

Kinetic resolution of 5-(hydroxymethyl)-3-phenyl-2-isoxazoline by using the 'low-temperature method' with porous ceramic-immobilized lipase

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Abstract—A significant enhancement of the enantioselectivity (E value = 249) in the lipase-catalyzed resolution of a primary alcohol, racemic 5-(hydroxymethyl)-3-phenyl-2-isoxazoline (\pm)-**1**, was obtained by using the 'low-temperature method' (-60°C) with porous ceramic-immobilized lipase (Amano PS-C II) and vinyl acetate in acetone.

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1. Introduction

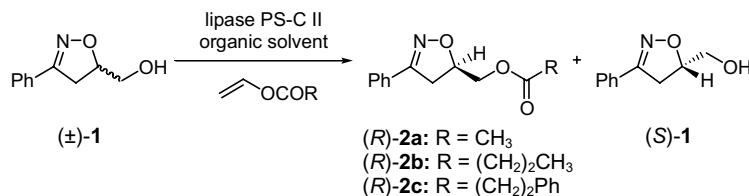
Optically active 5-(hydroxymethyl)-3-phenyl-2-isoxazoline **1** is a versatile key intermediate for the syntheses of β -hydroxy ketones,¹ γ -amino alcohols² and β -amino acids.³ Optically active isoxazolines have been synthesized mostly by asymmetric 1,3-dipolar cycloaddition of nitrile oxides to olefins.^{3,4} For example, the cycloaddition of nitrile oxide to allylic alcohols in the presence of an equimolar amount of (*R,R*)-diisopropyl tartrate (DIPT) gave the corresponding (*R*)-isoxazolines in 93% ee at best.^{4b} An interesting variant for the asymmetric 1,3-dipolar cycloaddition is the one using baker's yeast and β -cyclodextrin,^{4c} giving 3-(2,6-dichlorophenyl)-5-pyridylisoxazoline in 64% ee. Herein we have examined the lipase-catalyzed kinetic resolution of isoxazoline (\pm)-**1**, which has so far not been reported probably because of the low enantioselectivity expected for primary alcohols.⁵ We recently demonstrated a significant improvement in the efficiency and enantioselectivity for the primary alcohols in lipase-catalyzed resolutions by using the 'low-temperature method'^{6,7} with porous ceramic (Toyonite^{6d,e})-immobilized lipase (Amano PS-C II). The method is reliable, and widely applicable to the lipase reactions in a variety of organic solvents.^{6,7} We report herein that the low enantioselectivity (E^8 value = 4–5 in *i*-Pr₂O) in the lipase-catalyzed

resolution of (\pm)-**1** at room temperature could be markedly improved up to an E value of 249 at -60°C using lipase PS-C II in acetone. This is the first example of the lipase-catalyzed resolution of isoxazoline derivative (\pm)-**1** with a practically feasible level of enantioselectivity.

2. Results and discussion

Racemic compound (\pm)-**1** was readily prepared by the 1,3-dipolar cycloaddition of benzaldoxime with allyl alcohol in the presence of NCS in toluene-CH₂Cl₂.⁹ Resolution of (\pm)-**1** was first attempted with Celite-immobilized lipase PS (*Pseudomonas cepacia*) and vinyl acetate in *i*-Pr₂O at room temperature. However, the reaction proceeded too fast (100% conversion within 0.3 h). Next, the reaction was carried out at low temperature (-30°C) but a low enantioselectivity (E value = 5.9) was obtained, which could not be improved by screening other lipases (Scheme 1). The enantiomeric excess for each compound was determined by HPLC analysis after ester (*R*)-**2a** and the alcohol (*S*)-**1** were separated by silica gel column chromatography. The absolute configuration of (*S*)-**1** was determined by comparison of the specific rotation with the reported data.¹⁰ The faster-reacting enantiomers in the lipase-catalyzed reaction of 3-substituted 5-(hydroxymethyl)-2-isoxazolines **3a–d**^{11a–c} were reported to be (*R*), as shown in Figure 1. Interestingly, it has previously been reported that 3-phenyl-2*H*-azirine-2-methanol **4**,^{6a,e} which has an analogous cyclic imine structure, also shows

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Scheme 1. Resolution of (\pm) -5-(hydroxymethyl)-3-phenyl-2-isoxazoline $(\pm)\text{-1}$.

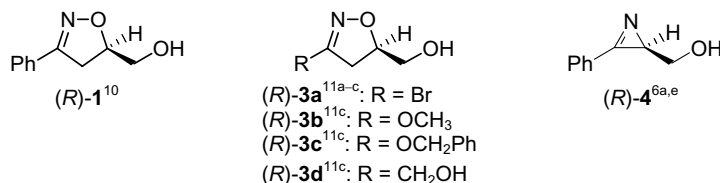


Figure 1. Faster-reacting enantiomers.

(R) -selectivity. Although the enantioselectivity for the primary alcohols has not been well established,⁵ these results seem to be useful references for the discussion of the enantioselectivity of the related compounds.

The observed low enantioselectivity forced us to explore more suitable reaction conditions. We initially examined the temperature effect by changing the organic solvent (*i*-Pr₂O, THF and acetone). The reactions were carried out in the presence of lipase PS-C II, because the lipase had been known to greatly enhance the reaction rate even at low temperatures.^{6,7} The results are shown in Figure 2. The *E* values in acetone were increased continuously by lowering the reaction temperature to reach the highest enantioselectivity (*E* = 249) at -60°C . In contrast, the temperature effects in THF or *i*-Pr₂O have the inversion temperature (T_{inv}),^{6,7} at which point the *E* values started to decrease upon further lowering of the temperature. The *E* value at T_{inv} (-40°C) in THF was 51, while that in *i*-Pr₂O at T_{inv} (-20°C) was 9.0.

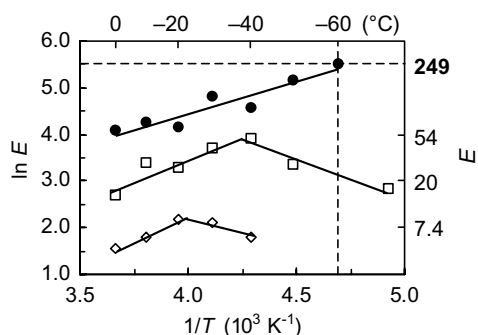


Figure 2. Temperature effect in lipase PS-C II catalyzed resolution of $(\pm)\text{-1}$ with vinyl acetate in acetone (black circle), THF (white square) and *i*-Pr₂O (white diamond).

We next examined the influence of acylating agents (vinyl acetate, vinyl butyrate and vinyl 3-phenylpropanoate¹²) on the temperature effect (Fig. 3). As reported previously,^{6,7} optimization of the acylating agent is essential to enhance the utility of the temperature effect. In contrast with the above results without having T_{inv}

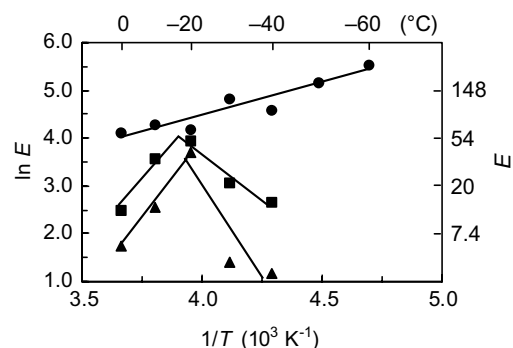


Figure 3. Temperature effect in lipase PS-C II catalyzed resolution of $(\pm)\text{-1}$ with vinyl acetate (black circle), vinyl butyrate (black square) and vinyl 3-phenylpropanoate (black triangle) in acetone.

for vinyl acetate, those for vinyl butyrate and vinyl 3-phenylpropanoate gave the T_{inv} both around -20°C . Thus, vinyl acetate was the optimal choice, but unexpected from the previous results for 3-phenyl-2*H*-azirine-2-methanol, in which vinyl butanoate was the best one.^{6c}

The comparison of lipase PS-C II with lipase PS is shown in Figure 4. At -20°C , both lipases (PS-C II

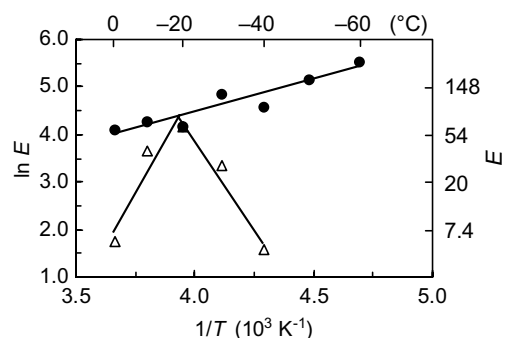


Figure 4. Temperature effect in the resolution of $(\pm)\text{-1}$ in the presence of lipase PS-C II (black circle) and lipase PS (white triangle) (acylating agent: vinyl acetate; solvent: acetone).

Table 1. Thermodynamic parameters and T_{inv} observed for the lipase-catalyzed resolution of (\pm)-**1** (vinyl acetate, lipase PS-C II)

| Solvent | T_{inv} (°C) | $T > T_{\text{inv}}$ | | $T_{\text{inv}} > T$ | |
|-----------------------------|-----------------------|--------------------------------------|---------------------------------------|--------------------------------------|---------------------------------------|
| | | $\Delta\Delta H^\ddagger$ (kcal/mol) | $\Delta\Delta S^\ddagger$ (cal/mol K) | $\Delta\Delta H^\ddagger$ (kcal/mol) | $\Delta\Delta S^\ddagger$ (cal/mol K) |
| THF | −38 | −3.46 ± 0.25 | −6.97 ± 0.50 | 3.26 ± 0.09 | 21.6 ± 0.61 |
| <i>i</i> -Pr ₂ O | −22 | −4.34 ± 0.03 | −12.8 ± 0.10 | 2.29 ± 0.10 | 13.5 ± 0.61 |
| Acetone ^a | −18 | −10.0 ± 0.45 | −31.4 ± 1.41 | 7.66 ± 0.22 | 37.9 ± 1.10 |
| Acetone ^b | −19 | −13.5 ± 0.04 | −45.9 ± 0.14 | 14.8 ± 1.53 | 65.1 ± 0.74 |
| Acetone ^c | −19 | −16.5 ± 0.94 | −56.5 ± 3.22 | 15.3 ± 0.25 | 68.9 ± 1.14 |

^a With vinyl butyrate.^b With vinyl 3-phenylpropanoate.^c In the presence of lipase PS.

and PS) showed the same E value ($E = 65$), although the reaction rate of the former was much higher [TTN/h (total turnover number per hour) = 16,000] than that of the latter (TTN/h = 640). In addition, the T_{inv} was observed only for lipase PS in the examined range of temperatures. Interestingly, the T_{inv} existed except for the reaction with lipase PS-C II and vinyl acetate in acetone. While these phenomena have been reported in some non-enzymatic reactions (e.g., *cis*-dehydroxylation of olefins,¹³ reduction of ketones,¹⁴ nucleophilic addition to imines¹⁵ and carbonyl compounds¹⁶ and Diels–Alder reaction¹⁷), only a few examples have been reported in enzymatic reactions.^{18a,b}

Thermodynamic parameters in the above reactions in Table 1 show that these reactions are enthalpy-driven at higher temperatures ($T > T_{\text{inv}}$) while they are entropy-driven^{19,20} at lower temperatures ($T_{\text{inv}} > T$). At the inversion temperature, the transition state structure can be changed; a temperature-induced structural change of the enzyme and/or a solute–solvent cluster change have been proposed.¹⁸ The phenomena of the presence of T_{inv} are interesting and now under current investigation.

3. Conclusion

The low enantioselectivity ($E = 5.9$) in the lipase-catalyzed resolution of isoxazoline (\pm)-**1** under the usual conditions (lipase PS, vinyl acetate, in *i*-Pr₂O) could be dramatically improved by up to $E = 249$ (at -60 °C) by controlling the temperature for the reaction with lipase PS-C II and vinyl acetate in acetone. Utility of the low-temperature method has been further expanded here. The encountered phenomena of T_{inv} are also interesting, and will be discussed elsewhere.

4. Experimental

4.1. General

Silica gel column chromatography was performed using Fuji Silysia BW-127 ZH (100–270 mesh). Thin-layer chromatography (TLC) was performed on Merck silica gel 60 F₂₅₄. Lipase PS-C II and lipase PS were purchased from Wako Pure Chemical Industries, Ltd, Osaka. The enantiomeric purities of **1**, **2a**, **2b** and **2c** were determined by HPLC using Chiralpak AS-H (**1**, **2a,b**) and

Chiralpak AD-H (**2c**) columns (Daicel Chemical Industries).

4.2. Typical lipase-catalyzed kinetic resolution of (\pm)-**1**

A mixture of (\pm)-**1** (60 mg) and lipase (20 mg) in acetone (5 mL) was stirred at -20 °C under atmospheric conditions for 30 min. Freshly distilled vinyl acetate (68 μ L, 2.0 equiv) was added and stirred for 1 h. The mixture was filtered with suction, and the filtrate concentrated in vacuo. The residual oil was separated by silica gel column chromatography [hexane/ethyl acetate = 1/2 (v/v)] to give ester (*R*)-**2** and the remaining alcohol (*S*)-**1** (white crystals). Enantiomeric purities of (*R*)-**2a–c** and (*S*)-**1** were determined by HPLC as follows (flow rate 0.5 mL/min, detection 254 nm). Other reaction conditions and results are summarized in Table 2.

(*S*)-**1**:¹¹ HPLC: Chiralpak AS-H, hexane/*i*-PrOH = 9/1, retention time (*R*) 41.4 min, (*S*) 56.6 min; $[\alpha]_{\text{D}}^{23} = +91.6$ (85% ee, c 1.0, CHCl₃).

(*R*)-**2a**: HPLC: Chiralpak AS-H, hexane/*i*-PrOH = 9/1, retention time (*R*) 50.9 min, (*S*) 64.9 min; ¹H NMR (500 MHz, CDCl₃) δ 2.08 (s, 3H), 3.31 (dd, $J = 7.0$, 10.5 Hz, 2H), 4.24 (dd, $J = 5.5$, 4.5 Hz, 2H), 4.94–5.00 (m, 1H), 7.40–7.68 (m, 5H); ¹³C NMR (50 MHz, CDCl₃) δ 20.8, 37.2, 64.8, 78.1, 126.6, 128.6, 129.0, 130.2, 156.1, 170.6; IR (KBr) 1736, 1247 cm⁻¹; $[\alpha]_{\text{D}}^{24} = -39.2$ (27% ee, c 1.0, CHCl₃).

(*R*)-**2b**:²¹ HPLC: Chiralpak AS-H, hexane/*i*-PrOH = 9/1, retention time (*R*) 41.3 min, (*S*) 48.1 min; ¹H NMR (500 MHz, CDCl₃) δ 0.93 (t, $J = 7.5$ Hz, 3H), 1.64 (sext, $J = 7.5$ Hz, 2H), 2.32 (t, $J = 7.5$ Hz, 2H), 3.31 (dd, $J = 7.0$, 11.0 Hz, 2H), 4.25 (dd, $J = 6.0$, 4.5 Hz, 2H), 4.95–5.00 (m, 1H), 7.41–7.68 (m, 5H); ¹³C NMR (50 MHz, CDCl₃) δ 13.65, 18.37, 35.96, 37.27, 64.69, 78.26, 126.64, 128.68, 129.13, 130.19, 156.08, 173.36; IR (KBr) 2963, 1732, 1190 cm⁻¹; $[\alpha]_{\text{D}}^{23} = -115$ (84% ee, c 1.0, CHCl₃); Anal. Calcd for C₁₄H₁₇NO₃: C, 68.00; H, 6.93; N, 5.66. Found: C, 68.19; H, 7.09; N, 5.42.

(*R*)-**2c**: HPLC: Chiralpak AD-H, hexane/*i*-PrOH = 20/1, retention time (*R*) 46.8 min, (*S*) 57.2 min; ¹H NMR (500 MHz, CDCl₃) δ 2.68 (t, $J = 7.5$ Hz, 2H), 2.94 (t, $J = 7.5$ Hz, 2H), 3.23 (dd, $J = 7.0$, 11.0 Hz, 2H), 4.24 (dd, $J = 5.5$, 4.5 Hz, 2H), 4.91–4.96 (m, 1H), 7.16–7.27 (m, 5H), 7.41–7.66 (m, 5H); ¹³C NMR (50 MHz,

Table 2. Lipase-catalyzed resolutions of (\pm)-1

| Solvent | Acylating agent ^a | Lipase | Temp (°C) | Ester ^b (% ee) | Alcohol ^b (% ee) | Conv. ^c (%) | <i>E</i> ^d | TTN/h |
|-----------------------------|------------------------------|---------|-----------|---------------------------|-----------------------------|------------------------|-----------------------|--------|
| <i>i</i> -Pr ₂ O | A | PS-C II | 0 | 53 | 40 | 43 | 4.8 | 44,000 |
| <i>i</i> -Pr ₂ O | A | PS-C II | -10 | 53 | 64 | 55 | 6.1 | 65,000 |
| <i>i</i> -Pr ₂ O | A | PS-C II | -20 | 72 | 39 | 35 | 9.0 | 21,000 |
| <i>i</i> -Pr ₂ O | A | PS-C II | -30 | 70 | 39 | 36 | 8.3 | 8500 |
| <i>i</i> -Pr ₂ O | A | PS-C II | -40 | 64 | 30 | 32 | 6.1 | 3200 |
| THF | A | PS-C II | 0 | 85 | 19 | 18 | 15 | 22,000 |
| THF | A | PS-C II | -10 | 88 | 64 | 42 | 30 | 25,000 |
| THF | A | PS-C II | -20 | 77 | 94 | 55 | 27 | 17,000 |
| THF | A | PS-C II | -30 | 89 | 78 | 47 | 41 | 12,000 |
| THF | A | PS-C II | -40 | 88 | 92 | 51 | 51 | 5000 |
| THF | A | PS-C II | -50 | 86 | 74 | 46 | 29 | 3000 |
| THF | A | PS-C II | -70 | 84 | 40 | 32 | 17 | 1400 |
| Acetone | A | PS-C II | 0 | 94 | 60 | 39 | 60 | 23,000 |
| Acetone | A | PS-C II | -10 | 89 | 97 | 52 | 72 | 21,000 |
| Acetone | A | PS-C II | -20 | 88 | 97 | 52 | 65 | 16,000 |
| Acetone | A | PS-C II | -30 | 96 | 82 | 46 | 125 | 6800 |
| Acetone | A | PS-C II | -40 | 97 | 41 | 30 | 98 | 3000 |
| Acetone | A | PS-C II | -50 | 96 | 94 | 49 | 175 | 3200 |
| Acetone | A | PS-C II | -60 | 99 | 23 | 19 | 249 | 850 |
| Acetone | B | PS-C II | 0 | 78 | 41 | 34 | 12 | 22,000 |
| Acetone | B | PS-C II | -10 | 78 | 98 | 56 | 36 | 22,000 |
| Acetone | B | PS-C II | -20 | 84 | 98 | 54 | 52 | 13,000 |
| Acetone | B | PS-C II | -30 | 85 | 54 | 39 | 21 | 7700 |
| Acetone | B | PS-C II | -40 | 75 | 81 | 52 | 14 | 2300 |
| Acetone | C | PS-C II | 0 | 36 | 90 | 71 | 5.8 | 21,000 |
| Acetone | C | PS-C II | -10 | 68 | 82 | 55 | 13 | 11,000 |
| Acetone | C | PS-C II | -20 | 93 | 40 | 30 | 41 | 6000 |
| Acetone | C | PS-C II | -30 | 15 | 97 | 87 | 4.1 | 8800 |
| Acetone | C | PS-C II | -40 | 11 | 94 | 90 | 3.2 | 4000 |
| Acetone | A | PS | 0 | 57 | 46 | 45 | 5.7 | 1200 |
| Acetone | A | PS | -10 | 91 | 58 | 39 | 38 | 1300 |
| Acetone | A | PS | -20 | 92 | 84 | 48 | 64 | 640 |
| Acetone | A | PS | -30 | 88 | 58 | 40 | 28 | 430 |
| Acetone | A | PS | -40 | 52 | 45 | 46 | 4.8 | 230 |

^a A: vinyl acetate; B: vinyl butyrate; C: vinyl 3-phenylpropanoate.

^b Determined by HPLC analysis.

^c Conv. = ee(s)/(ee(s) + ee(p)).⁸

^d Calculated by the literature method.⁸

CDCl₃) δ 30.86, 35.65, 37.23, 64.84, 78.13, 126.25, 125.65, 128.18, 128.44, 128.69, 129.08, 130.21, 140.15, 156.13, 172.54; IR (KBr) 3032, 1732, 1175 cm⁻¹; [α]_D²⁰ = -31.0 (36% ee, *c* 1.0, CHCl₃); Anal. Calcd for C₁₉H₁₉NO₃: C, 73.77; H, 6.19; N, 4.53. Found: C, 73.91; H, 6.30; N, 4.14.

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