Chiral *N*-Substituted Perylene-3,4-dicarboximides as Fluorescent Labeling Reagents

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Chiral *N*-substituted perylene-3,4-dicarboximides have been synthesized. These optically pure compounds were sufficiently soluble in acetonitrile used as fluorescent labeling reagents. Their excitation and emission maxima were observed at λ 500 and 550 nm in acetonitrile, respectively. Enantiomers in a racemic mixture could be analyzed by these reagents. Racemic Ibuprofen and alanine methyl ester were nicely separated by (*R*)-*N*-(2-hydroxy-1-phenylethyl)- and (*R*)-*N*-(α-carboxyphenethyl)perylene-3,4-dicarboximides in HPLC, respectively. The detection limits (S/N > 3) of Ibuprofen and alanine methyl ester were 1 and 13 pmol, respectively.

Fluorescent labeling reagents are required to have high sensitivity, appropriate excitation and emission maxima, sufficient solubility in the mobile phase, and a functional group smoothly reacting with target compounds. Many chiral fluorescent labeling reagents for amines, carboxylic acids, alcohols, phenols, aldehydes, and ketones have been proposed.[1,2] However, the fluorophores of most fluorescent labeling reagents are benzenes, naphthalenes, fluorenes, anthracenes, and pyrenes, whose fluorescence quantum yields (Φ) are 0.03–0.20, 0.19–0.40, 0.5–0.8, 0.25–0.35, and 0.3–0.7, respectively. Perylene is known to have highly fluorescence quantum yield ($\Phi > 0.9$). Though perylene-3,4:9,10-bis(dicarboximide)s are intensely fluorescent compounds, they are extremely less soluble in organic solvents and water. The chemistry of perylene-3,4-dicarboximides has been mainly developed by Nagao, Langhals, and Müllen. Perylene-3,4-dicarboximides which may be used as marking and printing toners, luminescence devices, and solar collectors, were obtained from perylene-3,4:9,10-tetracarboxylic 3,4:9,10-dianhydride,[3–5] 9,10-(iminodicarbonyl)perylene-3,4-dicarboxylic anhydride,[5,6] and perylene-3,4-dicarboxylic anhydride.[6] The synthesis of hydroxy-,[7] nitro-,[4] amino-,[4,7] 9-carboxy-,[7] 9-amino-,[7] and 9-bromoperylene-3,4-carboximides,[8] have been also reported. The 9-bromo derivatives are important precursors for near-infrared absorbing and emitting quaterrylenebis(dicarboximide)s.[9,10] Perylene-3,4-dicarboximides have been reported to be more stable than perylene-3,4:9,10-bis(dicarboximide)s under UV irradiation.[11] Thus, perylene-3,4-dicarboximides are very interesting compounds. We report here on the application of perylene-3,4-dicarboximides as chiral fluorescent labeling reagents.

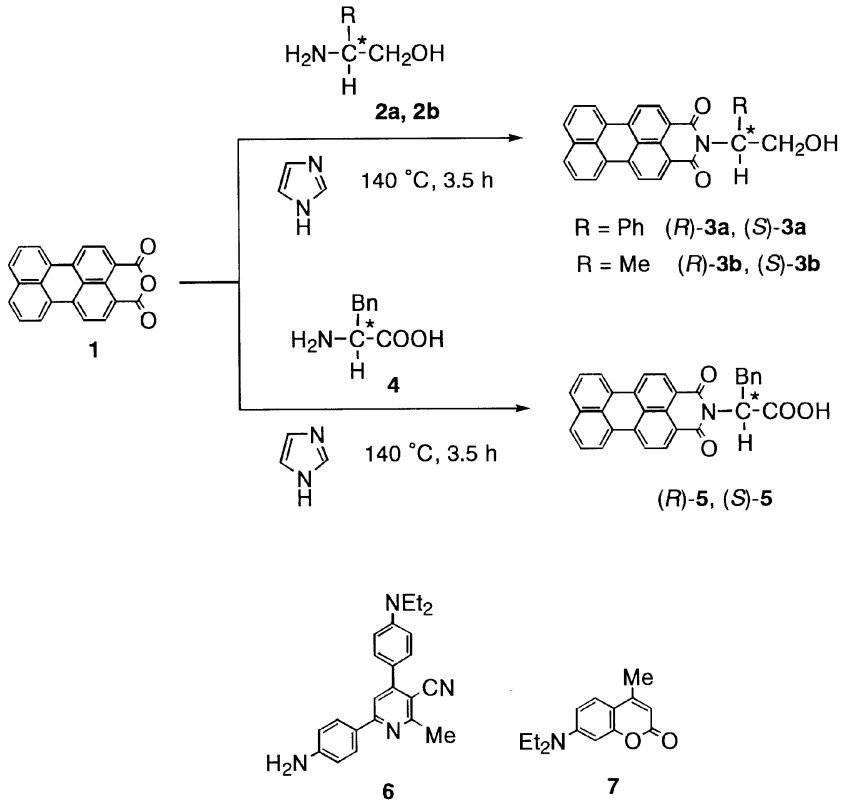
Results and Discussion

The syntheses of chiral *N*-substituted perylene-3,4-dicarboximides (*R*)-**3a**, (*S*)-**3a**, (*R*)-**3b**, (*S*)-**3b**, (*R*)-**5**, and (*S*)-**5** are shown in Scheme 1. Perylene-3,4-dicarboxylic anhydride (**1**) reacted with (*R*)-(–)-, (*S*)-(+)-2-phenylglycinols (**2a**) and (*R*)-(–)-, (*S*)-(+)-2-amino-1-propanols (**2b**) at 140 °C in the presence of imidazole under a nitrogen atmosphere to give the *N*-substituted perylene-3,4-dicarboximides (*R*)-**3a**, (*S*)-**3a**, (*R*)-**3b**, and (*S*)-**3b** in moderate yields, respectively. Perylene-3,4-dicarboxylic anhydride (**1**) also reacted with D- and L-phenylalanines (**4**) to afford the *N*-substituted perylene-3,4-dicarboximides (*R*)- and (*S*)-**5** in moderate yields, respectively.

The UV/Vis and fluorescence spectra of (*R*)-**3a** are shown in Fig. 1. The absorption maximum was observed at λ around 482 nm, being orange in color. The excitation and emission maxima were observed at λ 500 and 550 nm, respectively. Similar UV/Vis and fluorescence spectra were observed for (*S*)-**3a**, **3b**, and **5**.

The fluorescence spectra of perylene-3,4-carboximides **3a**, **3b**, **5**, and reference compounds **6** and **7** are summarized in Table 1. Compound **6** is a reference material in a series of our study on novel fluorescent labeling reagents.[12] The compound **7** is a typical fluorescent coumarin. Their structures are also shown in Scheme 1. All of the *N*-substituted perylene-3,4-dicarboximides **3a**, **3b**, and **5** showed their excitation and emission maxima at λ 500 and 550 nm in acetonitrile, respectively. Thus, both the excitation and emission maxima of **3a**, **3b**, and **5** were more bathochromic than those of **6** and **7**. The Stoke's shift in **3a**, **3b**, and **5** were small (50 nm), being characteristic for perylene derivatives. No remarkable difference in the relative fluorescence intensity (RFI) among **3a**, **3b**, and **5** was observed.

No remarkable difference in their solubility of the respective (*R*) and (*S*) derivatives was also observed. The phenyl derivatives **3a** were more soluble than the corresponding methyl derivatives **3b** in acetonitrile. Though the carboxy derivatives **5** were less soluble than the hydroxy derivatives **3**, they were soluble enough to be used as fluorescent labeling reagents.



Scheme 1.

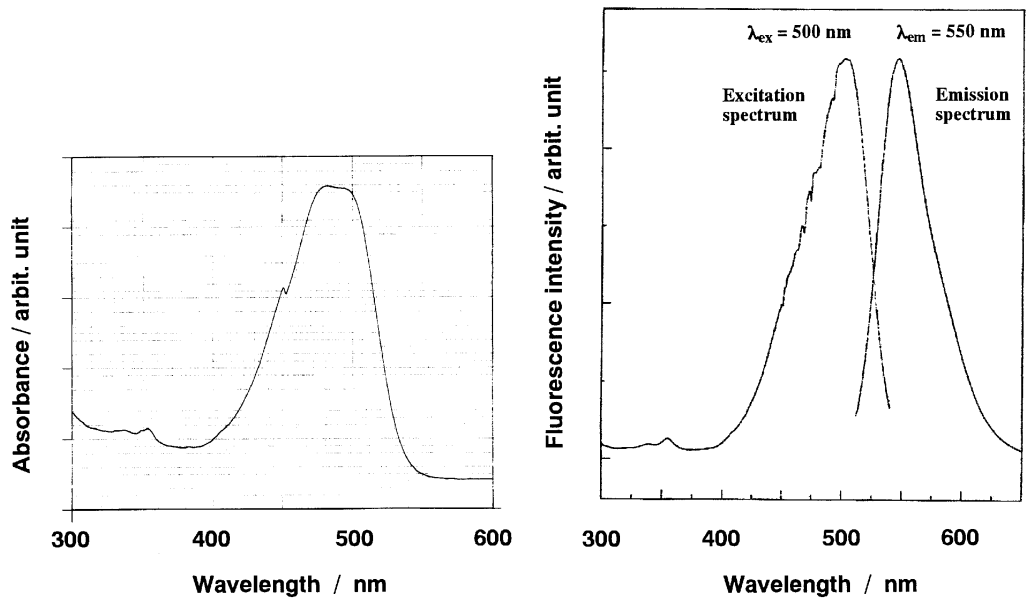


Fig. 1. UV/Vis and fluorescence spectra of (*R*)-**3a** in acetonitrile.

Since these reagents are used in reverse-phase HPLC, the effect of water on the RFI of (*R*)-**3a** and (*R*)-**5** was examined. The result is indicated in Fig. 2. The RFI of both the reference compounds **6** and **7** drastically decreased by the addition of water. While the decrease of RFI in (*R*)-**3a** and (*R*)-**5** was not so marked, being 49 and 61% retention, even in a 50% aqueous acetonitrile solution, respectively. The decrease in RFI in (*R*)-**3** and (*R*)-**5** could be attributed to a decrease in their solubility in an aqueous acetonitrile.

The analysis of racemic Ibuprofen by (*R*)-**3a** was investigated. (*S*)-Ibuprofen is known to show anti-inflammatoric and analgestic activities. The reaction of (*R*)-**3a** with racemic Ibupro-

Table 1. UV/Vis and Fluorescence Spectra of **3a, 3b, 5, 6,** and **7**

Compd	λ_{max} (ε)[a]	λ_{ex}[a]	λ_{em}[a]	RFI[b]	ee[c]/ %
	nm	nm	nm		
(*R*)-**3a**	482 (29500)	500	550	241	> 99
(*S*)-**3a**	482 (30000)	500	550	247	> 99
(*R*)-**3b**	482 (29500)	500	550	217	> 99
(*S*)-**3b**	482 (28500)	500	550	206	> 99
(*R*)-**5**	483 (29000)	500	550	205	> 99
(*S*)-**5**	483 (28500)	500	550	200	> 99
6	353 (53100)	353	508	100	–
7	367 (25300)	372	434	447	–

a) Measured in acetonitrile. b) Relative fluorescence intensity (RFI) measured in acetonitrile at 25 °C on 1×10^{-5} mol dm^{-3} of substrate. c) Determined by liquid chromatography (CHIROBIOTIC V, hexane–ethanol = 80 : 20), 0.4 cm^{-3} min^{-1}, detection: 550 nm (excitation: 500 nm).

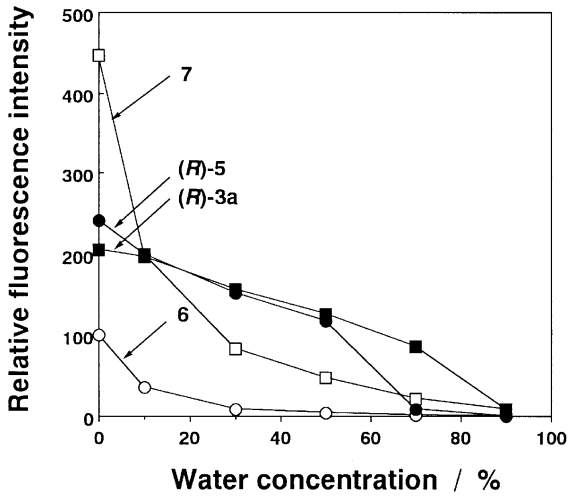


Fig. 2. Effect of water on relative fluorescence intensity of (*R*)-**3a**, (*R*)-**5**, **6**, and **7**.

fen (**8**) can produce (*R*)-**3a**-(*R*)-**8** and (*R*)-**3a**-(*S*)-**8** esters, as shown in Scheme 2.

The reactivity of (*R*)-**3a** with Ibuprofen is shown in Fig. 3. When (*R*)-**3a** was treated with racemic Ibuprofen at 60 °C for 6 h in the presence of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDC), a set of 1:1 diastereomers were formed and the yields of the products were highest under the conditions. The reaction of each enantiomeric Ibuprofen with (*R*)-**3a** predominantly gave the respective diastereomer as a product.

The chromatogram in the analysis of racemic Ibuprofen (**8**) by (*R*)-**3a** is shown in Fig. 4. The big peak at retention time around 4.8 min was attributed to (*R*)-**3a**. The peaks at around 29 and 32 min were attributed to the diastereomers derived from (*R*)- and (*S*)-Ibuprofens, respectively. These products were identified on the basis of the retention time of the respective (*R*)- and (*S*)-Ibuprofens reacted with (*R*)-**3a**. The detection limit, defined by a S/N ratio larger than 3, of Ibuprofen was 1 pmol.

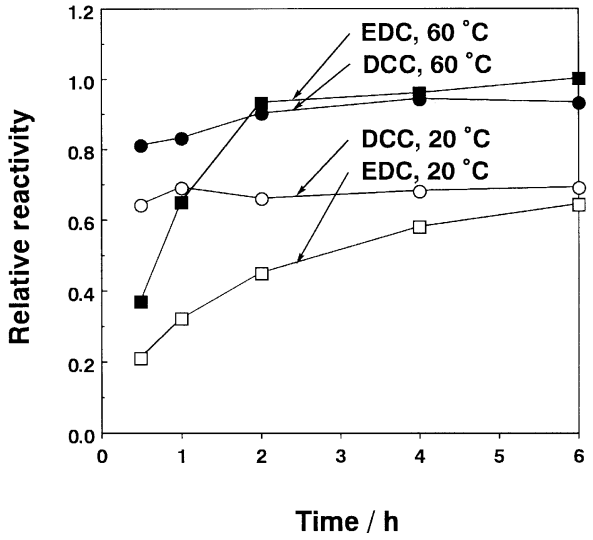
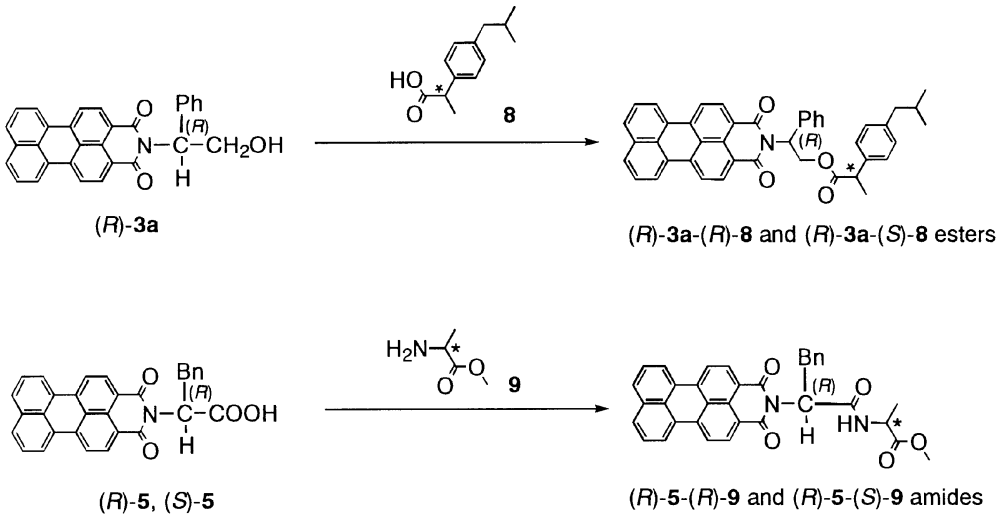


Fig. 3. Reactivity of (*R*)-**3a** with Ibuprofen.



Scheme 2.

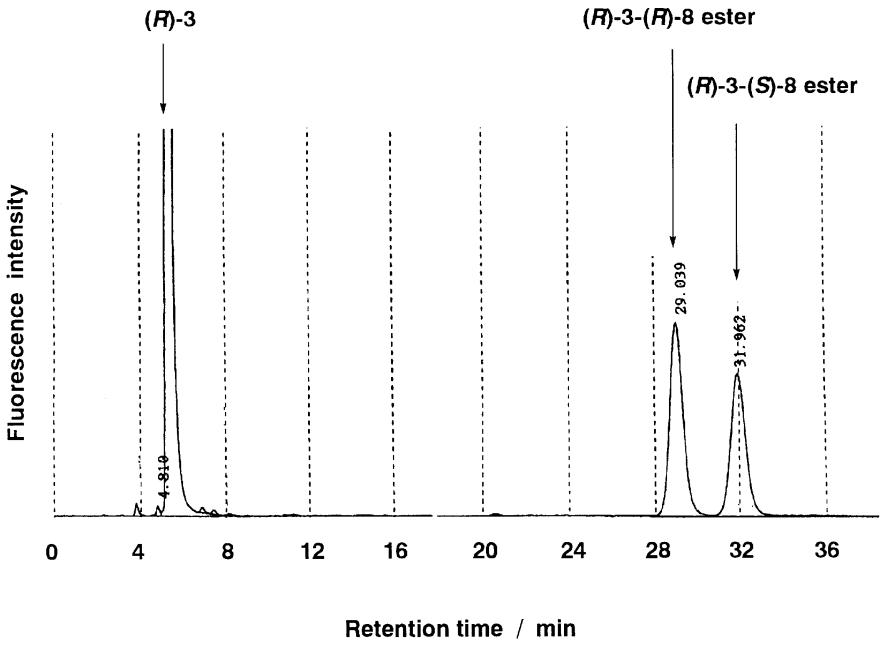


Fig. 4. HPLC analysis of racemic Ibuprofens by chiral fluorescent labeling reagent (*R*)-**3a**.

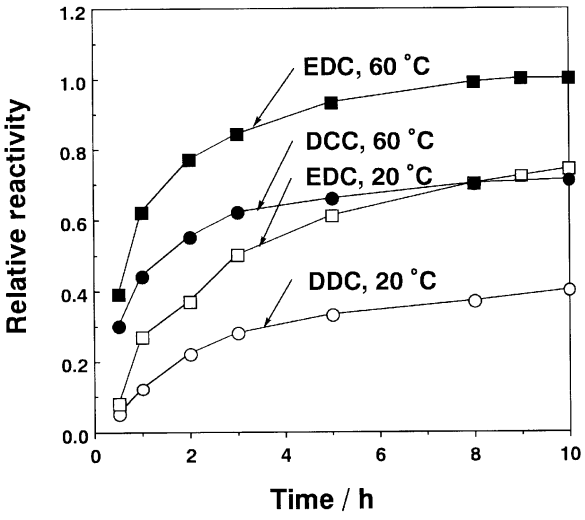


Fig. 5. Reactivity of (*R*)-**5** with alanine methyl ester.

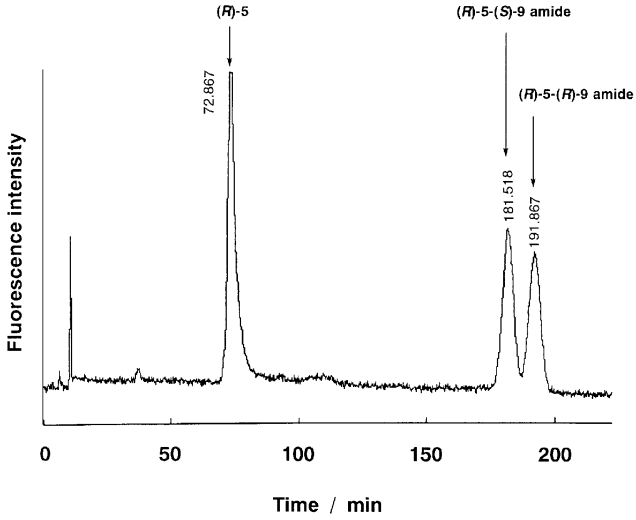


Fig. 6. HPLC analysis of DL-alanine methyl ester by chiral fluorescent labeling reagent (*R*)-**5**.

The analysis of DL-alanine methyl ester (**9**) by (*R*)-**5** was also investigated. The reaction of (*R*)-**5** with DL-alanine methyl ester (**9**) can afford the corresponding diastereomers (*R*)-**5**-(*R*)-**9** and (*R*)-**5**-(*S*)-**9** amides, as shown in Scheme 2.

Figure 5 summarizes the reactivity of (*R*)-**5** with alanine methyl ester. When alanine methyl ester was treated with (*R*)-**5** at 60 °C for 10 h in the presence of EDC, the product yields were highest.

Figure 6 shows the HPLC analysis of DL-alanine methyl ester (**9**) by (*R*)-**5**. The peaks at retention times of around 73, 182, and 192 min were attributed to (*R*)-**5**, the diasteremers derived from (*S*)- and (*R*)-alanine methyl esters, respectively. Thus, DL-alanine methyl ester reacted with (*R*)-**5** to give the corresponding diastereomers as 1:1 products, which were nicely analyzed by HPLC. The detection limit of alanine methyl ester was 13 pmol.

Conclusion

Chiral *N*-substituted perylene-3,4-dicarboximides, showing their absorption and emission maxima at λ 500 and 550 nm, respectively, were synthesized. They were sufficiently soluble in acetonitrile used as fluorescent labeling reagents. The detection limit, defined by a S/N ratio larger than 3, of Ibuprofen and alanine methyl ester by these reagents were 1 and 13 pmol, respectively. Thus, the chiral *N*-substituted perylene-3,4-dicar-

boximides could be used as fluorescent labeling reagents for the HPLC analysis of optically active amines and carboxylic acids.

Experimental

Instruments. Melting points were measured with a Yanagimoto MP-S2 micro-melting-point apparatus. NMR spectra were taken on JEOL α-400 and Varian Gemini-2000 spectrometers. EIMS spectra were recorded on a Shimadzu QP-1000 spectrometer. LCMS spectra were taken on a Shimadzu LC/MS-QP1100EX spectrometer. Fluorescence spectra was measured with a Hitachi F-4500 spectrometer. Liquid chromatography was performed with a Jasco Triroter-V instrument.

Materials. Perylene-3,4-dicarboxylic anhydride (**1**) was prepared as described in the literature.[4] (*R*)-(–)- and (*S*)-(+)-2-phenylglycinols (**2a**), (*R*)-(–)- and (*S*)-(+)-2-amino-1-propanols (**2b**), and D- and L-phenylalanines (**4**) were purchased from Aldrich Co., Ltd. Ibuprofen (**8**) and DL-alanine methyl ester hydrochlorides (**9**) were purchased from Research Biochemical International and Sigma, respectively.

Synthesis of *N*-(2-Hydroxy-1-phenyl- and -methylethyl)-perylene-3,4-dicarboximides 3. To perylene-3,4-dicarboxylic anhydride **1** (0.47 mmol, 150 mg) was added an amine **2a** or **2b** (1.12 mmol) and imidazole (0.93 mg) and heated at 140 °C under an argon atmosphere (**2a**: 2 h; **2b**: 3.5 h). After the reaction was completed, the mixture was poured into aqueous 10% hydrochloric acid (300 cm^{-3}). The resulting precipitate was washed with aqueous 10% potassium carbonate at 80 °C until the color of the filtrate became colorless. The precipitate was washed with water at 80 °C, dried, purified by silica gel column chromatography ($CHCl_3$: $CH_3COOC_2H_5$ = 1:1), and recrystallized from toluene. The physical and spectral data are shown below.

(*R*)-*N*-(2-Hydroxy-1-phenylethyl)perylene-3,4-dicarboximide ((*R*)-3a): Yield 65%; mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 4.35–4.39 (m, 1H), 4.43–4.49 (m, 1H), 5.08 (t, J = 6.8 Hz, 1H, disappears with D_2O), 6.30 (t, J = 6.8 Hz, 1H), 7.23–7.35 (m, 3H), 7.44–7.46 (m, 2H), 7.70 (t, J = 7.8 Hz, 2H), 8.04 (d, J = 7.8 Hz, 2H), 8.43 (d, J = 7.8 Hz, 2H), 8.65 (d, J = 7.8 Hz, 4H); EIMS (70 eV) *m*/*z* (rel intensity) 441 (M^+; 9) 321 (100); ORD (*c* 0.02521, $CHCl_3$, 25 °C) $[\alpha]_{584}$ –190.4°. Found: C, 81.41; H, 4.43; N, 3.18%. Calcd for $C_{30}H_{19}NO_3$: C, 81.62; H, 4.34; N, 3.17%.

(*S*)-*N*-(2-Hydroxy-1-phenylethyl)perylene-3,4-dicarboximide ((*S*)-3a): Yield 62%; mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 4.35–4.41 (m, 1H), 4.43–4.50 (m, 1H), 5.08 (t, J = 7.1 Hz, 1H, disappears with D_2O), 6.31 (t, J = 7.1 Hz, 1H), 7.23–7.35 (m, 3H), 7.44–7.46 (m, 2H), 7.69 (t, J = 7.8 Hz, 2H), 8.03 (d, J = 7.8 Hz, 2H), 8.41 (d, J = 7.8 Hz, 2H), 8.63 (d, J = 7.8 Hz, 4H); EIMS (70 eV) *m*/*z* (rel intensity) 441 (M^+; 17), 423 (100); ORD (*c* 0.02521, $CHCl_3$, 25 °C) $[\alpha]_{584}$ 183.5°. Found: C, 81.71; H, 4.47; N, 3.18%. Calcd for $C_{30}H_{19}NO_3$: C, 81.62; H, 4.34; N, 3.17%.

(*R*)-*N*-(2-Hydroxy-1-methylethyl)perylene-3,4-dicarboximide ((*R*)-3b): Yield 59%; mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 1.46 (d, J = 7.1 Hz, 3H), 3.72–3.78 (m, 1H), 4.02–4.09 (m, 1H), 4.83 (t, J = 7.1 Hz, 1H, disappears with D_2O), 5.23 (sextet, J = 7.1 Hz, 1H), 7.70 (t, J = 8.1 Hz, 2H), 8.05 (d, J = 8.1 Hz, 2H), 8.42 (d, J = 8.1 Hz, 2H), 8.64 (d, J = 8.1 Hz, 4H); EIMS (70 eV) *m*/*z* (rel intensity) 379 (M^+; 23), 322 (100). Found: C, 78.88; H, 4.60; N, 3.75%. Calcd for $C_{25}H_{17}NO_3$: C, 79.14; H, 4.52; N, 3.69%.

(*S*)-*N*-(2-Hydroxy-1-methylethyl)perylene-3,4-dicarboximide ((*S*)-3b): Yield 52%; mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 1.45 (d, J = 7.1 Hz, 3H), 3.72–3.77 (m, 1H), 4.02–4.08 (m, 1H), 4.83 (t, J = 7.1 Hz, 1H, disappears with D_2O), 5.23 (sextet, J = 7.1 Hz, 1H), 7.71 (t, J = 8.1 Hz, 2H), 8.05 (d, J = 8.1 Hz, 2H), 8.44 (d, J = 8.1 Hz, 2H), 8.66 (d, J = 8.1 Hz, 4H); EIMS (70 eV) *m*/*z* (rel intensity) 379 (M^+; 28), 322 (100). Found: C, 78.88; H, 4.60; N, 3.74%. Calcd for $C_{25}H_{17}NO_3$: C, 79.14; H, 4.52; N, 3.69%.

Synthesis of *N*-(α-Carboxyphenethyl)perylene-3,4-dicarboximides 5. To imidazole (0.62 mg) were added perylene-3,4-dicarboxylic anhydride **1** (100 mg, 0.31 mmol) and D- or L-phenylalanines **4** (124 mg, 0.75 mmol) and heated at 140 °C under an argon atmosphere for 1 h. After the reaction was completed, the mixture was poured into 10% aqueous hydrochloric acid (300 cm^{-3}). The resulting precipitate was filtered, washed with 10% aqueous potassium carbonate until the filtrate became colorless, dried, and recrystallized from toluene. The physical and spectral data are shown below.

(*R*)-*N*-(α-Carboxyphenethyl)perylene-3,4-dicarboximide ((*R*)-5): Yield 79%; mp > 300 °C; ^{1}H NMR ($CDCl_3$) δ 3.52–3.58 (m, 1H), 3.70–3.77 (m, 1H), 6.12 (dd, J = 9.6 and 5.7 Hz, 1H), 7.08–7.18 (m, 5H), 7.64 (t, J = 7.9 Hz, 2H), 7.92 (d, J = 7.9 Hz, 2H), 8.41 (d, J = 7.9 Hz, 2H), 8.45 (d, J = 7.9 Hz, 2H), 8.54 (d, J = 7.9 Hz, 2H); EIMS (70 eV) *m*/*z* (rel intensity) 469 (M^+; 10), 321 (100). Found: C, 79.39; H, 4.26; N, 3.04%. Calcd for $C_{31}H_{19}NO_4$: C, 79.31; H, 4.08; N, 2.98%.

(*S*)-*N*-(α-Carboxyphenethyl)perylene-3,4-dicarboximide ((*S*)-5): Yield 86%; mp > 300 °C; ^{1}H NMR ($CDCl_3$) δ 3.52–3.58 (m, 1H), 3.70–3.75 (m, 1H), 6.12 (dd, J = 9.8 and 5.6 Hz, 1H), 7.06–7.16 (m, 5H), 7.63 (t, J = 7.9 Hz, 2H), 7.91 (d, J = 7.9 Hz, 2H), 8.40 (d, J = 7.9 Hz, 2H), 8.44 (d, J = 7.9 Hz, 2H), 8.53 (d, J = 7.9 Hz, 2H); EIMS (70 eV) *m*/*z* (rel intensity) 469 (M^+; 8), 321 (100). Found: C, 79.66; H, 4.30; N, 2.99%. Calcd for $C_{31}H_{19}NO_4$: C, 79.31; H, 4.08; N, 2.98%.

Reactivity of (*R*)-3a with Ibuprofen and (*R*)-5 with Alanine Methyl Ester. To a dichloromethane solution (0.1 cm^{-3}) of Ibuprofen (**8**) or DL-alanine methyl ester hydrochlorides **9** (0.1 μmol) were added a dichloromethane solution (0.5 cm^{-3}) of (*R*)-**3a** (0.5 μmol) or (*R*)-**5** (0. 5 μmol) and a dichloromethane solution (0.1 cm^{-3}) of EDC (0.55 μmol) or DCC (0.55 μmol). In the case of Ibuprofen, a dichloromethane solution (0.1 cm^{-3}) of 4-pyrrolidinopyridine (0.01 μmol) was also added to the solution. The total amount of the solution was adjusted to 2.0 cm^{-3}. The reaction was carried out in a vial tube. After the reaction, the solution was analyzed by HPLC. Aflatoxin B_2 (6 nmol) was used as an internal standard. The conditions in HPLC were the same as their respective HPLC analysis shown below. The reactivity was calculated on the basis of the fluorescence intensity (intensity number in the detector) of the product.

HPLC Analysis of Ibuprofen by (*R*)-3a. To a dichloromethane solution (0.1 cm^{-3}) of racemic Ibuprofen (0.1 μmol) were added a dichloromethane solution (0.5 cm^{-3}) of (*R*)-**3a** (0.5 μmol) and a dichloromethane solution (0.1 cm^{-3}) of DCC (0.55 μmol). Then, a dichloromethane solution (0.1 cm^{-3}) of 4-pyrrolidinopyridine (0.01 μmol) was added to the solution. The mixture was heated at 60 °C for 4 h in a vial tube. After the reaction, the solution was analyzed by HPLC (column: CAPCELLPAK C_{18} (4.6 mm × 150 mm); mobile phase: CH_3CN–50 mM KH_2PO_4 (80:20), flow rate: 0.8 cm^{-3} min^{-1}; excitation: 500 nm, detection: 550 nm) (1 M = 1 mol dm^{-3}).

HPLC Analysis of Alanine Methyl Ester by (*R*)-5. To a dichloromethane solution (0.1 cm^{-3}) of racemic alanine methyl es-

ter (0.1 μmol) were added a dichloromethane solution (0.5 cm^{-3}) of (*R*)-**5** (0.5 μmol) and a dichloromethane solution (0.1 cm^{-3}) of EDC (0.55 μmol). The total amount of the solution was adjusted to 2.0 cm^{-3}. The mixture was heated at 60 °C for 4 h in a vial tube. After the reaction, the solution was analyzed by HPLC (column: Mightysil RP-18 GP (4.6 mm × 150 mm); mobile phase: CH_3CN–10 mM KH_2PO_4 (42:58), flow rate: 0.8 cm^{-3} min^{-1}; excitation: 500 nm, detection: 550 nm).

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