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Optical resolution of hexamethylbiphenol by cholesterol esterase and porcine pancreas lipase

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ABSTRACT

Both enantiomers of 2,2′-dihydroxy-4,4′,5,5′,6,6′-hexamethybiphenyl (**2**), a potentially useful chiral synthon, were obtained with >99% ee in high enantioselectivity by cholesterol esterase or porcine pancreas lipase (PPL)-mediated hydrolysis of the corresponding (±)-dipentanoate or (±)-dihexanoate, respectively. Absolute configuration of (*S*)-3-bromo-2,6′-dimethoxy-4,5,6,2′,3′,4′-hexamethyl-biphenyl (**2h**) was determined by X-ray analysis.

1. Introduction

Optically active 2,2′-dihydroxybiaryls are useful chiral synthons for asymmetric synthesis, supramolecular and molecular recognition chemistry [1–4]. Among them, 1,1′-binaphthyl-2,2′-diol (BINOL) has been extensively studied [3,4]. Although, optically active biphenyls are expected to show new properties, some studies have been made on optically active 2,2′-dihydroxybiphenyls [5–12] compared to corresponding binaphthyls because of multi steps or rather complicated organic synthetic methodology.

2,2′-Dihydroxy-4,4′,5,5′,6,6′-hexamethylbiphenyl (**2**) is a convenient chiral synthon to be possible easily substituted only at 3,3′ position. We have reported on the physicochemical properties in water of amphiphiles [13] incorporating (±)-**2** [14,15]. Optically active biphenol **2** was required for chiral recognition purpose as an extension of our work. Recently, Henschke et al. synthesized a new biphenol derivative that is useful for highly active and enantioselective hydrogenation catalysts [16]. In this case, however, biphenol (±)-**2** was resolved into constituent enantiomers by preparative chiral HPLC of high cost.

If a target substrate is especially well suited for enzyme, each enantiomer of the target is obtained with high efficiency under the mild catalytic condition. It is known that the enzymatic conversion of bulky and/or rigid compounds is difficult in general. Binaphthyls have been resolved with biocatalytic hydrolysis [17–21], acylation [22,23] and amidation [24,25]. Optically active biphenols were obtained by desymmetrization of diacetates by lipase-catalyzed hydrolysis [26]. Sanfilippo et al. have been reported the synthesis of a simple biphenyl, 2,2′-dihydroxy-6,6′-dimethoxy-1,1′-biphenyl with lipase from *Pseudomonas cepacia* (PSL) catalyzed acetylation in *tert*-butyl methyl ether [27]. It is known that in the case of cholesterol esterase catalyzed hydrolysis of binaphthol, the dipentanoate was chosen because it gave the best combination of enantioselectivity, rate of reaction, and ease of separation of diol and diester. So, we tried the comparison for stereoselective resolution of **1** between lipases and the cholesterol esterase. We report here the first example of kinetic resolution of axially chiral biphenols by enzyme-mediated hydrolysis for substrate **1**, which is bulky, rigid and hindered molecule.

Substrates, 2,2′-dipropanoyloxy-(±)-**1a**, 2,2′-dipentanoyloxy-(±)-**1b**, 2,2′-dihexanoyloxy-(±)-**1c**, 2,2′-diheptanoyloxy-(±)-**1d**, and 2,2′-dinonanoyloxy-(±)-**1e** were synthesized by known procedures.

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2. Experimental

2.1. General

PPL (Procine pancreas lipase (Type II Crude)), PLE (Pig liver esterase), CHE-BP: (Bovine Pancreas acetone powder), CCL (*Candida cylindrasea* Lipase) and Cholesterol esterase were purchased from SIGMA. Pancreatin F and Pancreatin (Hog pancreas lipase) were from Tokyo Kasei. Co. Newlase F (*Rhizopus niveus*) Amano F-AP15: (*Rhizopus japonicas*), Amano P (*Psedomonus fluorescence* Lipase), Amano M-10: (*Mucol Japonicus*), Amano A (*Aspergillus niger* lipase) and SAM-II (*Pseudomonas species* lipase) were gifted from Amano Pharm. Co.

Melting points were measured using a Yamato MP-21. Gas chromatographic analysis was performed using GC-column (DV-1; 25 m) equipped on Shimadzu GC-14A. Mass spectra were measured by using a HITACHI M-80 or JEOL JMX-SX 102A Shimadzu LC-MSQP8000. The enantiomeric excess determination by HPLC analysis was performed with a Shimadzu LC-6-AD instrument equipped with Shimadzu SPD-M10A detector and SUMICHIRAL OA 3200 column, eluted with *n*-hexane/2-propanol = 20:1. Optical rotation was measured with a Jasco DIP-1000. The IR spectra were measured on a PerkinElmer Spectrum One FT-IR spectrometer. The NMR spectra were measured on JEOL GNM-LA 500 FT NMR SYSTEM or JEOL JNM-LA 400 FT NMR SYSTEM. $CDCl_3$ or DMSO-d_6 with tetramethylsilane as the internal standard was used. CD spectra were measured on a JASCO J-725.

2.2. Biotransformation conditions

2.2.1. Typical procedure

Typical procedure 1: cholesterol esterase (6.0 g) was dissolved in phosphate buffer (0.1 M, pH 8) (1500 ml) and added substrate (±)-**1b** (3.0 g, 6.84 mmol) in ethanol (200 ml) for 1.5 h at 25 °C. After 8 h, the reaction mixtures were extracted with ether (500 ml × 3). The ethereal solutions were washed with a saturated solution of NaCl (100 ml × 3), water (100 ml × 3), dried over $MgSO_4$ and filtered. Subsequently, the solvent was removed under reduced pressure. Crystallization of the residue from hexane gave (*S*)-**2** (0.88 g, 3.26 mmol) (48%) with high enantioselectivity (>99% ee). The filtrate was evaporated in vacuo. The residue was purified by silica gel column chromatography (hexane–ethyl acetate 15:1). Next, treatment with 5% KOH/MeOH gave (*R*)-**2** (0.74 g, 2.74 mmol)(40%) with high enantioselectivity (>99% ee).

Typical procedure 2: PPL (5.25 g) was dissolved in phosphate buffer (0.1 M, pH 9) (1575 ml) and added under stirring by a dropwise substrate (±)-**1c** (2.1 g, 4.5 mmol) in ethanol (140 ml) for 20 min at 25 °C. After 1.5 h, the reaction mixtures were extracted with hexane/ethyl acetate (1:1) (500 ml × 3). The extracts were washed with a saturated solution of NaCl, water (100 ml × 3), dried over $MgSO_4$ and filtered. Subsequently, the solvent was removed under reduced pressure. Crystallization of the residue from hexane gave (*S*)-**2** (0.34 g) (99% ee). The filtrate was evaporated in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate 15:1) to give (*S*)-**2f** (0.17 g) and (*R*)-**1c** (1.0 g). Next, treatment with 5% KOH/MeOH gave (*S*)-**2** (0.06 g)(99% ee) and (*R*)-**2** (0.53 g) with high enantioselectivity (99% ee) (Scheme 1).

2.2.2. Chiral analysis

The enantiomeric excess was determined by chiral HPLC (SUMICHIRAL OA-3200, 5 μ, 4.6 mmφ × 25 cm). Separation factor α for biphenol (±)-**2** was calculated to be 2.18 at a flow rate 0.5 ml/min using solvent system *n*-hexane/2-propanol = 20:1.

2.3. Spectral data of compound

2.3.1. Compound **1a**: (±)-2,2′-dipropanoyloxy-4,4′,5,5′,6,6′-hexamethylbiphenyl

(±)-2,2′-Dihydroxy-4,4′,5,5′,6,6′-hexamethylbiphenyl (**2**) (5.00 g, 18.5 mmol) and propionic anhydride (25.5 ml, 200 mmol) were added and refluxed for 1.5 h. (±)-2,2′-Dipropanoyloxy-4,4′,5,5′,6,6′-hexamethylbiphenyl (**1a**) was obtained by the vacuum distillation of the reaction mixtures (1.1 Torr, 140–152 °C). Substrate **1a** was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 15:1) to afford 5.52 g (14.4 mmol) as white crystals. Yield: 78.0%. Mp: 89.0–90.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.83 (t, 6H, *J* = 7.6 Hz, —CH_2CH_3), 1.91 (s,6H, 6,6′-ArCH_3), 2.18 (s, 6H, 5,5′-ArCH_3), 2.31 (s, 6H, 4,4′-ArCH_3), 2.11 (dq, 2H, J_{gem} = 15.5 Hz, J_{vic} = 7.5 Hz, —COCH_2—), 2.16 (dq, 2H, J_{gem} = 15.5Hz, J_{vic} = 7.5 Hz, —COCH_2—), 6.80 (s, 2H, Ar—*H*). ^{13}C NMR (100 MHz, DMSO-d_6): δ 8.60 (—CH_2CH_3), 15.3, 16.6, 20.2 (ArCH_3), 26.8 (—$COCH_2$—), 120.9, 126.7, 132.0, 135.9, 136.3, 145.7 (C_6H_6), 171.9 (*C*O). IR(KBr): 2960, 2900 (Ar—H, Ar—CH_3, C—H), 1750 cm^{-1}(C=O). MS (APCI$^+$) *m*/*z* 383 (MH^+, 100), 327 (70), 271 (59).

2.3.2. Compound **1b**: (±)-2,2′-dipentanoyloxy-4,4′,5,5′,6,6′-hexamethylbiphenyl

(±)-2,2′-Dihydroxy-4,4′,5,5′,6,6′-hexamethylbiphenyl (**2**) (12.00 g, 44.4 mmol) and valeric anhydride (70 ml, 328 mmol) were added and refluxed for 5 h. (±)-2,2′-Dipentanoyloxy-4,4′,5,5′,6,6′-hexamethylbiphenyl (**1b**) was obtained during the vacuum distillation of the reaction mixtures (0.98 Torr, 170–180 °C). Substrate **1b** was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 15:1) to afford 17.5 g (39.9 mmol) as white crystals. Yield: 85.0%. Mp: 56.0–59.0 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.73–0.76 (t, 6H, —$CH_2CH_2CH_2CH_3$), 1.03–1.08 (m, 4H, —$CH_2CH_2CH_2CH_3$), 1.16–1.26 (m, 4H, —$CH_2CH_2CH_2CH_3$), 1.91 (s, 6H, 6,6′-ArCH_3), 2.10-2.14 (q, 4H, —$CH_2CH_2CH_2CH_3$), 2.16 (s, 6H, 5,5′-ArCH_3), 2.30 (s, 6H, 4,4′-ArCH_3), 6.78 (s, 2H, Ar—*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.7, 15.6, 16.9, 20.8, 21.9, 26.8, 33.9, 120.8, 127.3, 132.7, 136.4, 137.1, 146.2, 172.2. IR(KBr): 2959, 2930, 2873 (Ar—H, Ar—CH_3, C—H), 1756 cm^{-1}(C = O). MS (APCI$^+$) *m*/*z* 439 (MH^+, 68), 355 (100), 271 (10).

2.3.3. Compound **1c**: (±)-2,2′-dihexanoyloxy-4,4′,5,5′,6,6′-hexamethylbiphenyl

(±)-2,2′-Dihydroxy-4,4′,5,5′,6,6′-hexamethylbiphenyl (**2**) (3.00 g, 11.1 mmol) in ether (40 ml) and triethylamine (6 ml, 44.4 mmol) were cooled at 20 °C and solution of hexanoyl chloride (6 g, 44.4 mmol) was slowly added. And the reaction mixture was stirred for 4 h. To this was added water and extracted with ether. The organic layers were concentrated in vacuo to obtain (±)-2,2′-dihexanoyloxy-4,4′,5,5′,6,6′-hexamethylbiphenyl (**1c**) as pale yellow solids. Substrate **1c** was obtained by the vacuum distillation of the reaction mixtures (removal of hexanoic acid: 2.75 Torr, 109–155 °C). Substrate **1c** was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 15:1) to afford 5.15 g (11.0 mmol) as white crystals. Yield: 99.0%. Mp: 71.0–71.5 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.80–0.83 (t, 6H, —$CH_2CH_2CH_2CH_2CH_3$), 1.03–1.06 (m, 4H, —$CH_2CH_2CH_2CH_2CH_3$), 1.14–1.22 (8H, m, —$CH_2CH_2CH_2CH_2CH_3$), 1.91 (s, 6H, 6,6′-ArCH_3), 2.09-2.13 (q, 4H, —$CH_2CH_2CH_2CH_2CH_3$), 2.17 (s, 6H, 5,5′-ArCH_3), 2.30 (s, 6H, 4,4′-ArCH_3), 6.78 (s, 2H, Ar—*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ 13.8, 15.7, 16.9, 20.8, 22.2, 24.3, 31.0, 34.1, 120.8, 127.3, 132.6, 136.3, 137.1, 146.2, 172.2 cm^{-1}(C=O). MS (APCI$^+$) *m*/*z* 467 (MH^+, 100), 369 (25), 271 (25).

*2.3.4. Compound **1d**: (±)-2,2′-diheptanoyloxy-4,4′,5,5′,6,6′-hexamethylbiphenyl*

(±)-2,2′-Dihydroxy-4,4′,5,5′,6,6′-hexamethylbiphenyl (**2**) (5.10 g, 18.9 mmol) in ether (65 ml) and triethylamine (9.2 ml, 66.5 mmol) were cooled at 20 °C and solution of hexanoyl chloride (8.0 g, 53.8 mmol) was slowly added. And the reaction mixture was stirred for 4 h. To this was added water and extracted with ether. The organic layers were concentrated in vacuo to obtain (±)-2,2′-diheptanoyloxy-4,4′,5,5′,6,6′-hexamethylbiphenyl (**1d**) as a pale yellow liquid. Substrate **1d** was obtained by the vacuum distillation of the reaction mixtures (0.21 Torr, 188–200 °C). Substrate **1d** was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 15:1) to afford 8.65 g (17.5 mmol) as pale yellow oils. Yield: 93.0%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.84–0.87 (t, 6H, —$CH_2CH_2CH_2CH_2CH_2CH_3$), 1.06–1.23 (m, 16H, —$CH_2CH_2CH_2CH_2CH_2CH_3$), 1.90 (s, 6H, 6,6′-ArCH_3), 2.09-2.13 (q, 4H, —$CH_2CH_2CH_2CH_2CH_2CH_3$), 2.16 (s, 6H, 5,5′-ArCH_3), 2.30 (s, 6H, 4,4′-ArCH_3), 6.78 (s, 2H, Ar—*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 15.7, 17.0, 20.8, 22.4, 24.7, 28.6, 31.5, 34.2, 120.9, 127.3, 132.7, 136.4, 137.1, 146.2, 172.3 cm^{-1}(C═O). MS (APCI$^+$) *m*/*z* 495 (MH$^+$, 100), 383 (74), 271 (91).

*2.3.5. Compound **1e**: (±)-2,2′-dinonanoyloxy-4,4′,5,5′,6,6′-hexamethylbiphenyl*

(±)-2,2′-Dihydroxy-4,4′,5,5′,6,6′-hexamethylbiphenyl (**2**) (3.00 g, 11.1 mmol) in ether (40 ml) and triethylamine (4.8 ml, 44.4 mmol) were cooled at 20 °C and solution of octanoyl chloride (8.4 g, 44.4 mmol) was slowly added. And the reaction mixture was stirred for 4 h. To this was added water and extracted with ether. The organic layers were concentrated in vacuo to obtain (±)-2,2′-dinonanoyloxy-4,4′,5,5′,6,6′-hexamethylbiphenyl (**1e**) as a pale yellow liquid. Substrate **1e** was obtained by the vacuum distillation of the reaction mixtures (0.33 Torr, 200–220 °C). Substrate **1e** was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 15:1) to afford 5.29 g (9.60 mmol) as pale yellow oils. Yield: 87.0%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.92 (s, 6H, 6,6′-ArCH_3), 2.16 (s, 6H, 5,5′-ArCH_3), 2.31 (s, 6H, 4,4′-ArCH_3), 4.51 (s, 2H, Ar—O*H*), 6.77 (s, 2H, Ar—*H*). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.1, 15.7, 17.0, 20.8, 22.6, 24.7, 28.9, 29.1, 29.3, 31.9, 34.2, 120.9, 127.3, 132.7, 136.4, 137.1, 146.3, 172.3 cm^{-1}(C═O). MS (APCI$^+$) *m*/*z* 551 (MH$^+$, 100), 411 (42), 271 (57).

*2.3.6. Compound (S)-(-)-**2**: 2,2′-dihydroxy-4,4′,5,5′,6,6′-hexamethybiphenyl*

Mp: 243–244 °C; $[\alpha]_D^{27}$ −44 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (500MHz, $CDCl_3$): δ 1.91 (6H,s, 6,6′-ArCH_3), 2.16 (6H, s, 5,5′-ArCH_3), 2.30 (6H, s, 4,4′ArCH_3), 4.48 (2H,s, Ar—O*H*), 6.74 (2H,s, Ar—*H*); ^{13}C NMR (500 MHz, $CDCl_3$): δ 15.4, 16.7, 20.9 (Ar—CH_3), 114.3, 117.9, 127.7, 136.8, 138.4, 151.2 (C_6H_6); Mass (APCI$^+$) *m*/*z* [MH$^+$]: 271 (100).

*2.3.7. Compound (R)-(+)-**2**: 2,2′-dihydroxy-4,4′,5,5′,6,6′-hexamethybiphenyl*

Mp: 243–244 °C; $[\alpha]_D^{27}$ +44 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.91 (6H,s, 6,6′-ArCH_3), 2.16 (6H, s, 5,5′-ArCH_3), 2.30 (6H, s, 4,4′ArCH_3), 4.49 (2H,s, Ar—O*H*), 6.74 (2H,s, Ar—*H*); ^{13}C NMR (125 MHz, $CDCl_3$): δ 15.4, 16.9, 20.9 (Ar—CH_3), 114.4, 118.0, 127.7, 136.8, 138.4, 151.2 (C_6H_6); Mass (APCI$^+$) *m*/*z* [MH$^+$]: 271 (100).

*2.3.8. Compound (S)-(+)-**2g**: (S)-6,6′-dimethoxy-2,2′,3,3′,4,4′-hexamethyl-biphenyl*

(*S*)-2,2′-Dihydroxy-4,4′,5,5′,6,6′-hexamethylbiphenyl (**2**) (2.00 g, 7.40 mmol) and KOH (2.00 g) were dissolved in acetone (20 ml) and dichloromethane (20 ml). After dropwise addition of iodomethane (3.75 ml), the mixture was refluxed at 20 °C for 2 h and concentrated in vacuo to give a solid mixture. This was taken up with chloroform, neutralized with dilute HCl and washed with water. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give a white solid. Purification by silicagel column chromatography (*n*-hexane/chloroform = 2:1) afforded white crystals. Mp: 123–124 °C; $[\alpha]_D^{21.7}$ +7.40 (*c* = 0.513, $CHCl_3$); Yield: 2.09 g (95%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.86 (s, 6H, CH_3), 2.17 (s, 6H, CH_3), 35 (s, 6H, CH_3), 3.66 (s, 6H, OCH_3), 6.66 (s, 2H, Ar—*H*); ^{13}C NMR (500 MHz, $CDCl_3$): δ 15.4, 16.9, 21.3 (Ar—CH_3) 55.8 (—OCH_3) 110.5, 124.7, 127.0, 135.6, 136.5, 154.7 (C_6H_6); Mass (APCI$^+$) *m*/*z* [MH]$^+$: 298 (100).

*2.3.9. Compound (S)-(−)-**2h**: (S)-3-bromo-2,6′-dimethoxy-4,5,6,2′,3′,4′-hexamethyl-biphenyl*

Compound (*S*)-(+)-**2g** (1.20 g, 4.02 mmol) was vigorously stirred in acetonitrile (160 ml). To this was added dropwise *N*-Bromosuccinimide (0.358 g, 2.01 mmol) in acetonitrile (40.0 ml)

Table 1
Hydrolase-catalyzed hydrolysis of **1b**[a]

Entry	Enzyme	Yield (%)[b]
1	PPL	64
2	PLE	20
3	CHE-BP	13
4	Pancreatin F	7
5	Pancreatin	4
6	CCL	7
7	Newlase F	2
8	Amano F-AP 15	3
9	Amano P	1
10	Amano M-10	4
11	Amano A	4
12	SAM-II	3

[a] Reaction conditions: enzyme (15 mg, 5.0 mg/ml solvent) and substrate (6.8×10^{-2} mmol) in 0.1M phosphate buffer (pH 8.0) at 25 °C for 16h were employed.
[b] Determined by GC.

Table 2
Effects of acyl group for PPL-catalyzed hydrolysis of **1a**-**1e**[a]

rac-Substrate	Product (%)[b]	(*S*)-(-)-**2** (% ee)[c]
1a	34	63
1b	21	62
1c	19	91
1d	16	95
1e	5	99

[a] Reaction conditions: Enzyme (PPL)(30 mg, 3.1 mg/ml solvent) and substrate (6.8×10^{-2} mmol) in 0.1M phosphate buffer (pH 8.0) at 25 °C for 8 h were employed.
[b] Determined by GC.
[c] Determined by chiral HPLC(SUMICHIRAL OA-3200).

Table 3
Effect of pH for **1c** and **1d**[a]

rac-Substrate	pH	Product (%)[b]	Conversion (%)[c]	(*S*)-(-)-**2** (% ee)[d]	(*R*)-(+)-**2** (% ee)[d]	E_1 value[c]
1c	6.0	31	36	84	48	18
1c	7.0	30	42	79	58	15
1c	8.0	51	51	73	77	15
1c	9.0	49	43	94	71	69
1d	6.0	12	nd[e]	nd[e]	nd[e]	nd[e]
1d	7.0	20	43	77	57	14
1d	8.0	43	37	92	55	42
1d	9.0	39	30	95	40	58

[a] Reaction condition: enzyme (PPL) (30 mg, 3.1 mg/ml solvent) and substrate (6.4×10^{-2} mmol) in 0.1 M phosphate buffer (pH 8.0) at 25 °C for 24 h were employed.
[b] Determined by GC.
[c] $E = \ln[(1-c)(1-ee_S)]/\ln[(1-c)(1+ee_S)]$. Conversion: $c = ee_S/(ee_S + ee_P)$.
[d] Determined by chiral HPLC(SUMICHIRAL OA-3200).
[e] Not determined.

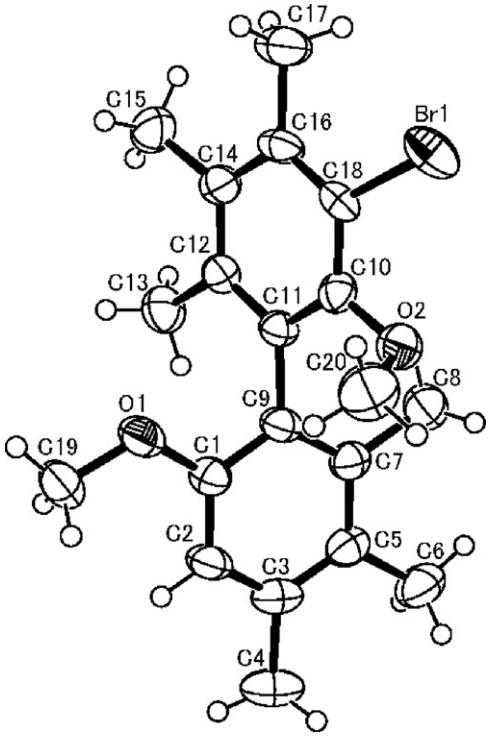


Fig. 1. ORTEP drawing of (*S*)-**2h**.

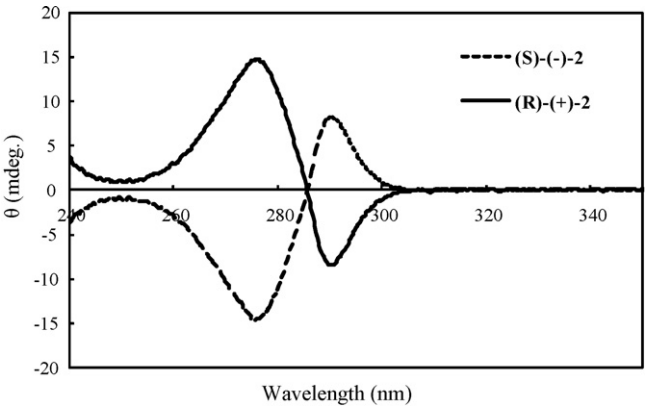


Fig. 2. The CD spectra of compound (*R*)- and (*S*)-**2** in hexane.

in ice bath (5 °C) for 30 min. After another 1.5 h, the mixture was filtered with suction and washed with CCl_4. The combined filtrate is concentrated in vacuo to give a yellowish white solid which was purified by silica gel column chromatography (*n*-hexane/chloroform = 1:2) to afford white crystals. Mp: 121.5–123 °C; $[\alpha]_D^{21.5}$ −31.2 (*c* = 0.524, $CHCl_3$); Yield: 0.569 g (67%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.85 (s, 3H, Ar—C*H*$_3$), 1.86 (s, 3H, Ar—C*H*$_3$), 2.16 (s, 3H, Ar—C*H*$_3$), 2.26 (s, 3H, Ar—C*H*$_3$), 2.36, (s, 3H, Ar—C*H*$_3$), 2.48 (s, 3H, Ar—C*H*$_3$), 3.39 (s, 3H, —OC*H*$_3$), 3.68 (s, 3H, —OC*H*$_3$),6.64 (s, H, Ar—*H*); ^{13}C NMR (125 MHz, $CDCl_3$): δ 15.35, 16.95, 17.12, 17.34, 20.70, 21.28 (*C*H$_3$) 55.41, 59.86, 109.8, 118.1, 121.2, 124.1, 127.0, 130.9, 132.0, 135.7, 135.8, 136.0, 136.2, 154.3 (C_6H_6); Mass(APCI$^+$) *m*/*z* [MH]$^+$: 377 (100).

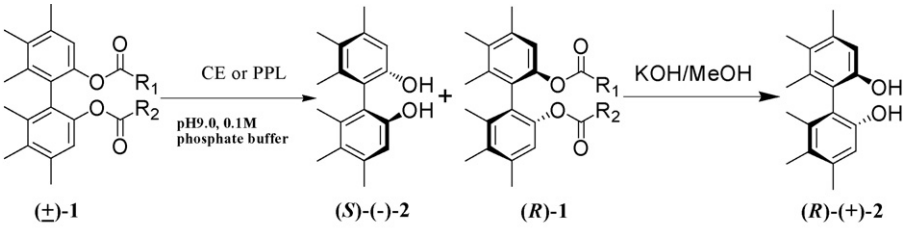
2.3.10. Single crystal X-ray analysis of (S)-(−)-*2h*

X-ray analysis were measured SMART (Bruker, 2001), ORTEPIII and MERCURY; Molecular formula: $C_{20}H_{25}BrO_2$. F_w: 377.31 space group C2. *a* = 20.859(2) Å. *b* = 7.2328(9) Å. *c* = 13.1472(16) Å. α = 90°. β = 113.300(2)°. γ = 90°V = 1821.7(4) Å^3 θ = 2.13–28.22°. *T* = 300(2) K. *Z* = 4. *F*(000) = 784. *D* = 1.376 Mg/m^3. Full-matrix least-squares

Table 4
Effect of temperature for *rac*-**1c** and **1d**[a]

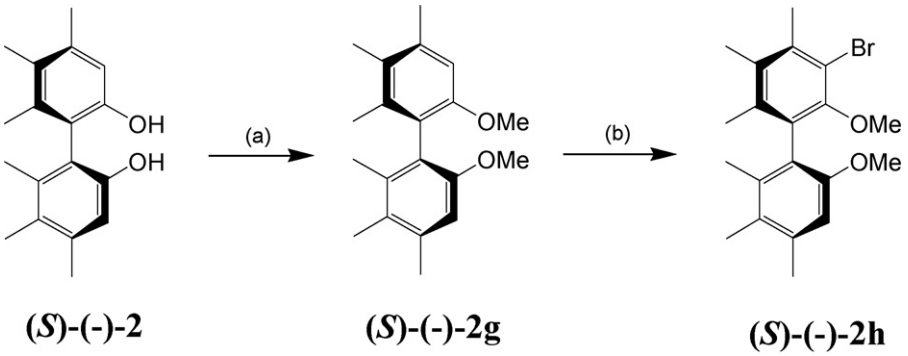
Substrate	Temperature (°C)	Product (%)[b]	Conversion (%)[c]	(*S*)-(-)-**2** (% ee)[d]	(*R*)-(+)-**2** (% ee)[d]	E_1 value[c]
1c	10	53	51	77	79	18
1c	20	49	50	83	82	27
1c	25	49	43	94	71	69
1c	30	31	30	97	42	99
1c	40	1	nd[e]	nd[e]	nd[e]	nd[e]
1d	20	44	35	82	44	15
1d	25	43	37	92	55	53
1d	35	27	18	96	21	55

[a] Reaction condition: enzyme (PPL) (30 mg, 3.1 mg/ml solvent) and substrate (6.4 10^{-2} mmol) in 0.1 M phosphate buffer (pH 8.0) at 25 °C for 24 h were employed.
[b] Determined by GC.
[c] $E = \ln[(1-c)(1-ee_S)]/\ln[(1-c)(1+ee_S)]$. Conversion: $c = ee_S/(ee_S + ee_P)$.
[d] Determined by chiral HPLC(SUMICHIRAL OA-3200).
[e] Not determined.



(±)-**1a**: R_1=R_2=CH_2CH_3
(±)-**1b**: R_1=R_2=$(CH_2)_3CH_3$
(±)-**1c**: R_1=R_2=$(CH_2)_4CH_3$
(±)-**1d**: R_1=R_2=$(CH_2)_5CH_3$
(±)-**1e**: R_1=R_2=$(CH_2)_7CH_3$
(±)-**1f**: R_1=$(CH_2)_4CH_3$, R_2=OH

Scheme 1.



Scheme 2. Synthesis of (*S*)-3-Bromo-2,6′-dimethoxy-4,5,6,2′,3′,4′-hexamethyl-biphenyl (**2h**) Reaction conditions: (a) CH_3I, KOH, acetone/CH_2Cl_2 and (b) NBS, CH_3CN, 0 °C.

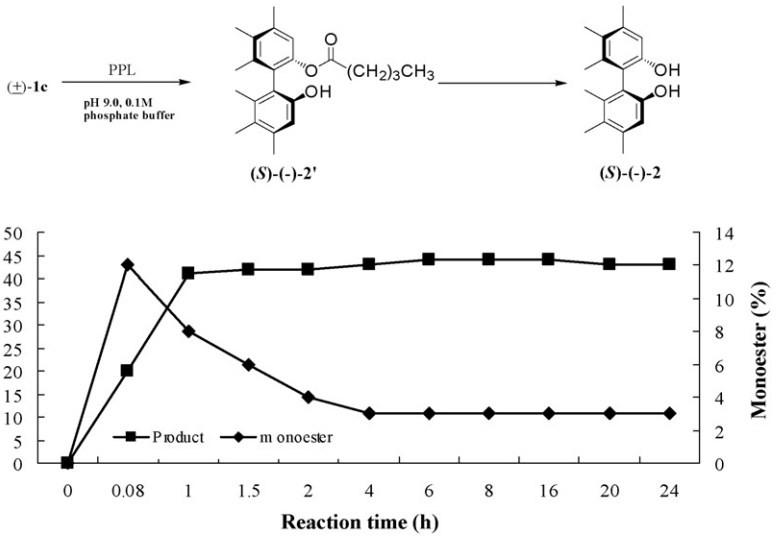


Fig. 3. Time course of product (*S*)-(-)-**2** (%) and monoester (*S*)-(-)-**2**′ (%) for PPL-catalyzed hydrolysis of **1c**. Reaction conditions: enzyme (PPL) (3.1 mg), substrate ((±)-**1c**) (1.2 mg) and ethanol (0.08 ml) in 0.1 M phosphate buffer (pH 9.0) at 25 °C for 24 h were employed. Determined by GC.

on F^2. R1 = 0.0362. wR2 = 0.0649. Goodness-of-fit = 0.834. Maximum and minimum peak in final difference map: 0.505 and −0.321 e Å^{-3}.

3. Results and discussion

Hydrolysis of (±)-2,2′-dipentanoyloxy-4,4′,5,5′,6,6′-hexamethylbiphenyl (**1b**) using cholesterol esterase gave biphenol (*S*)-**2** in good yield (48%) with high enantioselectivity (>99% ee) and (*R*)-**2** in good yield (40%) with high enantioselectivity (>99% ee). Moreover, in order to test several enzymes for hydrolysis, we tried for the asymmetric acylate (±)-**1a**. These results are shown in Table 1. Among them, porcine pancreas lipase (PPL) (Type II Crude, SIGMA) was found to catalyze for hydrolysis in moderate yield (64%). Next, we investigated the effect of the size of alkyl group for diol ester by PPL. These results are shown in Table 2. From these results, it was found that hydrolysis of 2,2′-dihexanoyloxy-(±)-**1c** and 2,2′-diheptanoyloxy-(±)-**1d** using PPL catalyst gave in high enantio selectivity 91% ee (yield 19%) and 95% ee (yield 16%), respectively. It seems that enantioselectivity of diacylated biphenol derivatives in the presence of PPL catalyst was affected by alkyl size of the substituted acyl group. So, the optimum conditions of (±)-**1c** and (±)-**1d** for synthesis of (*S*)- and (*R*)-**2** were investigated for reaction temperature, pH and reaction time. These results are shown in Tables 3 and 4 and Fig. 3. As shown in Table 3, it was found that pH effect for hydrolysis of (±)-**1c** and (±)-**1d** was pH 8 or pH 9. In the case of large scale procedure, the condition at pH 9 giving the high enantio selectivity for hydrolysis of **1c** and **1d** was adopted. Moreover, in Table 3, we optimized for the reaction temperature at 25 °C. In addition, as shown in Fig. 3, it was found that PPL-catalyzed hydrolysis of (±)-**1c** yields monoester (12%) after 1 h and then (*S*)-**2** was obtained. On the basis of these results, the scale-up reaction was carried out in order to obtain the isolated chiral (*R*)- and (*S*)-**2** [(*R*)-**2**, 25%; (*S*)-**2**, 33%](isolated yield) and [(*R*)-**2**, 99%; (*S*)-**2**, 99%](high enantioselectivity).

The structures of compound (*S*)-(-)-**2** was determined by X-ray diffraction (Fig. 1). The data also offer an additional and unambiguous proof for the structure of biphenol (*S*)-(-)-**2**. In order to measure X-ray of compound (*S*)-(-)-**2**, (*S*)-(-)-**2** was converted to the methoxy derivative (*S*)-(-)-**2g** and then the methoxy compound was treated with NBS to give the bromo derivative **2h** (Scheme 2).

On the basis of X-ray diffraction analysis data for compound **2h**, the absolute configuration was determined as (*S*)-form [28]. Also, this fact was supported by CD spectroscopy (Fig. 2) [29,30]. Moreover, the configuration has been supported from the elution order observed on chiral HPLC, which is identical to that observed for *R*-enantiomer first eluting and *S*-enantiomer second eluting [22] (Fig. 3).

4. Conclusion

This is the first time that a facile synthesis of high yield and enantiopure 2,2′-dihydroxy-4,4′,5,5′,6,6′-hexamethylbiphenyl (*R*)-

2 and (*S*)-**2** was accomplished. The absolute configuration of the latter was established by X-ray crystallography of the correlating bromoderivative **2h**. It is particularly noteworthy that cholesterol esterase (CE)-catalyzed hydrolysis reaction for pentanoate (±)-**1b** was more optimum method than using PPL.

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[28] Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-671694. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: int. code +44(1223)336-033; e-mail: deposit@ccdc.cam.ac.uk].
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