Enhancement of the Enantioselectivity in Lipase-Catalyzed Kinetic Resolutions of 3-Phenyl-2*H*-azirine-2-methanol by Lowering the Temperature to −40 °C

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We report here an efficient preparation of (*S*)-(+)-phenyl-2*H*-azirine-2-methanol ((*S*)-(+)-**1)** and its acetate ((*R*)-(−)-**2)** by a lipase-catalyzed kinetic resolution carried out preferentially at −40 °C in ether under unusual conditions for enzyme. We disclosed that a lipase from *Pseudomonas cepacia* (lipase PS) exerts its function at such a very low temperature to markedly enhance the enantioselectivity. In an enzymatic reaction, the enantioselectivity in the kinetic resolution is temperature dependent and obeys the following thermodynamic equation:[1]

$$\ln E = \Delta\Delta S^{\ddagger}/R - \Delta\Delta H^{\ddagger}/(RT) \qquad (1)$$

Hence, lowering the temperature can increase the enantioselectivity so far as the reaction is carried out below the racemic temperature T_r.[1] However, no report on exemplification of the theory by the enzymatic reaction below 0 °C is available[2] so far, because an enzyme is generally believed not to work effectively at such low temperatures. We found that the lipase-catalyzed reaction obeys the equation (eq 1) ranging from +30 to −50 °C. Recently, the chemoenzymatic synthesis has attracted much attention because of the demand for an environmentally-acceptable and total-cost-effective synthetic method. In these aspects, the lipase-catalyzed kinetic resolution[3] has been widely utilized as a reliable and readily available method for the resolution of racemic alcohols and carboxylic acid esters. In order to increase the enantioselectivity, a variety of methods, *e.g.*, reaction in appropriate organic media,[3g,4] use of additive,[5] choice of acyl donor,[6] and so on,[7] have been invented, while new and readily available methods for synthetic organic chemists are still being sought. One problem to be solved in the lipase-catalyzed resolution is low enantioselectivity for chiral primary alcohols, except the relatively successful case for *meso*-compounds,[8] where the methodologies devised for secondary alcohols are not readily applicable to primary ones.[9] We now propose that a simple cooling of the reaction system to −40 °C can be effective for enhancement of the enantioselectivity of the enzymatic reaction.

Azirine **1**[10,11] adopted here seems to be a useful chiral building block because the highly strained C=N double bond accepts a variety of chemical transformations such as a reduction to aziridine or an introduction of appropriate nucleophiles diastereoselectively.[11] Quite recently, the asymmetric synthesis of azirine derivatives has been the focus of several groups,[12] because naturally-occurring antibiotics containing the skeleton have been found. This class of compounds involves (*S*)-azirinomycin[13] and (*R*)-(−)-dysidazirine,[14] the latter of which has been recently synthesized by developing an asymmetric synthetic method of the azirine skeleton.[12b] The lipase-catalyzed resolution of chiral azirine has not been reported so far.

Racemic azirine (±)-**1** was prepared from cinnamyl alcohol by the reported method[15] with some modifications in 50% overall yield as shown in Scheme 1, which involves bromination of cinnamyl alcohol, reaction with NaN_3 in DMSO, and subsequent dehydrobromination and then thermolysis to (±)-**1**. In the final thermocyclization step, the reaction temperature should be carefully controlled not to exceed 100 °C until the evolution of nitrogen ceases. Chromatographic purification gave pale yellow crystals (mp 57−58 °C), which are stable enough to be stored in a refrigerator.

To begin, the conditions of the lipase-catalyzed transesterification of (±)-**1** were optimized according to the conventional method (Scheme 2). Thus, lipase PS was found to be a suitable lipase after screening commercially available lipases.[16] The reaction with an equimolar amount of vinyl acetate in diisopropyl ether at 30 °C was

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(16) Lipases showing the *E* values > 3 (origin, *E* value, fast-reacting enantiomer): CHIRAZYME L-2 (*Candida antarctica*, 8, *R*),CHIRAZYME L-7 (porcine pancreas, 5, *R*), lipase AK (*Pseudomonas fluorescens*, 4, *R*).

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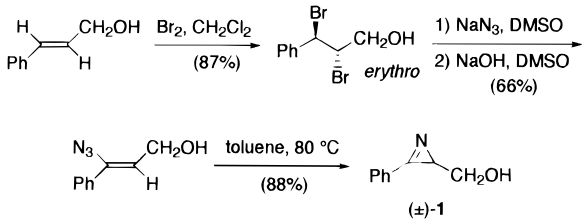
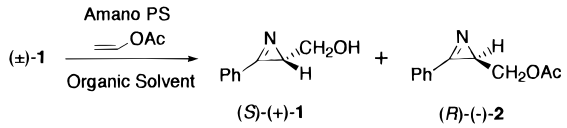
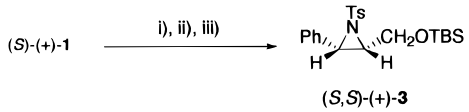
Scheme 1



Scheme 2



Scheme 3[a]



[a] Key: (i) TBS-Cl, imidazole, THF (34%); (ii) $LiAlH_4$, Et_2O (51%); (iii) TsCl, Et_3N, $CHCl_3$ (58%).

found to reach to a moderate conversion within a few hours, giving the *E* value[17] of 11 at best. Next, screening of the solvents[18] revealed that ether is the best choice, raising the *E* value to 17. In addition, vinyl acetate proved to give the highest selectivity among the several types of the acylating agents[6] examined. In spite of these efforts, the *E* value still remained around 17 and no other conventional means seemed to be applicable. The absolute configuration of (+)-**1**[10] thus obtained was determined to be *S* by correlation to authentic *N*-tosyl aziridine (*S*,*S*)-(+)-**3**[19] as shown in Scheme 3.

Then, we examined the temperature effect on the enantioselectivity in the prospects mentioned above. The reaction of (±)-**1** was carried out with lipase PS and vinyl acetate in ether at the temperature ranging from 30 to −60 °C as shown in Table 1. As the reaction system was cooled, the reaction rate was decreased as expected (convn h^{-1} lipase mg^{-1}), and thus, the amount of lipase was increased from 50 mg to 100, 200, and then 1600 mg at −60 °C so as to finish the reaction within a moderate time. The amount of the lipase, however, has no significant influence on the *E* value as examined at 30, 0, and −40 °C, respectively. Noteworthily, as the temperature was lowered the *E* values were markedly increased and reached up to 99 (at −40 °C), sufficient for practical use. Further cooling to −60 °C, however, began to lose the efficiency, decreasing the *E* value to 64.[20] The results listed in Table 1 are plotted in Figure 1 to give a straight line as a function of ln *E* and 1/*T* ranging from 30 to −50 °C. The parameters, $\Delta\Delta H^{\ddagger}$ and $\Delta\Delta S^{\ddagger}$, calculated according to the theoretical equation (eq 1) are −3.0 kcal mol^{-1} and −4.3 cal deg^{-1} mol^{-1}, respectively, and the racemic temperature T_r calculated is 425 °C.

Table 1. Temperature Modulation in the Lipase-Catalyzed Resolution[a]

entry	*T* (°C)	lipase (mg)	% yield[b] (% ee[c]) (*S*)-(+)-**1**	(*R*)-(−)-**2**	time (h)	*c*[d]	×10³*c*/h/ lipase mg	*E*[e]
1	30	50	50 (59)	41 (80)	2.0	0.42	4.2	17
2	30	200	44 (76)	45 (77)	0.5	0.50	5.0	18
3	20	50	45 (90)	55 (72)	4.3	0.56	2.6	19
4	0	50	54 (65)	45 (88)	6.0	0.43	1.4	31
5	0	100	42 (83)	49 (85)	3.0	0.49	1.6	31
6	0	200	57 (68)	43 (87)	1.4	0.44	1.6	28
7	−10	200	50 (77)	43 (86)	4.0	0.47	0.59	37
8	−20	200	49 (78)	42 (90)	4.5	0.46	0.51	46
9	−30	200	60 (54)	37 (94)	7.5	0.37	0.25	54
10	−40	200	66 (39)	22 (97)	8.0	0.29	0.18	84
11	−40	400	62 (46)	31 (97)	4.3	0.32	0.19	99
12	−50	400	60 (62)	38 (96)	8.5	0.39	0.11	86
13	−60	1600	55 (61)	37 (94)	6.0	0.39	0.041	64

[a] Conditions: (±)-**1** (50 mg, 0.34 mmol), vinyl acetate (29 mg, 0.34 mmol), dry Et_2O (5 mL). [b] Isolated yield. [c] Determined by HPLC analysis using a Chiralcel OB-H column for **1** and Chiralcel OD-H for **2**, respectively. [d] Conversion calculated from *c* = ee (**1**)/(ee (**1**) + ee (**2**)) according to ref 17. [e] Reference 17.

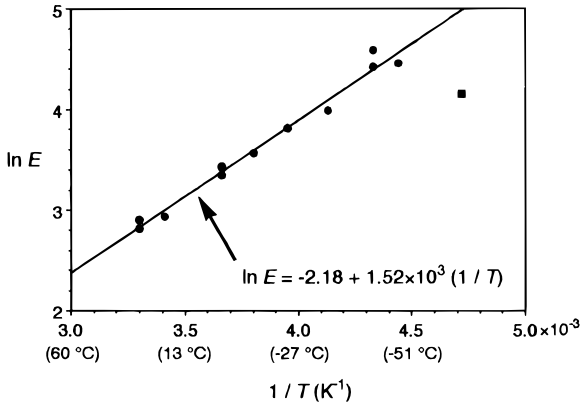


Figure 1. Temperature effect on the enantioselectivity.

These results suggest that the enantioselectivity of this lipase-catalyzed kinetic resolution is temperature-predictive even at such very low temperatures. Thus, lowering the temperature increases the *E* value, favoring the (*R*)-enantiomer in this case. The thermostability of the lipase in organic solvent[3g,4] enabled the reaction not only at higher temperatures[21] but also at such low temperatures. This temperature modulation will be a readily and generally applicable method to improve the enantioselectivity with theoretical prediction in the lipase-catalyzed resolution and should be a reliable method especially for primary alcohols. We are now actively investigating how to generalize the low-temperature modulating method by adopting to a variety of lipases and alcohols and how to utilize the chiral azirines (*S*)-(+)-**1** and (*R*)-(−)-**2** in organic synthesis.

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Supporting Information Available: Experimental procedures and spectral data for all compounds, including copies of ^1H and ^{13}C NMR spectra (18 pages).

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