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# Novel approach to determining the absolute configurations at the C3-positions of various types of sterols based on an induced circular dichroism

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ABSTRACT

Circular dichroism (CD) spectra of the 2,2′-binaphthyl ester derived from $\Delta^5$-sterols showed not bisignate CD but diagnostic CD bands at around 210 and 240 nm. These bands might be attributable to an interaction between an olefinic chromophore and a binaphthyl one. Various types of unsaturated sterols were thus derivatized followed by complete hydrogenation, to give saturated sterols. As a result, CD spectra of the binaphthyl derivatives of the saturated sterols showed bisignate curves centered at 240 nm (3*S*(β): positive chirality; 3*R*(α): negative one). This suggested a straightforward and practical method for discriminating the absolute stereogenic center at the C-3 positions of sterols based on an induced CD. This finding should contribute significantly to the analysis of metabolites of various types of sterols.


## 1. Introduction

Spectroscopic methods for elucidating absolute stereostructures, especially using nuclear magnetic resonance (NMR) and circular dichroism (CD), have advanced greatly in recent years. Among these methods, exciton-coupled circular dichroism (ECCD), a non-empirical approach to absolute stereochemical determination, has been applied to a wide variety of compounds [1–3]. Although its application has been limited mostly to compounds having two or more functional groups needed for exciton coupling, this method has also been extended to the analysis of molecules with a single functional group [4–11]. In the course of our stereochemical studies of chiral monofunctional molecules by the ECCD method, induced CD (ICD) was observed in dinitrodiphenic [7] and 2,2′-binaphthyl [8] esters of chiral secondary alcohols, indicating that the chirality of the substrates was effectively transferred to the biaryl chromophores. For 1,1′-binaphthyls, the relationship between the sign of the CD couplet and the dihedral angle between the two naphthalene chromophores was investigated in detail [12]. In contrast, the chiroptical properties of the 2,2′-binaphthyl chromophoric system have remained unexplored. We showed that the ICD of 2,2′-binaphthyl chromophores could be used to establish the absolute stereostructures of chiral mono-alcohols [8]. Moreover, we reported the successful application of the ICD of the binaphthyl chromophores in combination with molecular mechanics calculations, CONFLEX-MM2, to determine the absolute configurations of various types of natural products possessing a secondary alcohol [13]. Then we observed that the CD spectra of the 2,2′-binaphthyl esters (Fig. 1) derived from 3α- and 3β-cholestanols, which differ only in the configuration of the C-3 position, exhibited opposite exciton chirality from each other [8]. To our best knowledge, these compounds were discriminated only by complex formation with a dimeric porphyrin host [5]. Unfortunately, the CD spectra of di(1-naphthyl)acetic esters of epimeric sterols showed the same positive exciton Cotton effects, although there was a small difference between the amplitudes of their A values [4]. So far, the absolute configuration of steroids has been assigned by CD, modified Mosher's method, X-ray analysis, time-dependent density functional theory (TD-DFT), fluorescence-detected CD (FDCD), and so on [4,14–24]. Here we describe the successful application of a method to determine the absolute configuration at the C-3 positions of various sterols.

## 2. Experimental

Unless otherwise noted, the following procedures were adopted for characterization. The melting point (Mp) was determined by a

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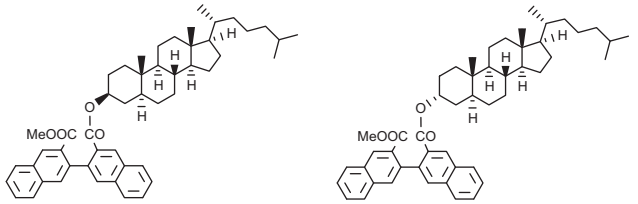



**Fig. 1.** 2,2′-Binaphthyl esters derived from 3β- and 3α-cholestanols.

Yanaco micro-hot-stage melting point apparatus and is uncorrected. $^1$H NMR spectra were recorded at 400 MHz in $CDCl_3$ with tetramethylsilane (TMS) as an internal reference (0.0 ppm). Chemical shifts are reported in ppm ($\delta$). Coupling constants $J$ are reported in Hz. The infrared spectra (IR) of samples in a $CHCl_3$ solution were recorded on a Shimadzu FTIR-8300 spectrometer. Peaks are reported in units of $cm^{-1}$ with the following relative intensities: br (broad), s (strong 67–100%), m (medium 33–67%), or w (weak 0–33%). Circular dichroism (CD) and ultraviolet (UV) spectra were taken on a JASCO J-725 spectrometer, and data were given in $\lambda_{ext}(\Delta\varepsilon)$(nm) and $\lambda_{max}(\varepsilon)$(nm). Mass spectra (MS) and high-resolution MS (HRMS) were recorded on a JEOL JMS-SX 102A QQ, Shimadzu GCMS-QP505, and JEOL JMS-GC-mate mass spectrometer. MS (EI) was recorded with an ionization voltage of 70 eV. MS (FAB) were recorded with nitrobenzyl alcohol (NBA) as a matrix. Data were reported in the form *m/e* (intensity relative to base = 100%). Column chromatography was carried out with silica gel (Wacogel C-200). Recycling high-performance liquid chromatography (RHPLC) was performed on a JAI LC-908 with a JAIGEL H column using $CHCl_3$ as a mobile phase. For thin-layer chromatography (TLC), Kieselgel 60 $F_{254}$ precoated silica gel plates (Merck) were used and spots were monitored under UV light (254 nm), then developed by spraying 10 % $H_2SO_4$ and/or 5% phosphomolybdic acid ethanol solution and heating the plate at 100 °C until coloration occurred. Preparative TLC (PTLC) was performed also with Kieselgel 60 $F_{254}$ plates (0.5 mm thick). Elemental analyses were performed by the Kyoto Pharmaceutical University. All spectral data were taken after purification with PTLC. Chemical purity was determined by HPLC (Shimadzu LC-2010C HT system; UV 254 nm, cosmosil C18AR-II 4.6 × 150 mm, 5 μm, MeOH in $H_2O$ 97.0%, 35 min.; flow rate 2.0 mL/min; 40 °C).

### 2.1. General derivatization procedure

To a solution of sterol (5–50 mg) and 3-cyanocarbonyl-3′-methoxycarbonyl-2,2′-binaphthalene **1** (1 mol equiv.) in acetonitrile (1–2 mL) or acetonitrile/dichloromethane (1–2 mL, 1/1 (v/v)) was added 4-(dimethylamino)pyridine (DMAP) (3 mmol equiv.), and the whole was stirred at room temperature for a specified time. After the removal of solvent in vacuo, the crude product was purified by silica gel column chromatography (eluent: *n*-hexane/ethyl acetate, 5/1) or preparative TLC (PTLC) to give the corresponding binaphthyl ester.

### 2.2. General catalytic hydrogenation procedure

To a solution of sterol (200 mg) in ethyl acetate (4 mL) was added 5% palladium-carbon (0.5 w/w). The whole was stirred at room temperature under a 0.5 MPa hydrogen atmosphere for a specified time. After the filtration of the palladium-carbon, the filtrate was concentrated. The crude product was purified by silica gel column chromatography (eluent: *n*-hexane/ethyl acetate, 5:1) to give the corresponding hydrogenated compound.

#### 2.2.1. (3S,5S,8R,9S,10S,13R,14S,17R)-10,13-dimethyl-17-[(2R)-5-methylhexan-2-yl]hexadecahydro-1H-cyclopenta[a]phenanthren-3-yl methyl 2,2′-binaphthalene-3,3′-dicarboxylate, 2b

Colorless gum; HPLC purity: 97.83%; $R_f$ (AcOEt/n-Hexane: 1/3) 0.67; $[\alpha]^{24}_D$ + 17.0° (c 0.0836, $CHCl_3$); UV(cyclohexane) $\lambda_{max}(\varepsilon)$ nm 243.0 (81 600), 339.0 (2 400); CD $\lambda_{ext}$ (nm)($\Delta\varepsilon$) (cyclohexane) 232.0 (−8.2), 239.9 (0), 245.6 (+8.0); IR (film, $CHCl_3$) Peaks ($cm^{-1}$) 2930 m, 2845 m, 1721 m, 1623 w, 1458 w, 1455 w, 1440 w, 1372 w, 1322 w, 1280 s, 1225 w, 1201 m, 1132 m, 1100 w, 1062 w, 997 w, 983 w, 950 w, 891 w; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ = 0.54 (m, 1H), 0.55 (s, 1.5H), 0.58 (s,1.5H), 0.62 (s,3H), 0.85 (d, $J$ = 6.4 Hz, 3H), 0.86 (d, $J$ = 6.4 Hz, 3H), 0.88 (d, $J$ = 6.4 Hz, 3H), 0.90–0.71 (m, 22H), 1.67–1.46 (m, 4H), 1.78 (m, 1H), 1.92 (m, 1H), 3.64 (s, 3H), 4.65 (m, 1H), 7.57 (m, 4H), 7.74 (s, 0.5H), 7.75 (s, 1H), 7.76 (s, 0.5H), 7.84 (d, $J$ = 8.3 Hz, 0.5H), 7.85 (d, $J$ = 8.3 Hz, 0.5H), 7.98 (d, $J$ = 8.3 Hz, 1H), 7.99 (d, $J$ = 8.3 Hz, 1H), 8.59 (s, 1H), 8.62 (s, 1H); MS EI *m/z*: 726 ($M^+$, 61), 370 (11), 357 (25), 356 (100), 339 (11), 312 (11), 311 (15), 297 (11), 295 (11), 281 (29), 280 (17); HRMS calcd for $C_{50}H_{62}O_4$: 726.4648, found 726.4653.

#### 2.2.2. (3R,5S,8R,9S,10S,13R,14S,17R)-10,13-dimethyl-17-[(2R)-5-methylhexan-2-yl]hexadecahydro-1H-cyclopenta[a]phenanthren-3-yl methyl 2,2′-binaphthalene-3,3′-dicarboxylate, 3b

Colorless gum; HPLC purity: 97.57%; $R_f$ (AcOEt/n-Hexane: 1/3) 0.64; $[\alpha]^{24}_D$ −13.7° (c 0.132, $CHCl_3$); UV(cyclohexane) $\lambda_{max}(\varepsilon)$ nm 244.5 (69 900), 339.0 (3 800); CD $\lambda_{ext}$ (nm)($\Delta\varepsilon$) (cyclohexane) 233.2 (+6.4), 240.0 (0), 246.8 (−4.5); IR (film, $CHCl_3$) Peaks ($cm^{-1}$) 3010 w, 2930 m, 2860 m, 1719 m, 1622 w, 1599 w, 1585 w, 1489 w, 1487 w, 1438 w, 1370 w, 1363 w, 1341 w, 1316 w, 1276 m, 1249 w, 1219 w, 1207 w, 1203 m, 1201 m, 1175 w, 1156 w, 1129 w, 1100 w, 1057 w, 998 w, 99 w, 970 w, 951 w, 928 w, 905 w, 889 w; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ = 0.32 (m, 1H), 0.44 (m, 1H), 0.58 (s, 6H), 0.62 (m,1H), 0.88 (d, $J$ = 6.4 Hz, 3H), 0.89 (d, $J$ = 6.4 Hz, 3H), 0.92 (d, $J$ = 6.8 Hz, 1.5H), 0.93 (d, $J$ = 6.8 Hz, 1.5H), 1.30–0.74 (m, 17H), 1.43–1.31 (m, 4H), 1.59–1.43 (m, 4H), 1.85 (m, 1H), 3.65 (s, 3H), 5.06 (m, 1H), 7.57 (m, 4H), 7.68 (s, 1H), 7.80 (s, 1H), 7.82 (s, 1H), 7.83 (s, 1H), 7.84 (d, $J$ = 8.3 Hz, 0.5H), 7.87 (d, $J$ = 8.3 Hz, 0.5H), 7.98 (d, $J$ = 8.3 Hz, 1H), 8.01 (d, $J$ = 8.3 Hz, 1H), 8.59 (s, 0.5H), 8.60 (s, 0.5H), 8.63 (s, 0.5H), 8.65 (s, 0.5H); MS EI *m/z*: 727 ($M^+$+1, 26), 726 ($M^+$, 47) 371 (18), 370 (49), 357 (55), 356 (100), 355 (16), 340 (12), 339 (38), 325 (18), 316 (16), 312 (19), 311 (27), 297 (19), 296 (19), 295 (25), 282 (12), 281 (56), 280 (32), 252 (19), 216 (11), 215 (21), 107 (12), 95 (11), 93 (12), 81 (10); HRMS calcd for $C_{50}H_{62}O_4$: 726.4648, found 726.4643.

#### 2.2.3. (3α,5β,7α,12α)-7,12-dihydroxy-24-methoxy-24-oxocholan-3-yl methyl 2,2′-binaphthalene-3,3′-dicarboxylate, 4b

Colorless gum; HPLC purity: 98.27%; $R_f$ (AcOEt/n-Hexane: 1/2) 0.49. UV(cyclohexane) $\lambda_{max}(\varepsilon)$ nm 243.6 (94 800), 338.6 (3 100); CD $\lambda_{ext}$ (nm)($\Delta\varepsilon$) (cyclohexane) 229.2 (+25.2), 238.6 (0), 247.2 (−18.8); IR (film, $CHCl_3$) Peaks ($cm^{-1}$) 2949 m, 2872 w, 2361 w, 2341 w, 1722 m, 1445 s, 1329 m, 1281 s, 1198 m, 1136 w, 1065 w, 895 w, 681 w; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ = 0.69 (s, 2H), 0.80–2.10 (m, 29H), 2.23–2.48 (m, 2H), 3.60 (S, 1.8H), 3.61 (s, 1.2H), 3.62 (s, 0.6H), 3.68 (s, 1.2H), 3.69 (s, 1.8H), 3.80 (s, 0.4H), 3.96 (s, 0.4H), 4.03 (s, 0.6H), 4.54–4.59 (m, 1H), 7.52–7.63 (m, 4H), 7.72–7.75 (m, 2H), 7.83–7.88 (m, 2H), 8.00–8.11 (m, 2H), 8.63 (d, $J$ = 8.24 Hz, 1H), 8.65 (s, 0.5H), 8.74(s, 0.5H); MS FAB (+) *m/z*: 783 ($M^+$+Na, 100); HRMS calcd for $C_{48}H_{56}O_8Na$ ($M^+$+Na): 783.3873, found 783.3870.

#### 2.2.4. (3α,5β,7α)-7-hydroxy-24-methoxy-24-oxocholan-3-yl methyl 2,2′-binaphthalene-3,3′-dicarboxylate, 5b

Colorless gum; HPLC purity: 99.67%; $R_f$ (AcOEt/*n*-Hexane: 1/2) 0.71. UV (cyclohexane) $\lambda_{max}(\varepsilon)$ nm 243.2 (99 700), 338.8 (3 100);

CD $\lambda_{ext}$ (nm)($\Delta\varepsilon$) (cyclohexane) 229.4 (+67.6), 238.6 (0), 246.0 (−50.7); IR (film, $CHCl_3$) Peaks ($cm^{-1}$) 2951 m, 2870 w, 1722 m, 1447 w, 1329 w, 1281 s, 1198 m, 1136 w, 1065 w, 986 w, 895 w, 687 w; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ = 0.65 (s, 1H), 0.68 (s, 2H), 0.81–2.01 (m, 29H), 2.24–2.49 (m, 2H), 3.59 (S, 1.8H), 3.63 (s, 1.2H), 3.68 (s, 1.2H), 3.69 (s, 1.8H), 3.91 (s, 0.4H), 4.02 (s, 0.6H), 4.63–4.73 (m, 1H), 7.53–7.62 (m, 4H), 7.71–7.77 (m, 2H), 7.83–7.89 (m, 2H), 8.04–8.05 (m, 2H), 8.63 (d, *J* = 7.80 Hz, 1H), 8.65 (s, 0.5H), 8.77 (s, 0.5H); MS FAB (+) *m/z*: 767 ($M^+$+Na, 100); HRMS calcd for $C_{48}H_{56}O_7Na$ ($M^+$+Na): 767.3924, found 767.3915.

### 2.2.5. (3α,5β)-24-methoxy-24-oxocholan-3-yl methyl 2,2′-binaphthalene-3,3′-dicarboxylate, 6b

Colorless gum; HPLC purity: 99.45%; $R_f$ (AcOEt/*n*-Hexane: 1/3) 0.69. UV (cyclohexane) $\lambda_{max}(\varepsilon)$ nm 242.6 (103 300), 338.0 (2 900); CD $\lambda_{ext}$ (nm)($\Delta\varepsilon$) (cyclohexane) 231.6 (+12.2), 240.8 (0), 245.8 (−8.8); IR (film, $CHCl_3$) Peaks ($cm^{-1}$) 2949 m, 2868 w, 1722 m, 1445 w, 1281 s, 1225 m, 1198 m, 1136 w, 1065 w, 895 w, 669 w; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ = 0.61 (d, *J* = 5.04 Hz, 3H), 0.77–1.98 (m, 30H), 2.24–2.46 (m, 2H), 3.64 (S, 3H), 3.69 (s, 3H), 4.64–4.73 (m, 1H), 7.52–7.62 (m, 4H), 7.72–7.88 (m, 4H), 7.97–8.02 (m, 2H), 8.62–8.65 (m, 2H); MS FAB (+) *m/z*: 751 ($M^+$+Na, 100); HRMS calcd for $C_{48}H_{56}O_6Na$ ($M^+$+Na): 751.3975, found 751.3979.

### 2.2.6. (3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R)-5-methylhexan-2-yl]-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl methyl 2,2′-binaphthalene-3,3′-dicarboxylate, 7b

Colorless gum; HPLC purity: 97.93%; $R_f$ (AcOEt/*n*-Hexane: 1/4) 0.77. UV (cyclohexane) $\lambda_{max}(\varepsilon)$ nm 242.2 (98 600), 338.2 (2 700); CD $\lambda_{ext}$ (nm)($\Delta\varepsilon$) (cyclohexane) 220.4 (+6.9), 242.6 (+6.9); IR (film, $CHCl_3$) Peaks ($cm^{-1}$) 2951 w, 2871 w, 1720 w, 1283 w, 1227 s, 1207 s, 1136 w, 1065 w, 895 w, 673 s; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ = 0.64–2.18 (m, 41H), 3.65 (s, 3H), 4.54–4.59 (m, 1H), 5.23 (d, *J* = 5.04 Hz, 1H), 7.53–7.62 (m, 4H), 7.75–7.77 (m, 2H), 7.84–7.87 (m, 2H), 7.97–8.02 (m, 2H), 8.60 (s, 1H), 8.62 (s, 1H); MS FAB (+) *m/z*: 747 ($M^+$+Na, 100); HRMS calcd for $C_{50}H_{60}O_4Na$ ($M^+$+Na): 747.4389, found 747.4396.

### 2.2.7. Methyl (3β)-stigmast-5-en-3-yl 2,2′-binaphthalene-3,3′-dicarboxylate, 8b

Colorless gum; HPLC purity: 98.95%; $R_f$ (AcOEt/*n*-Hexane: 1/3) 0.88. UV (cyclohexane) $\lambda_{max}(\varepsilon)$ nm 242.4 (83 300), 325.8 (4 000); CD $\lambda_{ext}$ (nm)($\Delta\varepsilon$) (cyclohexane) 221.2 (+5.4), 244.2 (+6.4); IR (film, $CHCl_3$) Peaks ($cm^{-1}$) 2936 m, 2855 m, 1720 s, 1445 w, 1283 s, 1207 m, 1136 m, 1063 m, 895 w, 683 m; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ = 0.64–2.17 (m, 47H), 3.64 (s, 3H), 4.54–4.59 (m, 1H), 5.23 (dd, *J* = 5.04 Hz, 1H), 7.53–7.62 (m, 4H), 7.75–7.77 (m, 2H), 7.84–7.86 (m, 2H), 7.97–8.00 (m, 2H), 8.60 (s, 1H), 8.62 (s, 1H); MS FAB (+) *m/z*: 775 ($M^+$+Na, 100); HRMS calcd for $C_{52}H_{64}O_4Na$ ($M^+$+Na): 775.4702, found 775.4708.

### 2.2.8. Methyl (3β)-spirost-5-en-3-yl 2,2′-binaphthalene-3,3′-dicarboxylate, 9b

Colorless gum; HPLC purity: 98.78%; $R_f$ (AcOEt/*n*-Hexane: 1/3) 0.69. UV (cyclohexane) $\lambda_{max}(\varepsilon)$ nm 242.4 (84 700), 326.4 (5 000); CD $\lambda_{ext}$ (nm)($\Delta\varepsilon$) (cyclohexane) 222.6 (+7.2), 238.6 (+6.3); IR (film, $CHCl_3$) Peaks ($cm^{-1}$) 2909 m, 2853 m, 1720 s, 1454 m, 1283 s, 1136 m, 1063 m, 980 m, 897 m, 685 w; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ = 0.76–2.19 (m, 36H), 3.36 (t, *J* = 5.50 Hz, 1H), 3.44–3.48 (m, 1H), 3.64 (s, 3H), 4.38 (q, *J* = 3.68 Hz, 1H), 4.52–4.60 (m, 1H), 5.23 (m, 1H), 7.53–7.62 (m, 4H), 7.75 (s, 2H), 7.84–7.86 (m, 2H), 7.97–8.00 (m, 2H), 8.60 (s, 1H), 8.62 (s, 1H); MS FAB (+) *m/z*: 775 ($M^+$+Na, 100); HRMS calcd for $C_{50}H_{56}O_6Na$ ($M^+$+Na): 775.3975, found 775.3978.

### 2.2.9. Methyl (3β,22E)-stigmasta-5,22-dien-3-yl 2,2′-binaphthalene-3,3′-dicarboxylate, 10b

Colorless gum; HPLC purity: 98.92%; $R_f$ (AcOEt/*n*-Hexane: 1/3) 0.83. UV (cyclohexane) $\lambda_{max}(\varepsilon)$ nm 242.4 (77 800), 338.4 (3 800); CD $\lambda_{ext}$ (nm)($\Delta\varepsilon$) (cyclohexane) 220.2 (+5.2), 243.6 (+6.4); IR (film, $CHCl_3$) Peaks ($cm^{-1}$) 2955 s, 2870 m, 1720 s, 1445 m, 1283 s, 1198 m, 1136 m, 1063 m, 974 w, 895 w, 692 w; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ = 0.64–2.18 (m, 43H), 3.64 (s, 3H), 4.54–4.59 (m, 1H), 4.97–5.03 (m, 1H), 5.11–5.25 (m, 2H), 7.53–7.62 (m, 4H), 7.75–7.76 (m, 2H), 7.84–7.86 (m, 2H), 7.97–8.01 (m, 2H), 8.60 (s, 1H), 8.62 (s, 1H); MS FAB (+) *m/z*: 773 ($M^+$+Na, 100); HRMS calcd for $C_{52}H_{62}O_4Na$ ($M^+$+Na): 773.4546, found 773.4543.

### 2.2.10. (3β)-cholesta-5,24-dien-3-yl methyl 2,2′-binaphthalene-3,3′-dicarboxylate, 11b

Colorless gum; HPLC purity: 99.50%; $R_f$ (AcOEt/*n*-Hexane: 1/4) 0.71. UV (cyclohexane) $\lambda_{max}(\varepsilon)$ nm 242.4 (132 100), 338.0 (4 000); CD $\lambda_{ext}$ (nm)($\Delta\varepsilon$) (cyclohexane) 220.4 (+6.6), 240.4 (+6.3); IR (film, $CHCl_3$) Peaks ($cm^{-1}$) 2945 m, 2855 w, 2341 w, 1720 m, 1445 w, 1283 s, 1196 w, 1136 w, 1065 w, 895 w, 781 m, 669 m; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ = 0.65–2.04 (m, 40H), 3.65 (s, 3H), 4.52–4.60 (m, 1H), 5.08 (t, *J* = 6.87 Hz, 1H), 5.21–5.26 (m, 1H), 7.53–7.62 (m, 4H), 7.75–7.76 (m, 2H), 7.84–7.86 (m, 2H), 7.97–8.01 (m, 2H), 8.60 (s, 1H), 8.62(s, 1H); MS FAB (+) *m/z*: 745 ($M^+$+Na, 100); HRMS calcd for $C_{50}H_{58}O_4Na$ ($M^+$+Na): 745.4233, found 745.4237.

### 2.2.11. (3β,5α)-4,4-dimethylcholesta-8,24-dien-3-yl methyl 2,2′-binaphthalene-3,3′-dicarboxylate, 12b

Colorless gum; HPLC purity: 98.96%; $R_f$ (AcOEt/*n*-Hexane: 1/3) 0.86. UV (cyclohexane) $\lambda_{max}(\varepsilon)$ nm 243.2 (55 800), 338.8 (4 300); CD $\lambda_{ext}$ (nm)($\Delta\varepsilon$) (cyclohexane) 223.4 (−15.9), 232.4 (0), 241.2 (+10.5); IR (film, $CHCl_3$) Peaks ($cm^{-1}$) 2930 w, 2341 w, 1720 w, 1281 w, 1224 m, 1202 m, 1065 w, 681 m; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ = 0.65–2.01 (m, 45H), 3.64 (s, 3H), 4.50–4.58 (m, 1H), 5.06–5.12 (m, 1H), 7.54–7.61 (m, 4H), 7.73–7.85 (m, 4H), 7.96–8.00 (m, 2H), 8.55–8.63 (m, 2H); MS FAB (+) *m/z*: 787 ($M^+$+Na, 100); HRMS calcd for $C_{53}H_{64}O_4Na$ ($M^+$+Na): 787.4702, found 787.4700.

### 2.2.12. Methyl (3β)-olean-12-en-3-yl 2,2′-binaphthalene-3,3′-dicarboxylate, 13b

Colorless gum; HPLC purity: 97.51%; $R_f$ (AcOEt/*n*-Hexane: 1/4) 0.77. UV (cyclohexane) $\lambda_{max}(\varepsilon)$ nm 243.2 (116 700), 338.2 (4 300); CD $\lambda_{ext}$ (nm)($\Delta\varepsilon$) (cyclohexane) 224.0 (−21.5), 233.6 (0), 247.6 (+15.0); IR (film, $CHCl_3$) Peaks ($cm^{-1}$) 2927 w, 2854 w, 1720 w, 1601 w, 1281 w, 1200 m, 1064 w, 704 m, 681 m, 664 m; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ = 0.77–2.20 (m, 47H), 3.63 (s, 3H), 4.50–4.61 (m, 1H), 5.13–5.17 (m, 1H), 7.50–7.61 (m, 4H), 7.74–7.85 (m, 4H), 7.96–8.01 (m, 2H), 8.55–8.63 (m, 2H); MS FAB (+) *m/z*: 787 ($M^+$+Na, 100); HRMS calcd for $C_{53}H_{64}O_4Na$ ($M^+$+Na): 787.4702, found 787.4698.

### 2.2.13. Methyl (3β,5α)-stigmastan-3-yl 2,2′-binaphthalene-3,3′-dicarboxylate, 14b

Colorless gum; HPLC purity: 99.05%; $R_f$ (AcOEt/*n*-Hexane: 1/4) 0.80. UV (cyclohexane) $\lambda_{max}(\varepsilon)$ nm 242.0 (107 300), 326.4 (3 600); CD $\lambda_{ext}$ (nm)($\Delta\varepsilon$) (cyclohexane) 232.2 (−4.2), 240.0 (0), 245.6 (+7.4); IR (film, $CHCl_3$) Peaks ($cm^{-1}$) 2936 m, 2870 m, 1720 s, 1445 m, 1281 s, 1198 m, 1136 m, 1065 w, 895 w, 681 m; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ = 0.55–1.93 (m, 50H), 3.63 (s, 3H), 4.62–4.67 (m, 1H), 7.53–7.61 (m, 4H), 7.75–7.74 (m, 2H), 7.83–7.85 (m, 2H), 7.97–8.00 (m, 2H), 8.58 (s, 1H), 8.62(s, 1H); MS FAB (+) *m/z*: 777 ($M^+$+Na, 100); HRMS calcd for $C_{52}H_{66}O_4Na$ ($M^+$+Na): 777.4859, found 777.4854.

*2.2.14. Methyl (3β,5α)-spirostan-3-yl 2,2′-binaphthalene-3,3′-dicarboxylate, 15b*

Colorless gum; HPLC purity: 98.78%; $R_f$ (AcOEt/*n*-Hexane: 1/4) 0.63. UV (cyclohexane) $\lambda_{max}(\varepsilon)$ nm 242.2 (110 000), 337.8 (3 600); CD $\lambda_{ext}$ (nm)($\Delta\varepsilon$) (cyclohexane) 232.2 (−4.2), 239.2 (0), 245.2 (+5.5); IR (film, $CHCl_3$) Peaks ($cm^{-1}$) 2930 s, 2851 m, 1720 s, 1454 m, 1281 s, 1196 m, 1134 s, 1063 s, 980 s, 897 w, 673 w; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ = 0.57–1.94 (m, 39H), 3.36 (t, *J* = 5.50 Hz, 1H), 3.44–3.49 (m, 1H), 3.64 (s, 3H), 4.36 (q, *J* = 3.90 Hz, 1H), 4.60–4.69 (m, 1H), 7.52–7.63 (m, 4H), 7.74–7.77 (m, 2H), 7.84–7.87 (m, 2H), 7.97–8.02 (m, 2H), 8.58 (s, 1H), 8.62(s, 1H); MS FAB (+) *m/z*: 777 ($M^+$+Na, 100); HRMS calcd for $C_{50}H_{58}O_6Na$ ($M^+$+Na): 777.4131, found 777.4127.

*2.2.15. Methyl (3β,5α)-stigmastan-3-yl 2,2′-binaphthalene-3,3′-dicarboxylate, 16b*

Colorless gum; HPLC purity: 99.55%; $R_f$ (AcOEt/*n*-Hexane: 1/4) 0.83. UV(cyclohexane) $\lambda_{max}(\varepsilon)$ nm 242.0 (120 500), 337.6 (4 500); CD $\lambda_{ext}$ (nm)($\Delta\varepsilon$) (cyclohexane) 232.2 (−8.2), 240.0 (0), 245.6 (+8.1); IR (film, $CHCl_3$) Peaks ($cm^{-1}$) 2936 m, 2870 m, 1720 s, 1445 w, 1281 s, 1196 w, 1136 w, 1065 w, 895 w, 677 m, 664; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ = 0.55–1.93 (m, 50H), 3.64 (s, 3H), 4.62–4.68 (m, 1H), 7.53–7.62 (m, 4H), 7.74–7.75 (m, 2H), 7.84–7.86 (m, 2H), 7.95–8.00 (m, 2H), 8.59 (s, 1H), 8.62(s, 1H); MS FAB (+) *m/z*: 777 ($M^+$+Na, 100); HRMS calcd for $C_{52}H_{66}O_4Na$ ($M^+$+Na): 777.4859, found 777.4855.

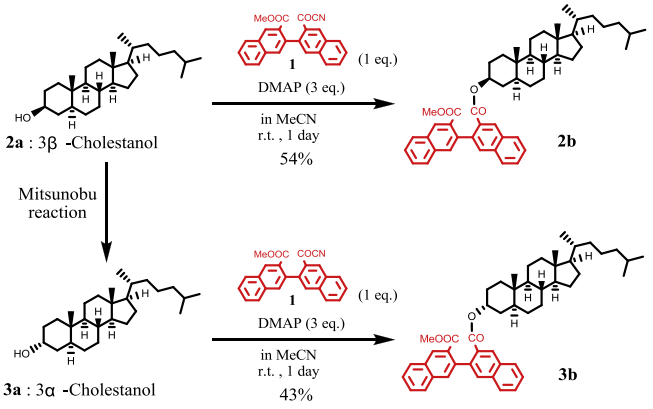
*2.2.16. (3β,5α)-4,4-dimethylcholest-8-en-3-yl methyl 2,2′-binaphthalene-3,3′-dicarboxylate, 17b*

Colorless gum; HPLC purity: 99.21%; $R_f$ (AcOEt/*n*-Hexane: 1/4) 0.77. UV (cyclohexane) $\lambda_{max}(\varepsilon)$ nm 242.8 (84 000), 337.6 (3 800); CD $\lambda_{ext}$ (nm)($\Delta\varepsilon$) (cyclohexane) 223.2 (−22.4), 233.6 (0), 247.6 (+15.0); IR (film, $CHCl_3$) Peaks ($cm^{-1}$) 2932 m, 2872 m, 1720 s, 1445 w, 1281 s, 1198 w, 1136 m, 1065 m, 968 w, 894 w, 712 w, 685 w; $^1H$ NMR (400 MHz, $CDCl_3$) $\delta$ = 0.42–1.99 (m, 48H), 3.63(s, 3H), 4.56 (dt, *J* = 4.12 Hz, 1H), 7.52–7.61 (m, 4H), 7.73–7.85 (m, 4H), 7.96–8.00 (m, 2H), 8.55–8.63 (m, 2H); MS FAB (+) *m/z*: 789 ($M^+$+Na, 100); HRMS calcd for $C_{53}H_{66}O_4Na$ ($M^+$+Na): 789.4859, found 789.4856.

## 3. Results and discussion

First, we investigated a commercially available 3β-cholestanol that is a dihydro derivative of cholesterol. 3β-Cholestanol was reacted with the chromophoric binaphthyl reagent **1** in the presence of DMAP in acetonitrile as a solvent at room temperature to give the corresponding 2,2′-binaphthyl ester **2b** [8] in 54% yield (Scheme 1). On the other hand, 3α-cholestanol [25] was prepared using the Mitsunobu reaction from 3β-cholestanol in two steps, and converted to the 2,2′-binaphthyl ester **3b** [8] in 43% yield under the same derivatization conditions. In these derivatizations, moderate yields were ascribed to the recovery of starting materials.

The solvent effect on the CD spectrum for **2b** was investigated (Fig. 2). Although the amplitude of the CD spectrum was changed depending upon the solvent used, the shape is identical. It is difficult to discuss the origin of the solvent difference in the amplitude in the current state. As seen in Fig. 2, cyclohexane is suitable to measure a CD spectrum of the compounds, and thus is used in the following experiments unless otherwise notified. CD spectra of 2,2′-binaphthyl ester **2b** and **3b** were shown in Fig. 3. The CD spectrum of the 2,2′-binaphthyl ester **2b**, which has the *S*-configuration at the C-3 position, showed bisignate curves centered at 240 nm, the UV absorption maxima, showing a positive exciton chirality. On the other hand, the CD spectrum of the 2,2′-binaphthyl ester **3b**, which has an *R*-configuration at the C-3 position, showed negative exciton chirality in opposition to that of **2b**. This fact definitely indicates that only the stereogenic center at C-3 affects the CD spectrum of the binaphthyl-ester derivatives of sterols through the transfer of stereogenic information, even though **2b** and **3b** were diastereomers to each other.




**Scheme 1.** Preparation of 3β-cholestanol derivative **2b** and 3α-cholestanol derivative **3b**.

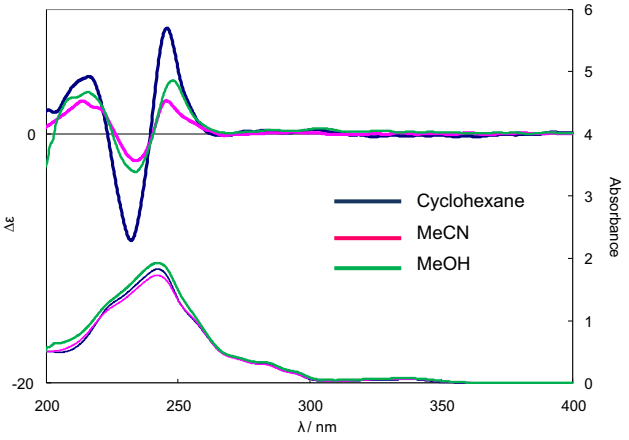



**Fig. 2.** CD and UV spectra of 3β-cholestanol derivative **2b** using various solvents.

Next, we applied this method to other sterols. Each sterol **4a**–**13a**, shown in Fig. 3, was derivatized with chromophoric reagent **1** followed by measurement of the CD spectrum. The results are summarized in Table 1. The epsilon value in UV and CD spectra seems to be influenced from the chemical structure attached to the naphthoate group. This may be caused by the change of the magnitude of transition moment depending upon the torsion angle between naphthalene groups as seen in the biaryl series [12].

As compiled in Table 1, there were two types of binaphthyl compounds, each showing a different pattern of the CD spectrum. One has a clear bisignate couplet at around 240 nm (type A), while the other does not (type B). The typical CD spectra of types A and B are shown in Fig. 4. For type A, one can determine the absolute configuration at C-3 by referring to the exciton chirality. For type B, however, the ECCD method is not applicable because the bisignate feature of the CD spectrum is lost. The CD spectrum of type A is seen in lanosterol derivative **12b** and β-amyrin derivative **13b**.

On the other hand, the CD spectrum of type B showed diagnostically two CD bands at around 210 and 240 nm, as shown in Fig. 5. The CD spectrum of type B is seen in sterols that have an olefine at the C-5 position ($\Delta^5$) such as cholesterol **7a**, sitosterol **8a**,

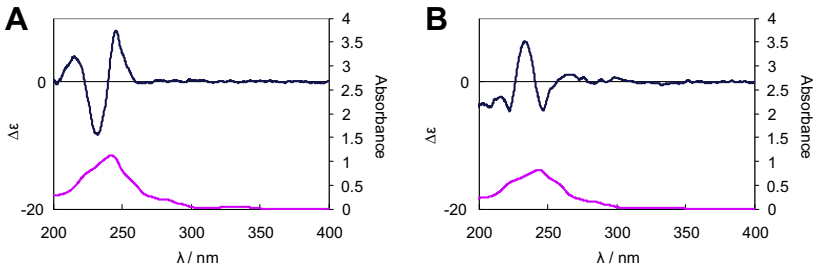



**Fig. 3.** CD and UV spectra of 3β-cholestanol derivative **2b** (A) and 3α-cholestanol derivative **3b** (B) in cyclohexane.

**Table 1**
UV and CD data of the binaphthyl esters derived from various sterols.

| Group | Binaphthyl esters[a] | Yield (%)[b] | UV$\lambda_{max}$, (nm)[c] | CD$\lambda_{ext}$ (As), (nm)[c] | Exciton chirality | Absolute config. at C3 |
|---|---|---|---|---|---|---|
| Saturated-Series | 2b | 54 | 243.0 (81600) 339.0 (2400) | 233.3 (−6.1) 238.2 (0) 247.0 (+9.3) | (+) | S |
| | 3b | 43 | 244.5 (69900) 339.0 (3800) | 232.9 (+8.3) 242.2 (0) 247.6 (−2.8) | (−) | R |
| | 4b | 89 | 243.6 (94800) 338.6 (3100) | 229.2 (+25.2) 238.6 (0) 247.2 (−18.8) | (−) | R |
| | 5b | 87 | 243.2 (99700) 338.8 (3100) | 229.4 (+67.6) 238.6 (0) 246.0 (−50.7) | (−) | R |
| | 6b | 46 | 242.6 (103300) 338.0 (2900) | 231.6 (+12.2) 240.8 (0) 245.8 (−8.8) | (−) | R |
| $\Delta^5$-series | 7b | 96 | 242.2 (98600) 338.2 (2700) | 220.4 (+6.9) 242.6 (+6.9) | – | S |
| | 8b | 25 | 242.4 (83300) 325.8 (4000) | 221.2 (+5.4) 244.2 (+6.4) | – | S |
| | 9b | 30 | 242.4 (84700) 326.4 (5000) | 222.6 (+7.2) 238.6 (+6.3) | – | S |
| | 14b | 24 | 242.0 (107300) 326.4 (3600) | 232.2 (−4.2) 240.0 (0) 245.6 (+7.4) | (+) | S |
| | 15b | 50 | 242.2 (110000) 337.8 (3600) | 232.2 (−4.2) 239.2 (0) 245.2 (+5.5) | (+) | S |
| $\Delta^{5,22}$-series | 10b | 14 | 242.4 (77800) 338.4 (3800) | 220.2 (+5.2) 243.6 (+6.4) | – | S |
| | 16b | 39 | 242.0 (120500) 337.6 (4500) | 232.2 (−8.2) 240.0 (0) 245.6 (+8.1) | (+) | S |
| $\Delta^{5,24}$-series | 11b | 43 | 242.4 (132100) 338.0 (4000) | 220.4 (+6.6) 240.4 (+6.3) | – | S |
| $\Delta^{8,24}$-series | 12b | 4 | 243.2 (55800) 338.8 (2800) | 223.4 (−15.9) 232.4 (0) 241.2 (+10.5) | (+) | S |
| | 17b | 17 | 242.8 (84000) 337.6 (3800) | 223.2 (−22.4) 231.6 (0) 239.2 (+17.1) | (+) | S |
| $\Delta^{12}$-series | 13b | 9 | 243.2 (116700) 338.2 (4300) | 224.0 (−21.5) 233.6 (0) 247.6 (+15.0) | (+) | S |

[a] Unless otherwise noted, 3.0 equiv. of DMAP was added to a solution of sterol and 1.0 equiv. of the reagent **1** in MeCN or MeCN–$CH_2Cl_2$.
[b] Conversion yield was 100% despite the low isolated yield in all cases examined.
[c] Measured in cyclohexane.

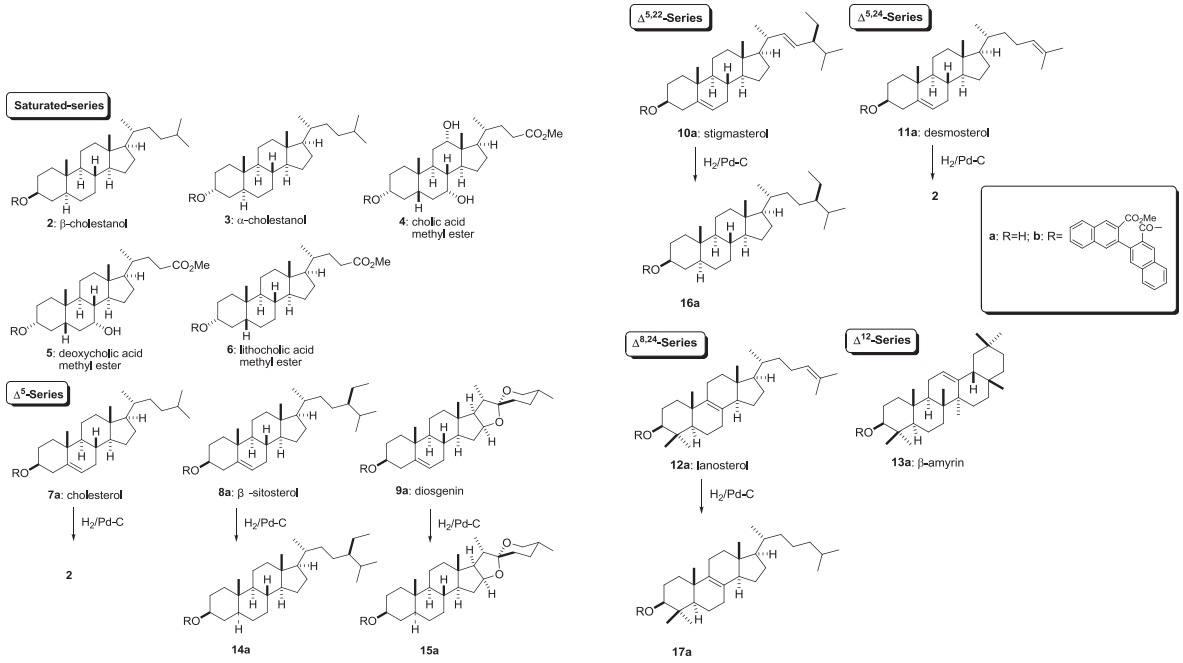



**Fig. 4.** Various sterols and their hydrogenated ones.

diosgenin **9a**, stigmasterol **10a** and demosterol **11a**. Interestingly, the cholesterol derivatives **2b** and **7b** show totally different CD spectra, even though their chemical structures are the same except for the olefin at C-5. It is possible to observe a CD signal arising

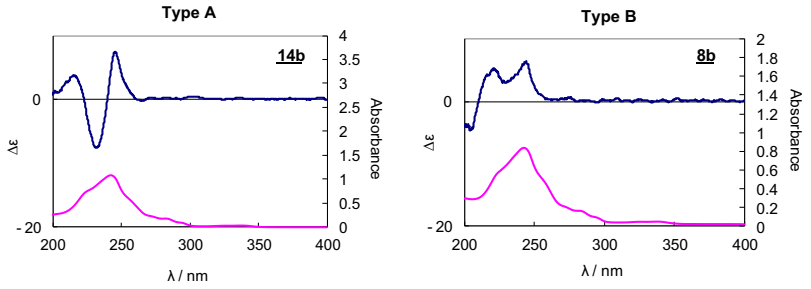



**Fig. 5.** CD spectra of type A (**14b**) (left) and type B(**8b**) (right).

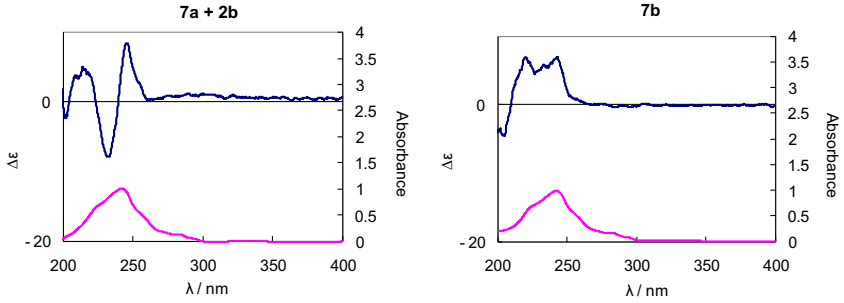
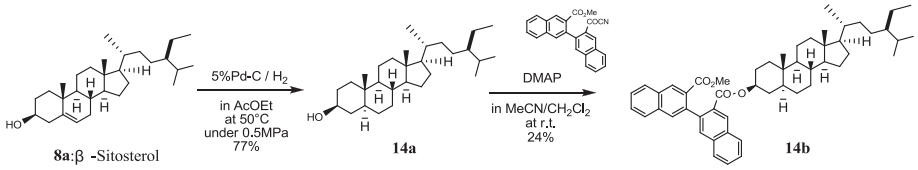



**Fig. 6.** CD spectra of combined spectrum of **7a** + **2b** (left) and **7b** (right).

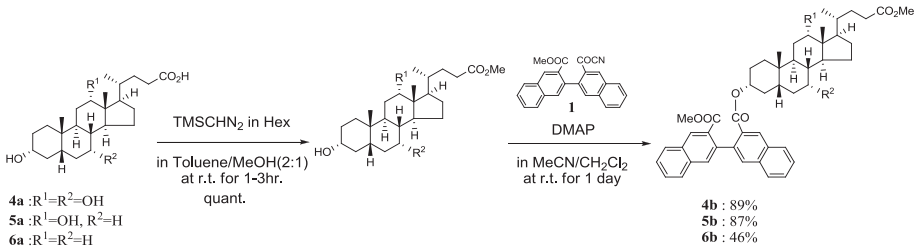



**Scheme 2.** Hydrogenation and derivatization of sitosterol **8a**.

from **7a** itself, because it contains the olefin in the structure. Therefore, we measured the CD spectrum for the mixture of **7a** and **2b**. By comparing the CD spectrum of the mixture with that of **7b,** we can readily conclude that the spectral change caused by the esterification. That is, the molecular orbital interaction between the C-5 olefin and naphthyl group may occur. The combined CD spectrum of **7a** and **2b** was not identical to that of **7b** (Fig. 6). Since the distance along the chemical bond from the C-5 olefin to the naphthyl group is far, it is difficult to assume that a through-bond interaction affects the transition moment of the chromophore. Thus, we presumed that a through-space interaction causes the changes in the CD spectrum between the C-5 olefin and naphthyl group. A high-level *ab initio* molecular orbital calculation is required to reveal the origin of this phenomenon, which is out of scope of this work.

To prove this hypothesis, an olefinic bond at the C-5 position of sitosterol **8a** was hydrogenated in the presence of 5% palladium-carbon, followed by derivatization to give saturated compound **14b** (Scheme 2). As we expected, the CD spectrum of **14b** showed the same split Cotton effect as that of type A (Fig. 4).

In addition, other $\Delta^5$-sterols **9a**–**11a** were similarly hydrogenated and derivatized. Hydrogenated product of $\Delta^5$-Sterol **11a** was found to be identical with 3β-cholestanol **2a** by comparison with those $^1$H NMR spectra [26]. Thus this result suggests that hydrogenation occurs from α-face of the C–C double bond at C-5 to give 5α-products in all $\Delta^5$-sterols examined in this study. The




**Scheme 3.** Preparation of cholic acid derivative **4b**–**6b**.

CD spectra of these derivatives (**15b**, **16b**, **2b**) showed the split Cotton effect, indicating positive exciton chirality (Table 1).

Next, we applied the procedure to not only 3α-hydroxycholic acid but also deoxycholic acid **5a** and lithocholic acid **6a**, which are metabolic compounds of cholic acid **4a**. First, **4a**–**6a** were converted to the corresponding methyl esters using trimethylsilyldiazomethane in quantitative yield, followed by derivatization to give the mono-binaphthyl derivatives **4b**–**6b** (Scheme 3). The CD spectra of the binaphthyl esters **4b**–**6b** showed negative exciton chirality, opposite that of the 3β-sterol derivatives (see Table 1).

In conclusion, the CD spectra of the 2,2′-binaphthyl ester derived from $\Delta^5$-sterols showed not a split CD but rather CD bands at around 210 and 240 nm. It was deduced that this result might be attributable to some interaction between the olefinic chromophore and the binaphthyl one. Various types of unsaturated sterols were thus hydrogenated completely, followed by derivatization to give saturated sterols. As a result, CD spectra of the binaphthyl derivatives of the saturated sterols showed bisignate curves centered at 240 nm (3*S*(β): positive chirality, 3*R*(α): negative one). This suggested a straightforward and practical method for discriminating the absolute stereogenic center at the C-3 positions of sterols based on an induced CD. This finding should contribute significantly to the analysis of the metabolites of various types of sterols.

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