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Effect of substituent on the enantioselectivity for lipase-catalyzed kinetic resolution of glycerol derivatives

Yasuhiro Kawanami,* Akira Honnma, Kayo Ohta and Naoko Matsumoto

Department of Biochemistry and Food Science, Faculty of Agriculture, Kagawa University, Miki-cho, Kagawa 761-0795, Japan

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Abstract—The lipase-catalyzed transesterifications of various substituted diphenyl 1,2-ketals of glycerol have been investigated. Efficient modification of the substrate structure with bis(4-bromophenyl) ketal was found to enhance the enantioselectivity up to $E=57$ at 0 °C.

1. Introduction

Chiral glycerol derivatives are useful as chiral building blocks for the syntheses of biologically active chiral compounds such as β-blockers[1] and PAF antagonists.[2] Although the efficient asymmetric syntheses of glycerol derivatives were achieved by the lipase-catalyzed transesterification of racemic 3-(aryloxy)-1,2-propanediols with lipase PS from *Pseudomonas cepacia* (PCL) and vinyl acetate,[3] the esterification of various 1,2-ketals of glycerol with succinic anhydride[4] and the hydrolysis of carboxylic esters of the 1,2-ketals of glycerol[5] were reported to proceed with low enantioselectivities. These results are consistent with the reported finding that the enantioselectivity of PCL toward primary alcohols is much lower than toward secondary alcohols.[6] However, Sakai et al. described that the *E* value for the kinetic resolution of 2,2-dimethyl-1,3-dioxolane-4-methanol **1** using lipase AK from *Pseudomonas fluorescens* (PFL) increased up to 55 (9 at 30 °C) by lowering the reaction temperature to −40 °C in *i*-Pr_2O,[7] although it needed a large amount of lipase and longer reaction time. As an alternative approach, we investigated the optimization of the structures of the glycerol derivatives by altering the protecting group because we recently found that the enantioselectivity during transesterification catalyzed by the immobilized PCL significantly depended on the position of the substituent on the aromatic ring, and the *N*-3,5-dimethylbenzyl group was found to transform the *trans*-2,5-substituted pyrrolidine derivative into an efficiently-resolved substrate.[8] Thus, we expected that 1,3-dioxolane-4-methanol with a variety of 4-substituted phenyl groups in place of the methyl group could improve the enantioselectivity (Fig. 1).

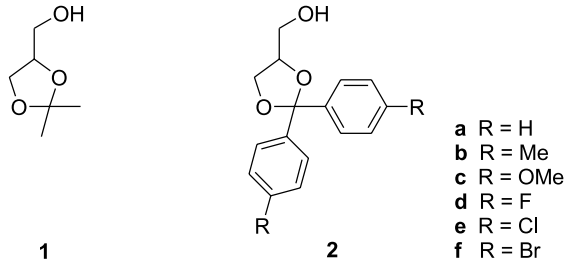

Figure 1.

In this paper, we describe the study of the substituent effect on the enantioselectivity for the lipase-catalyzed transesterification of various 2,2-bis(4-substituted phenyl)-1,3-dioxolane-4-methanols **2a**–**f**.

2. Results and discussion

The substrates **2a**–**f** were prepared by the cyclic ketal formation of glycerol and the corresponding 4-substituted benzophenones in the presence of *p*-toluenesufonic acid at reflux using a Dean–Stark apparatus as previously described[5] (Scheme 1). To investigate the solvent effect, the substrate **2e** was selected as a suitable substrate and the transesterifications catalyzed by lipase PS (PCL) and AK (PFL) were carried out in various solvents at 25 °C (Scheme 2). The enantiomeric excess (ee) values of alcohols **2e** and the resulting acetates **3e** were determined by an

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* Corresponding author. Tel.: +81 87 891 3088; fax: +81 87 891 3021; e-mail: kawanami@ag.kagawa-u.ac.jp

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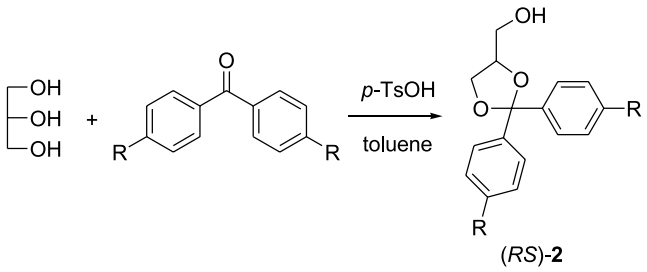


Scheme 1. Preparation of glycerol derivatives.

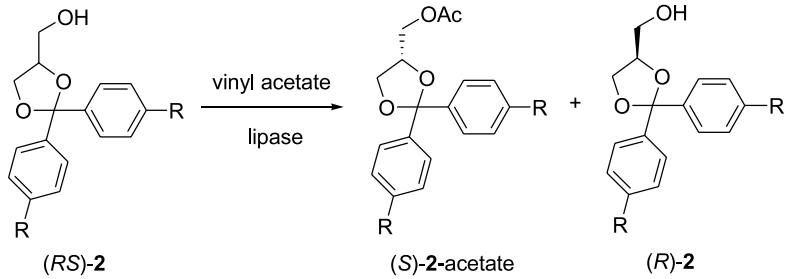
HPLC analysis using a chiral column. The absolute configurations of the remaining alcohols were determined to be *R*-form by comparison of the optical rotations, which were consistent with the empirical rule for chiral primary alcohols.[6] These results are summarized in Table 1.

Acylation of substrate **2e** with vinyl butyrate and lipase AK in *i*-Pr_2O afforded a slightly higher enantioselectivity ($E=11$) than that of **1** ($E=9$)[7] under the similar reaction conditions. Use of vinyl acetate as an acylating agent slightly increased the enantioselectivity ($E=18$). Dry hexane was found to be a better solvent ($E=18$) than wet-hexane for the kinetic resolution of the substrate **2e** using lipase AK. In the case of lipase PS, the similar solvent effect was observed and dry hexane was also proved to be the optimal solvent ($E=21$) among the solvents examined.

Next, the substituent effect on the enantioselectivity was examined using a series of substrates **2a–f** and vinyl acetate in dry hexane at 25 °C (Table 2).

Although the enantioselectivity of lipase AK for the substrates **2a–c** decreased as the size of the electron-donating group increased (Entries 1–3), those of the substrates **2d–f** increased as the size of the electron-withdrawing halogen group on the benzene ring far from the stereocenter increased (Entries 4–6). On the other hand, the enantioselectivity of lipase PS for the substrates **2a–c** and **2d–f** increased as the size of both substituent groups increased (Entries 7–12). Among them, the substrate **2f** (R=Br) showed the highest enantioselectivity ($E=36$). Thus, bis(4-bromophenyl) ketal as the protecting group was found to transform glycerol into an efficiently-resolved substrate.

Finally, the temperature effect on the enantioselectivity was examined using lipase PS in dry hexane at 0 °C as shown in Figure 2. Although the *E* values of the substrates **2a**, **2b**, and **2d** (R=H, Me, F) did not change at 0 °C, those of substrates **2e** and **2f** bearing an electron-withdrawing group (R=Cl, Br) increased from 21 to 25 and from 36 to 57, respectively. On the other hand, the *E* value decreased for substrate **2c** (R=OMe). This result suggests that the inversion of the temperature effect might be due to the strong electron-donating character of the methoxy group in the diphenyl ketals **2c**. It is noted that this effect of the substituent and temperature is quite opposite to that of the esterification of 2-(4-substituted phenoxy)propionic acids catalyzed by lipase MY from *Candida rugosa* ($E=120$, the substrate



Scheme 2. Lipase-catalyzed transesterification of glycerol derivatives.

Table 1. Solvent effect on the enantioselectivity in lipase-catalyzed acylations of **2e**[a]

Entry	Solvent	Lipase	Time (h)	ee_s(%)[b]	ee_p(%)[b]	Convn.(%)[c]	*E* value[c]
1	*i*-Pr_2O[d]	AK	8	61	71	46	11
2	*i*-Pr_2O	AK	3.5	71	79	47	18
3	THF	AK	7	63	63	30	6.0
4	Toluene	AK	6	48	61	44	7.0
5	Wet-hexane[e]	AK	2	48	44	65	7.0
6	Dry-hexane[f]	AK	2	69	94	58	18
7	*i*-Pr_2O	PS	5	33	62	35	5.9
8	THF	PS	8	15	56	21	4.1
9	Toluene	PS	8.5	35	70	33	8.0
10	Wet-hexane[e]	PS	1.5	76	69	52	10
11	Dry-hexane[f]	PS	4	67	83	45	21

[a] Reaction conditions: substrate (0.2 mmol), vinyl acetate (0.8 mmol), lipase (60 mg), solvent (2 ml), 25 °C.
[b] Determined by HPLC analysis using chiralcel OD column. ee_s; (*R*)-**2**, ee_p; (*S*)-**2**-acetate.
[c] Calculated using the equation in Ref. [9].
[d] Vinyl butyrate was used.
[e] Water-saturated hexane.
[f] Distilled from sodium.

Table 2. Subustituent effect on the enantioselectivity in lipase-catalyzed acylations of glycerol derivatives **2a**–**f**[a]

Entry	Substrate, R	Lipase	Time (h)	ee_s(%)[b]	ee_p(%)[b]	Convn. (%)[c]	*E* value[c]
1	**2a**, H	AK	1.5	95	68	58	20
2	**2b**, 4-Me	AK	2	49	69	41	9.1
3	**2c**, 4-OMe	AK	4	65	60	52	7.8
4	**2d**, 4-F	AK	2	83	42	66	5.9
5	**2e**, 4-Cl	AK	2	69	94	58	18
6	**2f**, 4-Br	AK	2	86	77	53	22
7	**2a**, H	PS	6	49	58	46	6.0
8	**2b**, 4-Me	PS	4	41	74	36	10
9	**2c**, 4-OMe	PS	4	67	83	45	21
10	**2d**, 4-F	PS	4	53	72	42	10
11	**2e**, 4-Cl	PS	4	67	83	45	21
12	**2f**, 4-Br	PS	2	81	87	48	36

[a] Reaction conditions: substrate (0.2 mmol), vinyl acetate (0.8 mmol), lipase (60 mg), dry-hexane (2 ml), 25 °C.
[b] Determined by HPLC analysis using chiralcel OD or OD-H column. ee_s; (*R*)-**2**, ee_p; (*S*)-**3**.
[c] Calculated using the equation in Ref. 9.

bearing a methoxy group, 57 °C).[10] Figure 3 shows the correlation between the enantioselectivity of **2a**–**f** and the Hammett σ_p value,[11] implying that the electron-withdrawing character might be the main factor to enhance the enantioselectivity. Figure 4 shows the correlation between the enantioselectivity and van der Waals radius.[12] The enantioselectivity of **2b** bearing the larger methyl group was much lower than that of **2e**,**f** (R = Cl, Br), which suggests that the electronic factor play a more important role than the steric factor in this PCL-catalyzed transesterification of the glycerol derivatives. On the other hand, a good correlation between the enantioselectivity and reactivity was observed as shown in Figure 5. These results suggest that the hydrophobic interaction between the bromo group and the active site of PCL allowed the fast-reacting substrate to strongly bind, leading to the enhancement of both the reactivity and enantioselectivity.

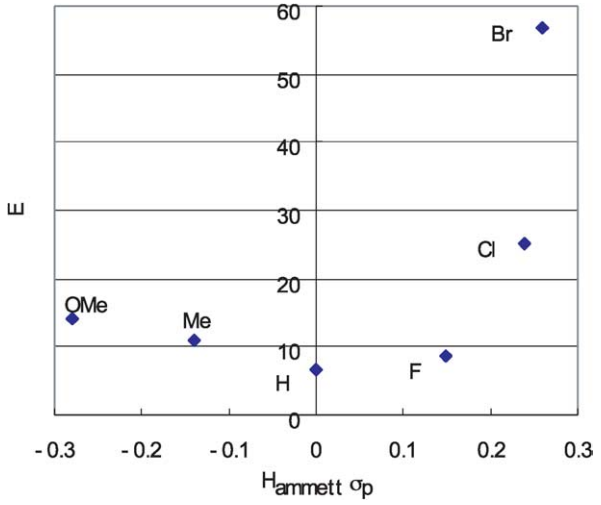


Figure 3. Relation between the enantioselectivity and Hammett σ_p.

Schrag et al. reported that the active-site cleft of PCL based on an X-ray structure analysis was ovoid (10 Å×25 Å across) and was about 15 Å deep and 4 Å wide at the bottom.[12] Figure 6 shows a cutaway view of the active-site cleft (Figure 2(d) in the literature[12]) and the supposed transition-state model for the fast-reacting (*S*)-bis(4-bromophenyl) ketal of glycerol. Although the detailed computer modeling of the X-ray crystal structure of PCL and conformations of the ketal have not yet been performed, the methylene group of dioxalane seems to nestle into the smaller hydrophobic pocket and one of the 4-bromophenyl groups seems to point toward the alternate hydrophobic pocket.[6] Therefore, this overlapping also supported the previous proposition that the additional hydrophobic interaction between the bromo group and the active-site cleft might enhance the reactivity and enantioselectivity.

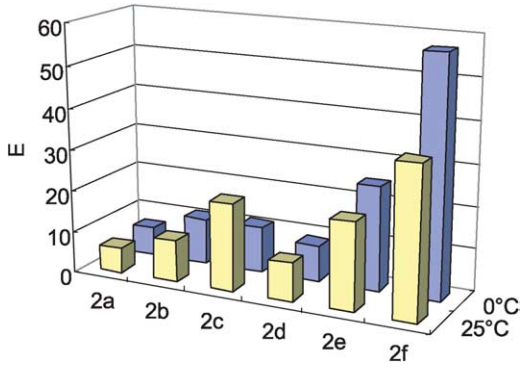


Figure 2. Temperature effect.

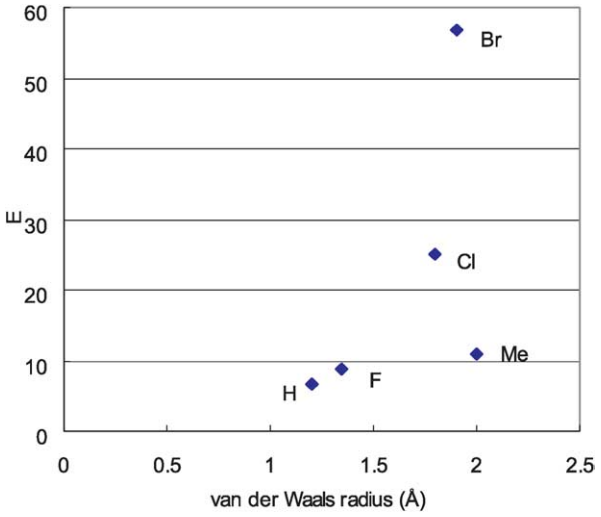


Figure 4. Relation between the enatioselectivity and van der Waals radius.

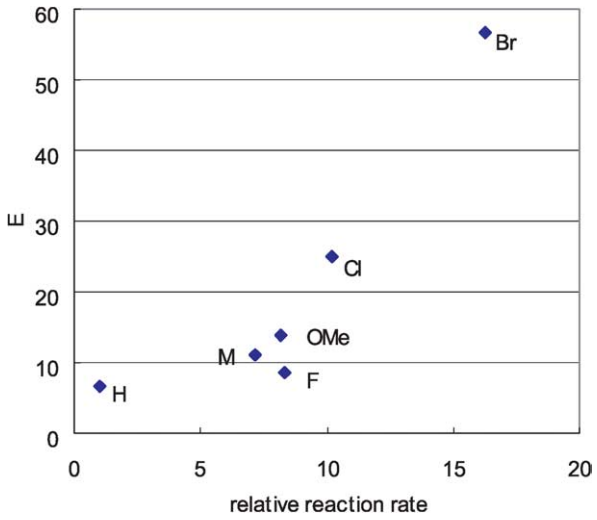


Figure 5. Relation between the enatioselectivity and reactivity. Relative reaction rate was calculated by comparing each (convn./time) of **2b–f** with that of **2a** (R=H).

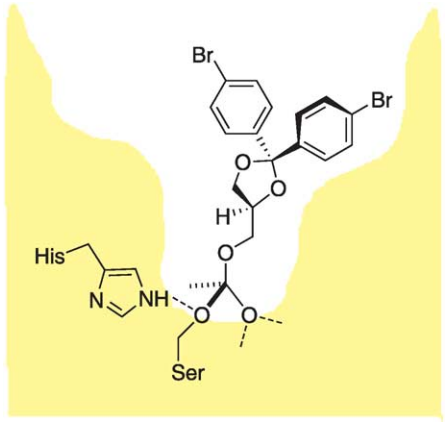


Figure 6. Supposed model of the fast-reacting (*S*)-enantiomer for PCL and the cutaway view of the active-site cleft of PCL. Larger hydrophobic pocket is located far away from the cutaway view and alternate hydrophobic pocket points toward this side.

3. Conclusion

In summary, we have demonstrated that the efficient modification of the substrate structure with bis(4-bromophenyl) ketal as the protecting group enhanced the enantioselectivity of the lipase-catalyzed kinetic resolution of glycerol derivatives at 0 °C. This substrate-tuning method by a protecting group would provide an alternative approach to improve the lipase-catalyzed kinetic resolution of primary alcohols.

4. Experimental

4.1. General

The IR spectra were determined using a JASCO A 302 FT-IR spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 and 100 MHz using a JNM-A400 spectrometer, respectively. The mass spectra were recorded using a JEOL JMS-SX102A mass spectrometer. The optical rotations were determined using a JASCO P-1010 polarimeter. The HPLC analysis was carried out using DAICEL Chiralcel OD and OD-H columns (0.46×25 cm) with a Shimadzu LC6A. TLC was carried out on Merck glass plates precoated with silica gel 60F-254 (0.25 mm) and the column chromatography was performed using Merck 23-400 mesh silica gel. All reagents were purchased from Wako Chemical Co., Tokyo Kasei Kogyo Co., Lancaster Synthesis, Ltd. or Aldrich Chemical.

4.2. Typical procedure for the synthesis of 1,2-ketal of glycerols

4.2.1. 2,2-Bis(4-methylphenyl)-1,3-dioxolane-4-methanol (2b). A mixture of glycerol (0.759 g, 8 mmol), 4,4′-dimethylbenzophenone (1.75 g, 8.2 mmol), and *p*-toluenesulfonic acid (18.2 mg) in toluene (5 ml) was refluxed for 24 h. After cooling, the reaction mixture was quenched with 0.1 M NaOH and extracted with ether. The combined organic layer was washed with saturated $NaHCO_3$ solution, dried over $MgSO_4$, and concentrated. The residue was washed with hexane and chromatographed (hexane/ethyl acetate, 3:1) to give a viscous oil (1.52 g, 64%); IR (neat) 3465 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.80 (1H, br s), 2.30 (3H, s), 2.32 (3H, s), 3.62 (1H, dd, J=5.1, 11.7 Hz), 3.78 (1H, dd, J=3.4, 11.7 Hz), 3.95 (1H, dd, J=6.1, 8.1 Hz), 4.00 (1H, t, J=8.1 Hz), 4.29 (1H, m), 7.10 (2H, d, J=8.0 Hz), 7.13 (2H, d, J=7.8 Hz), 7.34 (2H, d, J=8.0 Hz), 7.40 (2H, d, J=7.8 Hz); ^{13}C NMR ($CDCl_3$) δ 21.1, 63.2, 66.1, 76.8, 110.2, 125.9, 126.1, 128.8, 137.7, 137.9, 139.1, 139.2; EI HRMS $C_{18}H_{20}O_3$ (M^+) 284.1412. Found 284.1452.

4.2.2. 2,2-Bis(4-methoxyphenyl)-1,3-dioxolane-4-methanol (2c). Yield 36%; IR (neat) 3460 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.80 (1H, br s), 3.76–3.82 (2H, m), 3.78 (3H, s), 3.80 (3H, s), 3.96 (1H, dd, J=6.4, 8.0 Hz), 4.00 (1H, t, J=8.0 Hz), 4.30 (1H, m), 6.83 (2H, d, J=8.4 Hz), 6.87 (2H, d, J=8.4 Hz), 7.36 (2H, d, J=8.4 Hz), 7.42 (2H, d, J=8.0 Hz); ^{13}C NMR ($CDCl_3$) δ 55.3, 63.3, 66.1, 76.7, 110.2, 113.5, 127.7, 134.4, 159.4; EI HRMS $C_{18}H_{20}O_5$ (M^+) 316.1311. Found 316.1325.

4.2.3. 2,2-Bis(4-fluorophenyl)-1,3-dioxolane-4-methanol (2d). Yield 38%; IR (neat) 3455 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.81 (1H, dd, J=5.8, 6.8 Hz), 3.66 (1H, ddd, J=5.1, 6.8, 12.0 Hz), 3.81 (1H, ddd, J=3.6, 5.8, 11.7 Hz), 3.98 (1H, dd, J=6.1, 8.0 Hz), 4.02 (1H, t, J=8.3 Hz), 4.31 (1H, m), 6.98–7.10 (4H, m), 7.40–7.51 (4H, m); ^{13}C NMR ($CDCl_3$) δ 63.1, 66.3, 76.7, 109.5, 115.1, 115.3, 128.1, 128.2, 161.4, 163.9; EI HRMS $C_{16}H_{14}F_2O_3$ (M^+) 292.0911. Found 292.0870.

4.2.4. 2,2-Bis(4-chlorophenyl)-1,3-dioxolane-4-methanol (2e). Yield 42%; IR (neat) 3460 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.76 (1H, dd, J=5.2, 6.4 Hz), 3.65 (1H, ddd, J=5.6, 6.4, 12.0 Hz), 3.80 (1H, ddd, J=4.0, 5.2, 11.6 Hz), 3.98 (1H, dd, J=6.1, 8.1 Hz), 4.02 (1H, dd, J=7.1, 8.1 Hz), 4.31 (1H, m), 7.34–7.25 (4H, m), 7.47–7.37 (4H, m); ^{13}C NMR ($CDCl_3$) δ 62.6, 65.9, 76.7, 108.9, 127.0, 128.1, 133.9, 139.8; EI HRMS $C_{16}H_{14}Cl_2O_3$ (M^+) 324.0320. Found 324.0304.

4.2.5. 2,2-Bis(4-bromophenyl)-1,3-dioxolane-4-methanol (2f). Yield 43%; IR (neat) 3460 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ

1.70 (1H, dd, $J=5.8$, 6.8 Hz), 3.65 (1H, ddd, $J=5.1$, 6.8, 12.0 Hz), 3.80 (1H, ddd, $J=3.6$, 5.8, 12.0 Hz), 3.98 (1H, dd, $J=6.4$, 8.3 Hz), 4.02 (1H, dd, $J=7.6$, 8.3 Hz), 4.31 (1H, m), 7.40–7.31 (4H, m), 7.49–7.43 (4H, m); ^{13}C NMR ($CDCl_3$) δ 63.1, 66.4, 77.2, 109.4, 122.7, 127.8, 128.0, 131.6, 131.7, 140.8; EI HRMS $C_{16}H_{14}Br_2O_3$ (M^+) 413.9310. Found 413.9329.

4.3. Typical procedure for lipase-catalyzed tranesterification of 2a with vinyl acetate

Vinyl acetate (74 μl, 0.8 mmol) was added to a suspension of the 1,2-ketal of glycerol **2a** (25.6 mg, 0.1 mmol) and lipase (60 mg) in hexane (2 ml) and the mixture was stirred at 25 °C for 6 h. The reaction was monitored by TLC. The reaction mixture was filtered off on celite and washed with dichloromethane. The filtrate was evaporated under reduced pressure. Flash column chromatography (hexane/ethyl acetate 3:2) of the residue afforded the **2a**-acetate (HPLC with Chiralcel OD, hexane/*i*-PrOH 97:3, 0.7 ml/min^{-1}, retention time; 14 and 16 min for (*R*)- and (*S*)-enantiomer) and the alcohol **2a** (HPLC with Chiralcel OD, hexane/*i*-PrOH 97:3, 0.7 ml/min^{-1}, retention time; 32 and 37 min for (*R*)- and (*S*)-enantiomer). To establish the absolute configuration of the remaining alcohol **2a**, its optical rotation sign was compared with the reported value; (*R*)-(−)-**2a**; $[\alpha]_D^{21}$ −11.5 (*c*, 0.52, MeOH) (lit.,[5] $[\alpha]_D^{20}$ +22.5 (*c*, 0.36, MeOH) for (*S*)-isomer). The other absolute configurations of the remaining alcohols **2b-f** were assigned as (*R*) by comparison of the sign of the optical rotations and the order of retention times on an HPLC chiral column with that of (*R*)-(−)-**2a**. Moreover, those of the acetates were confirmed by transformation (10% acetic acid) into (*S*)-(+)-1-*O*-acetylglycerol.[13]

For **2b** and **2b**-acetate, HPLC with Chiralcel OD, hexane/*i*-PrOH 97:3, 0.7 ml/min^{-1}; **2c**, OD, hexane/*i*-PrOH 97:3, 0.6 ml/min; **2c**-acetate, OD-H, hexane/*i*-PrOH 97:3, 0.7 ml/min^{-1}; **2d**, OD, hexane/*i*-PrOH 96:4, 0.5 ml/min^{-1}; **2d**-acetate, OD-H, hexane/*i*-PrOH 200:1, 0.4 ml/min^{-1}; **2e**, OD, hexane/*i*-PrOH 96:4, 0.6 ml/min^{-1}; **2e**-acetate, OD-H, hexane/*i*-PrOH 200:1, 0.8 ml/min^{-1}; **2f**, OD, hexane/*i*-PrOH 98:2, 0.6 ml/min^{-1}; **2f**-acetate, OD-H, hexane/*i*-PrOH 200:1, 0.4 ml/min^{-1}.

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