

Practical Resolution of 3-Phenyl-2*H*-azirine-2-methanol at Very Low Temperature by Using Lipase Immobilized on Porous Ceramic and Optimized Acylating Agent

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A synthetically useful primary alcohol, 3-phenyl-2*H*-azirine-2-methanol, was resolved efficiently by a reaction at −40 °C with a lipase immobilized on the porous ceramic (*Toyonite*) and vinyl butanoate in ether (*E* value of 130 and TTN/h value of 7800).

We have recently proposed "the low-temperature method" for enhancing the enantioselectivity in the lipase-catalyzed resolutions;[1,2] this method was found to be especially effective for a variety of primary alcohols[3] involving 3-phenyl-2*H*-azirine-2-methanol (**1**), a versatile synthetic intermediate.[4] Among many fine-tuning methods for enhancing the enantioselectivity,[5] the low-temperature method is a theoretically reliable method[3] as usual chemical asymmetric reactions. After we proposed the method, it has been frequently utilized.[6] In previous papers,[1,2] we described that *Celite*-immobilized lipase-catalyzed resolution of azirine (±)-**1** with vinyl acetate in ether at 30 °C gave ester (*R*)-**2a** and alcohol (*S*)-**1** with an *E* value[7] of 17. The reaction rate was roughly estimated by the total turnover number per hour (TTN/h),[8] which was 4700 at 30 °C. In contrast, although lowering the reaction temperature to −40 °C enhanced the *E* value up to 99, the TTN/h value was lowered to 210. Thus, enhancing not only the enantioselectivity but also the activity even at such very low temperatures is an urgent subject for practical application. Here, we attained the purpose by using both an optimized acylating agent[9–11] and a lipase immobilized on a porous ceramic (*Toyonite*)[12,13] in the low-temperature reaction of (±)-**1** at −40 °C.

The porous ceramic support (*Toyonite*) is a new type of commercially available inorganic material for immobilization of enzymes. There are two types: *Toyonite* D-M and *Toyonite* 200M,[14] on which we immobilized lipase PS. In the present reactions, the temperature was kept at −40 °C, the temperature that gave the highest *E* value for **1**.[1] Results of the reactions using the *Toyonite*-immobilized lipase and various acylating agents are summarized in Table 1. As shown in entry 3, use of the *Toyonite* D-M-immobilized lipase and **3a** remarkably raised the TTN/h to 4200, 20 times larger than that of the reaction catalyzed by the *Celite*-immobilized lipase (entry 1). The immobilization of the lipase on *Toyonite* greatly enhanced the reaction rate, probably due to the high dispersion of lipase molecules on the highly porous support. However, it lowered the *E* value from 99 to 34. The immobilization may restrict the required conformational flexibility of the lipase.[15] Improvement of the *E* value by simply lowering the reaction temperature is obvious by comparison with the results for the reaction at 30 °C as shown in entry 2.

In order to adjust the two conflicting features, the reaction rate and the enantioselectivity, the length of the acylating agent was then examined. Elongation of the acyl chain to vinyl butanoate (**3b**) (entry 4) dramatically increased the *E* value up to 96, which is comparable to the value obtained by using the *Celite*-immobilized lipase (entry 1), and gave a sufficient TTN/h value of 3200. In contrast, a similar reaction using vinyl hexanoate (**3c**) somewhat lowered the *E* value (54) and the reaction rate (entry 5). Interestingly, use of two molar amounts of **3c** (entry 6) not only raised the TTN/h value but also raised the *E* value up to 102. Therefore, two molar amounts of the acylating agents were used in the following reactions with longer acylating agents **3d**–**3g**. Further elongation of the chain to vinyl octanoate (**3d**), however, suddenly decreased the *E* value to 35 (entry 7). In the case of vinyl dodecanoate (**3f**), both *E* value and TTN/h value were slightly recovered unexpectedly (entry 9). Vinyl 3-phenylpropanoate (**3g**), which is a suitable acylating agent for primary alcohols as reported by Kawasaki et al.,[10,11a] also gave a good *E* value of 71 and an acceptable TTN/h value (entry 10). Vinyl chloroacetate (**3h**) accelerated the reaction rate markedly, but gave a low *E* value (entry 11).

Similar reactions of (±)-**1** using the *Toyonite*-200M-immobilized lipase and acylating agents **3b** and **3c** were further examined. As shown in entry 12, the reaction using the *Toyonite*-200M-immobilized lipase and **3b** further increased both *E* value (130) and TTN/h value (7800), which are much higher than those obtained with the *Celite*-immobilized lipase (entry 1). Changing from **3b** to **3c** (entry 13) turned out to decrease the *E* value to 77, although TTN/h was the highest (8600). As the last attempt, the organic bridges were changed to 6-(propanoyloxy)hexylsilanetrioxyl groups, because we have recently found that this organic bridge gives the results suitable for the resolution of (±)-2-hydroxymethyl-1,4-benzodioxane.[13] However, it rather decreased the *E* and TTN/h values for compound **1** (entry 14).

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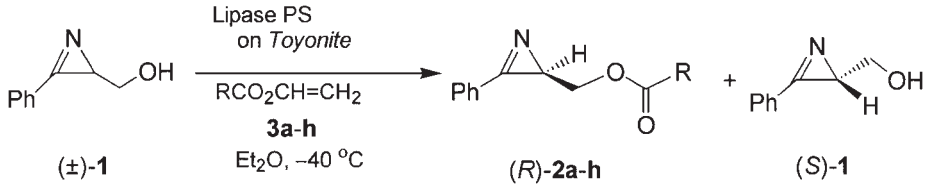
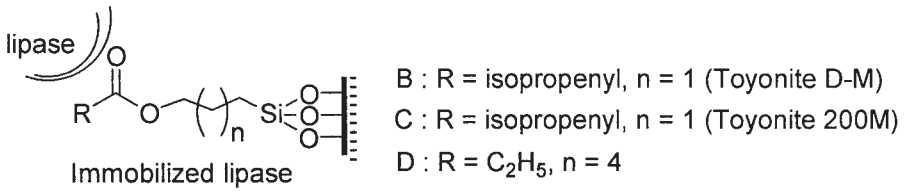
Table 1. *Toyonite*-Immobilized Lipase-Catalyzed Resolution of (±)-**1** at −40 °C



Entry	Acylating agents		Lipase[a]	Time	% Ee		Conv.	*E*	TTN/h[b]
	R			/h	(*R*)-**2**	(*S*)-**1**			
1[c]	CH_3	**3a** (1)[d]	A	4.3	97[f]	46[f]	0.32	99	210
2[e]	CH_3	**3a** (1)	B	0.4	63[f]	52	0.46	7	50000
3	CH_3	**3a** (1)	B	5.2	86[f]	81	0.49	34	4200
4	$CH_3(CH_2)_2$	**3b** (1)	B	6.0	96[f]	71	0.43	96	3200
5	$CH_3(CH_2)_4$	**3c** (1)	B	7.3	95[f]	33	0.26	54	1600
6	$CH_3(CH_2)_4$	**3c** (2)	B	12.0	91[f]	99	0.52	102	2000
7	$CH_3(CH_2)_6$	**3d** (2)	B	11.8	90[g]	62	0.41	35	1500
8	$CH_3(CH_2)_8$	**3e** (2)	B	11.0	91[f]	63	0.41	40	1500
9	$CH_3(CH_2)_{10}$	**3f** (2)	B	12.0	89[f]	81	0.47	44	1800
10	$PhCH_2CH_2$	**3g** (2)	B	8.0	95[h]	62	0.40	71	3600
11	$ClCH_2$	**3h** (1)	B	3.0	67[h]	37	0.36	7	5300
12	$CH_3(CH_2)_2$	**3b** (1)	C	2.0	97[f]	52	0.35	130	7800
13	$CH_3(CH_2)_4$	**3c** (2)	C	2.4	93[f]	85	0.44	77	8600
14	$CH_3(CH_2)_2$	**3b** (2)	D	11.0	95[f]	58	0.37	68	1500

a) A: Lipase PS on *Celite*; B: Lipase PS immobilized on *Toyonite* D-M with 3-(methacryloyloxy)propylsilanetrioxyl bridges; C: Lipase PS immobilized on *Toyonite* 200M with 3-(methacryloyloxy)propylsilanetrioxyl bridges; D: Lipase PS immobilized on *Toyonite* 200 with 3-(propanoyloxy)hexylsilanetrioxyl bridges.



b) See Ref. 8. c) Reported results in Ref. 1. d) Molar amounts of acylating agent. e) Reaction at 30 °C. f) Analyzed by *Chiralcel* OB-H. g) Analyzed by *Chiralcel* AS-H. h) Analyzed by *Chiralpak* OD-H.

In conclusion, these results suggest that the choice of acylating agent and the use of the *Toyonite*-immobilized lipase are critically important in the practical operation of the low-temperature reaction. A matched combination of these techniques will further enhance the utility of the low-temperature method.

Experimental

Lipase PS (Amano Enzyme Inc.) was purchased from Wako Pure Chemical Co. Ltd. Ether was distilled from sodium before use. Preparative column chromatography was carried out by using silica gel (Fuji Silysia BW-127 ZH, 100–270 mesh), and thin-layer chromatography was performed by using precoated silica-gel plate (Merck 60 PF_{254}, plate length 40 mm). ^{1}H NMR spectra were measured at 200 MHz. Enantiomeric purities were determined by HPLC analyses detected at 254 nm with a chiral column (*Chiralpak* OB-H, AS-H, or OD-H, Daicel Chemical Industries) as indicated in Table 1.

Immobilization of Lipase to the Ceramic Support (*Toyonite*) with Organic Bridges.[13] A suspension of commercially available lipase PS immobilized on *Celite* (15 g) in phosphate buffer (60 mL, pH 7.0, 10 mM) was stirred at room temperature for 3 h. After filtration, to the filtrate was added *Toyonite* (D-M or 200M) (1.5 g), and the mixture was shaken for 24 h at 25 °C. The resulting mixture was filtered, and the *Toyonite*-immobilized lipase was dried under vacuum (267 Pa) for 15 h and stored in a desiccator. Protein wt % was determined to be about 1.3 wt % by the difference between the amounts of the protein in the aqueous solutions before and after immobilization.[16]

Typical Lipase-Catalyzed Resolution of 3-Phenyl-2*H*-azirine-2-methanol ((±)-1) (entry 12). A mixture of (±)-**1** (50 mg, 0.34 mmol), *Toyonite*-200M-immobilized lipase PS (25 mg), and 5 mL of ether was cooled to −40 °C in a thermo-controlled cooling bath. Acylating agent **3b** (39 mg, 0.34 mmol) was injected quickly to the cooled mixture through a syringe. The reaction progress was monitored by TLC (hexane/ethyl acetate = 2/1). The mixture was filtered with suction to remove the lipase. The filtrate was concentrated, and the residual products were separated by column chromatography (silica-gel 5 g, hexane/ethyl acetate = 3/1–1/1). (*S*)-**1**: $[\alpha]_D^{27} = +123$ (*c* 1.00, $CHCl_3$); IR (KBr) 3333, 1736 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.15 (br s, 1H), 2.48 (dd, $J = 5.1$, 2.2 Hz, 1H), 3.69 (dd, $J = 10.2$, 5.1 Hz, 1H), 3.99 (dd, $J = 10.2$, 2.2 Hz, 1H), 7.49–7.58 (m, 3H), 7.86–7.90 (m, 2H). (*R*)-**2b**: $[\alpha]_D^{27} = -160$ (*c* 0.68, $CHCl_3$); IR (KBr) 1736 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.90 (t, $J = 3.7$ Hz, 3H), 1.60 (m, 2H), 2.27 (t, $J = 7.4$ Hz, 2H), 2.50 (dd, $J = 5.0$, 3.8 Hz, 1H), 4.20 (dd, $J = 11.8$, 5.0 Hz, 1H), 4.32 (dd, $J = 11.8$, 3.8 Hz,

1H) 7.53–7.65 (m, 3H), 7.87–7.90 (m, 2H). The conditions and the retention times in HPLC analyses (flow rate = 0.5 mL/min, otherwise indicated) for determination of the optical purity are as follows: (*S*)-**1**: hexane/*i*-PrOH = 9/1, flow rate = 0.8 mL/min, (*R*) 12.5 min, (*S*) 18.2 min. (*R*)-**2b**: hexane/*i*-PrOH = 20/1, (*R*) 22.7 min, (*S*) 26.0 min.

Data of HPLC Analyses for (*R*)-2a, (*R*)-2c–(*R*)-2h. (*R*)-**2a**: hexane/*i*-PrOH = 9/1, detection 254 nm, (*R*) 19.6 min, (*S*) 21.8 min. (*R*)-**2c**: hexane/*i*-PrOH = 100/1, (*R*) 29.2 min, (*S*) 32.2 min. (*R*)-**2d**: hexane/*i*-PrOH = 300/1, (*R*) 40.8 min, (*S*) 43.7 min. (*R*)-**2e**: hexane/*i*-PrOH = 300/1, (*R*) 33.8 min, (*S*) 37.6 min. (*R*)-**2f**: hexane/*i*-PrOH = 300/1, (*R*) 25.4 min, (*S*) 29.0 min. (*R*)-**2g**: hexane/*i*-PrOH = 100/1, flow rate = 0.7 mL/min, (*S*) 54.1 min, (*R*) 57.8 min. (*R*)-**2h**: hexane/*i*-PrOH = 9/1, flow rate = 0.7 mL/min, (*R*) 14.4 min, (*S*) 15.3 min.

Spectral Data. (*R*)-**2a**: IR (neat) 1742 cm^{-1}, ^1H NMR ($CDCl_3$) δ 2.03 (s, 3H), 2.49 (dd, $J = 5.4$, 3.7 Hz, 1H), 4.18 (dd, $J = 12.1$, 5.4 Hz, 1H), 4.27 (dd, $J = 12.1$, 3.7 Hz, 1H), 7.52–7.62 (m, 3H), 7.85–7.89 (m, 2H).

(*R*)-**2c**: IR (neat) 1735 cm^{-1}, ^1H NMR ($CDCl_3$) δ 0.86 (t, $J =$ 6.5 Hz, 3H), 1.25 (m, 4H), 1.53 (m, 2H), 2.28 (t, $J = 7.7$ Hz, 2H), 2.49 (dd, $J = 7.5$, 4.1 Hz, 1H), 4.23 (dd, $J = 10.5$, 7.5 Hz, 1H), 4.29 (dd, $J = 10.5$, 4.1 Hz, 1H), 7.56–7.61 (m, 3H), 7.86–7.90 (m, 2H).

(*R*)-**2d**: IR (neat) 1755 cm^{-1}, ^1H NMR ($CDCl_3$) δ 0.87 (t, $J =$ 8.0 Hz, 3H), 1.25 (m, 8H), 1.61 (m, 2H), 2.34 (t, $J = 6.6$ Hz, 2H), 2.50 (dd, $J = 6.8$, 4.6 Hz, 1H), 4.21 (dd, $J = 8.4$, 6.8 Hz, 1H), 4.28 (dd, $J = 8.4$, 4.6 Hz, 1H), 7.51–7.59 (m, 3H), 7.86–7.90 (m, 2H).

(*R*)-**2e**: IR (neat) 1755 cm^{-1}, ^1H NMR ($CDCl_3$) δ 0.88 (t, $J =$ 6.8 Hz, 3H), 1.26 (m, 12H), 1.62 (m, 2H), 2.38 (t, $J = 7.8$ Hz, 2H), 2.51 (dd, $J = 7.4$, 4.0 Hz, 1H), 4.23 (dd, $J = 13.2$, 7.4 Hz, 1H), 4.27 (dd, $J = 13.2$, 4.0 Hz, 1H), 7.56–7.59 (m, 3H), 7.86–7.90 (m, 2H).

(*R*)-**2f**: IR (neat) 1759 cm^{-1}, ^1H NMR ($CDCl_3$) δ 0.90 (t, $J = 6.8$ Hz, 3H), 1.28 (m, 16H), 1.64 (m, 2H), 2.40 (t, $J = 8.0$ Hz, 2H), 2.52 (dd, $J = 7.4$, 4.6 Hz, 1H), 4.14 (dd, $J = 16.6$, 7.4 Hz, 1H), 4.27 (dd, $J = 16.6$, 4.6 Hz, 1H), 7.58–7.61 (m, 3H), 7.88–7.93 (m, 2H).

(*R*)-**2g**: IR (KBr) 1738 cm^{-1}, ^1H NMR ($CDCl_3$) δ 2.49 (dd, $J = 9.0$, 4.5 Hz, 1H), 2.65 (t, $J = 8.2$ Hz, 2H), 2.90 (t, $J = 8.2$ Hz, 2H), 4.22 (dd, $J = 11.4$, 9.0 Hz, 1H), 4.28 (dd, $J = 11.4$, 4.5 Hz, 1H), 7.55–7.62 (m, 3H), 7.83–7.87 (m, 2H).

(*R*)-**2h**: IR (neat) 1740 cm^{-1}, ^1H NMR ($CDCl_3$) δ 2.54 (dd, $J = 5.4$, 3.8 Hz, 1H), 4.06 (s, 2H), 4.30 (dd, $J = 13.2$, 5.4 Hz, 1H), 4.39 (dd, $J = 13.2$, 3.8 Hz, 1H), 7.53–7.60 (m, 3H), 7.86–7.91 (m, 2H).

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References

1 T. Sakai, I. Kawabata, T. Kishimoto, T. Ema, and M. Utaka, *J. Org. Chem.*, **62**, 4906 (1997).

2 T. Sakai, T. Kishimoto, Y. Tanaka, T. Ema, and M. Utaka, *Tetrahedron Lett.*, **39**, 7881 (1998).

3 The temperature effect is an inherent phenomenon in the reactions of both primary and secondary alcohols. However, the secondary alcohols remarkably decrease the reaction rate at the low temperatures. Therefore, in a viewpoint of practical utilization, this method is useful for primary alcohols, which still have acceptable rate even at the low temperatures.

4 Review for azirines: F. Palacios, A. M. Ochoa de Retana, E. Martines de Marigorta, and J. Manuel de los Santos, *Org. Prep. Proced. Int.*, **34**, 219 (2002).

5 Review: E. Ljubović, M. Majeric-Elenkov, A. Avdagić, and V. Šunjić, *Food Technol. Biotechnol.*, **37**, 215 (1999).

6 a) U. T. Bornscheuer and R. J. Kazlauskas, "Hydrolases in Organic Synthesis," Wiley-VCH, Weinheim (1999). b) "Enzyme Catalysis in Organic Synthesis," ed by K. Drauz and H. Waldmann, Wiley-VCH, Weinheim (2002).

7 C.-S. Chen, Y. Fujimoto, G. Girdaukas, and J. C. Sih, *J. Am. Chem. Soc.*, **104**, 7294 (1982).

8 Total turnover number (TTN) is the term defined as the number of mole of product formed per mole of enzyme during the course of a complete reaction. Here, TTN/h shows the total turnover number per hour.

9 a) T. Ema, S. Maeno, Y. Takaya, T. Sakai, and M. Utaka, *J. Org. Chem.*, **61**, 8610 (1996). b) T. Ema, S. Maeno, Y. Takaya, T. Sakai, and M. Utaka, *Tetrahedron: Asymmetry*, **7**, 625 (1996).

10 M. Kawasaki, M. Goto, S. Kawabata, and T. Kometani, *Tetrahedron: Asymmetry*, **12**, 585 (2001).

11 Recent reports for the enzymatic resolution with various acylating reagents: a) M. Kawasaki, M. Goto, S. Kawabata, T. Kodama, and T. Kometani, *Tetrahedron Lett.*, **40**, 5223 (1999). b) K. Hirose, H. Naka, M. Yano, S. Ohashi, K. Naemura, and Y. Tobe, *Tetrahedron: Asymmetry*, **11**, 1199 (2000). c) Y. Kita, Y. Takebe, K. Murata, T. Naka, and S. Akai, *J. Org. Chem.*, **65**, 83 (2000). d) J. Ottosson and K. Hult, *J. Mol. Catal. B: Enzym.*, **11**, 1025 (2001). e) T. Itoh, E. Akasaki, and Y. Nishimura, *Chem. Lett.*, **2002**, 154. f) S. Akai, T. Naka, T. Fujita, Y. Takebe, T. Tsujino, and Y. Kita, *J. Org. Chem.*, **67**, 411 (2002).

12 a) M. Kamori, T. Hori, Y. Yamashita, Y. Hirose, and Y. Naoshima, *J. Mol. Catal. B: Enzym.*, **9**, 269 (2000). b) K. Kato, Y. Gong, T. Saito, and H. Kimoto, *J. Biosci. Bioeng.*, **90**, 332 (2000). c) T. Sakai, Y. Miki, M. Tsuboi, H. Takeuchi, T. Ema, K. Uneyama, and M. Utaka, *J. Org. Chem.*, **65**, 2740 (2000). d) M. Suzuki, C. Nagasawa, and T. Sugai, *Tetrahedron*, **57**, 4841 (2001).

13 T. Sakai, K. Hayashi, F. Yano, M. Takami, M. Ino, T. Korenaga, and T. Ema, *Bull. Chem. Soc. Jpn.*, **76**, 1441 (2003).

14 *Toyonite* D-M has 120 nm of mean pore size and *Toyonite* 200M has 60 nm mean pore size. Both of them have 3-(2-methacryloyloxy)propylsilanetrioxyl bridges on the surface. They are commercially available from Toyo Denka Kogyo Co. Ltd. *Toyonite*-immobilized lipase is also available from Amano Enzyme Inc. or Wako Pure Chemical Industries, Ltd. as Lipase PS-C "Amano" II.

15 J. M. Palomo, G. F.-Lorente, C. Mateo, M. Fuentes, R. F.-Lafuente, and J. M. Guisan, *Tetrahedron: Asymmetry*, **13**, 1337 (2002).

16 M. Bradford, *Anal. Biochem.*, **72**, 248 (1976).