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Enzyme-Mediated Enantioselective Hydrolysis of Dicarboxylic Acid Monoesters

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Abstract: The enzyme-mediated highly enantioselective hydrolysis of dicarboxylic acid monoesters was investigated. The racemic substrates, which were prepared by coupling of the corresponding alcohols with dicarboxylic anhydrides, were enantioselectively hydrolyzed by lipase from *Candida antarctica* (Novozym 435) in a buffer at 30 °C. The products were easily separated by a simple extraction procedure without laborious column chromatography to afford both enantiomers of the alcohols. We then determined that the dicarboxylic acid monoesters were suitable alternative substrates for the preparation of optically active alcohols.

Keywords: Dicarboxylic acid monoesters, enzymatic hydrolysis, kinetic resolution, lipase, optically active alcohols.

INTRODUCTION

The enzyme-mediated kinetic resolution of racemic alcohols and esters is one of the attractive methods for the preparation of optically active compounds [1-11]. During the reaction process, however, the products and the remaining substrates must be separated by column chromatography, which is the bottleneck to a sustainable production and an easy operation. The use of succinic anhydride as an acylating agent during enzymatic esterification could resolve this concern [12-22]. Although the separation of the resulting dicarboxylic monoesters from the remaining alcohols could be achieved by a simple extraction process, the procedure is not always satisfactory in terms of the enantioselectivity and the necessity for high amount of enzymes.

In our previous study, we succeeded in the enantioselective hydrolysis of monomethoxy poly(ethylene glycol) (MPEG; av MW 5000)-supported carboxylates using lipase from *Candida antarctica* (Novozym 435; CAL-B), and the separation of the reaction products was achieved by simple filtration because the MPEG-supported substrates were easily precipitated in Et_2O [23]. At the beginning of the study, we carried out the enzymatic hydrolysis of the MPEG-supported diester (±)-**1** (Fig. **1**). Unfortunately, Novozym 435 catalyzed the hydrolysis of two ester bonds, and the substrate was decomposed into several components. After the detail analysis of the products, however, we found that the MPEG ester part was first hydrolyzed to give the corresponding succinic acid monoester (±)-**2a**, because hydrolases generally preferred a primary ester. In addition, the resulting racemate (±)-**2a** was then enantioselectively hydrolyzed to the optically active **3**. We noted that the dicarboxylic acid monoesters could be a good substrate for hydrolytic enzymes, because the remaining dicarboxylic acid monoesters could be separated from the resulting alcohols by the same process as in the case of the enzymatic esterification with succinic anhydride as mentioned above. To the best of our knowledge, there have been only a very few reports on the enzyme-mediated enantioselective hydrolysis of dicarboxylic acid monoesters (Fig. **2**) [24, 25], and the *E* values (the ratio of the specificity constants of enantiomers (*R* and *S*); *E* value=$(V_R/K_R)/(V_S/K_S)$) [26] indicated that the enantioselectivities of the reactions using an industrial glutaryl acylase were low. However, the dicarboxylic acid monoesters are well known for use as prodrugs, which are hydrolyzed by hydrolases [27]. In addition, a free terminal carboxylate group could cause an interaction between the substrates and the enzyme active site residue through a hydrogen bonding [28-30]. We now disclose the enzyme-mediated enantioselective hydrolysis of dicarboxylic acid monoesters, and also report the structure of the acyl moiety that affects both the reactivity and enantioselectivity.

RESULTS AND DISCUSSION

We selected 1-phenylethanol (**3**) as the representative alcohol, and the racemate (±)-**3** was combined with succinic anhydride using DMAP in CH_2Cl_2 to give the corresponding substrate (±)-**2a** (Fig. **3**). The other substrates were synthesized by the same procedure.

At first, the enzymatic reaction using Novozym 435 of the succinic acid monoester (±)-**2a** was carried out (Fig. **4**). In a typical experiment, 89 mg of (±)-**2a** (sub. concn. 10 mmol L^{-1}) and 20 mg of Novozym 435 were added to 0.1 mol L^{-1} phosphate buffer (pH 6.5, 40 mL) in a recovery flask, and the reaction was stirred for 24 h at 30 °C. The water solubility of the substrate under suitable basic conditions

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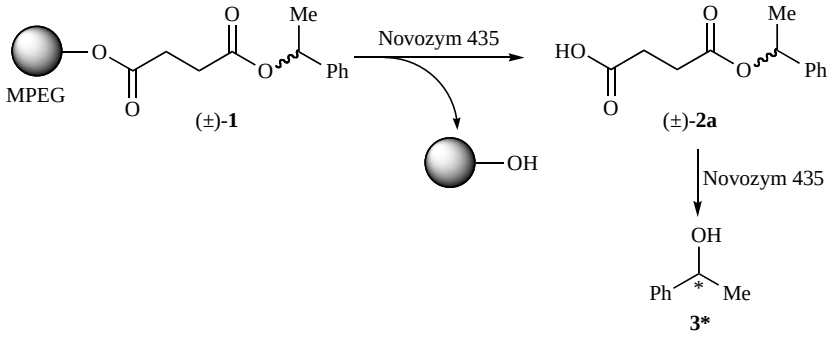

Fig. (1). Enzymatic hydrolysis of a MPEG-supported diester.

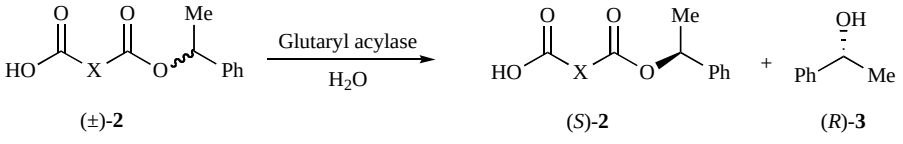

Fig. (2). Enzymatic hydrolysis of carboxylic acid monoesters with glutaryl acylase.

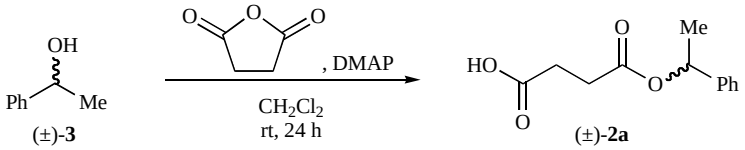

Fig. (3). Preparation of the substrate (±)-**2a**.

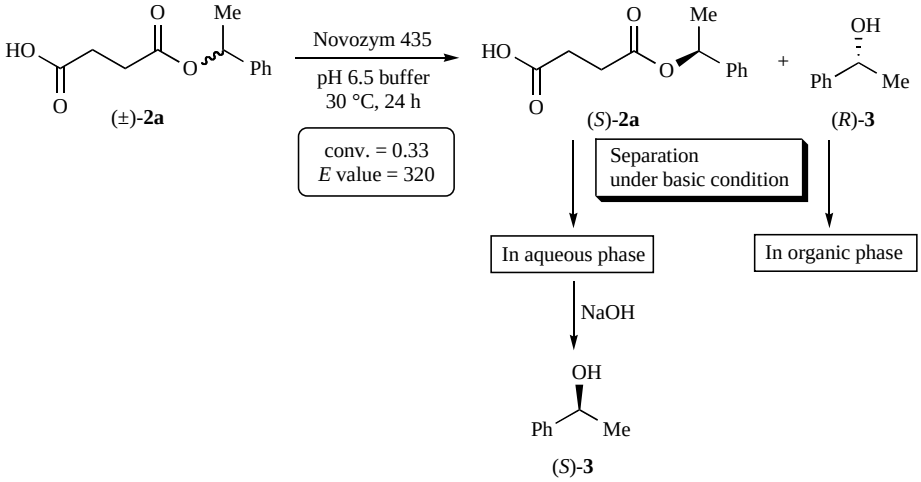

Fig. (4). Enzymatic hydrolysis of the succinic acid monoester (±)-**2a**.

(ca. pH 11) enabled us to establish a facile separation of the remaining **2a** and the resulting alcohol **3**. After acidification of the reaction mixture with 2 mol L^{-1} HCl, the first extraction process was performed with Et_2O. In this step, both the substrate **2a** and the alcohol **3** were extracted into the organic phase. In the following second extraction from the organic phase with 1 mol L^{-1} Na_2CO_3 aq, the substrate **2a** was then selectively obtained in the basic aqueous phase. The alcohol **3** successfully remained in the organic phase, and was isolated after evaporation. On the other hand, chemical hydrolysis of the substrate **2a** by the addition of 2 mol L^{-1} NaOH aq to the aqueous phase gave the corresponding **3**, which was also extracted with Et_2O. The yields of the compounds were determined after passing through a small amount of silica gel. The enantiomeric excesses (ee) were evaluated by a chiral GLC analysis. As expected, the hydrolysis proceeded with a high enantioselectivity to afford the optically active (*S*)-**3** (derived from (*S*)-**2a**; 45%, 48% ee) and (*R*)-**3** (15%, 99% ee). Although the reactivity was moderate (conv.=0.33; the conversion was calculated using $ee_s/(ee_s+ee_p)$; ee_s, ee of (*S*)-alcohol; ee_p, ee of (*R*)-alcohol), the enantioselectivity (*E* value=320; the *E* value was calculated using $\ln[(1-conv.)(1-ee_s)]/\ln[(1-conv.)(1+ee_s)]$) was very high.

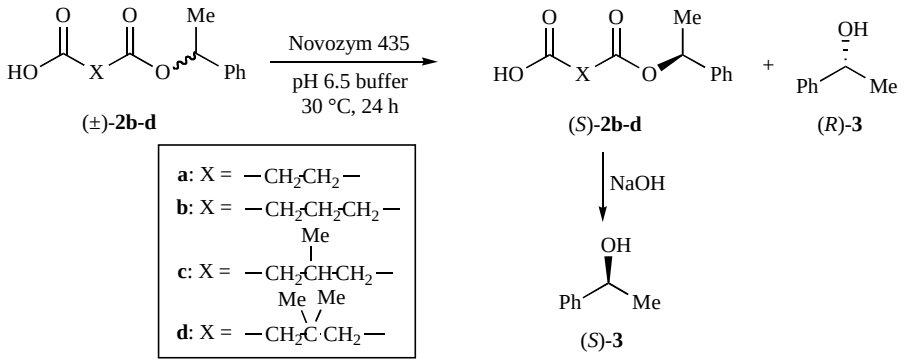

Fig. (5). Enzymatic hydrolysis of the substrates bearing various acyl parts.

Table 1. Enantioselective hydrolysis of dicarboxylic acid monoesters 2b-d with Novozym 435.[a]

Entry	Substrate	(*S*)-3		(*R*)-3		Conv.[b]	*E* value[c]
		Yield (%)	Ee (%)[d]	Yield (%)	Ee (%)[d]		
1	(±)-**2b**	34	91	38	>99	0.48	>640
2[e]	(±)-**2b**	42	96	41	>99	0.49	>790
3	(±)-**2c**	73	16	10	97	0.14	77
4	(±)-**2d**	No reaction				–	–

[a]The reaction was performed using 10 mM of the substrate with Novozym 435 (20 mg) in 0.1 M phosphate buffer (pH 6.5) for 24 h at 30 °C.
[b]Calculated using $ee_s/(ee_s+ee_p)$.
[c]Calculated using $\ln[(1-conv.)(1-ee_s)]/\ln[(1-conv.)(1+ee_s)]$.
[d]Determined by GC analysis.
[e]The reaction was performed on a gram scale of the substrate.

We next investigated changing the acyl group of the substrate in order to increase the conversion rate, because we speculated that a suitable acyl group could increase the affinity to the active site of the enzyme (Fig. **5**). The results are summarized in Table **1**. Surprisingly, changing the acyl group significantly affected not only the conversion, but also the enantioselectivity. The glutaric acid monoester (±)-**2b** was smoothly hydrolyzed, and the conversion was up to 0.48 (entry 1). In addition, an excellent enantioselectivity was observed (*E* value>640) to afford both almost optically pure enantiomers. This reaction was also useful as a preparative-scale operation (entry 2, >1 g of (±)-**2b**). Finally, both the reactivity and enantioselectivity in the case of the dicarboxylic acid monoester **2b** were eventually comparable to those for the enzymatic hydrolysis of the racemic 1-phenylethyl acetate ((±)-**4**), which was a typical substrate, under the same reaction conditions (conv.=0.50, *E* value=920). On the other hand, the reactivity of the 3-methylglutaric acid monoester (±)-**2c** significantly decreased (conv.=0.14) under the same reaction conditions, and the enantioselectivity (*E* value=77) was also lower than that for **2a** and **2b** (entry 3). As expected, the hydrolysis reaction of the compounds **2d** bearing a bulky substituent (3,3-dimethyl group) did not proceed at all (entry 4). Fiaud *et al*. reported the esterifications of (±)-**3** with dicarboxylic anhydride using the same enzyme in Et_2O for 24 h, and the enantioselectivities were almost same as those obtained by our hydrolysis version [22]. However, in this study, the enzymatic esterification of **3** with glutaric anhydride (conv.=0.23) was slower than that for the reaction with succinic anhydride (conv.=0.50). Interestingly, the results were apparently contrary to the effect of the acyl moiety in our cases.

In order to apply the concept of this reaction for the kinetic resolution of other secondary alcohols, we next examined the enzymatic hydrolysis of several succinic and glutaric acid monoesters (Fig. **6**), and these results are shown in Table **2**. In all cases, the reactions were performed for a longer reaction time (48 h) using a higher amount of the enzyme (40 mg), because the reactivities were lower than that in the case of the substrate **2b**. While the hydrolysis of the succinic acid monoester (±)-**5a** bearing an ethyl group as the R^2 substituent (R^1=Ph, R^2=Et) with a moderate enantioselectivity was very slow (entry 1; conv.=0.08, *E* value=72), changing the acyl moiety to the glutaric acid monoester ((±)-**5b**) drastically improved both the reactivity and enantioselectivity (entry 2; conv.=0.35, *E* value=340). In the case of (±)-**6b** bearing a phenylmethyl group (R^1=CH_2Ph, R^2=Me), the enzyme also catalyzed the hydrolysis of the compound with a higher enantioselectivity (conv.=0.46, *E* value=520) than that of (±)-**6a** (conv.=0.38, *E* value=180) to afford the highly optically active compounds (entries 3 and 4). On the other hand, the reactions of the substrates bearing a naphthyl group as the R^1 substituent also proceeded. Although the enantioselectivities of the esters ((±)-**8a** and **8b**, R^1=1-naphthyl, R^2=Et) of 1-(1-naphthyl)ethanol (**13**) were low (entries 7 and 8; *E* value = 16 and 18, respectively), both esters ((±)-**7a** and **7b**, R^1=2-naphthyl, R^2=Et) of 1-(2-

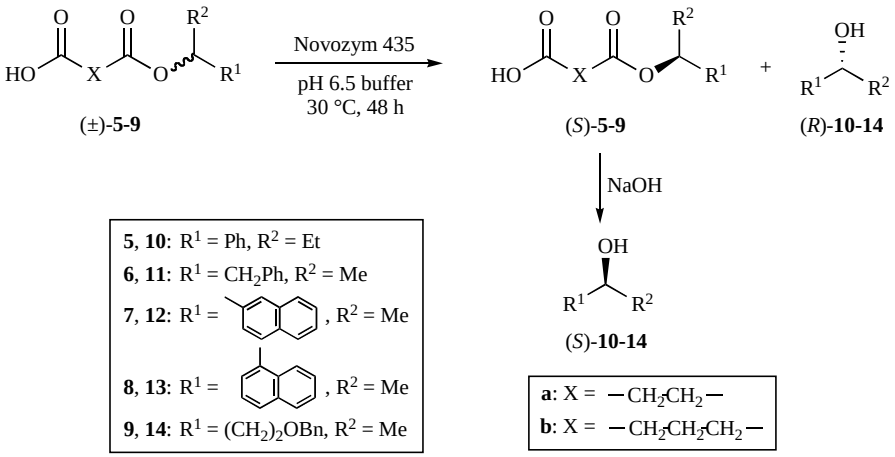

Fig. (6). Enzymatic hydrolysis of the various carboxylic acid monoesters.

Table 2. Enantioselective hydrolysis of dicarboxylic monoesters 5-9 with Novozym 435. [a]

Entry	Substrate	(*S*)-alcohol[b]		(*R*)-alcohol[b]		Conv.[c]	*E* value[d]
		Yield (%)	Ee (%)	Yield (%)	Ee (%)		
1	(±)-**5a**	64	9	10	97	0.08	72
2	(±)-**5b**	54	55	17	99	0.35	340
3	(±)-**6a**	48	59	22	98	0.38	180
4	(±)-**6b**	38	83	39	99	0.46	520
5	(±)-**7a**	50	97	49	99	0.49	840
6	(±)-**7b**	35	99	35	98	0.50	530
7	(±)-**8a**	72	9	10	87	0.09	16
8	(±)-**8b**	75	12	11	88	0.12	18
9	(±)-**9a**	48	98	44	99	0.50	920
10	(±)-**9b**	36	99	45	97	0.51	350

[a]The reactions were performed using 10 m mol L^{-1} of the substrate with Novozym 435 (40 mg) in 0.1 mol L^{-1} phosphate buffer (pH 6.5) for 48 h at 30 °C.
[b]The absolute configurations were determined by comparing the specific rotation signs with those reported.
[c]Calculated using $ee_s/(ee_s+ee_p)$.
[d]Calculated using $\ln[(1-conv.)(1-ee_s)]/\ln[(1-conv.)(1+ee_s)]$.

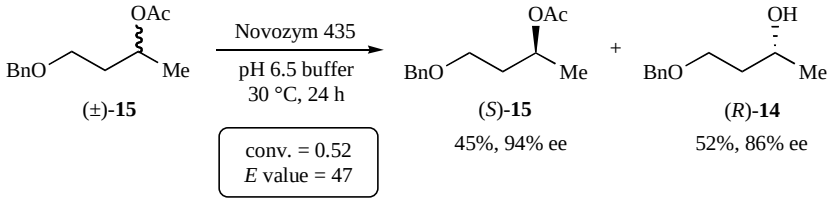

Fig. (7). Enzymatic hydrolysis of the acetate (±)-**15** with Novozym 435.

naphtyl)ethanol (**12**) were hydrolyzed with excellent enantioselectivities (entries 5 and 6; *E* value=840 and 530, respectively). It is noteworthy that the kinetic resolutions of (±)-**9a** and **9b**, which contain a benzyloxyethyl group (R^1=CH_2CH_2OBn, R^2=Me), were smoothly accomplished to afford almost optically pure compounds (entries 9 and 10; *E* value=920 and 350, respectively). We also examined the enzymatic hydrolysis of the acetate (±)-**15** for 24 h at 30 °C (Fig. **7**). Although the enzymatic hydrolysis smoothly proceeded, the enantioselectivity was only moderate (conv.=0.52, *E* value=47). These results indicated that the structure of the acyl moieties of **9** apparently affects the in-

teraction between the substrate and the active site of the enzyme.

Except for the substrate **13** bearing a 1-naphthyl group, all the reactions gave enough high *E* values (over 180), regardless of the size of the R^1 substituent. We then supposed that many aromatic and aliphatic R^1 substituents, which had a suitable size, could be acceptable for the substrates. We also speculated that a 1-naphthyl group would be too bulky for the interaction between the substrate and the enzyme due to the position of the naphthyl group. On the other hand, the conversions for the substrates with an ethyl group as the R^2 substituent (**5a** and **5b**) were lower than those for the substrates with a methyl group, and we supposed that the size of the R^2 substituent should be restricted. In order to improve the conversion for the practical synthesis, a much longer reaction time and a much higher amount of the enzyme could be required.

CONCLUSION

In conclusion, we succeeded in the enzyme-mediated enantioselective hydrolysis of dicarboxylic acid monoesters, and obtained several enantiomers of **3**, **10**, **11**, **12**, **13**, and **14**. We also have disclosed that the reactivity and eantioselectivity can be controlled using a suitable acyl group of the substrates. In our method, the separation of the reaction products was achieved by a simple extraction procedure. Further investigations into the reactions are now in progress.

EXPERIMENTAL

General

^{1}H (500 MHz or 300 MHz) and ^{13}C (125 MHz or 75 MHz) NMR spectra were measured on a JEOL α-500 or a JEOL JNM AL-300 with tetramethylsilane (TMS) as the internal standard. IR spectra were recorded with Shimadzu IR Prestige-21 spectrometers. Mass spectra were obtained with a JEOL EI/FAB mate BU25 Instrument by the EI method or a JEOL JMS-T100 by the ESI-TOF method. Optical rotations were measured with a Jasco DIP-1000 polarimeter. HPLC data were obtained on Shimadzu LC-10AD$_{VP}$, SPD-10A$_{VP}$, and sic 480II data station (System Instruments Co., Ltd.).

Preparation of the Substrate, (±)-3-((1-phenylethoxy)carbonyl)Propanoic Acid (2a)

Under an argon atmosphere, succinic anhydride (490 mg, 4.89 mmol) and DMAP (599 mg, 4.901 mmol) were added to a solution of 1-phenyletanol ((±)-**3**, 299.1 mg, 2.45 mmol) in CH_2Cl_2 (10 mL), and the solution was stirred for 2 h at room temperature. After the mixture was washed with 2 mol L^{-1} HCl, the products were extracted with CH_2Cl_2 (x4), and dried over Na_2SO_4. After evaporation *in vacuo*, the residue was purified by column chromatography on silica gel (hexane/AcOEt = 3/1) to give the (±)-**2a** as a colorless oil in 97% yield (528 mg); IR (neat) 2982, 2934, 1736, 1713, 1495, 1450, 1375, 1285, 1207, 1169, 1063, 959, 762, 700 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.53 (d, J = 6.5 Hz, 3H), 2.56-2.74 (m, 4H), 5.89 (q, J = 6.5 Hz, 1H), 7.21-7.41 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.1, 28.9, 29.1, 72.9, 126.0, 127.9, 128.5, 141.3, 171.4, 178.2; HRMS *m/z* (ESI) 245.0788 (calcd for $C_{12}H_{14}O_4Na$: 245.0790, M+Na$^+$).

The other substrates were synthesized by the same procedure.

Typical Experimental Procedure for Enantioselective Hydrolysis of Dicarboxylic Monoesters (1-phenylethanol (3))

To a recovery flask containing 88.9 mg of (±)-**2a** (sub. conc., 10 mmol L^{-1}) were added 40 mL of 0.1 mol L^{-1} phosphate buffer (pH6.5). To the mixture was added 20 mg of Novozym 435 (2.0 U/mg, using tributyrin at pH 8.0 and 40 °C), and the solution was stirred for 24 h at 30 °C. After addition of 2 mol L^{-1} HCl to the mixture, the products were extracted with Et_2O (x4), and dried over Na_2SO_4. After the organic phase was evaporated in order to reduce the volume, (*S*)-**2a** was selectively extracted with 1 mol L^{-1} Na_2CO_3 aq (x4). Evaporation of the remaining organic phase afforded (*R*)-**3**. On the other hand, to the water phase was added 2 mol L^{-1} NaOH aq (5 mL), and the mixture was stirred for 2 h at room temperature. The products were extracted with Et_2O (x4), and dried over Na_2SO_4. Evaporation of the organic phase afforded (*S*)-**3**. In order to determine the exact isolated yields and ees of the products, both enantiomers were purified by column chromatography on a small amount of silica gel (hexane/Et_2O = 2/1) to give (*S*)-**3** (22.0 mg, 45%, 48% ee) and (*R*)-**3** (7.5 mg, 15%, 99% ee), respectively. $[\alpha]_D^{28}$ = –43.0 (c 1.13, MeOH) (96% ee, (*S*)-form), $[\alpha]_D^{27}$ = +41.0 (c 1.30, MeOH) (>99% ee, (*R*)-form); lit. $[\alpha]_D^{20}$ = +45 (c 5.15, MeOH) ((*R*)-form) [31]. GC conditions: column, CP-Cyclodextrin-B-236-M19 (Chrompack), 0.25 mm x 50 m; injection, 140 °C; detection, 140 °C; oven, 120 °C; carrier gas, He; head pressure, 2.4 kg/cm^2; retention time, 14.6 (*R*) and 15.2 (*S*) min.

The reactions of the other substrates were carried out by the same procedure. The results were shown in the text. All the spectral data (^{1}H and ^{13}C NMR, IR, and MS) were in full agreement with those of the racemates, commercial sources, or those reported.

1-phenyl-1-propanol (10)

$[\alpha]_D^{25}$ = +42.8 (c 0.93, $CHCl_3$) (99% ee, (*R*)-form); lit. $[\alpha]_D^{20}$ = –47.0 (c 1.00, $CHCl_3$) ((*S*)-form) [32]. GC conditions: column, CP-Cyclodextrin-B-236-M19 (Chrompack), 0.25 mm x 50 m; injection, 140 °C; detection, 140 °C; oven, 120 °C; carrier gas, He; head pressure, 2.4 kg/cm^2; retention time, 22.6 (*R*) and 23.4 (*S*) min.

1-phenyl-2-propanol (11)

$[\alpha]_D^{28}$ = –35.4 (c 0.75, $CHCl_3$) (99% ee, (*R*)-form); lit. $[\alpha]_D^{20}$ = –37.6 (c 5.00, $CHCl_3$) ((*R*)-form) [33]. GC conditions: column, CP-Cyclodextrin-B-236-M19 (Chrompack), 0.25 mm x 50 m; injection, 130 °C; detection, 130 °C; oven, 110 °C; carrier gas, He; head pressure, 2.4 kg/cm^2; retention time, 27.1 (*R*) and 27.5 (*S*) min.

1-(2-naphthyl)ethanol (12)

Mp 68.2-68.8 °C (recrystallized from Et_2O-hexane); lit. 68-70 °C [34]. $[\alpha]_D^{28}$ = +38.3 (c 1.01, MeOH) (98% ee, (*R*)-form); lit. $[\alpha]_D$ = +34.6 (c 1.20, MeOH) ((*R*)-form) [35]. GC

conditions: column, CP-Cyclodextrin-B-236-M19 (Chrompack), 0.25 mm x 50 m; injection, 180 °C; detection, 180 °C; oven, 160 °C; carrier gas, He; head pressure, 2.4 kg/cm^2; retention time, 41.9 (*R*) and 42.9 (*S*) min.

1-(1-naphthyl)ethanol (13)

Mp 62.0-62.3 °C (recrystallized from CH_2Cl_2-hexane); lit. 64 °C [36]. $[\alpha]_D^{27}$ = +55.9 (c 0.64, MeOH) (88% ee, (*R*)-form); lit. $[\alpha]_D^{25}$ = +45.0 (c 2.00, MeOH) ((*R*)-form)[37]. GC conditions: column, CP-Cyclodextrin-B-236-M19 (Chrompack), 0.25 mm x 50 m; injection, 180 °C; detection, 180 °C; oven, 160 °C; carrier gas, He; head pressure, 2.4 kg/cm^2; retention time, 43.0 (*R*) and 44.3 (*S*) min.

4-benzyloxy-2-butanol (14)

$[\alpha]_D^{29}$ = –14.9 (c 1.08, MeOH) (99% ee, (*R*)-form); lit. $[\alpha]_D^{27}$ = +19.0 (c 0.95, MeOH) ((*S*)-form)[38]. HPLC conditions: column, CHIRALCEL OD-H (Daicel Chemical Industries, Ltd.); eluent, hexane/2-propanol = 90/10; flow rate, 0.5 mL/min; 254 nm; temperature, 25 °C; retention time, 12.8 (*S*) and 14.0 (*R*) min.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material (GC and HPLC charts) is available on the publishers website along with the published article.

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