (R)-**9** is shown in the Supporting Information.

Scheme 1. Acylation and Elimination of Nitroaldol



Scheme 2. Synthetic Scheme of Nitroaldol BODIPY(6) and Nitro Olefin BODIPY(8)[a]



[a] Reagents: (a) $NaBH_4$, THF, 25%; (b) acetic anhydride, pyridine, 67%; (c) (1) 2,4-dimethylpyrrole, TFA, CH_2Cl_2, (2) DDQ, (3) DIEA, $BF_3 \cdot Et_2O$, toluene, 26%; (d) NaOH aq, MeOH/CH_2Cl_2, quant; (e) PCC, CH_2Cl_2, 68%; (f) CH_3NO_2, NaOH aq, MeOH, 93%; (g) Ac_2O, pyridine, CH_2Cl_2, 86%.

With our novel HTS tool for kinetic resolution catalysts, we tested the enantioselectivity of some commercially available catalysts which have been used for acylation of chiral secondary alcohols. Ferrocene derivative[49] ((*R*)-**10**), histidine derivative[50] ((*S*)-**11**), and benzotetramisole[51−53] ((*R*)-**12**) were used as catalysts, and propionic anhydride, butyric anhydride, and isobutyric anhydride were used as acylating reagents. Acylation was performed in DMF, MeCN, or toluene at room temperature for 1 h, and a small portion of each reaction mixture was sampled and diluted 100 times with DMSO to 10 μM for fluorescence measurement. In the fluorescence assay, the fluorescence intensity cannot be measured at too high a dye concentration because self-quenching of the dye occurs; in the present case, 10 μM was appropriate, affording a linear calibration curve. The reaction yield in each case was obtained from the calibration curve by interpolation of the observed fluorescence intensity (see the Experimental Section and Supporting Information). The substrate **6** is strongly fluorescent, whereas product **8** is not fluorescent, so high fluorescence intensity corresponds to low reaction yield and low fluorescence intensity corresponds to high reaction yield.

As shown in Figure 4, when (*R*)-**12** was used as a catalyst in toluene, isobutyric anhydride was the best acylating reagent, and propionic anhydride and butyric anhydride gave lower enantioselectivity. Enantioselectivity of benzotetramisole is due to $\pi-\pi$ interaction between acylated benzotetramisole and substitute alcohol.[54] Thus, the fact that the bulky acylating reagent has good enantioselectivity is reasonable. To confirm the validity of our HTS system, we evaluated the results in these cases by HPLC analysis. For HPLC analysis, racemic **6** was treated with (*R*)-**12** and each acid anhydride in toluene, and after the reaction, the yield and % ee were determined by means of HPLC in a chiral column. The selectivity was estimated as follows.[55,56]

$$s = \frac{k_{\text{fast}}}{k_{\text{slow}}} = \frac{\ln[(1-c)(1-\text{ee})]}{\ln[(1-c)(1+\text{ee})]}$$

Calculated selectivity values are summarized in Table 1. As in the case of fluorescence HTS, isobutyric anhydride had the best selectivity value among the three acid anhydrides. Because this HPLC assay and our fluorescence assay provided the same result, our fluorescence HTS system appears to be suitable to evaluate the activity and enantioselectivity of kinetic resolution catalysts.

Moreover, a major advantage of the fluorescence probe is that this system can monitor the chemical reaction in situ, so we next examined real-time reaction monitoring with our probe. As shown in Figure 5, the reaction was performed in a fluorescence spectrometer and the fluorescence intensity of the reaction mixture was measured every 0.5 s. (*R*)- or (*S*)-**6** was dehydrated by propionic anhydride, butyric anhydride, or isobutyric anhydride in toluene in the presence of (*R*)-**12** as a catalyst. When propionic anhydride was used, the reaction was the fastest, but the enantioselectivity was low. When isobutyric anhydride was

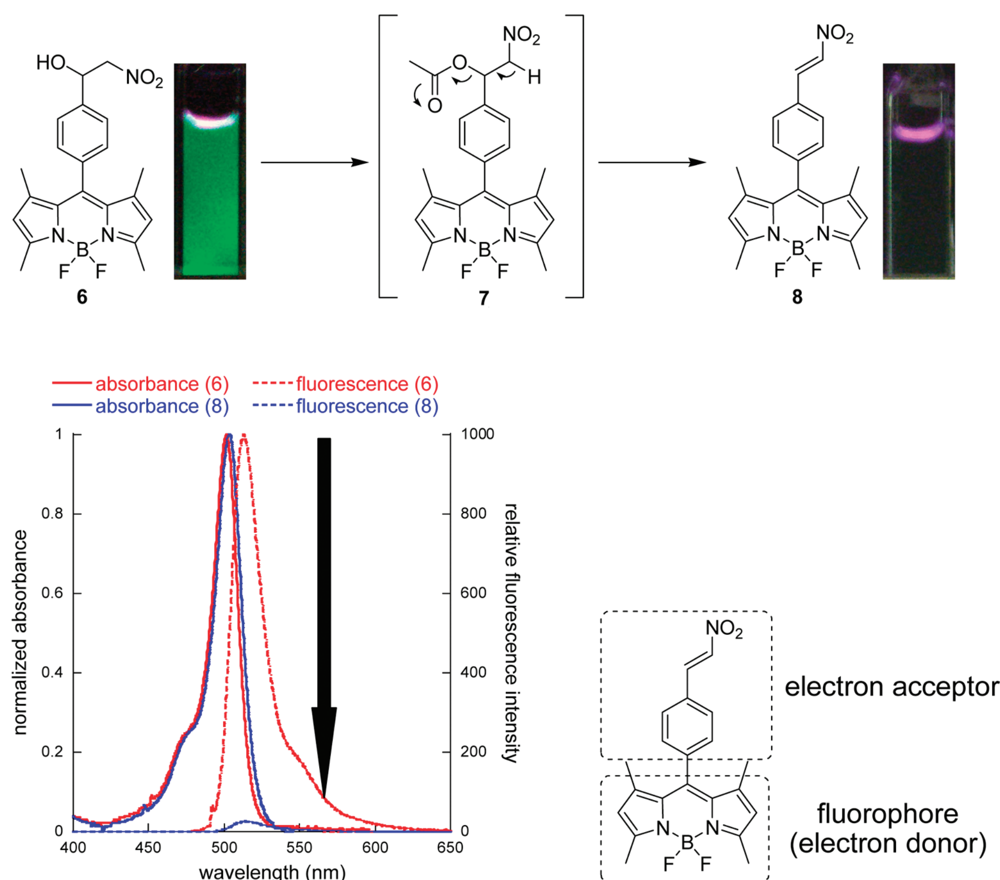


Figure 2. Fluorescence and absorbance spectra of **6** and **8**. The spectra of 1 μM dyes were measured in DMSO.

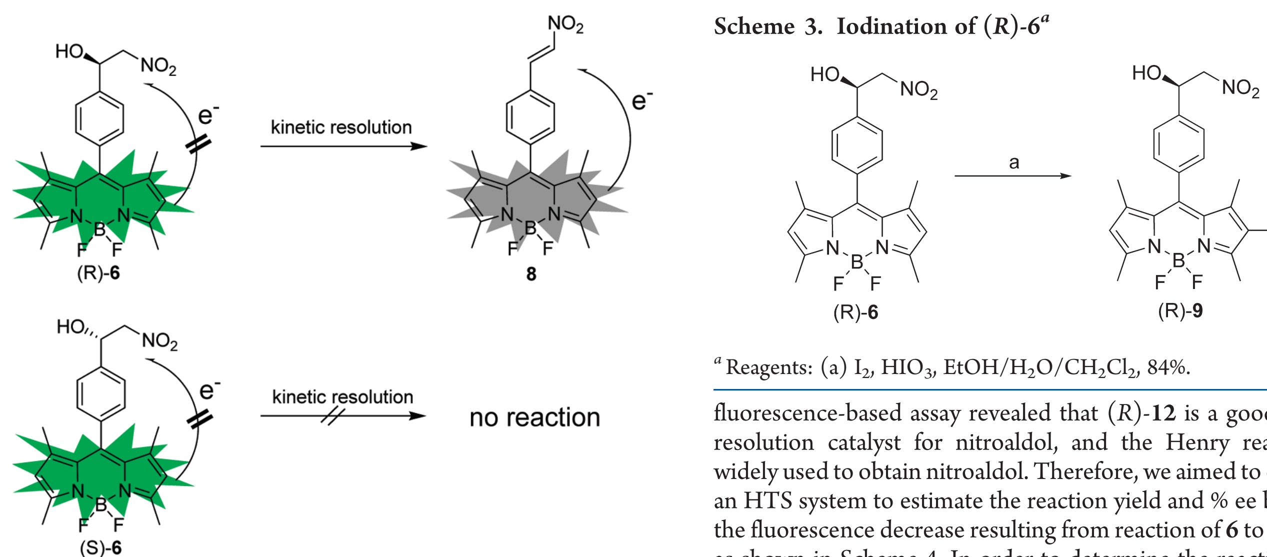
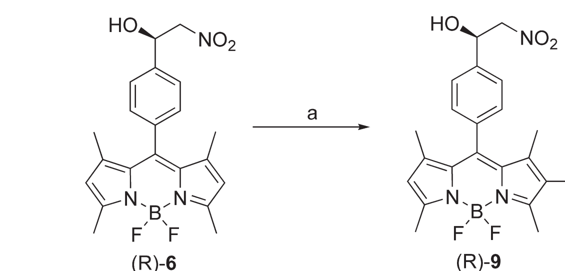


Figure 3. Schematic illustration of strategy of HTS system for kinetic resolution catalysts.

used, the reaction was the slowest, while enantioselectivity was high. This tendency is the same as in Table 1, confirming the usefulness of our fluorescence probe for kinetic study (see the Supporting Information).

HTS System for % ee Determination. Next, we tried to establish an HTS system to evaluate the reactivity and enantioselectivity of asymmetric catalysts for the Henry reaction. Our fluorescence-based assay revealed that (*R*)-**12** is a good kinetic resolution catalyst for nitroaldol, and the Henry reaction is widely used to obtain nitroaldol. Therefore, we aimed to establish an HTS system to estimate the reaction yield and % ee based on the fluorescence decrease resulting from reaction of **6** to afford **8**, as shown in Scheme 4. In order to determine the reactivity and enantioselectivity of a catalyst that catalyzes the first Henry reaction step, we need to measure the amounts of (*R*)-**6** and (*S*)-**6**. We thought that these amounts could be obtained from the fluorescence decrease in the second % ee determination step, similar to the enzymatic method for determining enantiomeric excess (EMDee).

Scheme 3. Iodination of (*R*)-6[a]



[a] Reagents: (a) I_2, HIO_3, $EtOH/H_2O/CH_2Cl_2$, 84%.

EMDee is well-known as one way to determine the % ee of a chiral compound,[57−60] based on the enantioselectivity of enzymatic kinetic resolution and a probe molecule to evaluate the activity. Because the reactivity of the enzyme is affected by the

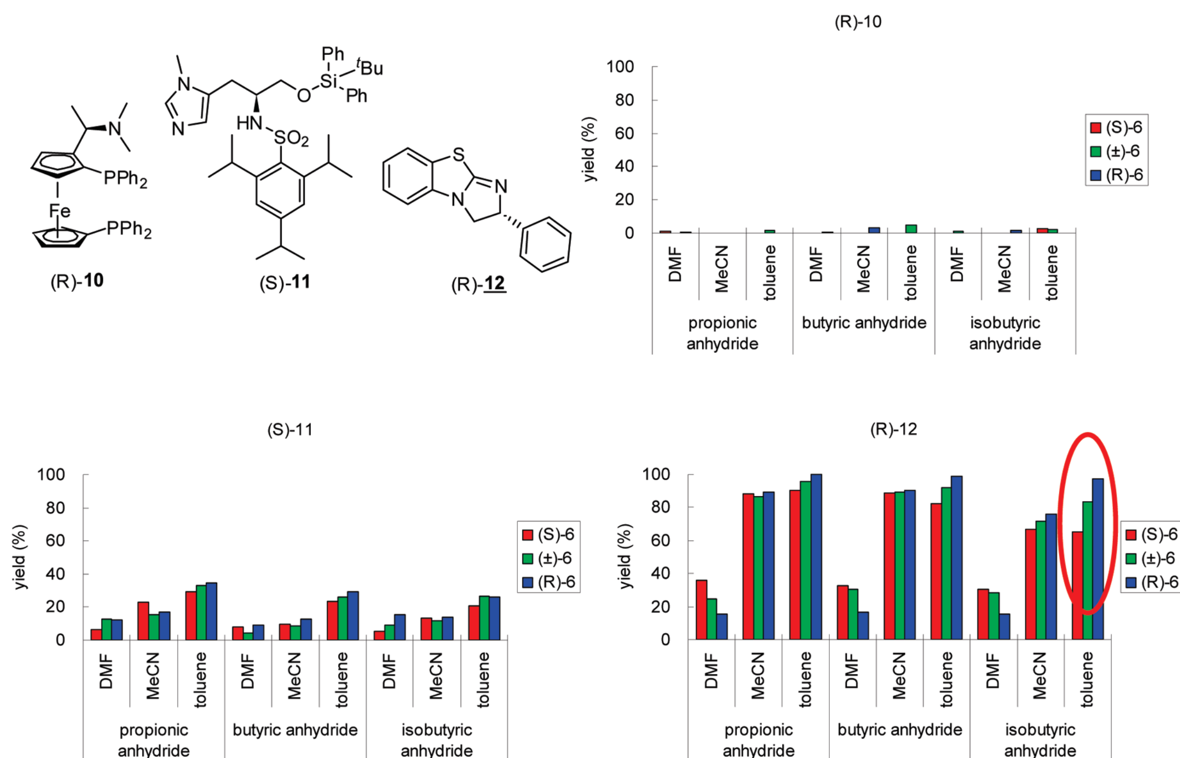


Figure 4. HTS of candidate kinetic resolution catalysts: 1 mM **6** was acylated with 5 mM acid anhydride in the presence of 5 mM catalyst in 300 μL of various solvents. Reaction mixtures were diluted 100 times with DMSO for measurement of fluorescence.

Table 1. Enantioselectivity of (*R*)-12 with Various Acid Anhydrides in Toluene[a]

entry	acid anhydride	yield[b] (%)	ee[b] (%)	selectivity	average
		44.2	63.1	16.8	
1	propionic anhydride	46.0	72.1	25.9	22.3
		46.3	72.1	24.3	
		35.4	49.8	34.6	
2	butyric anhydride	35.5	49.9	33.8	38.5
		34.2	48.5	47.0	
		31.7	44.1	59.2	
3	isobutyric anhydride	31.7	45.0	105.6	80.3
		31.8	44.7	76.2	

[a] 1 mM racemic **6**, 0.5 mM acid anhydride and 0.5 mM (R)-**12** were reacted in 1 mL of toluene at room temperature for 90 min. [b] Yield and ee were determined by HPLC from the peak area with absorbance detection at 500 nm.

chirality of the substrate, % ee of the substrate can be estimated from enzyme activity measured with the probe molecule. However, EMDee can estimate the amount of only one enantiomer; this is an essential limitation of enzymatic kinetic resolution, so a nonenzymatic kinetic resolution is desirable.[61]

Since we found that (*R*)-**12** with isobutyric anhydride in toluene is a superior kinetic resolution condition for nitroaldol among the conditions tested in Figure 4, it should be possible to establish an HTS system for % ee determination with (*R*)-**12** and (*S*)-**12**. (*R*)-**12** is commercially available, but (*S*)-**12** is not, so we synthesized (*S*)-**12** according to a conventional method employed to obtain (*R*)-**12** (see the Supporting Information).[62] We examined whether or not the fluorescence intensity of the reaction mixture would reflect the % ee. Compound **6** with various values of % ee was prepared by mixing solutions of (*R*)-**6** and (*S*)-**6**, followed by treatment with (*R*)- or (*S*)-**12** and isobutyric anhydride in toluene, and the fluorescence intensity of the reaction mixture was measured. The relation between fluorescence intensity and % ee is illustrated in Figure 6 for (*R*)-**12** and Figure 7 for (*S*)-**12**. (*R*)-**12** with isobutyric anhydride in toluene readily acylates (*R*)-**6** to produce nonfluorescent **8**, while it hardly acylates (*S*)-**6**, so the fluorescence intensity of the reaction mixture indicates the concentration of (*S*)-**6** which has not reacted. On the other hand, when (*S*)-**12** is used as the catalyst, the fluorescence intensity of the reaction mixture indicates the concentration of (*R*)-**6** which has not reacted. Namely, the fluorescence decrease at the second step for (*R*)- and (*S*)-**12** was measured and the sum of the fluorescence decreases in both reaction mixtures reflects the reaction yield of the first step, whereas the difference of fluorescence decrease between the two reaction mixtures reflects the % ee.

In both Figures 6 and 7, the fluorescence measurement error at higher fluorescence intensities is greater than that at lower fluorescence intensities. The reason for this is that at lower fluorescence intensities, the reaction is almost complete and the product **8** is predominant, so that the fluorescence intensity is stable. On the other hand, at higher fluorescence intensities, the substrate **6** is still predominant, the reaction is progressing, and so the fluorescence intensity is changing.

In this system, the solvent, catalyst, and nitromethane used in the first step would be present as impurities in the second step. Therefore, we confirmed that % ee determination at the second step is not affected by such impurities. As a model first-step reaction, we used 5 mM **5**, 100 equiv of nitromethane, 0.1 equiv of TEA as a catalyst, and DMF as a solvent, and the reaction

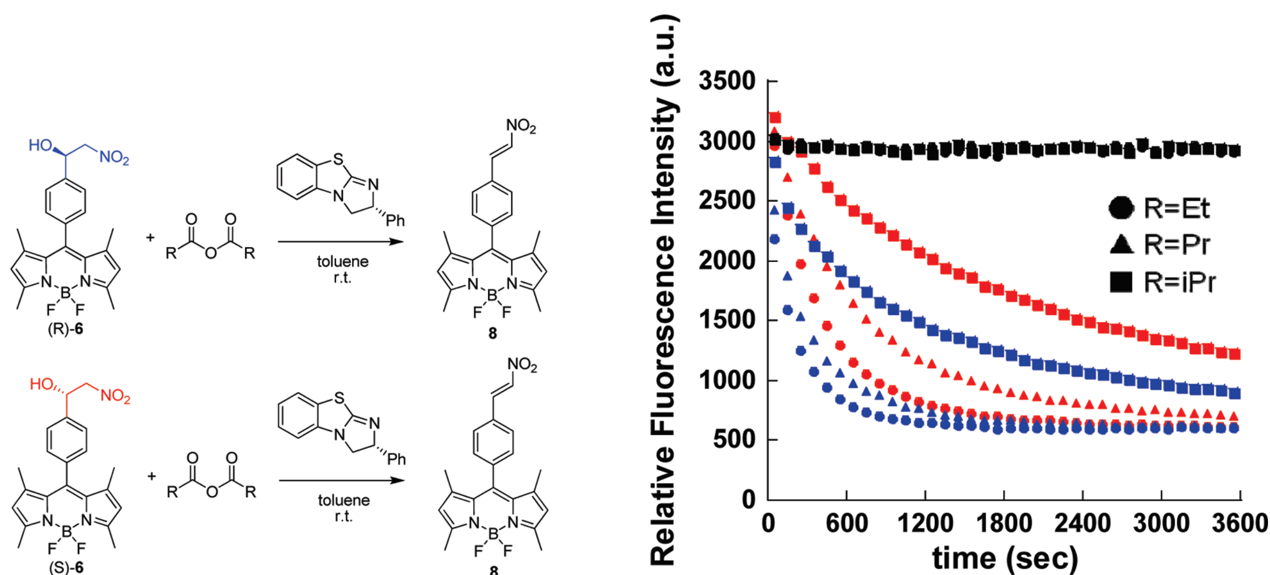
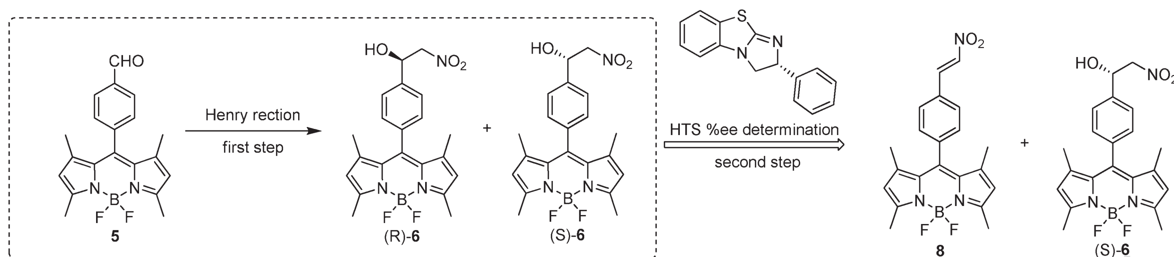


Figure 5. In situ reaction monitoring: 10 μM of (*R*)- or (*S*)-**6** was acylated with 20 mM acid anhydride in the presence of 20 mM (*R*)-**12** in 3 mL of toluene. The blue line shows the relative fluorescence intensity of (*R*)-**6**, the red line shows the relative fluorescence intensity of (*S*)-**6**, and the black line shows the relative fluorescence intensity of control (no catalyst). Propionic anhydride (●), butyric anhydride (▲), and isobutyric anhydride (■) were used. The reaction was performed in a fluorescence spectrometer, and fluorescence intensity was measured every 0.5 s (Ex 500 nm, Em 515 nm).

Scheme 4. HTS Procedure for Asymmetric Catalyst of Henry Reaction



mixture of the first step was diluted 10-fold with toluene solution containing (*R*)-**12** and isobutyric anhydride. Thus, compound **6** with various % ee values was treated with (*R*)-**12** and isobutyric anhydride in toluene containing DMF, nitromethane, and TEA as impurities. After the reaction, the fluorescence intensity of the reaction mixture was measured (Figure 8). Similarly to the case of Figure 6, the fluorescence intensity was well correlated with % ee. These results confirmed that our HTS system can evaluate yield and % ee based on measurement of fluorescence.

CONCLUSION

We have developed an HTS system for kinetic resolution catalysts of nitroaldol, employing a rationally designed fluorescence probe whose fluorescence is controlled by PET and changes upon reaction. With this HTS system, both reactivity and enantioselectivity could be evaluated simply by measuring the fluorescence intensity. Our novel HTS system revealed that (*R*)-**12** with isobutyric anhydride in toluene is a superior kinetic resolution condition. (*R*)-**12** with isobutyric anhydride in toluene acylated the hydroxyl group of (*R*)-**6** 80 times faster than that of (*S*)-**6**. Furthermore, we established an HTS system for asymmetric catalysts of the Henry reaction, employing (*R*)- or (*S*)-**12**. Such HTS systems based on nonenzymatic kinetic resolution may also be useful for discovery of superior asymmetric catalysts for other reactions.

EXPERIMENTAL SECTION

UV/vis and fluorescence analysis. For absorption or fluorescence measurement, compounds were dissolved in DMSO to obtain 1 mM stock solutions. These stock solutions were diluted with organic solvents as specified in the figure legends to the desired concentration. For determination of the fluorescence quantum yield (Φ_{fl}), fluorescein in 100 mM aq NaOH (Φ_{fl} = 0.85) was used as a fluorescence standard.

Fluorescence-Based HTS Assay for Determination of the Reaction Yield. To determine the reaction yield, a calibration curve of reaction yield versus fluorescence intensity was prepared. For preparation of the calibration curve, solutions of **6** and **8** (10 μM total concentration) in DMSO were mixed to obtain standard solutions corresponding to 0%, 20%, 40%, 60%, 80%, and 100% yields, respectively; these solutions were excited at 500 nm, and the fluorescence intensity was measured at 515 nm. For the HTS assay, 1 mM **6** was acylated with acid anhydride (5 equiv) in the presence of catalyst (5 equiv) in 300 μL of various solvents at room temperature for 1 h. Reaction mixtures were diluted 100 times with DMSO for measurement of fluorescence. The reaction yields were obtained from the calibration curve by interpolation of the measured fluorescence intensity of the samples.

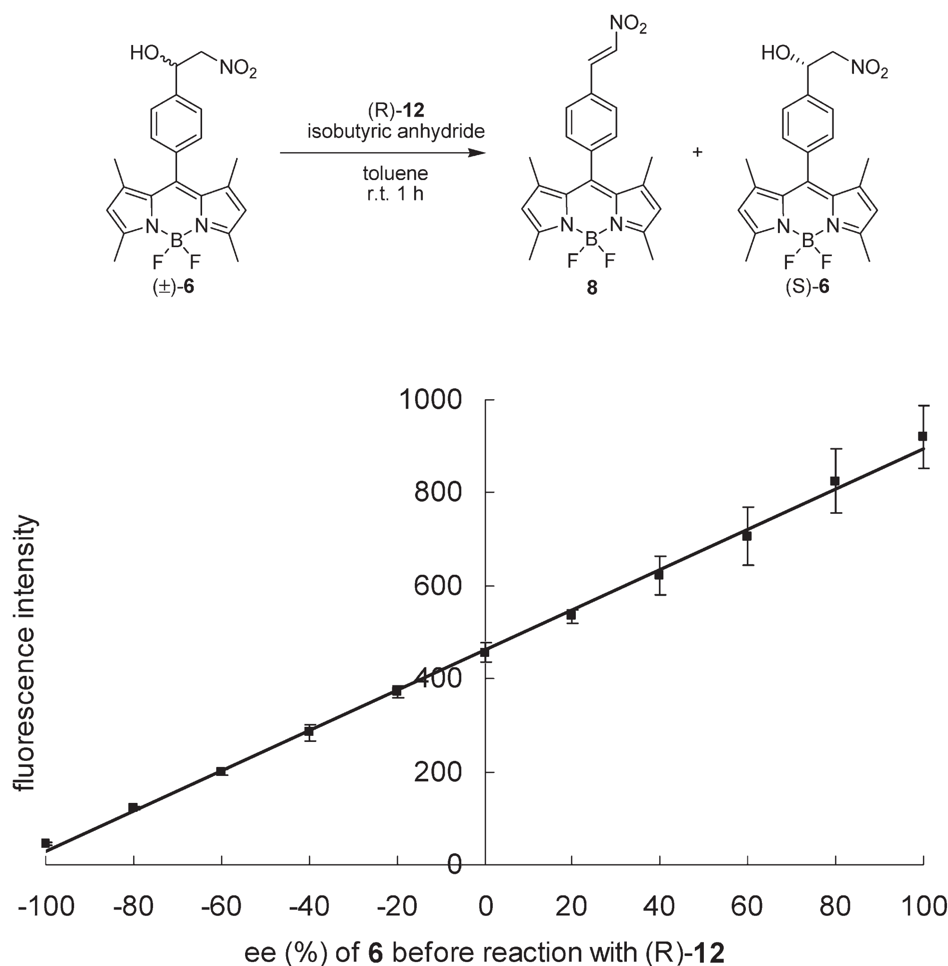


Figure 6. HTS system for % ee determination: 1 mM **6** (note that here 100% ee corresponds to (*S*)-**6**, while −100% ee corresponds to (*R*)-**6**) was treated with 3 mM (R)-**12** and 3 mM isobutyric anhydride in 1 mL of toluene at room temperature for 1 h. The reaction mixture was diluted 100 times with DMSO, and the fluorescence intensity was measured (Ex 500 nm, Em 515 nm) (n = 3).

Synthesis of 4-Hydroxymethylbenzaldehyde (1). Terephthalaldehyde (6.7 g, 50 mmol) was dissolved in 100 mL of dry THF. $NaBH_4$ (437 mg, 12.5 mmol) was added, and the solution was stirred at room temperature with a $CaCl_2$ tube for 1 h. The solvent was evaporated under reduced pressure. The residue was diluted with CH_2Cl_2 and washed with water 3 times. The organic solution was washed with brine and dried over Na_2SO_4. The compound was purified by silica gel column chromatography (eluent: CH_2Cl_2/MeOH = 19/1) to give a colorless oil (1.7 g, 25%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.09 (s, 1H), 4.74 (s, 2H), 7.54 (d, 2H, J = 8.1 Hz), 7.88 (d, 2H, J = 8.1 Hz), 10.01 (s, 1H).

Synthesis of 4-Acetoxymethylbenzaldehyde (2). Compound **1** (1.7 g, 12.5 mmol) was dissolved in 50 mL of dry pyridine, and 3 mL of acetic anhydride was added at 0 °C for 12 h. The reaction mixture was diluted with CH_2Cl_2, washed with water three times and brine once, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by silica gel column chromatography (eluent: CH_2Cl_2) to give a colorless oil (1.5 g, 67%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.15 (s, 3H), 5.19 (s, 2H), 7.52 (d, 2H, J = 8.0 Hz), 7.89 (d, 2H, J = 8.0 Hz), 10.03 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 20.9, 65.4, 128.2, 130.0, 136.1, 142.7, 191.7.

Synthesis of 8-(4-Acetoxymethylphenyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (3). Compound **2** (356 mg, 2.0 mmol) and 2,4-dimethylpyrrole (380 mg, 4.0 mmol) were dissolved in 350 mL of dry CH_2Cl_2 under an Ar atmosphere. One drop of TFA was added, and the solution was stirred at room temperature for 12 h. A solution of DDQ (545 mg, 2.0 mmol) in CH_2Cl_2 was added, and stirring was continued for 20 min. The reaction mixture was washed with water three times and brine once, dried over Na_2SO_4, filtered, and evaporated. The compound was purified by short column chromatography over alumina (eluent: CH_2Cl_2). The brown powder thus obtained and 5 mL of DIEA were dissolved in 200 mL of toluene under an Ar atmosphere. Then 5 mL of $BF_3 \cdot Et_2O$ was added, and the solution was stirred at room temperature for 30 min. The reaction mixture was washed with water three times and brine once, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by silica gel column chromatography (eluent: CH_2Cl_2/n-hexane = 1/1) to give an orange solid (202 mg, 26%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.37 (s, 6H), 2.16 (s, 3H), 2.55 (s, 6H), 5.20 (s, 2H), 5.98 (s, 2H), 7.28 (d, 2H, J = 8.2 Hz), 7.47 (d, 2H, J = 8.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.4, 14.6, 21.0, 65.6, 121.2, 128.2, 128.4, 131.4, 134.8, 137.1, 141.2, 143.0, 155.6, 170.7. HRMS (ESI$^+$): calcd for ([M + Na]$^+$) 419.1718, found 419.1730. Anal. Calcd for $C_{22}H_{23}BF_2N_2O_2$: C, 66.69; H, 5.85; N, 7.07. Found: C, 66.63; H, 5.80; N, 7.20.

Synthesis of 8-(4-Hydroxymethylphenyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (4). Compound **3** (200 mg, 0.51 mmol) was dissolved in 5 mL of CH_2Cl_2, and then 10 mL of MeOH and 2 mL of NaOH aq (0.1 M) were added. The reaction mixture was stirred at room temperature for 1 h, diluted with CH_2Cl_2, washed with water three times and brine once, dried over

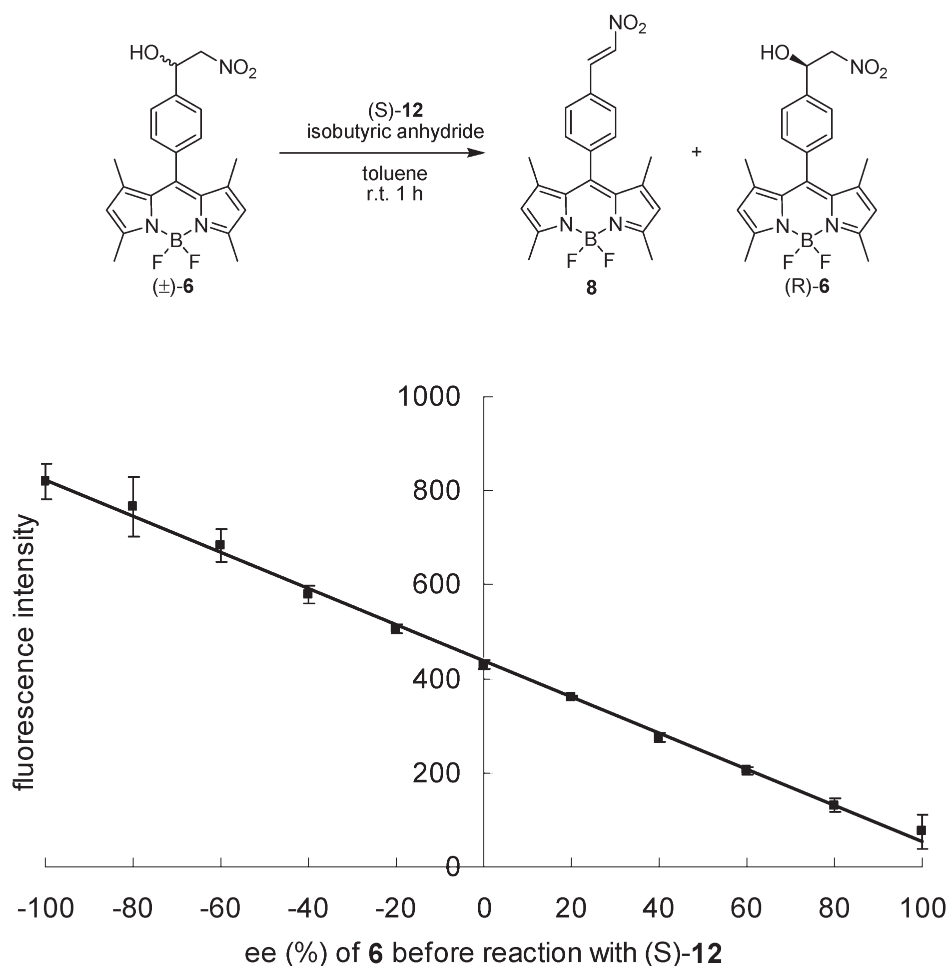


Figure 7. HTS system for % ee determination: 1 mM **6** (note that here 100% ee corresponds to (*S*)-**6**, while −100% ee corresponds to (*R*)-**6**) was treated with 3 mM (*S*)-**12** and 3 mM isobutyric anhydride in 1 mL of toluene at room temperature for 1 h. The reaction mixture was diluted 100 times with DMSO, and the fluorescence intensity was measured (Ex 500 nm, Em 515 nm) (n = 3).

Na_2SO_4, filtered, and evaporated. The residue was purified by silica gel column chromatography (eluent: CH_2Cl_2) to give an orange solid (217 mg, quant). ^1H NMR (300 MHz, $CDCl_3$): δ 1.38 (s, 6H), 1.93 (t, 1H, J = 5.7 Hz), 2.55 (s, 6H), 4.79 (d, 2H, J = 5.7 Hz), 5.98 (s, 2H), 7.27 (d, 2H, J = 8.4 Hz), 7.49 (d, 2H, J = 8.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.4, 14.5, 64.7, 121.2, 127.3, 128.1, 131.4, 134.2, 141.5, 141.9, 143.1, 155.4. HRMS (ESI$^+$): calcd for ([M + Na]$^+$) 377.1613, found 377.1660. Anal. Calcd for $C_{20}H_{21}BF_2N_2O$: C, 67.82; H, 5.98; N, 7.91. Found: C, 68.00; H, 6.07; N, 7.94.

Synthesis of 8-(4-Formylphenyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (5). Compound **4** (100 mg, 0.28 mmol) was dissolved in 50 mL of CH_2Cl_2 and PCC (120 mg, 0.56 mmol) was added, followed by 120 mg of $MgSO_4$. The reaction mixture was stirred at room temperature under an Ar atmosphere for 4 h, filtered through Celite, and purified by silica gel column chromatography (eluent: CH_2Cl_2) to give an orange solid (68 mg, y. 68%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.35 (s, 6H), 2.56 (s, 6H), 6.00 (s, 2H), 7.50 (d, 2H, J = 8.1 Hz), 8.03 (d, 2H, J = 8.1 Hz), 10.11 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.4, 14.5, 121.6, 129.1, 130.3, 130.7, 136.6, 139.6, 141.3, 142.7, 156.1, 191.4. HRMS (ESI$^+$): calcd for ([M + Na]$^+$) 375.1456, found 375.1470. Anal. Calcd for $C_{20}H_{19}BF_2N_2O$: C, 68.21; H, 5.44; N, 7.95. Found: C, 68.23; H, 5.53; N, 7.92.

Synthesis of 8-(4-(1-Hydroxy-2-nitroethyl)phenyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (6). Compound **5** (1.1 g, 3.1 mmol) and 3 mL of nitromethane were dissolved in 20 mL of CH_2Cl_2, and then 20 mL of MeOH and 0.1 mL of NaOH aq (2 M) were added at 0 °C. The reaction mixture was stirred at 0 °C for 4 h, diluted with CH_2Cl_2, washed with water three times and brine once, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by silica gel column chromatography (eluent: CH_2Cl_2) to give an orange solid (1.2 g, yield 93%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.34 (s, 6H), 2.55 (s, 6H), 3.01 (d, 1H, J = 4.2 Hz), 4.56 − 4.70 (m, 2H), 5.53 − 5.58 (m, 1H), 5.99 (s, 2H), 7.35 (d, 2H, J = 7.9 Hz), 7.55 (d, 2H, J = 7.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.4, 14.6, 70.5, 81.2, 121.4, 126.6, 128.8, 131.3, 135.8, 139.1, 140.5, 142.8, 155.8. HRMS (ESI$^+$): calcd for ([M + Na]$^+$) 436.1620, found 436.1651. Anal. Calcd for $C_{21}H_{22}BF_2N_3O_3$: C, 61.04; H, 5.37; N, 10.17. Found: C, 60.98; H, 5.37; N, 10.08.

Synthesis of 8-(4-((*E*)-2-Nitrovinyl)phenyl)-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene (8). Compound **6** (41 mg, 0.1 mmol) was dissolved in 10 mL of dry CH_2Cl_2, and then 2 mL of Ac_2O and 2 mL of dry pyridine were added. The reaction mixture was stirred at room temperature for 4 h, washed with water three times and brine once, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by silica gel column chromatography (eluent: CH_2Cl_2) to give a red solid (33 mg, yield 86%). ^1H NMR (300 MHz, $CDCl_3$): δ 1.39 (s, 6H), 2.57 (s, 6H), 6.00 (s, 2H), 7.42 (d, 2H, J = 8.1 Hz), 7.66 (d, 1H, J = 13.7 Hz), 7.70 (d, 2H, J = 8.1 Hz), 8.06 (d, 1H, J = 13.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.6, 14.7, 121.6, 129.5, 129.7, 130.8, 131.0, 137.9, 138.0, 139.1, 139.7, 142.7, 156.2. HRMS (ESI$^+$): calcd for ([M + Na]$^+$) 418.1514, found 418.1514. Anal. Calcd for

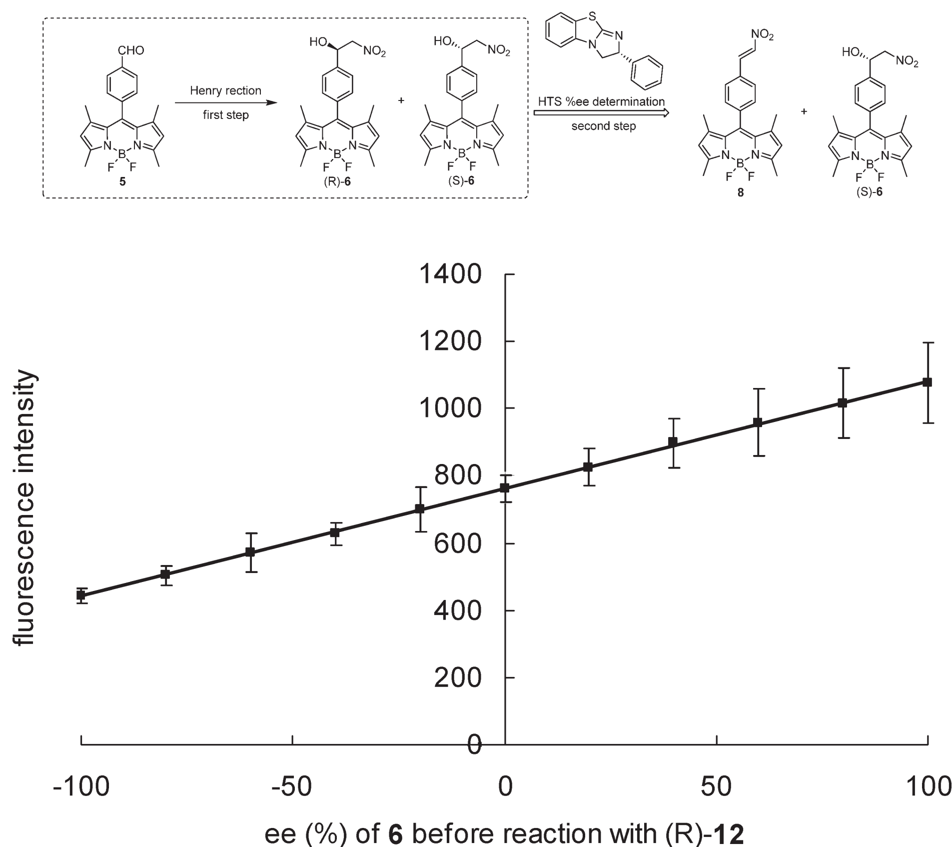


Figure 8. Effect of impurities on evaluation of % ee. A solution of 0.5 mM **6** (note that here 100%ee corresponds to (*S*)-**6**, while −100% ee corresponds to (*R*)-**6**) was treated with 5 mM (*R*)-**12** and 10 mM isobutyric anhydride in 1 mL of toluene solution which contained 10% DMF, 50 mM nitromethane, and 0.05 mM TEA as impurities at room temperature for 1 h. The reaction mixture was diluted 50 times with DMSO, and the fluorescence intensity was measured (Ex 500 nm, Em 15 nm) (n = 3).

$C_{21}H_{20}BF_2N_3O_2$: C, 63.82; H, 5.10; N, 10.63. Found: C, 63.53; H, 5.30; N, 10.54.

Synthesis of (*R*)-8-(4-(1-Hydroxy-2-nitroethyl)phenyl)-4,4-difluoro-2-iodo-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-*s*-indacene ((R)-9). (*R*)-**6** (51.3 mg, 0.12 mmol) and I_2 (15.7 mg, 0.062 mmol) were dissolved in 10 mL of CH_2Cl_2, and then 20 mL of EtOH was added followed by HIO_3 (10.9 mg, 0.062 mmol) dissolved in 10 mL of H_2O. The reaction mixture was stirred at 50 °C for 20 min and cooled to room temperature, and then 20 mL of saturated $Na_2S_2O_3$ aq was added and the whole was extracted with CH_2Cl_2. The organic layer was washed with water three times and brine once, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by silica gel column chromatography (eluent: CH_2Cl_2) to give a red solid (56.4 mg, yield 84%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.35 (s, 6H), 2.57 (s, 3H), 2.63 (s, 3H), 3.02 (d, 1H, J = 4.4 Hz), 4.60 (dd, 1H, J = 13.2 Hz, 3.7 Hz), 4.67 (dd, 1H, J = 13.2 Hz, 8.0 Hz), 5.55 − 5.60 (m, 1H), 6.06 (s, 1H), 7.34 (d, 2H, J = 8.1 Hz), 7.57 (d, 2H, J = 8.1 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.7, 14.8, 15.8, 16.7, 70.5, 81.2, 84.5, 122.5, 126.8, 128.7, 130.8, 131.7, 135.6, 139.4, 140.3, 143.0, 144.8, 154.9, 158.2. HRMS (ESI$^-$): calcd for ([M − H]$^-$) 538.0611, found 538.0606. Anal. Calcd for $C_{21}H_{21}BF_2IN_3O_3$: C, 46.78; H, 3.93; N, 7.79. Found: C, 46.43; H, 3.96; N, 7.67.

■ ASSOCIATED CONTENT

Supporting Information. Fluorescence properties, HPLC conditions, ORTEP, calibration curve, NMR spectra, and CIF. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
*E-mail: tlong@mol.f.u-tokyo.ac.jp.

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