Enhancement of the Efficiency of the Low Temperature Method for Kinetic Resolution of Primary Alcohols by Optimizing the Organic Bridges in Porous Ceramic-Immobilized Lipase

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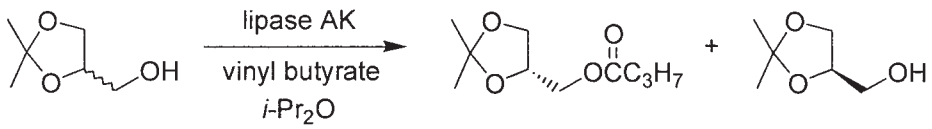
For the enhancement of enantioselectivity and acceleration of the reaction rate in the lipase-catalyzed resolution of primary alcohols, the use of a very low reaction temperature (−30 °C) and an immobilized lipase on organic bridges-coated porous ceramic support was found to be highly effective. Furthermore, the structure of the organic bridges greatly influenced the temperature effect between $\ln E$ and $1/T$ as well as the reaction rate. Among the organic bridges examined in the resolution of (±)-2-hydroxymethyl-1,4-benzodioxane, the 6-(2-methylpropanoyloxy)hexylsilanetrioxyl bridge was the best choice for both the E value and the reaction rate at −30 °C.

We have recently established a low-temperature method in the lipase-catalyzed kinetic resolution[1,2] of primary alcohols as a reliable means for fine tuning of the enantioselectivity.[3] By lowering the reaction temperatures, E values[4] are increased progressively until −40 °C, retaining the theoretical linear correlation between $\ln E$ and $1/T$, as usually observed in chemical[5] asymmetric reactions. The generality of these apparently unusual temperature effects in the lipase-catalyzed reactions is confirmed by application to several types of alcohols, lipases, and organic solvents.[2] Our findings are the first examples of the enzymatic reaction carried out at very low temperatures (to −60 °C) for the synthesis of optically active compounds. Since our proposal of the low-temperature method for the enzymatic reaction, the utilities have been widely substantiated by many chemists.[6,7] For example, Šunjić et al.[8] have resolved 3-hydroxymethyl-1,4-benzodiazepin-2-one effectively at low temperatures and discussed them in detail in their review.[9] In recent enzymatic reactions, other than those using lipases,[10,11] the theoretical understanding of the temperature dependence of enantioselectivity has been an important subject.

In contrast to the above mentioned utilities of the low-temperature method, its inevitable problem of retarding the reaction rate has been posed. For example, as previously reported in our lipase AK (*Pseudomonas fluorescens*)-catalyzed resolution of solketal (2,2-dimethyl-1,3-dioxolane-4-methanol) (**1**),[2] the E value (9 at 30 °C, Table 1, entry 1) is increased up to 55 by lowering the temperature to −40 °C, although 10 times the amount of lipase and 8 fold of the reaction time are required, as compared with those in the conditions at 30 °C. Thus, the acceleration of the reaction rate is an urgent subject to make the low-temperature method practical.

Table 1. *Toyonite*-Immobilized Lipase-Catalyzed Resolution of Solketal (±)-**1**[a)]



(±)-**1** (*R*)-**1a** (*R*)-**1**

Entry	Lipase	Organic bridge	*E*		TTN/h	
			30 °C	−40 °C	30 °C	−40 °C
1[b)]	AK	*Celite*	9.0	55	11000	110
2	AK	*Toyonite*[c)]	3.2	21	53000	1400
3[b)]	PS	*Celite*	6.8	15	6400	22
4	PS	*Toyonite*[c)]	6.8	15	110000	1700

a) Reaction conditions: compound (±)-**1** (50 mg, 0.38 mmol), vinyl butyrate (87 mg, 0.76 mmol), isopropyl ether (3 mL). b) Reaction procedure and the results were reported in Ref. 2. At 30 °C : Lipase AK was used, 20 mg at 30 °C (3 h); 200 mg at −40 °C (24 h). c) Lipases are immobilized ca. 1.5 wt % on the *Toyonite*, which is pre-coated with organic bridge **3a**. The immobilized lipase was used, 3 mg at 30 °C and 100 mg at −40 °C.

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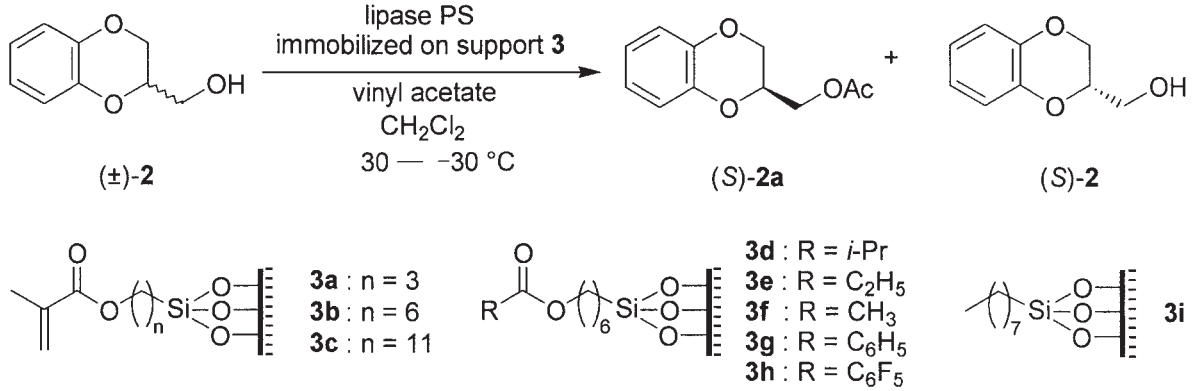
Recently, we have reported that porous ceramic support (*Toyonite*)-immobilized lipase[12] highly accelerates the reaction rate in the resolution of 2-hydroxy-2-(pentafluorophenyl)acetonitrile. *Toyonite*[13] is a commercially available ceramic support, and the utilities for great acceleration of the reaction rate and/or enantioselectivity have been recently reported.[14–16] Immobilization of an enzyme on appropriate supports greatly affects the enantioselectivity and activity. The chemical and physical natures of the supports may alter the conformation and the rigidity of enzyme, the so-called conformational engineering.[17] In this paper, we report that: a) *Toyonite*-immobilized lipase can be usable at very low temperatures to −30 °C to highly accelerate the reaction rate, b) the functionalities of the organic bridges on the surface of *Toyonite* were found to have great influence on the efficiency (rate and E value) and the temperature effect ($\ln E$ and $1/T$), and c) the requisite structural features of the bridges were elucidated.

The reaction rate was roughly estimated by using TTN/h (total turnover number per hour) at a stage of similar conversion (Table 1). In the previous paper on the lipase AK (*Pseudomonas fluorescens*)-catalyzed resolution of solketal,[2] the TTN/h of 11000 at 30 °C decreased to 110 at −40 °C despite an increase in E value (entry 1). In contrast, reactions with *Toyonite*-immobilized lipase AK increased the TTN/h up to 53000 at 30 °C and 1400 at −40 °C, respectively (entry 2). The E value, however, decreased from 55 to 21 at −40 °C. On the other hand, the reaction with lipase PS (*Pseudomonas cepacia*) showed a lower E value and a lower reaction rate at −40 °C, as reported (entry 3).[2] However, the use of *Toyonite* dramatically increased the TTN/h (entry 4) with the same E value at −40 °C as that in entry 3. These results suggest that *Toyonite*-immobilized lipase markedly accelerates the reaction rate, although the change in the E value depends on the kind of lipases.

In order to examine the effect of organic bridges on *Toyonite* and the applicability to other alcohols, similar reactions of 2-hydroxymethyl-1,4-benzodioxane ((±)-**2**) were carried out by using three types of lipases (Table 2): a) commercially available lipase PS immobilized on *Celite* (entry 1), b) lipase PS on *Toyonite* without organic bridges (entry 2), and c) that on *Toyonite* with organic bridges (entry 3). In the case of entry 2, the reaction is accelerated, as shown in the TTN/h, from 26 to 52 with retention of the E value. Attachment of organic bridges (entry 3) further increased the TTN/h up to 130, while the E value was lowered to 18. These results indicate that the immobilization of lipase on *Toyonite* certainly enhances the reaction rate, and the organic bridges attached play a significant role.[17]

For further optimization of the efficiency in both rate and enantioselectivity, the commercially available 3-(2-methylpropenoyloxy)propylsilanetrioxyl bridge **3a** was replaced by various others **3b**–**3i**, which were prepared by the patented method[18] or its modifications. Effects of the length of aliphatic chain of the bridges were examined by using organic bridges **3b** and **3c**. Necessity of the olefinic moiety for binding with lipase was surveyed by the use of **3d**, **3e**, and **3f**. Interaction between electron-rich and electron-deficient[19] phenyl rings in

Table 2. Effect of Organic Bridges in the *Toyonite*-Immobilized Lipase-Catalyzed Resolution of (±)-**2**



Entry	Organic bridge	*E* 30 °C	*E* −30 °C	TTN/h 30 °C	TTN/h −30 °C	Bridge wt %	Protein[a] wt %
1	none[b]	18	33	3100	26	—	—
2	none[c]	19	31	5300	52	—	0.75
3	**3a**	8.6	18	7300	130	5.3	1.3
4	**3b**	10	17	14000	280	5.7	1.3
5	**3c**	6.6	9.3	14000	230	6.5	1.6
6	**3d**	7.9	28	8700	280	5.5	1.6
7	**3e**	4.8	21	27000	840	5.3	1.1
8	**3f**	11	28	12000	100	5.3	1.3
9	**3g**	6.8	12	57000	940	6.9	1.1
10	**3h**	5.9	6.8	7200	61	7.7	1.6
11	**3i**[d]	—	—	—	—	6.4	0.23

a) Determined by the Bradford method. See Ref. 20. b) Commercially available lipase PS immobilized on *Celite*. c) *Toyonite* without organic bridges. d) No reaction even under prolonged reaction time.

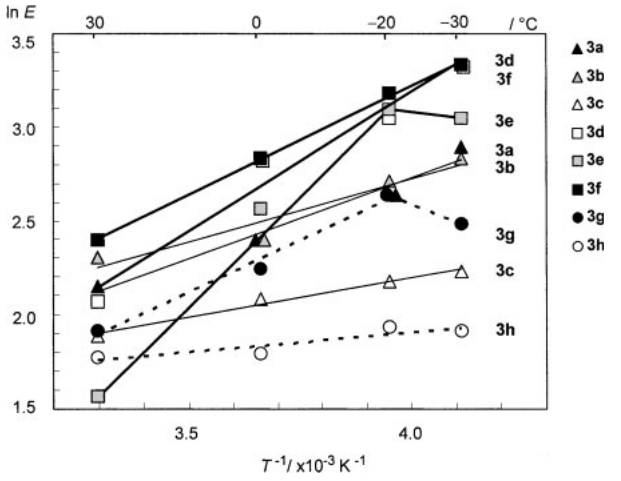


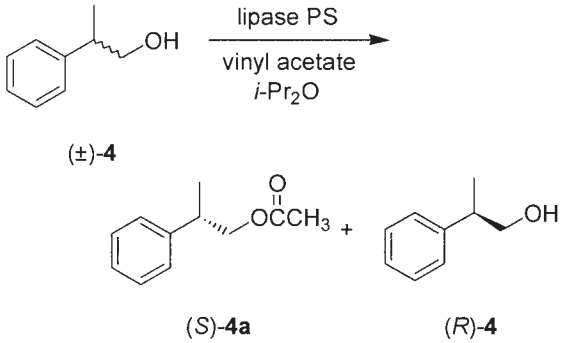
Fig. 1. Temperature effect in the *Toyonite*-immobilized lipase-catalyzed resolution of (±)-**2** by varying the organic bridges (**3a–c** : —; **3d–f** : —; **3g**, **h** : ••••).

the binding ability towards lipase was examined by the use of **3g** and **3h**. Furthermore, the role of the carbonyloxy moiety was checked by using **3i**. Table 2 shows amounts of the bridges attached on *Toyonite* and that of immobilized lipases. The latter was estimated by the protein weight %,[20] which ranges between 1.1 and 1.6, except for the very low case of **3i** (0.23). The low immobilization ability of **3i** suggests that the alkoxycarbonyl moiety is requisite and plays a major role in the immobilization ability towards lipase, probably by hydrogen bonding. Thus, the terminal conjugate olefin in **3a–3c** would not have a special role in the binding to lipase.

The resulting *Toyonite*-immobilized lipases with **3a–i** were examined in the resolution of alcohol **2** with a range of temperatures between 30 and −30 °C. The results are shown in Table 2 and Fig. 1, revealing the significant role of the bridges. From the viewpoint of the *E* value, *Toyonite* with **3f** (R = CH_3) is the best choice between 30 and −30 °C. The plot for *Toyonite* with **3e** (R = Et) shows the steepest line, exhibiting its highest enantiomeric excess (ee) at −20 °C. However, that at −30 °C deviates from the line to give a lowered ee. These results also indicate that the olefinic moiety, as seen in **3a**, **3b**, and **3c**, is not a requisite structural factor. Furthermore, neither elongation of the aliphatic chain from **3a** to **3b** and **3c**, nor fluorination of the aromatic ring **3h** improves the *E* values. Besides the enantioselectivity, we emphasize here that the choice of organic bridges is crucially important to accelerate the rate in the low temperature reaction, as seen in the case of **3d**, **3e**, and **3g**. By taking into account both the *E* value and the reaction rate, **3d** is the most suitable bridge for the low temperature reaction of **2**. It is surprising that such small structural differences as seen in **3d**, **3e**, and **3f** dramatically affect the results. The *E* values for **3e** and **3g** at −30 °C are decreased from the lines between 30 and −20 °C. These irregularities in the temperature effect of enantioselectivity may involve some intrinsic problems, which we wish to discuss elsewhere.

In order to confirm the applicability of *Toyonite*-immobilized lipase with **3d**, it was used for a similar reaction of 2-phenylpropanol ((±)-**4**), which had been reported to exhibit

Table 3. *Toyonite*-Immobilized Lipase-Catalyzed Resolution of (±)-**4**



Entry	Organic bridge	*E*		TTN/h	
		30 °C	−30 °C	30 °C	−30 °C
1	**3a**	2.1	3.4	22000	61
2	**3d**	2.4	5.4	85000	4200

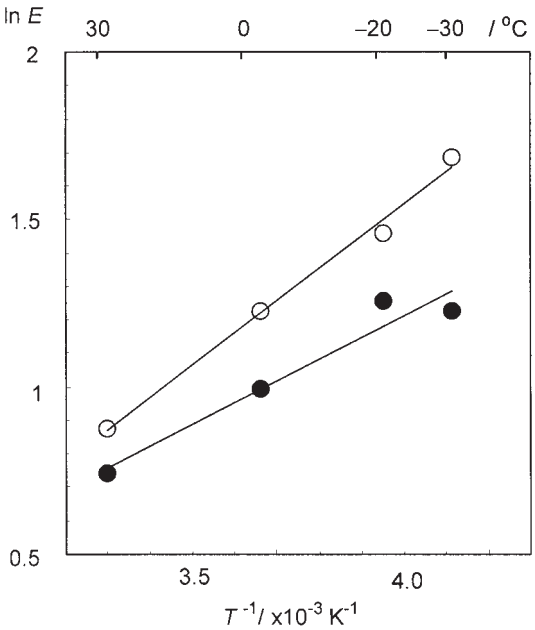


Fig. 2. Temperature effect in the *Toyonite*-immobilized lipase-catalyzed resolution of (±)-**4** (●: organic bridge **3a**; ○: organic bridge **3d**).

a low *E* value (only 2.1) at 30 °C.[21] As shown in Table 3 and Fig. 2, the lipase with **3d** also showed higher *E* values and a markedly improved TTN/h at −30 °C than those with **3a**, suggesting its applicability to other substrates.

In conclusion, the wide utility of the temperature effect ($\ln E$–$1/T$) was confirmed in the kinetic resolution with porous ceramic support (*Toyonite*)-immobilized lipase. The lipase was found to dramatically accelerate the reaction rate even at −30 °C in diisopropyl ether. The temperature effect was highly influenced by the variation in organic bridges attached to *Toyonite*. The 6-(2-methylpropanoyloxy)hexylsilanetrioxyl bridge is the best choice for both the reaction rate and the *E* value at −30 °C. This system is quite useful in the practical use of the low-temperature reaction in a lipase-catalyzed resolution. The utility of the carbonyl group of organic bridges

for hydrogen bonding with lipase and the hydrophobicity of the chain may strongly influence the conformational engineering of lipase.[17] Details on the relation between the functionality of the bridges and the efficiency of lipase are now under investigation.

Experimental

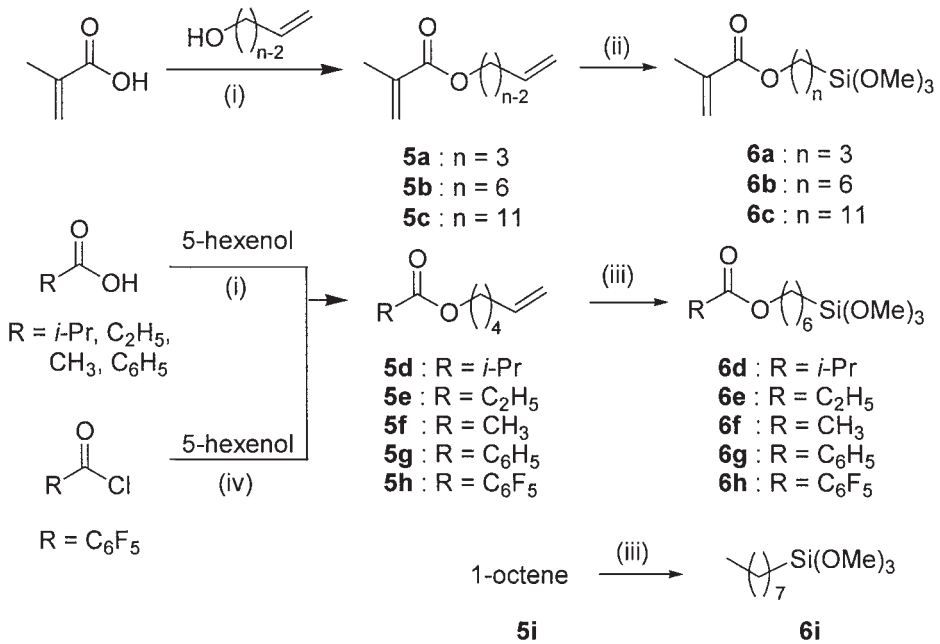
Diisopropyl ether and dichloromethane were distilled from sodium and calcium hydride, respectively, before use. Preparative column chromatography was carried out by using silica-gel (Fuji Silysia BW-127 ZH, 100–270 mesh) and thin-layer chromatography was performed by using precoated silica-gel plates (Merck 60 PF_{254}, plate length 40 mm). Boiling points are oven temperatures in the bulb-to-bulb distillation and are incorrect. ^{1}H and ^{13}C NMR spectra were measured at 200 and 50 MHz, respectively, and chemical shifts are recorded relative to tetramethylsilane. 3-(Methacryloyloxy)propyltrimethoxysilane **6a** was purchased from Shin-Etsu Chemical Co. Ltd. and the trimethoxysilane compounds **6b–i** were prepared from the corresponding olefinic esters **5b–h** and 1-octene (**5i**) by the application or modification of the patented method for **6a**[18] (Scheme 1).

Typical Synthetic Method for Olefinic Esters 5b–g.[22] Typical synthetic procedures for **5e** and **5h** are shown below.

5-Hexenyl Propanoate (5e): A mixture of 5-hexen-1-ol (1.00 g, 10 mmol), propanoic acid (1.48 g, 20 mmol), *p*-toluenesulfonic acid monohydrate (95 mg, 0.5 mmol), and toluene (50 mL) was heated under reflux with stirring for 20 h using a Dean-Stark apparatus. The resulting mixture was treated in the usual manner, and purified by column chromatography on silica-gel (20 g, hexane/ethyl acetate) to give the corresponding ester (1.10 g, 71% yield) as a colorless oil: IR (neat) 1738, 1640 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.14 (t, $J = 7.6$ Hz, 3H), 1.42–1.55 (m, 2H), 1.58–1.73 (m, 2H), 2.00 (q, $J = 6.7$ Hz, 2H), 2.30 (q, $J = 7.6$ Hz, 2H), 4.08 (t, $J = 6.5$ Hz, 2H), 4.95–5.05 (m, 2H), 5.70–5.90 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 9.09, 25.2, 27.6, 28.0, 33.2, 64.2, 114.7, 138.3, 174.5.

5-Hexenyl Pentafluorobenzoate (5h): This compound could not be prepared by the above esterification and was thus prepared as follows. A mixture of pentafluorobenzoic acid (0.59 g, 2.8 mmol), DMF (0.05 mL, 0.65 mmol), and $SOCl_2$ (0.84 g, 7.1 mmol) was stirred at 80–90 °C for 17 h. After excess $SOCl_2$ was removed by evaporation, the resulting pentafluorobenzoyl chloride was added dropwise to a mixture of 5-hexen-1-ol (0.28 g, 2.8 mmol), pyridine (0.51 g, 6.5 mmol), 4-(dimethylamino)pyridine (0.13 g, 1.1 mmol), and CH_2Cl_2 (4 mL) at 0 °C. The mixture was stirred at room temperature overnight. Water and 10% aqueous HCl were added subsequently to acidify the mixture (pH 3), and the organic layer was extracted with ether three times. The combined organic layer was treated in the usual manner. The residual oil was distilled (bp 100 °C, 8 mmHg) to give ester **5h** (0.79 g, 96% yield): IR (neat) 1743, 1652 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.54 (m, 2H), 1.78 (m, 2H), 2.12 (q, $J = 7.0$ Hz, 2H), 4.39 (t, $J = 6.5$ Hz, 2H), 4.92–5.09 (m, 2H), 5.70–5.93 (m, 1H).

Typical Synthetic Method for Silane Bridges 6a–c. 3-(Methacryloyloxy)propyltrimethoxysilane (6a):[23] Reaction vessels were dipped in 5% aqueous NaOH overnight and then dried under vacuum with heating. In a flask, gelation inhibitor [1,3,5-trimethyl-2,4,6-tris(3,5-di-*t*-butyl-4-hydroxybenzyl)-benzene] (25 mg, 0.032 mmol) and polymerization inhibitor [2,6-di-*t*-butyl-4-methylphenol] (24 mg, 0.11 mmol) were charged and purged with N_2 atmosphere. 2-Propenyl methacrylate (1.89 g, 15 mmol) and $H_2PtCl_6 \cdot 6H_2O$ (20 mg, 0.038 mmol) in *i*-PrOH (0.05 mL) were added. Next, $HSiCl_3$ (4.06 g, 30 mmol) and toluene (4 mL) were added dropwise over a period of 5 min. The reaction mixture was stirred in an oil bath with a thermostat at 50 °C for 3 h, and then for 3 h at room temperature. The reaction vessel was cooled in an ice-water bath, and urea (8.11 g, 135 mmol) and then MeOH (5.8 mL, 180 mmol) were added subsequently. The reaction mixture was stirred at 50 °C overnight. Distillation of the mixture (85 °C/10 mmHg) yielded



Scheme 1. Conditions : (i) *p*-TsOH·H_2O, toluene; (ii) gelation inhibitor [1,3,5-trimethyl-2,4,6-tris(3,5-di-*t*-butyl-4-hydroxybenzyl)benzene], polymerization inhibitor [2,6-di-*t*-butyl-4-methylphenol], H_2PtCl_6–*i*-PrOH, $HSiCl_3$–toluene, then urea, methanol; (iii) H_2PtCl_6–*i*-PrOH, $HSiCl_3$–toluene, then urea, methanol; (iv) DMAP, pyridine, CH_2Cl_2.

6a (1.4 g, 36% yield) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.65–0.74 (m, 2H), 1.71–1.88 (m, 2H), 1.95 (s, 3H), 3.58 (s, 9H), 4.12 (t, $J = 6.8$ Hz, 2H), 5.55 (s, 1H), 6.09 (s, 1H).

6-(Methacryloyloxy)hexyltrimethoxysilane (6b) and 11-(Methacryloyloxy)undecyltrimethoxysilane (6c): These compounds were prepared in a similar way to those for **6a**.[23] Compounds **6d–i**, without an olefin functional group in the starting esters, were prepared by the modified procedure. The typical procedure for **6d** and the spectral data for new compounds **6d–h** are shown below. The prepared silane compounds after distillation still have small amounts of impurities after distillation, which could not be removed by repeated distillations. However, after their use for attachment on *Toyonite*, only the impurities remained unreacted.

Typical Synthetic Method for Silane Bridges 6d–i. Trimethoxy-6-(2-methylpropanoyloxy)hexylsilane (6d): Reaction vessels were dipped in 5% aqueous NaOH overnight and then dried under vacuum with heating. To a mixture of ester **5d** (1.19 g, 7.0 mmol) and $H_2PtCl_6 \cdot H_2O$ (20 mg, 0.038 mmol)–*i*-PrOH (0.06 mL) were added toluene (2.1 mL) and $HSiCl_3$ (1.90 g, 14 mmol) at room temperature under N_2. After being stirred at 50 °C for 18 h, urea (1.92 g, 32 mmol) and MeOH (1.34 g, 42 mmol) were added, and then further stirred at the same temperature for 29 h. Distillation of the mixture (85 °C/10 mmHg) gave **6d** (1.29 g, 63% yield) as a colorless oil: IR (neat) 1738, 1504, 1090 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.60–0.68 (m, 2H), 1.13–1.24 (m, 6H), 1.29–1.80 (m, 6H), 2.47–2.60 (m, 1H), 3.54–3.60 (m, 9H), 4.04 (t, $J = 6.6$ Hz, 2H).

Trimethoxy-6-(propanoyloxy)hexylsilane (6e): 60% yield; IR (neat) 1738, 1463, 1086 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.64 (t, $J = 6.0$ Hz, 2H), 1.13 (t, $J = 7.7$ Hz, 3H), 1.18–1.67 (m, 8H), 2.26–2.88 (m, 2H), 3.54–3.60 (m, 9H), 4.05 (t, $J = 6.6$ Hz, 2H).

6-Acetoxyhexyltrimethoxysilane (6f): 78% yield, bp 110 °C; IR (neat) 1739, 1194 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.58–0.72 (m, 2H), 1.19–1.70 (m, 9H), 2.04 (s, 3H), 3.55–3.59 (m, 9H), 4.04 (t, $J = 6.3$ Hz, 2H).

6-(Benzoyloxy)hexyltrimethoxysilane (6g): 92% yield; IR (neat) 1720, 1603, 1452, 1275, 1090 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.60–0.73 (m, 2H), 1.19–1.85 (m, 8H), 3.50–3.62 (m, 9H), 4.31 (t, $J = 6.6$ Hz, 2H), 7.40–7.60 (m, 3H), 8.02–8.06 (m, 2H).

Trimethoxy-6-(pentafluorobenzoyloxy)hexylsilane (6h): 60% yield; bp 90 °C (1 mmHg); IR (neat) 1741, 1652, 1525, 1502, 1328, 1232, 1085, 1008 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.57–0.91 (m, 2H), 1.16–1.84 (m, 8H), 3.56 (m, 9H), 4.37 (t, $J = 6.4$ Hz, 2H).

Typical Procedure for Attachment of Organic Bridge to Ceramic Support.[13] The ceramic support (*Toyonite* 200, 2.0 g) was added to a toluene (3 mL) solution of trimethoxy-6-(2-methylpropanoyloxy)hexylsilane (**6d**) (114 mg, 0.39 mmol). The mixture was stirred for 20 min at room temperature and then filtered. The resulting *Toyonite* with organic bridges was dried in vacuo (2 mmHg) for 5 h. ^{1}H NMR analysis of the filtrate showed that all of the trimethoxysilyl compound was consumed. Therefore, bridge wt % shown in Table 2 is calculated based on the amount of the bridge consumed in this procedure.

Immobilization of Lipase to the Ceramic Support (*Toyonite*) with Organic Bridges.[13] A suspension of commercial lipase PS (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 lipase solution was added *Toyonite* (1.5 g), and the mixture was shaken for 24 h at 25 °C. The resulting mixture was filtered, and the lipase-immobilized *Toyonite* was dried under vacuum (2 mmHg) for 15 h and stored in a desiccator. Protein wt % shown in Table 2 was determined from the difference between the amount of protein in the lipase solution before and after immobilization.[20]

Table 4. *Toyonite*-Immobilized Lipase-Catalyzed Resolution of (±)-**2**

Organic bridge	Temp. °C	Lipase PS mg	Alcohol[a] % ee	Ester[a] % ee	Conv.[b] %	E[b]
3a	30	6.0	19	76	20	8.6[c]
	0	45	28	79	27	11
	−20	100	27	82	31	14
	−30	150	32	86	27	18[c]
3b	30	6.0	48	74	39	10[c]
	0	45	60	72	46	11
	−20	100	55	79	41	15
	−30	150	52	82	39	17[c]
3c	30	6.0	54	59	48	6.6[c]
	0	45	45	68	40	8.0
	−20	100	43	71	37	8.8
	−30	150	42	72	37	9.3[c]
3d	30	6.0	54	65	45	7.9[c]
	0	45	73	78	48	17
	−20	100	52	85	38	21
	−30	150	57	88	39	28[c]
3e	30	6.0	17	60	22	4.8[c]
	0	45	28	81	25	13
	−20	100	27	89	23	22
	−30	150	41	87	32	21[c]
3f	30	6.0	34	77	31	11[c]
	0	45	57	82	41	17
	−20	100	48	88	35	24
	−30	150	58	88	39	28[c]
3g	30	6.0	31	67	32	6.8[c]
	0	45	30	75	28	9.4
	−20	100	27	83	24	14
	−30	150	32	80	28	12[c]
3h	30	6.0	26	64	29	5.9[c]
	0	45	27	65	29	6.0
	−20	100	31	67	31	6.9
	−30	150	30	67	31	6.8[c]

a) Determined by HPLC analysis. b) Calculated by the literature method.[4] c) Shown in Table 2.

Typical Procedure for the Lipase-Catalyzed Reactions. Resolution of 2-Hydroxymethyl-1,4-benzodioxane ((±)-2): To a cooled mixture of (±)-**2** (50 mg, 0.30 mmol), lipase (6 mg in the reaction at 30 °C, 45 mg at 0 °C, 100 mg at −20 °C, and 150 mg at −30 °C) and CH_2Cl_2 (3 mL) was added freshly distilled vinyl acetate (47 mg, 0.60 mmol). Progress of the reaction was monitored by TLC (SiO_2) analysis, and the reaction was stopped by filtration with suction at an appropriate conversion. The product mixture was purified by silica-gel column chromatography (SiO_2 1.5 g, EtOAc, ϕ 1.1 cm × 4.0 cm). Optical purities (% ee) of alcohol (*S*)-**2** and acetate (*S*)-**2a** without isolation were determined by HPLC analysis with a chiral column [**2a**: Daicel Chiralcel OB-H, hexane/*i*-PrOH (100:1), flow rate: 1.0 mL/min, detector 254 nm, retention time = 55 min for (*S*)-**2a** and 62 min for (*R*)-**2a** or Daicel Chiralcel OJ, hexane/*i*-PrOH (50:1), flow

rate: 1.0 mL/min. retention time = 30 min for (*R*)-**2a** and 43 min. for (*S*)-**2**; **2**: Daicel Chiralcel OD-H, hexane/*i*-PrOH (20:1), flow rate: 0.5 mL/min; retention time = 43 min for (*R*)-**2** and 49 min for (*S*)-**2**]. Details (*E* value, ee, and calculated conversion) are summarized in Table 4.

(*S*)-2-Hydroxymethyl-1,4-benzodioxane ((*S*)-2):[24] IR (neat) 3253 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.03 (t, 1H), 3.89 (dd, $J = 4.8$, 2.6 Hz, 2H), 4.05–4.16 (m, 1H), 4.21–4.33 (m, 2H), 6.80–6.92 (m, 4H); $[\alpha]_D^{26}$ 28.4 (*c* 1.1, EtOH, 77% ee).

(S)-2-Acetoxymethyl-1,4-benzodioxane ((*S*)-2a): IR (neat) 1743 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.11 (s, 3H), 4.06 (dd, $J = 4.8$, 2.6 Hz, 1H), 4.21–4.33 (m, 2H), 4.30–4.45 (m, 2H), 6.80–6.92 (m, 4H).

Lipase-Catalyzed Resolution of 2-Phenylpropanol ((±)-4).[2,22] Freshly distilled vinyl acetate (32 mg, 0.37 mmol) was added to a cooled mixture of (±)-**4** (50 mg, 0.37 mmol), lipase (6 mg in the reaction at 30 °C, 45 mg at 0 °C, 100 mg at −20 °C, and 150 mg at −30 °C), and *i*-Pr_2O (3 mL). Progress of the reaction was monitored by TLC analysis (SiO_2, hexane/EtOAc (4:1)), and the reaction was stopped by filtration with suction at a suitable conversion. The product mixture was purified by silica-gel column chromatography (SiO_2 4 g, 1.1 × 11 cm, hexane/EtOAc (50:1)) to yield (*S*)-**4a** and (*R*)-**4**. Then, a mixture of (*S*)-**4a** and KOH (0.1 mL, 0.25 M) in MeOH (0.1 mL) was stirred at room temperature for 12 h and treated in the usual manner to give (*S*)-**4**. Optical purities (% ee) of (*S*)-**4** and (*R*)-**4** thus obtained were determined by HPLC analyses with a chiral column (Daicel Chiralcel OB-H, hexane/*i*-PrOH (50:1), flow rate 0.5 mL/min, detector 254 nm; (*S*)-**4**: retention time (rt) = 27.7 min; (*R*)-**4**: 30.3 min.) Data for HPLC analyses in Table 3: for entry 1: $E = 2.1$: (*S*)-**4**: 26% ee; (*R*)-**4**: 23% ee; $E = 3.4$: (*S*)-**4**: 43% ee; (*R*)-**4**: 33% ee; for entry 2: $E = 2.4$: (*S*)-**4**: 29% ee; (*R*)-**4**: 27% ee; $E = 5.4$: (*S*)-**4**: 50% ee; (*R*)-**4**: 41% ee.

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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 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).

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

5 D. Cainelli, D. Giacomini, and P. Galletti, *Chem. Commun.*, **1999**, 567.

6 K. Baczko and C. J. Larpent, *J. Chem. Soc., Perkin Trans. 2*, **2000**, 521.

7 C. Pichon, C. Hubert, C. Alexandre, and F. Huet, *Tetrahedron: Asymmetry*, **11**, 2429 (2000).

8 a) A. Avdagić, A. Lesac, Z. Majer, M. Hollosi, and V. Šunjić, *Helv. Chim. Acta*, **81**, 1567 (1998). b) A. Avdagić and V. Šunjić, *Helv. Chim. Acta*, **81**, 85 (1998).

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

10 a) R. S. Phillips, *Trends. Biotechnol.*, **14**, 13 (1996). b) C. Heiss, M. Laivenieks, J. G. Zeikus, and R. S. Phillips, *J. Am. Chem. Soc.*, **123**, 345 (2001). The linear temperature effect on enantioselectivity ($\ln E$ and $1/T$) is not attributable to the conformational change of enzyme, but meaning that the rigid transition state for enantio-discrimination is preserved under the temperatures examined.

11 B. Galunsky, S. Ignatova, and V. Kasche, *Biochim. Biophys. Acta*, **1343**, 130 (1997): temperature dependence of enantioselectivity for α-chymotrypsin.

12 T. Sakai, Y. Miki, M. Tsuboi, H. Takeuchi, T. Ema, K. Uneyama, and M. Utaka, *J. Org. Chem.*, **65**, 2740 (2000).

13 Here we used *Toyonite* type 200 without organic bridges on the surface: a new type of spherical porous ceramics support having 155 ± 5 μm average diameter and 60 nm average sized pores (Y. Yamashita, M. Kamori, H. Takenaka, and J. Takahashi, *Jpn. Kokai Tokkyo Koho*, JP 09313179 (1997); U. S. Patent 6004786). *Toyonite* 200 was supplied by Toyo Denka Kogyo Co. Ltd., Kochi, Japan. For the lipase-catalyzed reactions, we attached the appropriate organic bridges and lipases on *Toyonite* by ourselves as shown in experimental section. Commercially available *Toyonite* 200M is the one that 3-(2-methylpropenoyloxy)propylsilanetrioxyl groups are attached on *Toyonite* 200. Lipase PS-immobilized *Toyonite* 200M is also available from Amano Pharmaceutical Co., Ltd. or Wako Pure Chemical Industries, Ltd. as Lipase PS-C "Amano" II. See also Ref. 12.

14 M. Kamori, T. Hori, Y. Yamashita, Y. Hirose, and Y. Naoshima, *J. Mol. Catal. B: Enzym.*, **9**, 269 (2000).

15 K. Kato, Y. Gong, T. Saito, and H. Kimoto, *J. Biosci. Bioeng.*, **90**, 332 (2000).

16 M. Suzuki, C. Nagasawa, and T. Sugai, *Tetrahedron*, **57**, 4841 (2001).

17 For a current example: a) J. M. Palomo, G. F.-Lorente, C. Mateo, M. Fuentes, R. F.-Lafuente, and J. M. Guisan, *Tetrahedron: Asymmetry*, **13**, 1337 (2002). b) M. Terreni, C. Mateo, A. Bastida, P. Dalmases, L. Hugust, and J. M. Guisan, *Enzyme Microb. Technol.*, **28**, 389 (2001).

18 M. Yamatani and T. Yamasaki, (Shin-Etsu Chemical) *Jpn. Tokkyo Koho*, JP 06005077 (1994).

19 J. Williams, *Acc. Chem. Res.*, **26**, 593 (1993). See also Ref. 12.

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

21 A. N. E. Weissfloch and R. J. Kazlauskas, *J. Org. Chem.*, **60**, 6959 (1995).

22 **5a**: F. Iu and G. Liu, *Macromolecules*, **34**, 1302 (2001); **5b**: C. W. Lee and R. H. Grubbs, *J. Org. Chem.*, **66**, 7155 (2001); **5c**: S. Warwel, G. Steinke, and M. R. Klass, *Biotechnol. Tech.*, **10**, 283 (1996); **5d**: M. Hilker and S. Schulz, *J. Chem. Ecol.*, **20**, 1075 (1994); **5f**: E. Hata, T. Yamada, and T. Mukaiyama, *Bull. Chem. Soc. Jpn.*, **68**, 3629 (1995); **5g** : T. Ikeda, S. Yue, and C. R. Hutchinson, *J. Org. Chem.*, **50**, 5193 (1985).

23 **6a**: M. Yamatani and T. Yamasaki, (Shin-Etsu Chemical), *Jpn. Tokkyo Koho*, JP 06005077 (1994); **6b**: M. Yonekura, M. Noda, and H. Yamanaka, *Jpn. Tokkyo Koho*, JP 09085891 (1997); **6c**: M. Kawashima, I. Komura, and J. Yamauchi, *Jpn. Tokkyo Koho*, JP 01050888 (1989).

24 S. Antus, A. Gottsegen, J. Kajtar, T. Kovacs, T. S. Toth, and H. Wagner, *Tetrahedron: Asymmetry*, **4**, 339 (1993). (*S*)-**2**: $[\alpha]_D^{r.t}$ 34.3 (*c* 0.7, EtOH); >99% ee.