Novel Chiral Fluorescent Labeling Reagent—4-Substituted 7*H*-benzo[*de*]benzoimidazo[2,1-*a*]isoquinolin-7-ones—

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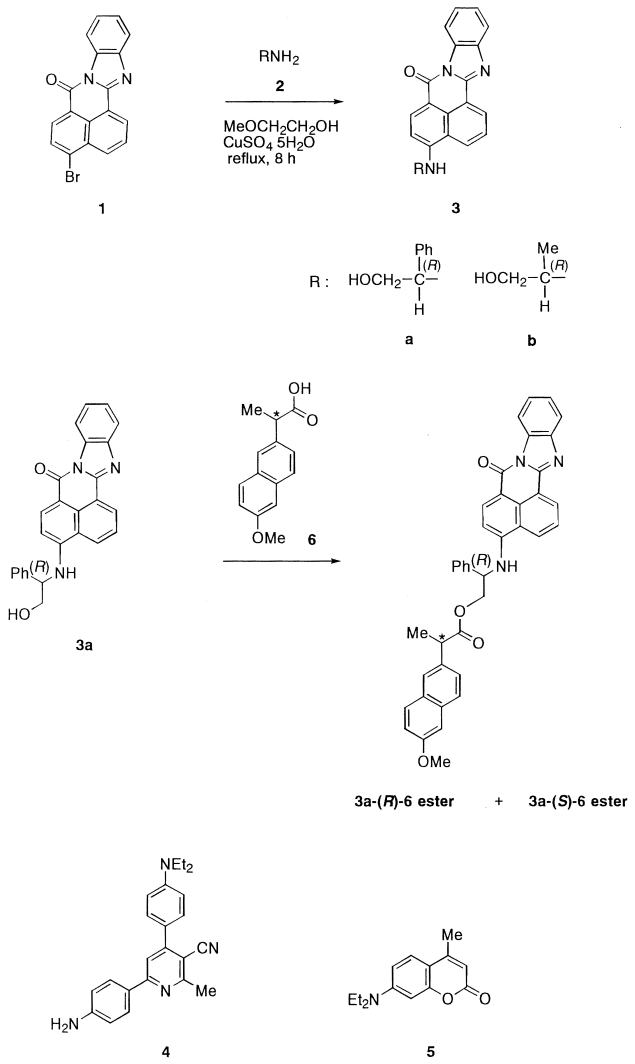
(*R*)-4-(2-Hydroxy-1-phenylethylamino)-7*H*-benzo[*de*]benzoimidazo[2,1-*a*]isoquinolin-7-one was used as a fluorescent labeling reagent for Naproxen. The excitation and emission maxima of this reagent were observed at λ 453 and 508 nm in acetonitrile, respectively. This reagent reacted with racemic Naproxen in the presence of dicyclohexylcarbodiimide to afford the corresponding diastereomers, which were nicely separated in HPLC. The detection limit (S/N $>$ 3) of Naproxen was ca. 5 fmol.

Bathochromicity in the excitation and emission maxima, high sensitivity, good solubility into the mobile phase, good stability during storage, good reactivity with target compounds, and good separation in HPLC are required for fluorescent labeling reagents.[1,2] 7*H*-Benzo[*de*]benzoimidazo[2,1-*a*]isoquinolin-7-ones are known in dye chemistry. Arient et al. have reported on the synthesis of 7*H*-benzo[*de*]benzoimidazo[2,1-*a*]isoquinolin-7-ones.[3–6] Peters et al. have also reported on the synthesis, color, and dyeing properties on synthetic fibres by heterocyclic compounds derived from 1,8-naphthalenedicarboxylic anhydride.[7–9] Qian et al. have studied the Stoke's shift of compounds derived from 1,8-naphthalenedicarboxylic anhydride.[10] Thus, 7*H*-benzo[*de*]benzoimidazo[2,1-*a*]isoquinolin-7-ones are interesting fluorescent compounds. In our previous paper, 4-(2-aminoethylamino)-7*H*-benzo[*de*]benzoimidazo[2,1-*a*]isoquinolin-7-one was reported to show excellent properties as a fluorescent labeling reagent.[11] When a chiral moiety is introduced into 7*H*-benzo[*de*]benzoimidazo[2,1-*a*]isoquinolin-7-ones, novel optically active and highly sensitive chiral labeling reagents can be obtained. We report here on the synthesis, properties, and application of 4-substituted 7*H*-benzo[*de*]benzoimidazo[2,1-*a*]isoquinolin-7-ones as chiral fluorescent labeling reagents.

Results and Discussion

The synthesis of 4-substituted 7*H*-benzo[*de*]benzoimidazo[2,1-*a*]isoquinolin-7-ones **3** is indicated in Scheme 1. 4-Bromo-7*H*-benzo[*de*]benzoimidazo[2,1-*a*]isoquinolin-7-one (**1**) was prepared by the reaction of 4-bromo-1,8-naphthalenedicarboxylic anhydride with *o*-phenylenediamine, as described in our previous paper.[11] The bromo derivative **1** reacted with chiral amines **2** in the presence of copper(II) sulfate pentahydrate to afford **3** in moderate yields.

The UV/vis absorption and fluorescence spectra of **3a** and **3b** are shown in Figs. 1 and 2, respectively. No remarkable difference in their UV/vis absorption and fluorescence spectra was observed between **3a** and **3b**. The shape of the absorption and fluorescence spectra indicates that the emitted light is scarcely absorbed by the compounds.



Scheme 1.

The fluorescence spectra of **3** are summarized in Table 1. The excitation and emission maxima of **3** were observed at λ

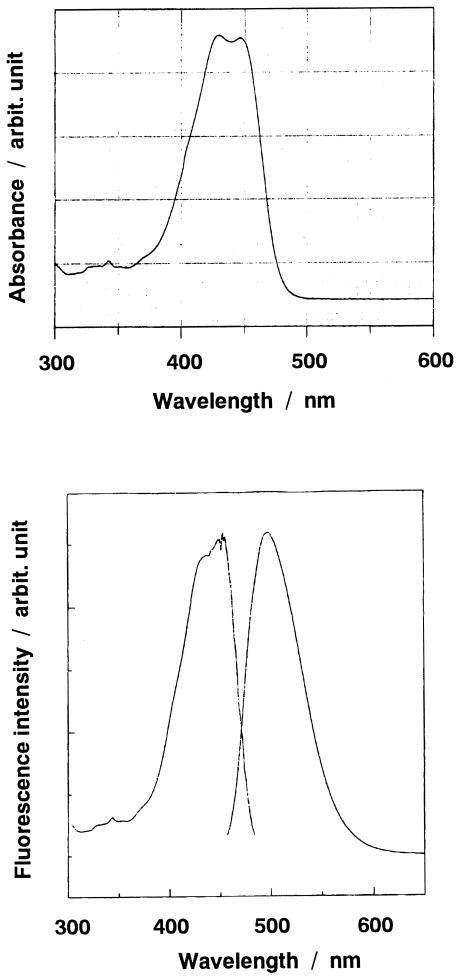


Fig. 1. UV/vis absorption and fluorescence spectra of **3a** in acetonitrile.

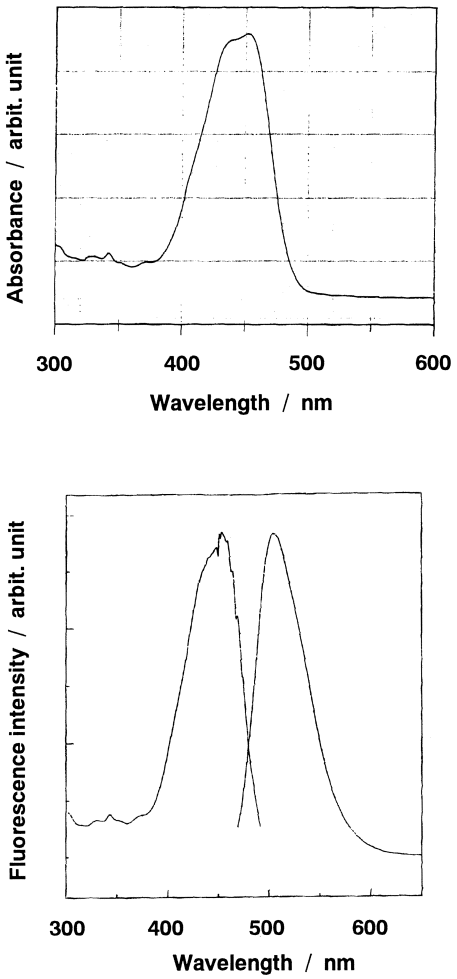


Fig. 2. UV/vis absorption and fluorescence spectra of **3b** in acetonitrile.

around 454 and 507 nm in acetonitrile, respectively, being more bathochromic than reference compounds, 6-(4-aminophenyl)-4-[4-(diethylamino)phenyl]-2-methylpyridine-3-carbonitrile (**4**) and 7-diethylamino-4-methylcoumarin (**5**). Compound **4** was a standard material throughout our study on the novel fluorescent labeling reagents.[12] Compound **5** is a typical fluorescent compound. The relative fluorescence intensities (RFI) of **3** in acetonitrile were higher than that of **4** and lower than **5**. No remarkable difference in RFI between **3a** and **3b** was observed.

Since the HPLC analysis of amino acids is usually performed in a reverse phase, the effect of water on RFI was examined. The result is shown in Fig. 3. The RFI of reference compounds **4** and **5** was drastically decreased by the addition of water. Since no remarkable difference in the UV/vis absorption spectra of **4** and **5** in 0–70% aqueous acetonitrile solutions was observed, the decrease of RFI in **4** and **5** could be attributed to the decrease in their fluorescence quantum yields in the solutions. Interestingly, the RFI of **3** did not decrease up to 70% aqueous acetonitrile. No remarkable difference in the λ_{max} and ε values was observed for **3** in 0–70% aqueous acetonitrile solutions. Both compounds **3a** and **3b** were sufficiently soluble in the acetonitrile used as fluorescent labeling reagents. However, compound **3a**, having a more bulky phenyl group adjacent to a chiral center, was ca. 5-times more soluble than **3b**. Therefore, compound **3a** seems to be better than **3b** used as a fluorescent labeling reagent.

Table 1. UV/vis Absorption, Fluorescence Spectra, and Optical Purity of **3**

Compd	λ_{max} (ε)[a]	λ_{ex}[b]	F_{max}[b]	RFI[b]	ee%[c]
	nm	nm	nm		
3a	447(30800)	453	508	297	>99
3b	450(23600)	455	506	279	>99
4[d]	353(53100)	353	508	100	—
5[e]	367(25300)	372	434	447	—

a) Measured in acetonitrile at 25°C. b) Measured in acetonitrile at 25°C (1×10^{-5} mol dm^{-3}). c) Analyzed by HPLC (CHIROBIOTEC V, hexane–ethanol = 80:20, excitation: 453 nm, detection: 508 nm, 0.8 mL min^{-1}). d) 6-(4-Aminophenyl)-4-[4-(diethylamino)phenyl)-2-methylpyridine-3-carbonitrile. e) 7-(Diethylamino)-4-methylcoumarin.

Naproxen (**6**) acts as an inhibitor for cyclooxygenase. The reaction of **3a** with Naproxen can produce the corresponding esters, **3a**-(*R*)-**6** and **3a**-(*S*)-**6** esters, as shown in Scheme 1. The reactivity of **3a** with **6** is summarized in Table 2. Though

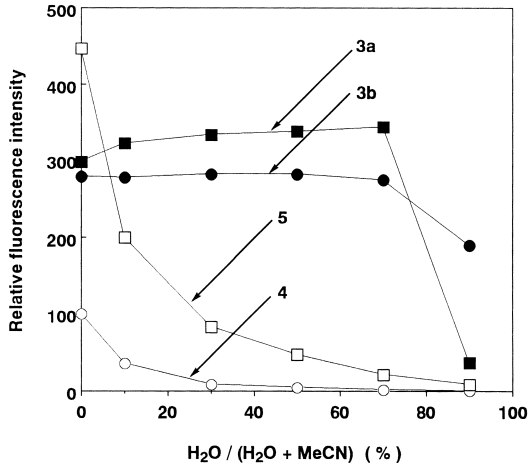


Fig. 3. Effect of water on relative fluorescence intensity.

Table 2. Reactivity of **3a** with Naproxen

Run	Additive	Time/h	Relative reactivity
1	DEPC[a)]	6	1
2	DPDS–TPP[b)]	6	2
3	DPPA[c)]	6	12
4	EDC[d)]	6	48
5	CMP–TEA[e)]	6	68
6	DCC[f)]	6	81
7	DCC[f)]	7	100
8	DCC[f)]	9	100

a) Diethyl phosphorocyanidate. b) 2,2′-Dipyridyl disulfide-triphenylphosphine. c) Diphenyl phosphorazidate. d) 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide. e) 2-Chloro-1-methylpyridinium iodide-triethylamine. f) Dicyclohexylcarbodiimide.

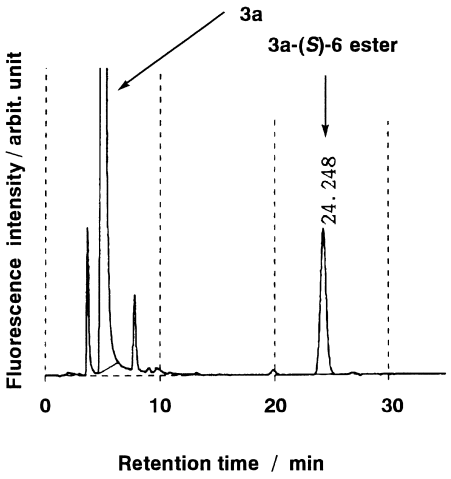


Fig. 4. HPLC analysis of labeled (*S*)-Naproxen by **3a**.

several catalysts were used for the reaction of **3a** with **6**, the highest yield was obtained when the reaction was carried out in the presence of dicyclohexylcarbodiimide (DCC) at 80 °C for 9 h.

HPLC analyses of the respective enantiomeric Naproxens reacted with **3a** are indicated in Figs. 4 and 5. The diastereomers derived from (*S*)- and (*R*)-Naproxens were observed at retention times of 24.248 and 26.708 min, respectively. The HPLC analysis of racemic Naproxens (**6**) by **3a** is also shown in Fig. 6. The labeled diastereomers derived from (*S*)- and (*R*)-Naproxens were observed at retention times of 24.260 and 26.879 min, respectively. The LCMS analysis of both the diastereomers showed their MH^+ ion peaks at *m*/*z* 618 together with their base peaks at *m*/*z* 185 formed by a cleavage between the carbonyl and methine carbons.

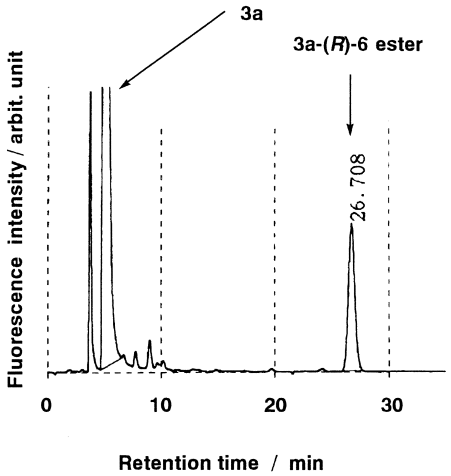


Fig. 5. HPLC analysis of labeled (*R*)-Naproxen by **3a**.

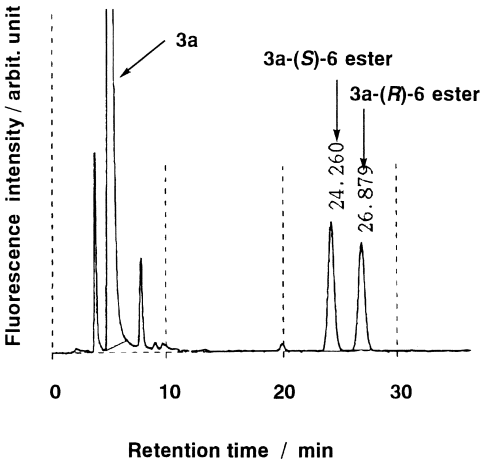


Fig. 6. HPLC analysis of labeled racemic Naproxen by **3a**.

Conclusion

We prepared 4-substituted 7*H*-benzo[*de*]benzoimidazo[2,1-*a*]isoquinolin-7-ones having a chiral center in a molecule. These compounds showed good properties as the fluorescent labeling reagent for carboxylic acids used in reverse-phase HPLC. Racemic Naproxens were nicely analyzed in HPLC by reacting with (*R*)-4-(2-hydroxy-1-phenylethylamino)-7*H*-benzo[*de*]benzoimidazo[2,1-*a*]isoquinolin-7-one. The detection limit (S/N $>$ 3) of Naproxen was ca. 5 fmol.

Experimental

Instruments. The melting points were measured with a Yanagimoto MP-S2 micro-melting-point apparatus. Fluorescence

spectra was measured with a Jasco FP-777 spectrometer. NMR spectra were taken on a Varian Inova 400 spectrometer. EIMS spectra were taken on a Shimadzu QP-1000 spectrometer. LCMS spectra were recorded on a Micromass Quatro II spectrometer. Liquid chromatography was performed with a Shimadzu LC-10AD instrument.

Materials. (*R*)-(−)-2-Amino-2-phenylethanol (**2a**), (*R*)-(−)-2-amino-1-propanol (**2b**), and (*S*)-2-(6-methoxy-2-naphthyl)propionic acid ((*S*)-Naproxen) were obtained from Wako Pure Chemical Industries, Ltd, ACROS Organics, and Sigma-Aldrich Co. Ltd., respectively. 7-Diethylamino-4-methylcoumarin (**5**), (±)-2-(6-methoxy-2-naphthyl)propionic acid (Naproxen, **6**), diethyl phosphorocyanidate (DEPC), 2,2′-dipyridyl disulfide-triphenylphosphine (DDDS–TPP), diphenyl phosphorazidate (DPPA), and 2-chloro-1-methylpyridinium iodide-triethylamine (CMP–TEA) were purchased from Tokyo Kasei Co., Ltd. 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide (EDC) and dicyclohexylcarbodiimide (DCC) were obtained from Nakalai Tesque, Inc. (*R*)-2-(6-Methoxy-2-naphthyl)propionic acid ((*R*)-Naproxen) was isolated from (±)-2-(6-methoxy-2-naphthyl)propionic acid (**6**) by preparative HPLC.

Synthesis of or (*R*)-4-(2-Hydroxy-1-phenylethylamino and 2-hydroxy-1-methylethylamino)-7*H*-benzo[*de*]benzoimidazo[2,1-*a*]isoquinolin-7-ones (3). To a 2-methoxyethanol solution (40 mL) of 4-bromo-7*H*-benzo[*de*]benzoimidazo[2,1-*a*]isoquinolin-7-one **1** (100 mg, 0.3 mmol) were added copper(II) sulfate pentahydrate (0.03 mmol, 7.5 mg) and an amine **2** (1.5 mmol). The mixture was refluxed for 8 h. After the reaction was completed, the product was extracted with chloroform (100 mL × 2), purified by column chromatography (SiO_2, $CHCl_3$ then $CHCl_3$–AcOEt (1:1)), and recrystallized from methanol. The physical and spectral data are given below.

(*R*)-4-(2-Hydroxy-1-phenylethylamino)-7*H*-benzo[*de*]benzoimidazo[2,1-*a*]isoquinolin-7-one (3a). Yield 35%; mp 265–266 °C; ^{1}H NMR ($CDCl_3$) δ 3.79–3.84 (m, 1H), 4.09–4.15 (m, 2H), 6.04 (d, J = 8.4 Hz, 1H), 6.26 (br s, 1H), 7.08–7.10 (m, 2H), 7.27–7.28 (m, 3H), 7.39–7.45 (m, 2H), 7.60 (t, J = 7.4 Hz, 1H), 7.94–7.96 (m, 1H), 8.00 (d, J = 8.2 Hz, 1H), 8.26 (d, J = 8.4 Hz, 1H), 8.65 (d, J = 7.4 Hz, 1H), 8.77 (d, J = 7.4 Hz, 1H); EIMS (70 eV) *m*/*z* (rel intensity) 405 (M^+; 20), 374 (100). Anal. Calcd for $C_{26}H_{19}N_3O_2$: C, 77.02; H, 4.72; N, 10.36%. Found: C, 76,98; H, 4.82; N, 10.57%. ORD (*c* 0.08288, $CHCl_3$, 25 °C) $[\alpha]_{584}$ 413°.

(*R*)-4-(2-Hydroxy-1-methylethylamino)-7*H*-benzo[*de*]benzoimidazo[2,1-*a*]isoquinolin-7-one (3b). Yield 37%; mp 263–265 °C; ^{1}H NMR ($CDCl_3$) δ 1.41 (d, J = 6.7 Hz, 3H), 3.70–3.84 (m, 1H), 3.96–3.99 (m, 2H), 5.66 (br s, 1H), 6.73 (d, J = 8.5 Hz, 1H), 7.46–7.49 (m, 2H), 7.59 (t, J = 8.2 Hz, 1H), 7.88–7.90 (m, 1H), 7.94 (d, J = 8.5 Hz, 1H), 8.55 (d, J = 8.2 Hz, 1H), 8.61–8.62 (m, 1H), 8.80 (d, J = 8.2 Hz, 1H); EIMS (70 eV) *m*/*z* (rel intensity) 343 (M^+; 46), 312 (100). Anal. Calcd for $C_{21}H_{17}N_3O_2$: C, 73.45; H, 4.99; N, 12.24%. Found: C, 73.18; H, 5.10; N, 12.31%. ORD did not measured due to low solubility in chloroform.

Reactivity of 3a with Naproxen (6). To a dichloromethane solution (1 mL) of racemic Naproxens (0.02 μmol) was added a dichloromethane solution (20 μL) of **3a** (0.1 μmol). To the mixture was added a dichloromethane solution (30 μL) of an additive (0.03 μmol). The reaction was carried out at 80 °C in a vial tube. The mixture was then analyzed by HPLC (column: Mightysil RP-18 GP (4.6 × 150 mm), mobile phase: MeCN–H_2O (67.5:32.5), 0.8 mL min^{-1}, detection: 508 nm (excitation: 453 nm).

HPLC Analysis of Enantiomeric and Racemic Naproxens. A dichloromethane solution of Naproxens (1 μg mL^{-1}) was prepared. After evaporating 10 μL of this solution, a dichloromethane solution (500 μL) of **3a** (0.05 μmol) and a dichloromethane solution (15 μL, 1 μmol mL^{-3}) of DCC (0.015 μmol) were added. To the mixture was added pyridine (10 μL). The mixture was reacted in a vial tube for 9 h at 80 °C. The solution was then analyzed by HPLC (column: Mightysil RP-18 GP (4.6 × 150 mm), mobile phase: MeCN–H_2O (67.5:32.5), 0.8 mL min^{-1}, detection: 508 nm (excitation: 453 nm).

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