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Comparison of the chiral recognition of prochiral substrates in the acetylation reaction by a novel lipase (CSL) from the yeast, *Cryptococcus* spp. S-2 with immobilized PPL

Enzyme-catalyzed desymmetrization and asymmetrization of prochiral 2-substituted 1,3-propanediols by CSL and immobilized PPL

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Abstract

In order to elucidate the nature of a novel lipase (CSL), isolated from the yeast *Cryptococcus* spp. S-2, in chiral recognition by comparison with that of immobilized PPL, the desymmetrization and asymmetrization of prochiral 2-phenyl-1,3-propanediol (**1a**), 2-benzyl-1,3-propanediol (**1b**), 2-methyl-2-phenyl-1,3-propanediol (**1c**), 2-benzyl-2-methyl-1,3-propanediol (**1d**), 2-ethyl-2-phenyl-1,3-propanediol (**1e**), and 2-benzyl-2-ethyl-1,3-propanediol (**1f**) by acetylation was investigated. Acetylation of **1a** with excess vinyl acetate by the CSL-enzyme catalyst gave the corresponding monoacetate **2a** with high enantioselectivity (80% ee) in 46% yield. Very high levels of desymmetrization were observed in the tertiary systems of **1c**–**f**, giving the corresponding monoacetates **2c**–**f**, respectively, in >97%. In the desymmetrization of diols **1a**, **1c**, **1d**, and **1f**, the sense of chiral differentiation of CSL was opposite to that of immobilized porcine pancreatic lipase (PPL).

Keywords: Lipase; Desymmetrization; Asymmetrization; 2-Substituted 1,3-propanediol; *Cryptococcus*

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1. Introduction

Enzyme-catalyzed esterification of prochiral diols is an attractive method for the preparation of chiral building blocks for the synthesis of complex compounds of biological and pharmacological interest. Since a number of biologically important natural products contain chiral quaternary carbon center(s) [1], many enzyme-based transformation involving the formation of such asymmetric centers have been investigated. Among these, the enzyme-catalyzed hydrolysis of prochiral disubstituted malonates [2], the hydrolysis of prochiral diesters derived from diols [3], and the reduction of prochiral 2,2-disubstituted 1,3-diones [4] have achieved a certain degree of success. An efficient lipase-catalyzed desymmetrization of prochiral 2,2-disubstituted 1,3-propanediols using 1-ethoxyvinyl 2-furoate with lipase MY from *Candida rugosa* [5] has also been developed.

Among the known biocatalysts, lipases have been the most widely applied in the synthesis of enantiomerically pure compounds [6]. Recently, a new addition has been made to this family of enzymes. A novel lipase (CSL) has been isolated from the yeast, *Cryptococcus* spp. S-2 and it has been demonstrated to have good potential for the hydrolysis of vegetable oils, which are industrially and economically important for the production of biodiesel fuel [7]. In connection with our interest in the desymmetrization of prochiral compounds [8], we have carried out an examination of this novel CSL-enzyme as a possible alternative for the enantioselective monoesterification of prochiral 2,2-disubstituted 1,3-propanediols by chiral recognition. Previously, several efficient lipase-catalyzed desymmetrizations of prochiral 2-monosubstituted 1,3-propanediols have been reported [9–13].

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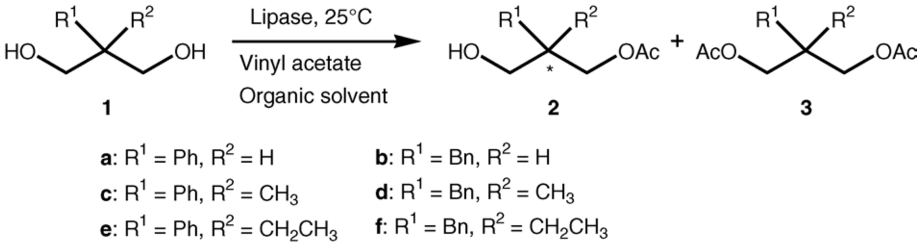


Scheme 1.

In contrast, there are few examples on the desymmetrization of prochiral 2,2-disubstituted 1,3-propanediols furnishing chiral quaternary carbons [5,14].

In this paper, we describe the desymmetrization and asymmetrization of prochiral 2-monosubstituted and 2,2-disubstituted 1,3-propanediols (**1a**–**f**) by CSL catalyzed acetylation (Scheme 1), and compare the reactivity and enantioselectivity of CSL with that of immobilized porcine pancreatic lipase (PPL).

2. Experimental

2.1. General procedures

^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded in $CDCl_3$ with JEOL NM-ECP500 and NM-LA500 spectrometers using tetramethylsilane as the internal standard. Optical rotations were measured on a Jasco DIP-370 polarimeter. Enantioselectivity (%ee) was determined by HPLC analysis of the reaction mixture with a system consisting of a Jasco 880-PU pump, a Jasco 875-UV detector and a Daisel CHIRALCEL AD and OD column (4.6 mm × 250 mm).

2.2. Lipases

Preparation of the lipase (CSL) from the yeast, *Cryptococcus* spp. S-2 was done by a reported method [7]. Immobilized porcine pancreatic lipase on Hyflo Super Celite was prepared by a reported method [12]. The lipases, CSL, and immobilized PPL, to be used in experiments were stored at 4 °C.

2.3. Prochiral 2-substituted and 2,2-disubstituted 1,3-propanediols

2.3.1. 2-Phenyl-1,3-propanediol (**1a**)

A solution of commercially available diethyl phenylmalonate (4.8 g, 19.3 mmol) in Et_2O (20 mL) was added dropwise to a suspension of $LiAlH_4$ (1.11 g, 29 mmol) in Et_2O (20 mL) at 0 °C. The reaction mixture was stirred for 12 h at room temperature. The mixture was then cooled to 0 °C and treated with water until no more gas evolution could be observed. The ether layer was filtered and the cake was washed with Et_2O. After concentration under reduced pressure, the resulting residue was purified by column chromatography on silica gel using $CHCl_3$–MeOH (97:3) to yield 2.3 g (76%) of 2-phenyl-1,3-propanediol (**1a**) as colorless crystals. **1a**: mp 51.5–52.0 °C; ^{1}H NMR ($CDCl_3$) δ 7.34–7.20 (m, 5H), 3.99 (dd, $J = 7.6$, 10.8 Hz, 2H), 3.92 (dd, $J = 5.5$, 10.8 Hz, 2H), 3.09 (m, 1H), the hydroxy protons were not observed due to broadening of the corresponding signal; ^{13}C NMR ($CDCl_3$) δ 139.2, 128.8 (×2), 128.0 (×2), 127.2, 66.1 (×2), 49.8; FAB-HRMS calcd for $C_9H_{13}O_2$ $[M+H]^+$, 153.0916; found 153.0914; Anal. calcd for $C_9H_{12}O_2$: C 71.03, H 7.95; found: C 71.15, H 7.81.

2.3.2. 2-Benzyl-1,3-propanediol (**1b**)

To a suspension of NaH (2.42 g, 80 mmol) in dry THF (100 mL) was added dropwise the starting material, freshly distilled dimethyl malonate (10.4 mL, 91 mmol), at 0 °C. After evolution of gas seized, benzyl chloride (7.0 mL, 61 mmol) was added at 0 °C and the reaction mixture was stirred for 1 h at room temperature, followed by refluxing for 6 h. The resulting mixture was treated with a saturated aqueous solution of NH_4Cl and extracted with EtOAc (2× 20 mL). The organic layer was dried over anhydrous $MgSO_4$ and evaporated at reduced pressure to give an oil which was purified by column chromatography (silica gel, hexane–ethyl acetate, 95:5), to afford dimethyl 2-benzylmalonate (6.9 g, 51%) as a colorless liquid: ^{1}H NMR ($CDCl_3$) δ 7.27–7.16 (m, 5H), 3.67 (s, 6H), 3.66 (t, $J = 7.8$ Hz, 1H), 3.20 (d, $J = 7.8$ Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 169.2 (×2), 137.7, 128.7 (×2), 128.5 (×2), 126.7, 53.5 (×2), 52.5, 34.7; EI-HRMS calcd for $C_{12}H_{14}O_4$ $[M]^+$, 222.0892; found 222.0891.

Dimethyl 2-benzylmalonate (496 mg, 2.2 mmol) was converted to 2-benzyl-1,3-propanediol **1b** (300 mg, 81%) as colorless crystals by reduction using $LiAlH_4$ (170 mg, 4.5 mmol) in ether: mp 66.0–66.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.29–7.16 (m, 5H), 3.79 (dd, $J = 3.7$, 10.5 Hz, 2H), 3.67 (dd, $J = 6.9$, 10.5 Hz, 2H), 2.61 (d, $J = 7.3$ Hz, 2H), 2.04 (m, 1H), the hydroxy protons were not observed due to broadening of the corresponding signal; ^{13}C NMR ($CDCl_3$) δ 139.8, 129.0 (×2), 128.5 (×2), 126.1, 65.6 (×2), 43.8, 34.2; FAB-HRMS calcd for $C_{10}H_{15}O_2$ $[M+H]^+$, 167.1072; found 167.1075; Anal. calcd for $C_{10}H_{14}O_2$: C 72.26, H 8.49; found: C 72.30, H 8.47.

2.3.3. 2-Methyl-2-phenyl-1,3-propanediol (**1c**)

To a suspension of NaH (0.88 g, 26 mmol) in dry THF (50 mL) was added dropwise diethyl phenylmalonate (4.73 g, 20 mmol) at 0 °C. After evolution of gas seized, iodomethane (1.6 mL, 26 mmol) was added to the reaction mixture at 0 °C. The reaction conditions and the work-up method were similar to the previous procedure for the preparation of dimethyl 2-benzylmalonate. Column chromatography on silica gel (hexane–ethyl acetate, 95:5) afforded diethyl 2-methyl-2-

phenylmalonate (4.8 g, 97%) as a colorless liquid: ^{1}H NMR ($CDCl_3$) δ 7.28–7.16 (m, 5H), 4.13 (m, 4H), 1.76 (s, 3H), 1.51 (t, $J = 7.1$ Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 171.5 (×2), 138.3, 128.1 (×2), 127.5, 127.4 (×2), 61.6 (×2), 58.8, 22.3, 13.9(×2); FAB-HRMS calcd for $C_{14}H_{19}O_4$ $[M+H]^+$, 251.1283; found 251.1281.

Diethyl 2-methyl-2-phenylmalonate (4.8 g, 19.3 mmol) was converted to 2-methyl-2-phenyl-1,3-propanediol **1c** (2.4 g, 75%) as colorless crystals by reduction with $LiAlH_4$ (1.16 g, 30 mmol): mp 82.5–83.0 °C; ^{1}H NMR ($CDCl_3$) δ 7.33–7.11 (m, 5H), 3.84 (d, $J = 11.0$ Hz, 2H), 3.70 (d, $J = 11.0$ Hz, 2H), 1.17 (s, 3H), the hydroxy protons were not observed due to broadening of the corresponding signal; ^{13}C NMR ($CDCl_3$) δ 142.9, 128.7 (×2), 126.7, 126.7 (×2), 70.1 (×2), 44.6, 20.7; FAB-HRMS calcd for $C_{10}H_{15}O_2$ $[M+H]^+$, 167.1072; found 167.1080; Anal. Calcd for $C_{10}H_{14}O_2$: C 72.26, H 8.49; found: C 72.19, H 8.63.

2.3.4. *2-Benzyl-2-methyl-1,3-propanediol (**1d**)*

To a suspension of NaH (0.39 g, 11.3 mmol) in dry THF (25 mL) was added dropwise dimethyl 2-benzylmalonate (2.34 g, 10.5 mmol) in dry THF (5 mL) at 0 °C. After evolution of gas seized, iodomethane (0.72 mL, 11.5 mmol) was added to the reaction mixture at 0 °C. The reaction conditions and the work-up procedures were similar to those for the preparation of dimethyl 2-benzylmalonate. Column chromatography on silica gel (hexane–ethyl acetate, 95:5) afforded dimethyl 2-benzyl-2-methylpropanedicarboxylate (97%) as a colorless liquid: ^{1}H NMR ($CDCl_3$) δ 7.26–7.07 (m, 5H), 3.71 (s, 6H), 3.21 (s, 2H), 1.33 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 172.3 (×2), 136.0, 130.1 (×2), 128.2 (×2), 126.9, 54.9 (×2), 52.4, 41.2, 19.7; EI-HRMS calcd for $C_{13}H_{16}O_4$ $[M]^+$, 236.1049; found 236.1055.

Reduction of dimethyl 2-benzyl-2-methylpropanedicarboxylate (2.26 g, 9.6 mmol) with $LiAlH_4$ gave diol **1d** (1.58 g, 92%) as colorless crystals: mp 67.0–67.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.28–7.19 (m, 5H), 3.55 (d, $J = 10.8$ Hz, 2H), 3.52 (d, $J = 10.8$ Hz, 2H), 2.69 (s, 2H), 0.74 (s, 3H), the hydroxy protons were not observed due to broadening of the corresponding signal; ^{13}C NMR ($CDCl_3$) δ 137.8, 130.6 (×2), 128.0 (×2), 126.1, 70.1 (×2), 40.1, 39.8, 18.6; FAB-HRMS calcd for $C_{11}H_{17}O_2$ $[M+H]^+$, 181.1229; found 181.1232; Anal. Calcd for $C_{11}H_{16}O_2$: C 73.30, H 8.95; found: C 73.23, H 9.02.

2.3.5. *2-Ethyl-2-phenyl-1,3-propanediol (**1e**)*

To a suspension of NaH (0.27 g, 11.3 mmol) in dry THF (25 mL) was added dropwise diethyl 2-phenylmalonate (2.36 g, 10.0 mmol) in dry THF (5 mL) at 0 °C. After evolution of gas seized, iodoethane (1.0 mL, 12.5 mmol) was added to the reaction mixture at 0 °C. The reaction conditions and the work-up procedures were similar to those for the preparation of diethyl 2-benzylmalonate. Column chromatography on silica gel (hexane–ethyl acetate, 95:5) afforded diethyl 2-ethyl-2-phenylpropanedicarboxylate (65%) as a colorless liquid: ^{1}H NMR ($CDCl_3$) δ 7.44–7.26 (m, 5H), 4.27–4.17 (m, 4H), 2.35 (q, $J = 7.3$ Hz, 2H), 1.23 (t, $J = 7.1$ Hz, 6H), 0.89 (t, $J = 7.3$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 170.7 (×2), 136.9, 128.1 (×2), 128.0 (×2), 127.3, 63.0, 61.3 (×2), 28.8, 13.9 (×2), 9.2; FAB-HRMS calcd for $C_{15}H_{21}O_4$ $[M+H]^+$, 265.1440; found 265.1450.

Reduction of diethyl 2-ethyl-2-phenylpropanedicarboxylate (1.32 g, 5.0 mmol) with $LiAlH_4$ gave diol **1e** (0.92 g, 99%) as colorless crystals: mp 72.0–72.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.39–7.23 (m, 5H), 4.10 (d, $J = 11.0$ Hz, 2H), 3.92 (d, $J = 11.0$ Hz, 2H), 1.68 (q, $J = 7.6$ Hz, 2H), 0.68 (t, $J = 7.6$ Hz, 3H), the hydroxy protons were not observed due to broadening of the corresponding signal; ^{13}C NMR ($CDCl_3$) δ 141.2, 128.7 (×2), 127.1 (×2), 126.5, 68.5 (×2), 47.4, 26.8, 7.9; FAB-HRMS calcd for $C_{11}H_{17}O_2$ $[M+H]^+$, 181.1229; found 181.1220.

2.3.6. *2-Benzyl-2-ethyl-1,3-propanediol (**1f**)*

To a suspension of NaH (0.16 g, 6.8 mmol) in dry THF (25 mL) was added dropwise dimethyl 2-benzylmalonate (1.00 g, 4.5 mmol) in dry THF (5 mL) at 0 °C. After evolution of gas seized, iodoethane (0.40 mL, 5.0 mmol) was added to the reaction mixture at 0 °C. The reaction conditions and the work-up procedures were similar to those for the preparation of diethyl 2-benzylmalonate. Column chromatography on silica gel (hexane–ethyl acetate, 95:5) afforded dimethyl 2-benzyl-2-ethyl-propanedicarboxylate (79%) as colorless crystals: mp 50.0–50.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.27–7.06 (m, 5H), 3.71 (s, 6H), 3.24 (s, 2H), 1.84 (q, $J = 7.6$ Hz, 2H), 0.90 (t, $J = 7.6$ Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 171.7 (×2), 136.1, 129.8 (×2), 128.2 (×2), 126.9, 59.5, 52.2 (×2), 37.7, 24.9, 8.7; FAB-HRMS calcd for $C_{14}H_{19}O_4$ $[M+H]^+$, 251.1283; found 251.1281.

Reduction of dimethyl 2-benzyl-2-ethylpropanedicarboxylate (0.87 g, 3.5 mmol) with $LiAlH_4$ gave diol **1f** (0.61 g, 89%) as a colorless liquid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29–7.20 (m, 5H), 3.60 (d, $J = 10.8$ Hz, 2H), 3.57 (d, $J = 10.8$ Hz, 2H), 2.68 (s, 2H), 1.27 (q, $J = 7.6$ Hz, 2H), 0.94 (t, $J = 7.6$ Hz, 3H), the hydroxy protons were not observed due to broadening of the corresponding signal; ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.8, 130.4 (×2), 128.1 (×2), 126.1, 68.1 (×2), 42.4, 36.7, 23.4, 7.5; FAB-HRMS calcd for $C_{12}H_{19}O_2$ $[M+H]^+$, 195.1385; found 195.1384; Anal. calcd for $C_{12}H_{18}O_2$: C, 74.19; H, 9.24, found: C, 74.18; H 9.48.

2.4. *General procedure for lipase-catalyzed desymmetrization*

In the case of CSL, to a solution of 0.2 mmol of **1a–f** and 54 mg of lipase CSL in 2.0 mL of organic solvent, 0.037 mL of vinyl acetate (2 equiv.) were added. In the case of immobilized PPL, 216 mg of immobilized PPL and 0.11 mL of vinyl acetate (6 equiv.) were used. The suspension was magnetically stirred at room temperature and the time-courses of the reaction were monitored by TLC analysis. The reaction was terminated by filtration of the enzyme through a Celite pad and the filtrate was evaporated under reduced pressure. The residue was characterized by ^{1}H NMR and HPLC on a chiral column and then the residue was separated by chromatography on silica gel columns using hexane–ethyl acetate (7:3) as an eluent to give the monoacetate, the diacetate and recovered starting material.

2.4.1. *3-Acetoxy-2-phenylpropanol (**2a**)*

Colorless oil; IR (neat) cm^{-1} 3417 (OH), 2954 (C–H), 1735 (C=O); ^{1}H NMR ($CDCl_3$) δ 7.34–7.22 (m, 5H), 4.37 (dd, J = 6.4, 11.2 Hz, 1H), 4.35 (dd, J = 6.4, 11.2 Hz, 1H), 3.84 (dd, J = 6.4, 11.2 Hz, 1H), 3.82 (dd, J = 6.4, 11.2 Hz, 1H), 3.13 (quint, J = 6.4 Hz, 1H), 2.03 (s, 3H), the hydroxy proton was not observed due to broadening of the corresponding signal; ^{13}C NMR ($CDCl_3$) δ 171.3, 138.9, 128.7 (×2), 128.1 (×2), 127.3, 64.9, 63.8, 47.2, 20.9; FAB-HRMS calcd for $C_{11}H_{15}O_3$ $[M+H]^+$, 195.1021; found 195.1025.

2.4.2. *3-Acetoxy-2-benzylpropanol (**2b**)*

Colorless oil, IR (neat) cm^{-1} 3400 (OH), 2927 (C–H), 1735 (C=O); ^{1}H NMR ($CDCl_3$) δ 7.29–7.16 (m, 5H), 4.17 (dd, J = 4.6, 11.2 Hz, 1H), 4.06 (dd, J = 6.4, 11.2 Hz, 1H), 3.59 (dd, J = 4.6, 11.2 Hz, 1H), 3.49 (dd, J = 4.6, 11.2 Hz, 1H), 2.67 (dd, J = 7.6, 13.8 Hz, 1H), 2.61 (dd, J = 7.6, 13.8 Hz, 1H), 2.11(m, 1H), 2.07 (s, 3H), the hydroxy proton was not observed due to broadening of the corresponding signal; ^{13}C NMR ($CDCl_3$) δ 171.7, 139.3, 129.0 (×2), 128.5 (×2), 126.3, 64.0, 62.1, 42.5, 34.3, 20.9; FAB-HRMS calcd for $C_{12}H_{17}O_3$ $[M+H]^+$, 209.1178; found 209.1175.

2.4.3. *3-Acetoxy-2-methyl-2-phenylpropanol (**2c**)*

Colorless oil; IR (neat) cm^{-1} 3448 (OH), 2938 (C–H), 1735 (C=O); ^{1}H NMR ($CDCl_3$) δ 7.38–7.22 (m, 5H), 4.34 (d, J = 11.5 Hz, 1H), 4.33 (d, J = 11.5 Hz, 1H), 3.73 (d, J = 11.5 Hz, 1H), 3.72 (d, J = 11.5 Hz, 1H), 2.04 (s, 3H), 1.34 (s, 3H), the hydroxy proton was not observed due to broadening of the corresponding signal; ^{13}C NMR ($CDCl_3$) δ 171.5, 142.5, 128.6 (×2), 126.8, 126.4 (×2), 68.5, 67.9, 43.7, 20.9, 20.6; FAB-HRMS calcd for $C_{12}H_{17}O_3$ $[M+H]^+$, 209.1178; found 209.1186.

2.4.4. *3-Acetoxy-2-benzyl-2-methylpropanol (**2d**)*

Colorless oil, IR (neat) cm^{-1} 3450 (OH), 2935 (C–H), 1735 (C=O); ^{1}H NMR ($CDCl_3$) δ 7.28–7.14 (m, 5H), 3.95 (d, J = 11.2 Hz, 1H), 3.91 (d, J = 11.2 Hz, 1H), 3.32 (d, J = 11.7 Hz, 1H), 3.28 (d, J = 11.7 Hz, 1H), 2.60 (d, J = 13.3 Hz, 1H), 2.56 (d, J = 13.3 Hz, 1H), 2.11 (s, 3H), 0.81 (s, 3H), the hydroxy proton was not observed due to broadening of the corresponding signal; ^{13}C NMR ($CDCl_3$) δ 171.9, 137.0, 130.6 (×2), 128.1 (×2), 126.3, 67.6, 66.1, 40.1, 39.9, 20.9, 18.5; FAB-HRMS calcd for $C_{13}H_{19}O_3$ $[M+H]^+$, 223.1334; found 223.1326.

2.4.5. *3-Acetoxy-2-ethyl-2-phenylpropanol (**2e**)*

Colorless oil, IR (neat) cm^{-1} 3452 (OH), 2965 (C–H), 1739 (C=O); ^{1}H NMR ($CDCl_3$) δ 7.38–7.23 (m, 5H), 4.50 (d, J = 11.2 Hz, 1H), 4.45 (d, J = 11.2 Hz, 1H), 3.83 (d, J = 11.5 Hz, 1H), 3.79 (d, J = 11.5 Hz, 1H), 2.06 (s, 3H), 1.78–1.70 (m, 2H), 0.70 (t, J = 7.6 Hz, 3H), the hydroxy proton was not observed due to broadening of the corresponding signal; ^{13}C NMR ($CDCl_3$) δ 171.6, 140.9, 128.6 (×2), 126.7 (×2), 126.6, 65.8, 65.8, 46.8, 26.3, 20.9, 7.7; FAB-HRMS calcd for $C_{13}H_{19}O_3$ $[M+H]^+$, 223.1334; found 223.1330.

2.4.6. *3-Acetoxy-2-benzyl-2-ethylpropanol (**2f**)*

Colorless oil, IR (neat) cm^{-1} 3444 (OH), 2931 (C–H), 1736 (C=O); ^{1}H NMR ($CDCl_3$) δ 7.30–7.18 (m, 5H), 3.97 (d, J = 11.5 Hz, 1H), 3.94 (d, J = 11.5 Hz, 1H), 3.35 (d, J = 11.7 Hz, 1H), 3.30 (d, J = 11.7 Hz, 1H), 2.63 (d, J = 13.5 Hz, 1H), 2.54 (d, J = 13.5 Hz, 1H), 2.12 (s, 3H), 1.29–1.15 (m, 2H), 0.97 (t, J = 7.3 Hz, 3H), the hydroxy proton was not observed due to broadening of the corresponding signal; ^{13}C NMR ($CDCl_3$) δ 171.8, 137.1, 130.4 (×2), 128.1 (×2), 126.3, 65.9, 65.9, 42.7, 36.2, 22.7, 20.9, 7.4; FAB-HRMS calcd for $C_{14}H_{21}O_3$ $[M+H]^+$, 237.1491; found 237.1483.

2.4.7. *1,3-Diacetoxy-2-phenylpropane (**3a**)*

Colorless oil; IR (neat) cm^{-1} 2962 (C–H), 1743 (C=O); ^{1}H NMR ($CDCl_3$) δ 7.33–7.21 (m, 5H), 4.33 (d, J = 11.2 Hz, 2H), 4.30 (d, J = 11.2 Hz, 2H), 3.29 (m, 1H), 2.00 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 170.9, 138.3, 128.7 (×2), 127.9 (×2), 127.4, 64.8 (×2), 43.7, 20.8 (×2); FAB-HRMS calcd for $C_{13}H_{17}O_4$ $[M+H]^+$, 237.1127; found 237.1128.

2.4.8. *1,3-Diacetoxy-2-benzylpropane (**3b**)*

Colorless oil, IR (neat) cm^{-1} 2954 (C–H), 1739 (C=O); ^{1}H NMR ($CDCl_3$) δ 7.29–7.13 (m, 5H), 4.06 (dd, J = 5.3, 11.2 Hz, 2H), 4.00 (dd, J = 6.4, 11.2 Hz, 2H), 2.68 (d, J = 7.6 Hz, 2H), 2.31 (m, 1H), 2.04 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 171.0 (×2), 138.7, 129.0 (×2), 128.6 (×2), 126.4, 63.7 (×2), 39.1, 34.6, 20.8 (×2); FAB-HRMS calcd for $C_{14}H_{19}O_4$ $[M+H]^+$, 251.1283; found 251.1292.

2.4.9. *1,3-Diacetoxy-2-methyl-2-phenylpropane (**3c**)*

Colorless oil, IR (neat) cm^{-1} 2977 (C–H), 1743 (C=O); ^{1}H NMR ($CDCl_3$) δ 7.33–7.21 (m, 5H), 4.28 (d, J = 11.2 Hz, 2H), 4.27 (d, J = 11.2 Hz, 2H), 2.00 (s, 6H), 1.38 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.9 (×2), 142.0, 128.5 (×2), 126.9, 126.2 (×2), 68.4 (×2), 41.6, 21.0, 20.8 (×2); FAB-HRMS calcd for $C_{14}H_{19}O_4$ $[M+H]^+$, 251.1283; found 251.1291.

2.4.10. *1,3-Diacetoxy-2-benzyl-2-methylpropane (**3d**)*

Colorless oil, IR (neat) cm^{-1} 2927 (C–H), 1739 (C=O); ^{1}H NMR ($CDCl_3$) δ 7.27–7.07 (m, 5H), 3.89 (d, J = 11.2 Hz, 2H), 3.86 (d, J = 11.2 Hz, 2H), 2.64 (s, 2H), 2.07 (s, 6H), 0.86 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 170.8 (×2), 136.4, 130.4 (×2), 128.1 (×2), 126.5, 67.3 (×2), 40.3, 38.0, 20.8 (×2), 19.0; FAB-HRMS calcd for $C_{15}H_{21}O_4$ $[M+H]^+$, 265.1440; found 265.1433.

2.5. *Absolute configuration of monoacetates **2c**, **2d**, and **2f** produced by the CSL-catalyzed acetylation*

According to a reported method [14d], to a solution of **2c** {17.1 mg, 0.082 mmol; $[\alpha]D^{25}$ + 7.3 (c 1.2, CH_2Cl_2)} produced from **1c** by CSL in acetone (1.0 mL) was added the Jones reagent (0.20 mL) at room temperature and the reaction mixture was stirred for 3 h. Isopropanol (0.2 mL) was added and the solution was diluted with ether (10 mL). The resulting mixture was filtered through Celite and the filtrate was evaporated. The residue was purified by chromatography on silica gel column using hexane–ethyl acetate (7:3) as an eluent to give 3-acetoxy-2-methyl-2-phenyl-propionic acid **4** (12.5 mg, 71%) as colorless oil: $[\alpha]D^{25}$–9.2 (*c* 1.2, $CHCl_3$), IR (neat) cm^{-1} 2992 (C–H), 1735 (C=O), 1704 (C=O); ^1H NMR ($CDCl_3$) δ 7.39–7.27 (m, 5H), 4.59 (d, *J* = 11.0 Hz, 1H), 4.35 (d, *J* = 11.0 Hz, 1H), 2.03 (s, 3H), 1.66 (s, 3H), the carboxylic proton was not observed due to broadening of the corresponding signal; ^{13}C NMR ($CDCl_3$) δ 179.8, 170.8, 139.1, 128.7 (×2), 127.8, 126.1 (×2), 68.9, 50.1, 20.8, 20.4; FAB-HRMS calcd for $C_{12}H_{15}O_4$ $[M+H]^+$, 223.0970; found 223.0960.

To a stirred solution of the acid **4** (12.5 mg, 0.056 mmol) in MeOH (1 mL) was added a 0.2 M aqueous solution of LiOH (0.30 mL, 0.060 mmol) and the resulting mixture was stirred at room temperature for 6 h. The solvent was evaporated and the crude product was purified by column chromatography ($CHCl_3$–MeOH, 95:5) to afford 3-hydroxy-2-methyl-2-phenyl-propionic acid **7** (5.1 mg, 51%) as a colorless oil: $[\alpha]D^{25}$–11 (*c* 0.51, EtOH); IR (neat) cm^{-1} 3386 (OH), 2938 (C–H), 1704 (C=O); ^1H NMR ($CDCl_3$) δ 7.37–7.26 (m, 5H), 4.09 (d, *J* = 11.2 Hz, 1H), 3.65 (d, *J* = 11.2 Hz, 1H), 1.67 (s, 3H), the hydroxy and carboxylic protons were not observed due to broadening of the corresponding signals; ^{13}C NMR ($CDCl_3$) δ 180.8, 139.6, 128.7 (×2), 127.6, 126.3(×2), 69.1, 52.4, 20.1; FAB-HRMS calcd for $C_{10}H_{13}O_3$ $[M+H]^+$, 181.0865; found 181.0862.

To a solution of **2d** {17.5 mg, 0.079 mmol; $[\alpha]D^{25}$ + 7.4 (*c* 1.9, $CHCl_3$)} produced from **1d** by CSL in acetone (1.0 mL) was added the Jones reagent (0.19 mL) at room temperature and the reaction mixture was stirred for 3 h. The reaction conditions and the work-up procedures were similar to those for the preparation of acid **4**. Column chromatographic separation on silica gel (hexane–ethyl acetate, 7:3) of the crude product afforded 2-acetoxymethyl-2-methyl-3-phenylpropionic acid **5** (46%) as a colorless liquid: $[\alpha]D^{25}$ + 2.3 (*c* 0.86, $CHCl_3$); IR (neat) cm^{-1} 2923 (C–H), 1735 (C=O), 1700 (C=O); ^1H NMR ($CDCl_3$) δ 7.28–7.12(m, 5H), 4.14 (d, *J* = 11.0 Hz, 1H), 4.09 (d, *J* = 11.0 Hz, 1H), 2.97 (d, *J* = 13.5 Hz, 1H), 2.92 (d, *J* = 13.5 Hz, 1H), 2.08 (s, 3H), 1.20 (s, 3H), the carboxylic proton was not observed due to broadening of the corresponding signal; ^{13}C NMR ($CDCl_3$) δ 180.8, 170.7, 135.9, 130.2 (×2), 128.3 (×2), 127.0, 67.8, 47.0, 41.1, 20.8, 19.5; FAB-HRMS calcd for $C_{13}H_{17}O_4$ $[M+H]^+$, 237.1127; found 237.1138.

To a solution of the acid **5** (7.6 mg, 0.032 mmol) in MeOH (1 mL) was added a 0.2 M aqueous solution of NaOH (0.17 mL, 0.034 mmol) and the resulting mixture was stirred at room temperature for 6 h. The solvent was evaporated and the crude product was purified by column chromatography ($CHCl_3$–MeOH, 95:5) to afford 2-benzyl-3-hydroxy-2-methyl-propionic acid **8** (4.7 mg, 75%) as colorless crystals: mp 97.5–98.5 °C; $[\alpha]D^{25}$–5.4 (*c* 0.47, MeOH); IR (film) cm^{-1} 3390 (OH), 2927 (C–H), 1712 (C=O); ^1H NMR ($CDCl_3$) δ 7.29–7.18 (m, 5H), 3.60 (d, *J* = 11.5 Hz, 1H), 3.56 (d, *J* = 11.5 Hz, 1H), 3.03 (d, *J* = 13.3 Hz, 1H), 2.90 (d, *J* = 13.3 Hz, 1H), 1.14 (s, 3H), the hydroxy and carboxylic protons were not observed due to broadening of the corresponding signals; ^{13}C NMR ($CDCl_3$) δ 181.9, 136.2, 130.4 (×2), 128.2 (×2), 126.8, 66.6, 48.5, 40.6, 19.2; FAB-HRMS calcd for $C_{11}H_{15}O_3$ $[M+H]^+$, 195.1021; found 195.1021.

To a solution of **2f** {14.1 mg, 0.060 mmol; $[\alpha]D^{25}$ + 1.4 (*c* 1.5, CH_2Cl_2)} produced from **1f** by CSL in acetone (1.0 mL) was added the Jones reagent (0.2 mL) at room temperature and the reaction mixture was stirred for 3 h. The reaction conditions and the work-up procedures were similar to those for the preparation of acid **4**. Column chromatographic separation on silica gel (hexane–ethyl acetate, 7:3) of the crude product afforded 2-acetoxymethyl-2-benzyl-butyric acid **6** (91%) as a colorless liquid: $[\alpha]D^{25}$ −1.5 (*c* 1.2, $CHCl_3$); IR (neat) cm^{-1} 2969 (C–H), 1739 (C=O), 1705 (C=O); ^1H NMR ($CDCl_3$) δ 7.28–7.12 (m, 5H), 4.11 (d, *J* = 11.5 Hz, 1H), 4.08 (d, *J* = 11.5 Hz, 1H), 3.07 (d, *J* = 13.8 Hz, 1H), 2.94 (d, *J* = 13.8 Hz, 1H), 2.12 (s, 3H), 1.74–1.60 (m, 2H), 0.96 (t, *J* = 7.6 Hz, 3H), the carboxylic proton was not observed due to broadening of the corresponding signal; ^{13}C NMR ($CDCl_3$) δ 177.4, 170.6, 136.4, 130.0 (×2), 128.3 (×2), 126.8, 63.7, 50.8, 38.6, 26.2, 20.9, 8.6; FAB-HRMS calcd for $C_{14}H_{19}O_4$ $[M+H]^+$, 251.1283; found 251.1296.

To a solution of the acid **6** (12.8 mg, 0.051 mmol) in MeOH (1 mL) was added a 0.2 M aqueous solution of LiOH (0.30 mL, 0.060 mmol) and the resulting mixture was stirred at room temperature for 6 h. The solvent was evaporated and the crude product was purified by column chromatography ($CHCl_3$–MeOH, 95:5) to afford 2-benzyl-2-hydroxymethyl-butyric acid **9** (8.5 mg, 80%) as a colorless liquid: $[\alpha]D^{25}$–1.2 (*c* 0.85, MeOH); IR (film) cm^{-1} 3417 (OH), 2935 (C–H), 1697 (C=O); ^1H NMR (CD_3OD) δ 7.29–7.20 (m, 5H), 3.58 (s, 2H), 2.97 (d, *J* = 13.3 Hz, 1H), 2.92 (d, *J* = 13.3 Hz, 1H), 1.63 (q, *J* = 7.6 Hz, 2H), 0.97 (t, *J* = 7.6 Hz, 3H), the hydroxy and carboxylic protons were not observed due to broadening of the corresponding signals; ^{13}C NMR (CD_3OD) 5 179.0, 139.0, 131.3 (×2), 129.0 (×2), 127.4, 62.4, 53.8, 39.3, 26.6, 9.0; FAB-HRMS calcd for $C_{12}H_{17}O_3$ $[M+H]^+$, 209.1178; found 209.1179.

3. Results and discussion

*3.1. Lipase-catalyzed desymmetrization of prochiral 2-substituted and 2,2-disubsutituted 1,3-propanediols **1a–f** by CSL and immobilized PPL*

The lipase-catalyzed desymmetrization was examined in the reaction of prochiral 2-substituted and 2,2-disubstituted 1,3-propanediols **1a–f** with vinyl acetate as the acyl donor using the lipases CSL and immobilized PPL (Scheme 1). Comparisons of the desymmetrization by CSL and immobilized PPL are summarized in Table 1. In the reaction of **1a**, although significant amounts of diacetate **3a** was formed, the enantioselectivity of **2a** was considerably high with 80% ee and 94% ee for CSL and immobilized PPL, respectively. Noteworthy is that the configuration of the major enantiomer of **2a** in the reaction catalyzed by CSL was opposite to that by immobilized PPL (Table 1, entries 1 and 2). The absolute configuration of the major isomer of **2a** from the CSL-catalyzed desymmetrization was determined to be *S* by a direct comparison of the optical rotation with a reported value [15]. The reactivity and enantioselectivity in the immobilized

Table 1
Lipase-catalyzed acetylation of **1a–f** at 25 °C

Entry	Diol[a]	Lipase[b]	Reaction time (h)	Yield (%)[c]		Ratio of enantiomers of **2**[d]		% e.e. of **2**[c]	$[\alpha]_D^{25}$ of **2**
				2	**3**	*S*	*R*		
1	**1a**	CSL[e]	24	46	54	90	10	80	−11.4
2	**1a**	PPL[e]	6	84	15	3	97	94	+15.7
3	**1b**	CSL[f]	72	71	29	67	33	34	−9.6
4	**1b**	PPL[g]	12	49	51	71	29	42	−10.3
5	**1c**	CSL[e]	72	46	1	30	70	40	+7.3
6	**1c**	PPL[e]	72	2	–	51	49	2	n.d.
7	**1d**	CSL[f]	72	46	1	29	71	42	+7.4
8	**1d**	PPL[g]	72	63	1	76	24	52	−8.1
9	**1e**	CSL[f]	72	14	–	31	69	38	+2.5
10	**1e**	PPL[g]	72	<1	–	–	–	–	–
11	**1f**	CSL[f]	72	13	–	46	54	8	+0.68
12	**1f**	PPL[g]	72	5	–	55	45	10	n.d.

[a] The concentration of **1a–f** was 0.1 M. For other details see Section 2.

[b] CSL: lipase from yeast, *Cryptococcus* spp. S-2. PPL: immobilized porcine pancratic lipase on Hyflo Super Celite. Weight of CSL and PPL was 1.5 and 6.0 w/w of substrate, respectively.

[c] Yield based on ^{1}H NMR.

[d] Enantiomeric ratio of **2** was determined by HPLC using a Daisel CHIRALCEL OD (hexane–*i*PrOH, 50:1 for **2a**) or an AD (hexane–EtOH, 30:1 for **2b**, **2e**, and **2f**; hexane–*i*PrOH, 30:1 for **2c** and **2d**) column.

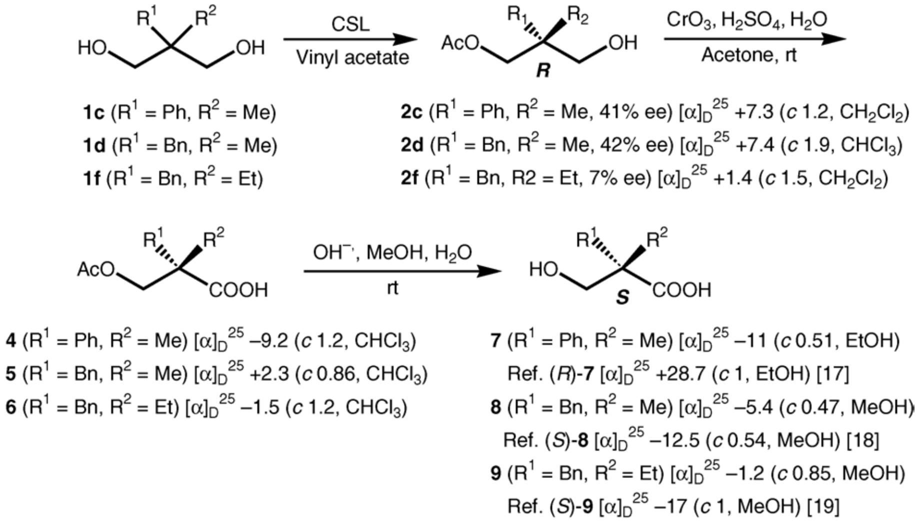
[e] Solvent was vinyl acetate.

[f] Two equivalents of vinyl acetate as acyl donor was added.

[g] Six equivalents of vinyl acetate as acyl donor was added.

PPL-catalyzed acetylation of **1a** were higher than those of CSL-catalyzed acetylation. In the reaction of diol **1b** catalyzed by CSL and immobilized PPL, the major enantiomer of monoacetate **2b** was of the same configuration (Table 1, entries 3 and 4) and the degree of asymmetrization was almost the same. The absolute configuration of the major product of **2b** was determined to be *S* by a direct comparison of the optical rotation with reported values [12,16]. Previously, there have been some examples on the enantioselective transesterification of diol **1b** using several lipases originating from microorganisms (*Pseudomonas fluorecens* [9], *P. cepacia* [10], *Rhizomucor miehei* [11]). All of the acetylations of diol **1b** by the lipases isolated from these microorganisms yielded (*R*)-**2b** rather than (*S*)-**2b**. It is interesting that opposite enantioselectivity (*S* selectivity) was observed in the monoacetylation of diol **1b** by CSL, even though the CSL-enzyme was isolated from a microorganism.

The monoacetate **2c** from CSL-catalyzed desymmetrization of diol **1c** was obtained in 46% with 40% ee, while only a little of diol **1c** was transformed to the monoacetate **2c** by immobilized PPL (Table 1, entries 5 and 6). The enantioselectivity and reactivity in the CSL-catalyzed desymmetrization of **1c** was lower than those of **1a**, however diacetate **3c** was hardly produced even though the reaction time was longer than in the case of **1a** (Table 1, entries 1 and 5). Previously, there have been only a few examples on the formation of **2c** by lipase-catalyzed desymmetrization of **1c** [14b,14c]. Thus, the asymmetric desymmetrization of **1c** to **2c** catalyzed by CSL is a notable example.



Scheme 2. Determination of absolute configration of monoacetate **2c**, **2d**, and **2f**.

In order to determine the absolute configuration of the product **2c**, it was transformed to alcohol **7** of which absolute stereochemistry has been established. Oxidation with Jones' reagent of the monoacetate **2c** from the reaction catalyzed by CSL afforded acid **4**, and subsequent hydrolysis gave alcohol **7** (Scheme 2). Comparison of the optical rotation of **7** {$[\alpha]D^{25}$–11 (*c* 0.51, EtOH)} thus obtained with the reported value {optically pure (*R*)-**7**: $[\alpha]D^{25}$ + 28.7 (*c* 1, EtOH)} [17] indicated the absolute configuration of chiral carbon in the major isomer of **7** to be *S*. Thus, the absolute configuration of the major product **2c** in the reaction catalyzed by CSL was determined to be *R*.

Acetylation of **1d** catalyzed by CSL and immobilized PPL gave the corresponding monoacetate **2d**. The configuration of the major product by CSL was opposite to that produced by immobilized PPL (Table 1, entries 7 and 8), as in the case of the asymmetrization of **1a** to **2a** (Table 1, entries 1 and 2). In order to determine the absolute configuration of the major monoacetate of **2d**, monoacetate **2d** from the CSL-catalyzed acetylation was converted to the alcohol **8** (Scheme 2). Comparison of the optical rotation of **8** {$[\alpha]D^{25}$–5.4 (*c* 0.47, MeOH)} thus afforded with the reported value {(*S*)-**8**: $[\alpha]D^{25}$–12.5 (*c* 0.54, MeOH)} [18] indicated the absolute configuration of the chiral carbon in the major isomer of **8** to be *S*. Thus, the absolute configurations of the major monoacetate **2d** in the reactions catalyzed by CSL and immobilized PPL were determined to be *R* and *S*, respectively.

The monoacetate **2e** from the CSL-catalyzed acetylation of diol **1e** was yielded in 14% with 38% ee, while immobilized PPL did not catalyze the reaction of diol **1e** (Table 1, entries 9 and 10). The absolute stereochemistry of the major isomer of monoacetate **2e** from the CSL-catalyzed acetylation was determined to be *R* by direct comparison of the optical rotation with the reported value [14b].

Acetylation of **1f** catalyzed by CSL and immobilized PPL gave the corresponding monoacetate **2f** (Table 1, entries 11 and 12), but in low yield and with low asymmetric induction. The absolute configuration of the major isomer of **2f** from the CSL-catalyzed acetylation was determined to be *R* by comparison of the optical rotation of the corresponding alcohol **9** {$[\alpha]D^{25}$–1.2 (*c* 0.85, MeOH)}, obtained similarly to **7**, with the reported value {(*S*)-**9**: $[\alpha]D^{25}$–17 (*c* 1, MeOH)} [19] (Scheme 2). As in the case of **1d**, the configuration of the major product by CSL was opposite to that produced by immobilized PPL.

As shown in Table 1, the absolute configuration at C-2 of the major isomer produced from the tertiary substrates **1a** and **1b** ($R^2 = H$) by the CSL-catalyzed acetylation was *S*, whereas that from the quaternary substrates **1c** and **1d** ($R^2 = Me$) and **1e** and **1f** ($R^2 = Et$) was *R*. These results imply that the active site of CSL recognizes the hydrophobic group at C-2 of 1,3-propanediol and that the methyl group better fits the pocket.

Table 2 shows the desymmetrization and asymmetrization of **1a** and **1b** as functions of reaction time. In the CSL-catalyzed acetylation of **1a**, the yields of **2a** and **3a** varied and the enantiomeric excess of **2a** increased with the reaction time. At the reaction time of 12 h, the relative amount of the monoester **2a** was about at a peak and the enantiomeric excess was 50% (Table 2, entry 2). On the other hand, the desymmetrization of diol **1a** to monoacetate **2a** by immobilized PPL proceeded in high chemical and optical yields (data not shown), and these results were in good agreement with previously reported results [15].

Table 2
CSL-catalyzed acetylation of **1a** and **1b** at 25 °C

Entry	Diol[a]	Reaction time (h)	Yield (%)[b]		Ratio of enantiomers of **2**[c]	
			2	**3**	*S*	*R*
1	**1a**[d]	6	58	2	72	28
2	**1a**[d]	12	84	7	75	25
3	**1a**[d]	18	84	15	78	22
4	**1a**[d]	24	46	54	90	10
5	**1b**[e]	12	33	<1	61	39
6	**1b**[e]	24	71	1	61	39
7	**1b**[e]	36	90	4	62	38
8	**1b**[e]	48	85	13	63	37
9	**1b**[e]	72	71	29	67	33

[a] The concentration of **1a, b** was 0.1 M. For other detail see Section 2.
[b] Yield based on ^{1}H NMR.
[c] Enantiomeric ratio of **2** was determined by HPLC analysis using a Daisel CHIRALCEL OD (hexane–*i*PrOH, 50:1 for **2a**) or an AD (hexane–EtOH, 30:1 for **2b**) column.
[d] Solvent was vinyl acetate.
[e] 2 equiv. of vinyl acetate as acyl donor was added.

In the CSL-catalyzed acetylation of **1b**, the yields of **2b** and **3b** and the enantiomeric excess of **2b** varied with reaction time similarly as in the case of **1a**, with the change in ee being relatively smaller. At the reaction time of 36 h, the relative amount of monoester **2a** was about at its maximum with the enantiomeric excess being 24% ee (Table 2, entry 7).

3.2. *Influence of diisopropyl ether in the desymmetrization of **1d** by CSL*

The influence of diisopropyl ether as solvent was examined in the monoacetylation of **1d** with vinyl acetate by CSL. The results are summarized in Table 4. The yields of monoacetate **2d** progressively increased from 46% to 92% with increasing equivalents of vinyl acetate, whereas the enantioselectivity decreased from 43% ee to 19% ee. The conversion yield of monoacetate **2d** in diisopropyl ether solutions with 10 equiv. or more of vinyl acetate was practically the same as that in vinyl acetate alone (entries 4 and 7). When the reaction time was shortened to 12 h, the yield of **2d** was only 22%, however, the enantiomeric excess of **2d** was as high as 54% (entries 1 and 2).

In order to gain an understanding of this phenomenon, the time-course of the desymmetrization of **1d** catalyzed by CSL and immobilized PPL was examined. The progress of the reaction was monitored by the measuring the integration of the signals of monoacetate **2d**, diacetate **3d**, and starting material **1d** by NMR, and the enantiomeric excess of monoacetate **2d** was analyzed by HPLC with a chiral column. The time-course of the reaction as a function of conversion and ee are depicted in Fig. 1. As seen in Fig. 1(A), the monoacetylation of **1d** catalyzed by CSL in excess vinyl acetate as both solvent and acyl donor successfully proceeded in 90% conversion after 60 h. However, the enantiomeric excess of monoacetate **2d** decreased from 41% ee

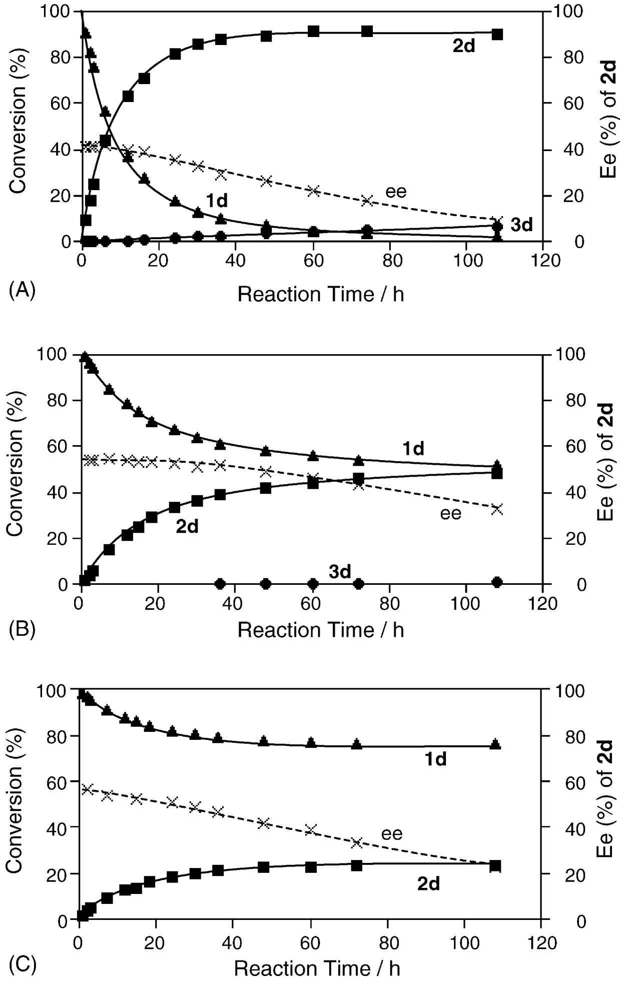


Fig. 1. Time-courses of the CSL and PPL catalyzed desymmetrization of **1d**. Percents of **1d** (▲), **2d** (■), and **3d** (●) were based on the ^{1}H NMR analysis. Enantiomeric excess of **2d** (×) was determined by HPLC using a chiral column. (A) Enzyme: CSL (1.5 w/w), solvent: vinyl acetate. (B) Enzyme: CSL (1.5 w/w), solvent: diisopropyl ether, 2 equiv. of vinyl acetate. (C) Enzyme: PPL (6.0 w/w), solvent: diisopropyl ether, 2 equiv. of vinyl acetate.

to 9% ee upon progression of the reaction. When diisopropyl ether was used as the solvent, as shown in Fig. 1(B and C), the depression of the enantiomeric ratio of the monoacetate **2d** was reduced. Furthermore, the production of diacetate **3d** could hardly be observed. On the other hand, the extent of the conversion of **1d** decreased upon changing the solvent from vinyl acetate to diisopropyl ether.

Table 3
CSL-catalyzed acetylation of (*R*)-**2d**[a] at 25 °C

Entry	Equivalents of vinyl acetate (VA)	Reaction time (h)	Yield (%)[b]			%e.e. of **2d**[c]
			1d	**2d**	**3d**	
1	0	24	3.0	96.7	0.3	30
2	0	72	7.0	92.4	0.6	28
3	2	24	0.1	92.5	7.4	25
4	2	72	0.1	88.1	11.8	19
5	10	24	0.1	86.3	13.6	21
6	10	72	0.2	81.7	18.1	11

[a] The concentration of (*R*)-**2d** (32% ee) was 0.1 M.
[b] Yield based on ^{1}H NMR analysis.
[c] Enantiomeric excess of **2d** was determined by HPLC using a Daisel CHIRALCEL AD (hexane–*i*PrOH 30:1) column.

Since the reason for the decrease in the enantiomeric excess of **2d** upon progression of the enzymatic acetylation could be racemization via intramolecular 1,3-transesterification as often seen in reactions under acidic conditions, the influences of solvent, acyl donor, and enzyme on the enantiomeric excess of isolated optically active **2d** (32% ee) were studied (Table 3). In the presence of CSL in diisopropyl ether without the acyl donor present, the gradual formation of the hydrolyzed diol **1d** and a small amount of diacetate **3d** was observed. During this process, only a slight decrease in ee of **2d** was observed (32% ee to 28% ee over 72 h). Therefore, we believe that the intramolecular pathway is not the main cause for the racemization observed in the reaction starting with diol **1d**. Addition of the acyl donor to the mixture lead to the accelerated formation of diacetate **3d** as compared with the reaction starting with diol **1d**, with the slight accompaniment of the hydrolyzed diol **1d**. Interestingly, under these conditions, a larger amount of the acyl donor led to a higher extent of **2d** racemization (11% ee with 10 equiv. of vinyl acetate at 72 h). In addition, the rate of the hydrolysis of diacetate **3d** was found to be much smaller than that of monoacetate **2d**.

*3.3. Influence of organic solvent in the desymmetrization of **1d** by CSL*

The influence of organic solvent was next investigated on the enantioselectivity and conversion yield in the esterification

Table 4
Influence of solvent in monoacetylation of **1d**[a]

Entry	Solvent	Equivalents of vinyl acetate (VA)	Reaction time (h)	Yield (%)[b]		%e.e. of **2d**[c]
				2d	**3d**	
1	*i*Pr$_2$O	2	72	46	1	42
2	*i*Pr$_2$O	2	12	22	<1	54
3	*i*Pr$_2$O	6	72	78	3	37
4	*i*Pr$_2$O	10	72	90	5	27
5	VA/*i*Pr$_2$O (1:2)	33	72	91	8	27
6	VA/*i*Pr$_2$O (2:1)	67	72	91	7	25
7	VA	100	72	92	6	19

[a] The concentration of **1d** was 0.1 M.
[b] Yield based on ^{1}H NMR analysis.
[c] Enantiomeric excess of **2d** was determined by HPLC using a Daisel CHIRALCEL AD (hexane–*i*PrOH 30:1) column.

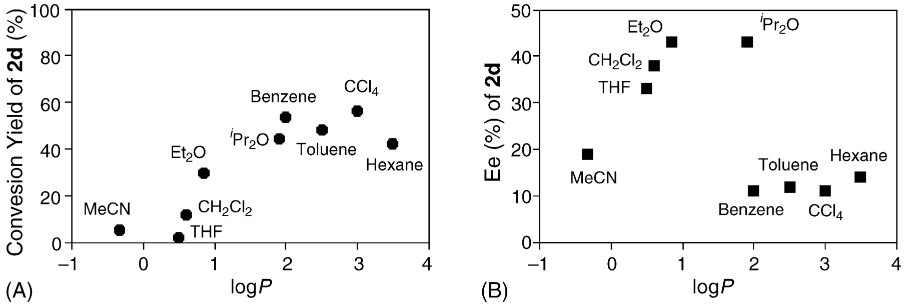


Fig. 2. Correlation between hydrophobic palameter, log *P* and conversion yield of **2d** and enantiomeric excess of **2d** in the CSL-catalyzed desymmetrization of **1d**. log *P* is logarithm of the partition coefficient in *n*-octanol–water system. Source data were cited in references [20]. Yield of **2d** based on ^{1}H NMR analysis. Enantiomeric excess of **2d** was determined by HPLC using a chiral column. (A) Correlation between log *P* and yield of **2d**. (B) Correlation between log *P* and ee of **2d**.

catalyzed by CSL. As shown in Table 5, the remarkable influence of organic solvent was observed. Higher enantioselectivity was observed in diisopropyl ether (entry 5) and diethyl ether (entry 6) than that in the other organic solvents. Interestingly, an examination of the hydrophobicity coefficients log *P* [20], which is the logarithm of the *n*-octanol–water partition coefficient of organic substances, of the solvents revealed that the higher the hydrophobicity was, the higher conversion yields were as shown in Fig. 2(A). When a solvent having a log *P* value between 0.6 and 2.0 was used, the enantioselectivity in **2d** was relatively higher in solvents of higher hydrophobicity [Table 5 and Fig. 2(B)]. This influence of solvent on enantioselectivity in the desymmetrization of **2d** by CSL resembled the tendency reported for several other lipases [21]. Moreover, when a mixture of hexane and diisopropyl ether was used as the solvent, although there was no difference in conversion yield compared with runs using either hexane or diisopropyl ether alone, the enantioselectivity of the monoacetate **2d** was higher with higher proportions of diisopropyl ether (Table 5, entries 10 and 11). The slightly increase of the selectivity may be explained by the conformational change in the active site of CSL with lower hydrophobicity of hexane-*i*Pr$_2$O (1:2) solvent [22].

Table 5
CSL-catalyzed monoacetylation of **1d** in various organic solvents[a]

Entry	Solvent[b]	Log *P*[c]	Yield (%)[d]		%e.e. of **2d**[e]
			2d	**3d**	
1	Hexane	3.5	42	<1	14
2	CCl$_4$	3.0	56	<1	11
3	Toluene	2.5	48	<1	12
4	Benzene	2.0	54	<1	11
5	*i*Pr$_2$O	1.9	46	1	42
6	Et$_2$O	0.85	30	<1	43
7	CH$_2$Cl$_2$	0.60	12	<1	38
8	THF	0.49	2	<1	16
9	MeCN	−0.33	6	<1	19
10	Hexane/*i*Pr$_2$O (2:1)		43	<1	21
11	Hexane/*i*Pr$_2$O (1:2)		44	<1	36

[a] The concentration of **1d** was 0.1 M. Reaction time is 72 h.
[b] 2 equiv. of vinyl acetate as acyl donor was added.
[c] Logarithm of the octanol–water partition coefficient of the solvent [20].
[d] Yield based on ^{1}H NMR analysis.
[e] Enantiomeric excess of **2d** was determined by HPLC using a Daisel CHIRALCEL AD (hexane–*i*PrOH 30:1) column.

In conclusion, with the present work we have demonstrated that the novel lipase CSL has a good potential for the desymmetrization of prochiral 2,2-disubstituted 1,3-propanediols in organic solvents. Especially noteworthy is that in many cases using CSL, such as in the desymmetrization of **1a**, **b**, **d**, and **f**, to monoacetate **2a**, **b**, **d**, and **f**, respectively, the product had stereochemistry opposite to that obtained in the reaction of commonly used immobilized PPL.

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