Pergamon

Tetrahedron: *Asymmetry* 11 (2000) 1199–1210

TETRAHEDRON: *ASYMMETRY*

Improvement of enantioselectivity in kinetic resolution of a primary alcohol through lipase-catalyzed transesterification by using a chiral acyl donor

Keiji Hirose,* Hiroyuki Naka, Masashi Yano, Sozaburo Ohashi, Koichiro Naemura and Yoshito Tobe

Department of Chemistry, Faculty of Engineering Science, Osaka University, Toyonaka, Osaka 560-8531, Japan

Received 28 December 1999; accepted 4 February 2000

Abstract

The enantioselectivity of the kinetic resolution of a primary alcohol which has a chiral center at the β-position of the hydroxyl group was substantially improved by employing a racemic mixture of a chiral acyl donor for enantioselective transesterification catalyzed by a lipase. The combination of the lipase, solvent, and acyl donor was also effective for the enantioselectivity. In addition, the important effect of the chirality of an acyl donor on kinetic resolution was investigated by using enantiomerically pure acyl donors. Here we demonstrate the effective kinetic resolution ($E=98$) of a primary alcohol, 2-phenyl-1-propanol, by using a racemic mixture of a chiral acyl donor, vinyl 3-phenylbutanoate.

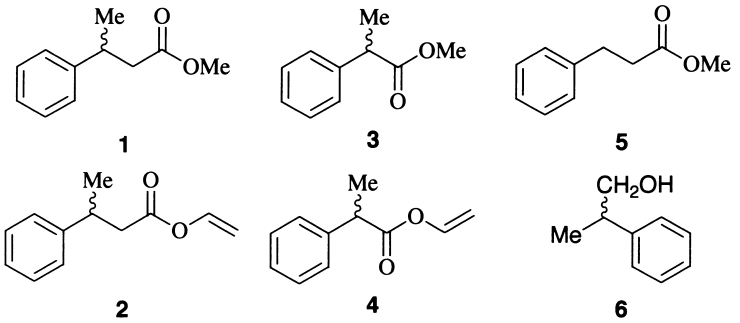
1. Introduction

The importance of enzyme-catalyzed reactions has been well recognized during the last decade as a promising method for the preparation of enantiomerically pure compounds.[1] Particularly, transesterification catalyzed by lipases has become a popular method for the preparation of enantiomerically pure secondary alcohols.[2] However, the enantioselectivities of lipase-catalyzed transesterification of primary alcohols are relatively low, in most cases unsatisfactorily low so as to preclude preparation of enantiomerically pure primary alcohols for synthetic purposes. In this respect, it is desirable to devise measures to improve the enantioselectivity of the kinetic resolution of primary alcohols. Examples of the measures that have been taken in order to improve the enantioselectivity of transesterification gave: screening of enzymes, changing the solvent[3] and/or temperature,[4] adding an additive,[5] microwave irradiation,[6] immobilization of enzyme on support,[7] site-directed mutagenesis,[8] and changing the acyl donors.[9] We focused our attention on the

* Corresponding author. E-mail: hirose@chem.es.osaka-u.ac.jp

0957-4166/00/$ - see front matter
PII: S0957-4166(00)00060-4

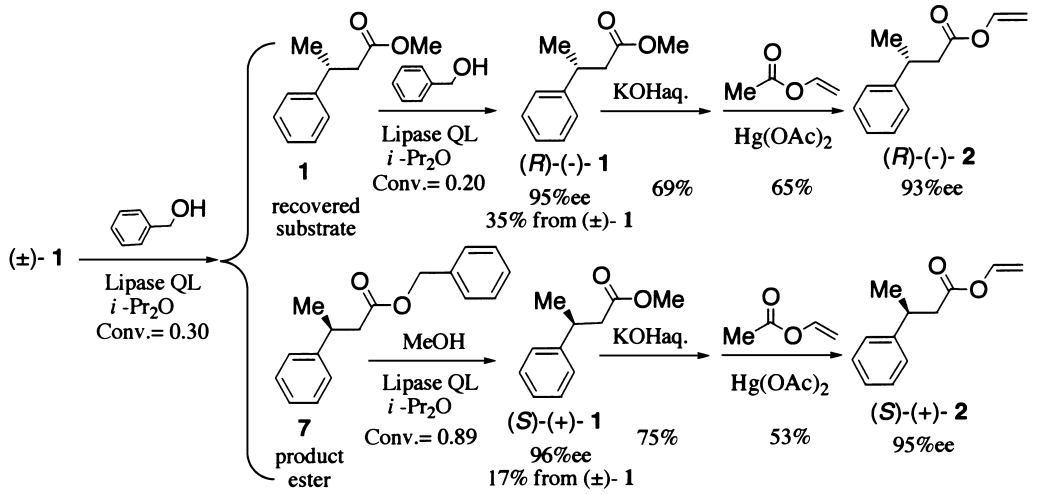
importance of the acylated enzyme which is the key intermediate for enzymatic reaction. The steric environment of the acyl moiety in the acylated enzyme would be readily modified by simply employing different acyl donors. The method to change the acyl donors could be considered as active-site-directed transient chemical modification of an enzyme,[10] which might lead to an improvement of the enantioselectivity for the kinetic resolution of primary alcohols. Indeed, when the widely employed achiral and small acyl donor, isopropenyl acetate, was replaced by methyl 3-phenylpropanoate **5**, a moderate improvement of enantioselectivities was observed.[11] Recently, the effect of an acyl donor which has an aromatic ring in the acyl moiety on the enantioselectivity of lipase-catalyzed transesterification of 2-phenyl-1-propanol **6** was reported.[12] However, there seems to be no report on the use of a chiral acyl donor in the transesterification catalyzed by lipase for the resolution of primary alcohols.[13] It is, therefore, interesting to investigate the effect of a chiral acyl donor on the enantioselectivity of the transesterification.



In this paper, we report improvement of the enantioselectivity of lipase-catalyzed transesterification of primary alcohol **6** by using chiral acyl donors, methyl 3-phenylbutanoate **1**, vinyl 3-phenylbutanoate **2** and methyl 2-phenylpropanoate **3**, vinyl 2-phenylpropanoate **4**, possessing a stereocenter at the α- and β-positions of the carbonyl group, respectively, together with the effect of chirality of acyl donor **1** and **2** on the enantioselectivity. Furthermore, the importance of a combination between chiral acyl donors and lipases, Lipase QL from *Alcaligenes* sp., Lipase SL from *Pseudomonas cepacia*, Lipase TL from *Pseudomonas stutzeri*, and Lipase UL from *Rhizopus* sp., is also described.

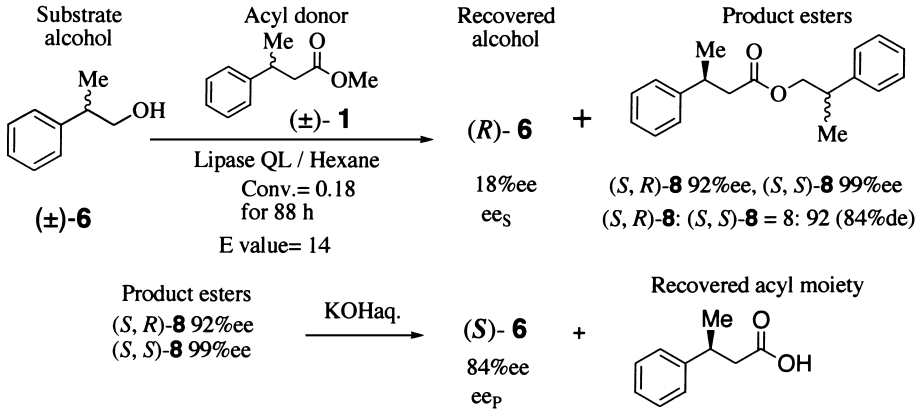
2. Results and discussion

The substrate alcohol 2-phenyl-1-propanol **6** was chosen in order to compare the results with previous studies using achiral acyl donors.[14] Of the acyl donors, methyl esters (±)-**1** and (±)-**3** were prepared according to previously reported procedures.[15] Vinyl esters (±)-**2** and (±)-**4** were prepared by transesterification with vinyl acetate catalyzed by mercury(II) acetate.[16] The syntheses of optically active acyl donors (*R*)-**1**, (*S*)-**1**, (*R*)-**2** and (*S*)-**2** were carried out by repeated Lipase QL-catalyzed transesterification using benzyl alcohol, as shown in Scheme 1. Thus, (±)-**1** was treated with benzyl alcohol in the presence of Lipase QL and the recovered starting material was subjected to the same procedure to give (*R*)-**1** enriched to 95% ee. On the other hand, the product of the initial reaction **7** was treated with methanol in the presence of Lipase QL to afford (*S*)-**1** with 96% ee. Both (*R*)-**1** and (*S*)-**1** were converted to the corresponding vinyl esters (*R*)-**2** and (*S*)-**2** by hydrolysis followed by transesterification catalyzed by $Hg(OAc)_2$.



Scheme 1. The syntheses of optically active chiral acyl donors **1** and **2**

The enantioselective transesterification of alcohol (±)-**6** with 2 mol equivalents of an acyl donor was carried out in an organic solvent at 30°C using lipase as a chiral catalyst. The progress of the reaction was monitored by GLC, and the reactions were terminated by removal of the enzyme by filtration. The recovered alcohol, recovered acyl donor and product esters were separated by column chromatography on silica gel. The product esters were obtained as a mixture of two diastereomers. The enantiomeric excesses (ees) of the recovered acyl donor and recovered alcohol (ee_S) were directly determined by HPLC using a chiral column detected by a UV detector at 240 nm. The absolute configuration of the recovered alcohol was confirmed by comparison of its specific rotation with that reported in the literature.[17] The enantiomeric excesses of the product esters were determined by HPLC using a chiral column for each diastereomer, and the stereochemical assignment was established by comparison of the retention volumes with those of authentic samples prepared independently. A representative result using (±)-**1** as an acylating reagent is shown in Scheme 2.



Scheme 2. Representative kinetic resolution using a chiral acyl donor (±)-**1**. The *E* values were calculated from the enantiomeric excesses (ee_s and ee_p)[18]

To compare the efficiency of resolution, E values are generally used for enantioselective reaction. In order to apply the concept of E values to the present reactions, in which the products were obtained as mixtures of two diastereomeric esters, the enantiomeric excess of the alcohol moiety in the product esters (ee_P) was used in the equation described in the literature.[18] When **1** or **2** was used as an acyl donor, the four components (*R*,*R*)-, (*R*,*S*)-, (*S*,*S*)- and (*S*,*R*)-**8** were separated effectively using the HPLC conditions described in the Experimental section. The ee_P was calculated based on the enantiomeric excesses of both of the diastereomeric product esters and diastereomeric excesses of the product esters, which were obtained by HPLC using a chiral column. When **3** or **4** was used as an acyl donor, the ee_P wase obtained by the hydrolysis of the mixture of diastereomeric product esters, because effective separation among the four components of the product esters was not observed.

First, the effect of an acyl donor on enantioselectivity for transesterification was investigated by using esters **1** and **2** which have a methyl group on the β-position of the carbonyl group of 3-phenylpropanoate **5**. The results are shown in Table 1. For comparison, the result using achiral acylating agent **5** is also listed in Table 1. The introduction of a methyl group does not seem to affect the E values (entries 1 and 10), but the reaction required more time than that for **5**. Since the methyl group interferes with the transesterification of both enantiomers of the substrate alcohol (±)-**6**, it is possible to control the reactivities by changing the reaction conditions. Indeed, when hexane instead of diisopropyl ether was used as a solvent, substantial improvement of selectivity ($E=14$) was observed together with reduction of the reaction time (entry 2). Accordingly, the following investigations were carried out in hexane as a general solvent.

Table 1
The effect of chirality of acyl donors **1** and **2** on E values for the kinetic resolution of primary alcohol (±)-**6** using Lipase QL[a)]

Entry	Acyl donor	Reaction time(h)	conv.(%)	Recovered Alcohol			Product esters			E-value
				chirality	(yield %)	ee_s(%)[b]	chirality	(yield %)	ee_p(%)[c]	
1	(±)- **1**	189	36	(*R*)	(64)	26	(*S*)	(20)	52	4
2	(±)- **1**	88	18	(*R*)	(47)	18	(*S*)	(21)	84	14
3	(*S*)- **1**	69	30	(*R*)	(47)	35	(*S*)	(17)	82	15
4	(*R*)- **1**	no reaction			-			-		
5	(±)- **2**	15	51	(*R*)	(33)	87	(*S*)	(51)	84	32
6	(*S*)- **2**	1	58	(*R*)	(42)	99	(*S*)	(58)	71	30
7	(*R*)- **2**	41	27	(*R*)	(39)	19	(*S*)	(31)	52	4
8	(±)- **3**	no reaction			-			-		
9	(±)- **4**	10	33	(*R*)	(60)	2	(*S*)	(36)	4	1
10	**5**	3	39	(*R*)	(59)	40	(*S*)	(8)	62	6

a: All reactions were carried out in hexane as solvent except for entries 1 and 10 where diisopropyl ether was employed. b: Enantiomeric excess of recovered alcohol **6**. c: Enantiomeric excess of reacted alcohol **6**.

As shown in entries 2 and 3, the E value obtained by using (±)-**1** is the same as that obtained by using (*S*)-**1**. This result indicates that one of the enantiomers of acyl donor (*S*)-**1** contributes predominantly to determine the enantioselectivity of the transesterification of **6** when the racemic mixture of **1** is employed as an acyl donor. Acyl donor (*R*)-**1** is practically inactive, or may act as an inhibitor for this transesterification, because longer reaction times were required when the

racemic acyl donor was employed than in the case when (*S*)-**1** was employed. The reaction did not proceed when (*R*)-**1** was used as an acyl donor (entry 4).

When vinyl ester **2** was used, the reaction became much faster than in the case of **1** (entries 5–7). In addition, an improvement of the *E* value was observed. As in the case of **1**, the *S*-enantiomer of **2** plays a predominant role in the determination of reactivity and selectivity. Since (*R*)-**2** gave a low *E* value after a prolonged reaction time, and the use of (±)-**2** resulted in a longer reaction time than when (*S*)-**2** was used, the *R*-enantiomer may also act as an inhibitor of the reaction.

It is worth emphasizing here that the improvement of enantioselectivity was attained up to a satisfactorily high level ($E > 20$)[19] with chiral acyl donor **2**, and the extent of enantioselectivity for the reaction with racemic acyl donor (±)-**2** ($E = 32$) was comparable with that for the reaction with optically active (*S*)-**2** ($E = 30$). Accordingly, an enantiomerically pure acyl donor is not necessary for effective resolution.

The position of the stereogenic center in the acyl donor affected the reactivity and enantioselectivity of transesterification reaction. The transesterification results using esters **3** and **4** which have a stereogenic center on the α-position of the carbonyl group of 3-phenylpropanoate **5** are summarized in entries 8 and 9 in Table 1. When methyl ester **3** was used, the transesterification reaction did not proceed. When vinyl ester **4** was used, the reaction proceeded but the *E* value was low.

Next, the effect of the enzyme was investigated using four lipases: Lipase QL, Lipase SL, Lipase TL and Lipase UL. In all cases when Lipase UL or acyl donor **3** was employed, the reactions did not proceed. Table 2 summarizes the results when isopropenyl acetate, **2** and **4** were used as acyl donors. When isopropenyl acetate was employed, the reaction took place rapidly but *E* values were small (around 1 to 3; entries 11–13). On the other hand, when chiral acyl donor (±)-**2** was employed, the reaction took place relatively slowly and the enantioselectivities were markedly improved (entries 14–16). When (±)-**4** was employed, the reaction proceeded more slowly but the *E* values were not markedly improved (entries 17–19).

Table 2
The effect of lipase and acyl donor on kinetic resolutions of primary alcohol (±)-**6** using acyl donors isopropenyl acetate (IPAc), (±)-**2** and (±)-**4** in hexane

Entry	Lipase	Acyl donor	Reaction time(h)	conv.(%)	Recovered Alcohol chirality	(yield %)	ee_s(%)	Product esters chirality	(yield %)	ee_p(%)	E-value
11	QL	IPAc	3	57	(*R*)	(37)	8	(*S*)	(42)	6	1
12	SL	IPAc	9	55	(*R*)	(44)	45	(*S*)	(25)	37	3
13	TL	IPAc	4	57	(*R*)	(33)	4	(*S*)	(46)	3	1
14	QL	(±)-**2**	15	51	(*R*)	(33)	87	(*S*)	(51)	84	32
15	SL	(±)-**2**	40	52	(*R*)	(28)	93	(*S*)	(52)	84	40
16	TL	(±)-**2**	2	51	(*R*)	(31)	45	(*S*)	(49)	43	4
17	QL	(±)-**4**	10	33	(*R*)	(60)	2	(*S*)	(36)	4	1
18	SL	(±)-**4**	10	39	(*R*)	(37)	47	(*S*)	(35)	73	10
19	TL	(±)-**4**	77	21	(*R*)	(43)	7	(*S*)	(25)	26	2

Finally, the effect of solvent was investigated for the reaction with chiral acyl donor (±)-**2** using Lipase QL and SL as catalyst. The results are summarized in Table 3. For both lipases, the reaction was the slowest in benzene and the fastest in diisopropyl ether. In contrast to the reaction

Table 3
The solvent effect on *E* values of kinetic resolutions of primary alcohol (±)-**6** by Lipase QL- or SL-catalyzed transesterification with chiral acyl donor (±)-**2**

Entry	Solvent	Lipase			Recovered Alcohol			Product esters			E-value
			conv.(%)	time(h)	chirality	(yield %)	ee_s(%)	chirality	(yield %)	ee_p(%)	
20	BEN	QL	37	26	(*R*)	(51)	51	(*S*)	(41)	86	23
21	HEX	QL	51	15	(*R*)	(33)	87	(*S*)	(51)	84	32
22	DIPE	QL	53	2	(*R*)	(45)	98	(*S*)	(42)	85	57
23	BEN	SL	no reaction								
24	HEX	SL	52	40	(*R*)	(28)	93	(*S*)	(52)	84	40
25	DIPE	SL	47	1.5	(*R*)	(31)	85	(*S*)	(39)	95	98

Hex: hexane, BEN: benzene, DIPE: diisopropyl ether

using (±)-**1** as an acyl donor (entries 1 and 2 in Table 1), the enantioselectivity in the reaction using diisopropyl ether was much better than that of the reaction in hexane. Thus, the best *E* value (98) was obtained when the reaction was performed with Lipase SL in diisopropyl ether as solvent.

As mentioned above, the present results demonstrate that the combination of the acyl donor, lipase and solvent is important to achieve high enantioselectivities. In particular, the use of acyl donor **2**, which has a stereogenic center at the β-position of the carbonyl group, resulted in a dramatic improvement of enantioselectivity. Moreover, the configuration of the acyl donor affected the reactivity remarkably. One enantiomer of the acyl donor was practically inactive towards this transesterification so that it was not necessary to use an enantiomerically pure acyl donor. The *E* value was improved up to 98 using a racemic mixture of acyl donor (±)-**2**. In all cases described in this article, the chirality of a fast-reacting substrate is unchanged; (−)-(*S*)-**6** always reacts faster than (+)-(*R*)-**6**.

The enantioselectivities of lipase-catalyzed transesterification might be caused by the diastereomeric interaction between the acylated lipase and substrate in the acylated enzyme–substrate complex and/or by the intramolecular interaction of the tetrahedral intermediates in the transition state. A large acyl moiety makes the binding site more crowded than a smaller acyl moiety does. Such spatial constraints for the fast- and slow-reacting substrates in the enzyme–substrate complexes and/or following transition states might influence the enantioselectivities. A full explanation of the enantioselectivities of our results is not possible at this moment. Recently, Hæffner and Norin reported the prediction of enantioselectivities using molecular modeling of a lipase-catalyzed reaction.[20] The molecular modeling of an acylated enzyme–substrate complex and/or transition state might give an explanation of the enantioselectivities, including the position of the stereogenic center, the chirality and all of the other structural relationships between a substrate and an acyl moiety.

3. Experimental

3.1. General

^{1}H NMR spectra were recorded at 270 MHz on a JEOL JNM-MH-270 spectrometer for solutions in $CDCl_3$ with $SiMe_4$ as an internal standard, and *J* values are given in hertz. Mass

spectra were recorded with 3-nitrobenzyl alcohol as a matrix on a JEOL-DX-303-HF spectrometer. IR spectra were measured on JASCO FT/IR-410 spectrometer. UV and visible spectra were measured on a HITACHI 260-10 spectrometer. Optical rotations were measured using a JASCO DIP-40 polarimeter and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. HPLC analyses were carried out on a Shimadzu LC-6A using a chiral column CHIRAL PAK AD (250×4.6 mm) (Daicel) or a chiral column Opti-Pak XC (Waters), 250 mm×4.6 mm column. GLC analyses were carried out on a Shimadzu GC 8A chromatograph using an SE-52 on Uniport HP 2 m×2.6 mm column. Lipase QL, Lipase SL, Lipase TL, and Lipase UL were supplied by Meito Sangyo and used without further purification. Diisopropyl ether, benzene, and hexane were stocked on molecular sieves 4 Å after drying on $CaCl_2$ followed by distillation.

3.2. (±)-Vinyl 3-phenylbutanoate **2**

Mercury(II) acetate (242 mg, 0.80 mmol) was added to a solution of (±)-3-phenylbutanoic acid (10 g, 61 mmol) in freshly distilled vinyl acetate (31.5 g, 365 mmol). After stirring at room temperature for 30 min, a mixture of 98% sulfuric acid and fuming sulfuric acid (20 μl, 59:41 v/v) was added and the mixture was refluxed for 4 h with stirring. The reaction mixture was neutralized with sodium acetate and extracted with ether. The extract was washed with saturated aqueous NaCl solution and dried over anhydrous $MgSO_4$. Chromatography on silica gel (hexane:ethyl ether, 98:2, as eluent) gave (±)-vinyl 3-phenylbutanoate **2** (5.5 g, 48%) as a colorless oil; IR (neat) 1754, 1644, 1149 cm^{-1}; δ_H ($CDCl_3$) 1.32 (3H, d, J=6.9, CH_3), 2.74–2.55 (2H, m, CH_2), 3.37–3.25 (1H, m, CH), 4.54 (1H, dd, J=6.3, 1.6, (*E*)-vinyl CH_2), 4.84 (1H, dd, J=11.7, 1.6, (*Z*)-vinyl CH_2), 7.33–7.18 (6H, m, ArH and vinyl CH).

3.3. (±)-Vinyl 2-phenylpropanoate **4**

Esterification of (±)-2-phenylpropanoic acid (5.8 g, 39 mmol) with mercury(II) acetate (154 mg, 0.50 mmol) in 20.0 g (232 mmol) of vinyl acetate was carried out as described for the preparation of (±)-**2**. Purification by silica gel chromatography (hexane:ethyl ether, 98:2, as eluent) afforded 3.7 g (54%) of (±)-**4** as a colorless oil; IR (neat) 1751, 1645, 1150 cm^{-1}; δ_H ($CDCl_3$) 1.54 (3H, d, J=7.1, CH_3), 3.78 (1H, q, J=7.1, CH), 4.55 (1H, dd, J=6.2, 1.6, (*E*)-vinyl CH_2), 4.85 (1H, dd, J=14.0, 1.6, (*Z*)-vinyl CH_2) and 7.24–7.36 (6H, m, ArH and vinyl CH).

3.4. Resolution of (±)-**1**

A mixture of (±)-**1** (10.0 g, 56.1 mmol), Lipase QL (from *Alcaligenes* sp.) (3.0 g) and benzyl alcohol (24.3 g, 0.255 mol) in diisopropyl ether (600 ml) was stirred for 14 days at 30°C. The reaction was terminated by the filtration of the enzyme at ca. 30% conversion monitored by GLC. Then the volatile materials were evaporated under reduced pressure. The same reactions by the same procedures were carried out three times. Distillation of the combined reaction product (125 g) under reduced pressure (90–110°C/38 mmHg) gave 97.1 g of a distillate A consisting of benzyl alcohol, unreacted (−)-**1** (31.5% ee by HPLC), and (+)-benzyl ester in a ratio of 82.2, 16.4 and 0.3%, respectively, by GLC. And the residue A (6.7 g) consisted of (+)-benzyl ester and unreacted (−)-**1** in a ratio of 94.2 and 5.8%, respectively, by GLC. A portion of the distillate A (48.8 g), which contained about 8.0 g of (−)-**1** and 40.1 g of benzyl alcohol, Lipase QL (4.0 g) and diisopropyl ether (600 ml) was stirred for 16 days at 30°C. The reaction was terminated

by filtration of the enzyme and volatile materials were evaporated under reduced pressure. The same reaction was carried out using the rest of the distillate A (48.3 g) under similar conditions. Distillation of the combined reaction mixture (97.0 g) under reduced pressure (106°C/25 mmHg–135°C/5 mmHg) gave a distillate B (80.6 g) composed of benzyl alcohol (65 g) and unreacted (−)-**1** (15 g, 66% ee by HPLC) and a residue B (6.0 g) composed of (+)-**7** (5.4 g, 96% ee by HPLC).

In order to obtain enantiomerically pure (*R*)-**1**, the same reaction was repeated using the distillate B (80.7 g), Lipase QL (7.5 g) in the same solvent (700 ml) for 45 days at 30°C. After a similar work-up, distillation under reduced pressure (120–130°C/25 mmHg) gave (*R*)-**1** (11.5 g, 35% yield based on (±)-**1**) as a colorless oil (95% ee by HPLC); $[\alpha]_D^{24}$ −25.3 (*c* 1.07, $CHCl_3$).

The combined residue A + B obtained from the distillations after the enzymatic reactions for the synthesis of (*R*)-(−)-**1** described above was distilled again under reduced pressure (142–178°C/5 mmHg) to give (+)-benzyl 3-phenylbutanoate **7** (11.9 g) as a colorless oil. A mixture of (+)-**7** (2.5 g, 9.8 mmol), methanol (1.3 g, 39.0 mmol), and Lipase QL (1.25 g) in diisopropyl ether (150 ml) was stirred for 13 days at 30°C. The reaction was terminated by filtration and the volatile materials were evaporated under reduced pressure. The same reactions were carried out twice by the same procedures employing different amounts (4.0 and 3.0 g, respectively) of the starting material. All the reaction products were combined and purified by distillation under reduced pressure (130–139°C/50 mmHg) followed by column chromatography on silica gel (hexane:ethyl ether, 9:1, as eluent) to afford (*S*)-(+)-**1** (5.5 g, 17% yield based on (±)-**1**) as a colorless oil (96% ee by HPLC); $[\alpha]_D^{25}$ +29.6 (*c* 1.35, $CHCl_3$).

3.5. (R)-(−)-Vinyl 3-phenylbutanoate 2

To the solution of (*R*)-(−)-**1** (5.0 g, 28 mmol) in methanol (65 ml) was added 45% aqueous KOH solution (61 ml), and the solution was stirred at room temperature for 14 h. The solvent was evaporated under reduced pressure until its volume became 1/3. After acidification of the mixture with 6N hydrochloric acid, the mixture was extracted with ether. Chromatography on silica gel (hexane:ethyl ether, 8:2, as eluent) gave (*R*)-(−)-3-phenylbutanoic acid (3.2 g, 69%); $[\alpha]_D^{22}$ −48.3 (*c* 1.07, benzene). Esterification of (−)-3-phenylbutanoic acid (2.8 g, 17 mmol) with mercury(II) acetate (68 mg, 0.21 mmol) in 9.5 g (110 mmol) of vinyl acetate was carried out as described for the preparation of (±)-**2**. Purification by silica gel chromatography (hexane:ethyl ether, 98:2, as eluent) afforded 2.1 g (65%) of (*R*)-(−)-vinyl 3-phenylbutanoate as a colorless oil (93% ee); $[\alpha]_D^{22}$ −19.8 (*c* 1.01, $CHCl_3$).

3.6. (S)-(+)-Vinyl 3-phenylbutanoate 2

The reaction was carried out using the same procedure described for the preparation of (*R*)-(−)-vinyl 3-phenylbutanoate **2**. To a solution of (*S*)-(+)-**1** (3.0 g, 17 mmol) in methanol (65 ml) was added 45% aqueous KOH solution (36 ml), and the solution was stirred at room temperature for 2.5 h. The solvent was evaporated under reduced pressure until its volume became 1/3. After acidification of the mixture with 6N hydrochloric acid, the mixture was extracted with ether. Chromatography on silica gel (hexane:ethyl ether, 8:2, as eluent) gave (*S*)-(+)-3-phenylbutanoic acid (2.1 g, 75%); $[\alpha]_D^{22}$ +52.2 (*c* 1.00, benzene). Esterification of (+)-3-phenylbutanoic acid (1.9 g, 12 mmol) with mercury(II) acetate (48 mg, 0.25 mmol) in 7.5 g (87 mmol) of vinyl acetate was carried out as described for the preparation of (±)-**2**. Purification by silica gel chromatography

(hexane:ethyl ether, 98:2, as eluent) afforded 1.2 g (53%) of (*R*)-(−)-vinyl 3-phenylbutanoate as a colorless oil (95% ee); $[\alpha]_D^{22}$ +20.7 (*c* 1.08, $CHCl_3$).

3.7. Lipase-catalyzed acylation of alcohols

3.7.1. General procedure for lipase-catalyzed acylation

Entry 1—A mixture of (±)-**2**-phenylpropanol **6** (400 mg, 2.9 mmol), (±)-methyl 3-phenylbutanoate **1** (517 mg, 2.9 mmol) and Lipase QL (400 mg) in diisopropyl ether (20 ml) was stirred at 30°C and the progress of the reaction was monitored by GLC. After stirring for 189 h, the enzyme was removed by filtration and the volatile materials were evaporated under reduced pressure. Silica gel chromatography of the residue gave recovered substrate (*R*)-(+)-**6** (26% ee) and the mixture of product esters, (*R*)-2-phenyl-1-propyl (*S*)-3-phenylbutanoate (*S*,*R*)-**8** (90% ee) and (*S*)-2-phenyl-1-propyl (*S*)-3-phenylbutanoate (*S*,*S*)-**8** (94% ee). The ee values of the recovered substrate (*R*)-(+)-**6**, the acylated substrate (*S*,*R*)-**8** and (*S*,*S*)-**8**, and the recovered acyl donor (*R*)-**1** were determined by HPLC using a chiral column. De values of acylated substrate (*S*,*R*)-**8** and (*S*,*S*)-**8** were 54% which were estimated by the same chromatogram of HPLC. The analysis of HPLC was performed under the following conditions; hexane:isopropyl alcohol (99:1) as eluent, Opti-Pak XC (Waters) as column, 0.2 ml/min as flow rate, and 1,3,5-tri-*tert*-butylbenzene as inner standard at ambient temperature. The capacity factors for (*R*,*R*)-, (*R*,*S*)-, (*S*,*S*)-, and (*S*,*R*)-**8** were 2.08, 2.23, 3.73, and 4.40, respectively.

3.7.2. General procedure for the hydrolysis of product esters

A mixture of the product esters and 45% aqueous KOH solution (8.3 ml) in methanol (15 ml) was stirred for 3 h under reflux. After concentration, the mixture was extracted with ether. The combined organic layer was washed with saturated aqueous NaCl solution and dried over anhydrous $MgSO_4$. Evaporation of the solvent gave alcohol (*S*)-**6**. The original aqueous layer was acidified (pH 1) with concentrated HCl and extracted with ether. The organic layer was washed with saturated aqueous NaCl solution and dried over anhydrous $MgSO_4$. Evaporation of the solvent gave an acyl moiety as carboxylic acid.

Entry 2—Reaction of (±)-**6** (40 mg, 0.29 mmol), (±)-**1** (100 mg, 0.56 mmol) with Lipase QL (150 mg) in hexane (40 ml) at 30°C for 88 h gave (*R*)-**6** (17 mg, 47% yield, 18% ee) and a mixture of product esters (*S*,*R*)-**8** and (*S*,*S*)-**8** (17 mg, 21% yield). The ee values of (*S*,*R*)-**8** and (*S*,*S*)-**8** were 92 and 99%, respectively, and the diastereomeric excess of (*S*,*S*)-**8** over (*S*,*R*)-**8** was 84%. The acyl donor (*R*)-**1** (65 mg, 65% yield, 11% ee) was also recovered.

Entry 3—Reaction of (±)-**6** (40 mg, 0.29 mmol), (*S*)-**1** (100 mg, 0.56 mmol) with Lipase QL (150 mg) in hexane (40 ml) at 30°C for 69 h gave (*R*)-**6** (18 mg, 47% yield, 35% ee) and a mixture of (*S*,*R*)-**8** and (*S*,*S*)-**8** (14 mg, 17% yield). The ee values of the (*S*,*R*) and (*S*,*S*) product esters were 85 and 99%, respectively, and the diastereomeric excess of (*S*,*S*)-**8** over (*S*,*R*)-**8** was 82%. The acyl donor (*S*)-**1** (75 mg, 75% yield, 99% ee) was also recovered.

Entry 4—Reaction of (±)-**6** (40 mg, 0.29 mmol), (*R*)-**1** (100 mg, 0.56 mmol) with Lipase QL (150 mg) in hexane (40 ml) at 30°C proceeded too slowly to be analyzed (3% conversion for 86 h). There was practically no reaction.

Entry 5—Reaction of (±)-**6** (107 mg, 0.79 mmol), (±)-**2** (300 mg, 1.58 mmol) with Lipase QL (150 mg) in hexane (22 ml) at 30°C for 15 h gave (*R*)-**6** (35 mg, 33% yield, 87% ee) and a mixture of (*S*,*R*)-**8** and (*S*,*S*)-**8** (112 mg, 51% yield). The ee values of (*S*,*R*)-**8** and (*S*,*S*)-**8** were 24 and 96%, respectively, and the diastereomeric excess of (*S*,*S*)-**8** over (*S*,*R*)-**8** was 80%. The acyl donor (*R*)-**1** (130 mg, 43% yield, 36% ee) was also recovered.

Entry 6—Reaction of (±)-**6** (107 mg, 0.79 mmol), (*S*)-**2** (300 mg, 1.58 mmol) with Lipase QL (150 mg) in hexane (22 ml) at 30°C for 1 h gave (*R*)-**6** (45 mg, 42% yield, 99% ee) and a mixture of (*S*,*R*)-**8** and (*S*,*S*)-**8** (130 mg, 58% yield). The ee values of (*S*,*R*)-**8** and (*S*,*S*)-**8** were 99 and 99%, respectively, and the diastereomeric excess of (*S*,*S*)-**8** over (*S*,*R*)-**8** was 72%. The acyl donor (*S*)-**1** (130 mg, 43% yield, 92% ee) was also recovered.

Entry 7—Reaction of (±)-**6** (107 mg, 0.79 mmol), (*R*)-**2** (300 mg, 1.58 mmol) with Lipase QL (150 mg) in hexane (22 ml) at 30°C for 41 h gave (*R*)-**6** (42 mg, 39% yield, 19% ee) and a mixture of (*S*,*R*)-**8** and (*S*,*S*)-**8** (69 mg, 31% yield). The ee values of (*R*,*S*)-**8** and (*R*,*R*)-**8** were 99 and 38%, respectively, and the diastereomeric excess of (*R*,*R*)-**8** over (*R*,*S*)-**8** was 31%. The acyl donor (*R*)-**1** (211 mg, 1.11 mmol, 70% yield, 99% ee) was also recovered.

Entry 8—Reaction of (±)-**6** (107 mg, 0.79 mmol), (±)-**3** (300 mg, 1.58 mmol) with Lipase QL (150 mg) in hexane (22 ml) at 30°C did not proceed.

Entry 9—Reaction of (±)-**6** (116 mg, 0.85 mmol), (±)-**4** (300 mg, 1.70 mmol) with Lipase QL (160 mg) in hexane (23 ml) at 30°C for 10 h gave (*R*)-**6** (69 mg, 60% yield, 2% ee), recovered acyl donor (*R*)-**4** (184 mg, 61% yield) and a mixture of product esters (83 mg, 36% yield), which was hydrolyzed by the procedures described in *General procedure for the hydrolysis of product esters*. The enantiomeric excess of alcohol (*S*)-**6** was 4% as determined by HPLC using a chiral column.

Entry 10—Reaction of (±)-**6** (400 mg, 2.9 mmol), methyl 3-phenylpropanoate **5** (3.1 g, 17.4 mmol) with Lipase QL (400 mg) in diisopropyl ether (20 ml) for 2.5 h followed by silica gel chromatography of the products gave (+)-(*R*)-**6** (40% ee), $[\alpha]_D$ +3.0 (*c* 1.43, MeOH) and (*S*)-(−)-2-phenylpropanol 3-phenylpropanoate (62% ee), $[\alpha]_D$ −6.3 (*c* 1.07, $CHCl_3$), which was hydrolyzed by the procedures described in *General procedure for the hydrolysis of product esters*. The enantiomeric excess of alcohol (*S*)-**6** was 62% as determined by HPLC using a chiral column.

Entry 11—Reaction of (±)-**6** (200 mg, 1.47 mmol), isopropenyl acetate (300 mg, 2.99 mmol) with Lipase QL (200 mg) in hexane (90 ml) at 30°C for 3 h gave (*R*)-**6** (73 mg, 37% yield, 8% ee) and the product ester (*S*)-2-phenyl-1-propyl acetate (110 mg, 42% yield), which was hydrolyzed by the procedures described in *General procedure for the hydrolysis of product esters*. The enantiomeric excess of alcohol (S)-**6** was 6% as determined by HPLC using a chiral column.

Entry 12—Reaction of (±)-**6** (200 mg, 1.47 mmol), isopropenyl acetate (300 mg, 2.99 mmol) with Lipase SL (200 mg) in hexane (90 ml) at 30°C for 9 h gave (*R*)-**6** (87 mg, 44% yield, 45% ee) and the product ester (*S*)-2-phenyl-1-propyl acetate (65 mg, 25% yield), which was hydrolyzed by the procedures described in *General procedure for the hydrolysis of product esters*. The enantiomeric excess of alcohol (*S*)-**6** was 37% as determined by HPLC using a chiral column.

Entry 13—Reaction of (±)-**6** (200 mg, 1.47 mmol), isopropenyl acetate (300 mg, 2.99 mmol) with Lipase TL (200 mg) in hexane (90 ml) at 30°C for 4 h gave (*R*)-**6** (66 mg, 33% yield, 4% ee) and the product ester (*S*)-2-phenyl-1-propyl acetate (120 mg, 46% yield), which was hydrolyzed by the procedures described in *General procedure for the hydrolysis of product esters*. The enantiomeric excess of alcohol (*S*)-**6** was 3% as determined by HPLC using a chiral column.

Entry 14—Identical to entry 5.

Entry 15—Reaction of (±)-**6** (107 mg, 0.79 mmol), (±)-**2** (300 mg, 1.58 mmol) with Lipase SL (150 mg) in hexane (22 ml) at 30°C for 40 h gave (*R*)-**6** (30 mg, 28% yield, 93% ee) and a mixture of product esters (*S*,*R*)-**8** and (*S*,*S*)-**8** (116 mg, 52% yield). The ee values of (*S*,*R*)-**8** and (*S*,*S*)-**8** were 46 and 95%, respectively, and the diastereomeric excess of (*S*,*S*)-**8** over (*S*,*R*)-**8** was 84%. The acyl donor (*R*)-**1** (126 mg, 42% yield, 40% ee) was also recovered.

Entry 16—Reaction of (±)-**6** (107 mg, 0.79 mmol), (±)-**2** (300 mg, 1.58 mmol) with Lipase TL (150 mg) in hexane (22 ml) at 30°C for 2 h gave (*R*)-**6** (33 mg, 31% yield, 45% ee) and a mixture

of product esters (*S*,*R*)-**8** and (*S*,*S*)-**8** (110 mg, 49% yield). The ee values of (*S*,*R*)-**8** and (*S*,*S*)-**8** were 85 and 98%, respectively, and the diastereomeric excess of (*S*,*S*)-**8** over (*S*,*R*)-**8** was 40%. The acyl donor (*R*)-**1** (184 mg, 61% yield, 36% ee) was also recovered.

Entry 17—Identical to entry 9.

Entry 18—Reaction of (±)-**6** (116 mg, 0.85 mmol), (±)-**4** (300 mg, 1.70 mmol) with Lipase SL (160 mg) in hexane (23 ml) at 30°C for 10 h gave (*R*)-**6** (43 mg, 37% yield, 47% ee), recovered (*R*)-**4** (195 mg, 65% yield) and a mixture of product esters (80 mg, 35% yield), which was hydrolyzed with the procedures described in *General procedure for the hydrolysis of product esters*. The optical purity of alcohol (*S*)-**6** was 73% ee which was determined by HPLC using a chiral column.

Entry 19—Reaction of (±)-**6** (116 mg, 0.85 mmol), (±)-**4** (300 mg, 1.70 mmol) with Lipase TL (160 mg) in hexane (23 ml) at 30°C for 77 h gave (*R*)-**6** (50 mg, 43% yield, 7% ee), recovered (*R*)-**4** (181 mg, 60% yield) and a mixture of product esters (56 mg, 25% yield), which was hydrolyzed with the procedures described in *General procedure for the hydrolysis of product esters*. The optical purity of alcohol (*S*)-**6** was 26% ee which was determined by HPLC using a chiral column.

Entry 20 —Reaction of (±)-**6** (36 mg, 0.26 mmol), (±)-**2** (100 mg, 0.53 mmol) with Lipase QL (50 mg) in benzene (8 ml) at 30°C for 26 h gave (*R*)-**6** (18 mg, 50% yield, 51% ee) and a mixture of (*S*,*R*)-**8** and (*S*,*S*)-**8** (30 mg, 41% yield). The ee values of (*S*,*R*)-**8** and (*S*,*S*)-**8** were 33 and 96%, respectively, and the diastereomeric excess of (*S*,*S*)-**8** over (*S*,*R*)-**8** was 85%. The acyl donor (*R*)-**1** (42 mg, 42% yield, 36% ee) was also recovered.

Entry 21—Identical to entry 5.

Entry 22—Reaction of (±)-**6** (36 mg, 0.26 mmol), (±)-**2** (100 mg, 0.53 mmol) with Lipase QL (50 mg) in diisopropyl ether (8 ml) at 30°C for 2 h gave (*R*)-**6** (16 mg, 45% yield, 98% ee) and a mixture of (*S*,*R*)-**8** and (*S*,*S*)-**8** (41 mg, 45% yield). The ee values of (*S*,*R*)-**8** and (*S*,*S*)-**8** were 72% and 99%, respectively, and the diastereomeric excess of (*S*,*S*)-**8** over (*S*,*R*)-**8** was 84%. The acyl donor (*R*)-**1** (48 mg, 0.25 mmol, 49% yield, 36% ee) was also recovered.

Entry 23—Reaction of (±)-**6** (36 mg, 0.26 mmol), (±)-**2** (100 mg, 0.53 mmol) with Lipase QL (50 mg) in benzene (8 ml) at 30°C did not proceed.

Entry 24—Identical to entry 15.

Entry 25—Reaction of (±)-**6** (36 mg, 0.26 mmol), (±)-**2** (100 mg, 0.53 mmol) with Lipase SL (50 mg) in diisopropyl ether (8 ml) at 30°C for 1.5 h gave (*R*)-**6** (11 mg, 31% yield, 85% ee) and a mixture of (*S*,*R*)-**8** and (*S*,*S*)-**8** (29 mg, 39% yield). The ee values of (*S*,*R*)-**8** and (*S*,*S*)-**8** were 48 and 99%, respectively, and the diastereomeric excess of (*S*,*S*)-**8** over (*S*,*R*)-**8** was 94%. The acyl donor (*R*)-**1** (59 mg, 60% yield, 36% ee) was also recovered.

Acknowledgements

The authors thank Meito Sangyo Co. Ltd, for kindly providing the lipases. This work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

References

1. Crout, D. H. G.; Chrisen, M. In *Modern Synthetic Methods*; Scheffold, R., Ed.; Springer-Verlag: Berlin, 1989; Vol. 5, pp. 1–114. Davies, H. G.; Green, R. H.; Kelly, D. R.; Roberts, S. M. *Biotransformation in Preparative Organic Chemistry*; Academic Press: London, 1990. Wong, C. H.; Whitesides, G. W. *Enzymes in Synthetic Organic*

Chemistry. *Tetrahedron Organic Chemistry Series*; Pergamon: Oxford, 1994; Vol. 12. Naemura, K.; Fukuda, R.; Murata, M.; Konishi, M.; Hirose, K.; Tobe, Y. *Tetrahedron: Asymmetry* **1995**, *6*, 2385. Naemura, K.; Kittaka, K.; Murata, M.; Ida, H.; Hirose, K.; Tobe, Y. *Enantiomer* **1996**, *1*, 219.

2. Kazlauskas, R. J.; Weissfloch, A. M. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656. Naemura, K.; Fukuda, R.; Konishi, M.; Hirose, K.; Tobe, Y. *J. Chem. Soc., Perkin Trans. 1* **1994**, 1253. Naemura, K.; Fukuda, R.; Murata, M.; Konishi, M.; Hirose, K.; Tobe, Y. *Tetrahedron: Asymmetry* **1995**, *6*, 2385. Tuomi, W. V.; Kazlauskas, R. J. *J. Org. Chem.* **1999**, *64*, 2638.
3. Wescott, C. R.; Klibanov, A. M. *Biochim. Biophys. Acta* **1994**, *1206*, 1. Koskinen, A. M. P. *Enzymatic Reactions in Organic Media*; Klibanov, A. M., Ed.; Blackie Academic: Glasgow, 1996. Ke, T.; Wescott, C. R.; Klibanov, A. M. *J. Am. Chem. Soc.* **1996**, *118*, 3366. Tanaka, K.; Yasuda, M. *Tetrahedron: Asymmetry* **1998**, *9*, 3275.
4. Sakai, T.; Kishimoto, T.; Tanaka, Y.; Ema, T.; Utaka, M. *Tetrahedron Lett.* **1998**, *39*, 7881. Sakai, T.; Kawabata, I.; Kishimoto, T.; Ema, T.; Utaka, M. *J. Org. Chem.* **1997**, *62*, 4906.
5. Guo, Z.-W.; Sih, C. J. *J. Am. Chem. Soc.* **1989**, *111*, 6836. Itoh, T.; Hiyama, Y.; Betchaku, A.; Tsukube, H. *Tetrahedron Lett.* **1993**, *34*, 2617. Itoh, T.; Mitsukura, K.; Kanphai, W.; Takagi, Y.; Kihara, H.; Tsukube, H. *J. Org. Chem.* **1997**, *62*, 9165.
6. Lin, G.; Lin, W.-Y. *Tetrahedron Lett.* **1998**, *39*, 4333.
7. Berger, B.; Faber, K. *J. Chem. Soc., Chem. Commun.* **1991**, 1198.
8. Hirose, Y.; Kiriya, K.; Nakanishi, Y.; Kurono, Y.; Achiwa, K.; *Tetrahedron Lett.* **1995**, *36*, 1063.
9. Berglund, P.; Holmquist, M.; Hult, K.; Hogberg, H. E. *Biotechnology Lett.* **1995**, *17*, 55. Ema, T.; Maeno, S.; Takaya, Y.; Sakai, T.; Utaka, M. *J. Org. Chem.* **1996**, *61*, 8610.
10. Ema, T.; Maeno, S.; Takaya, Y.; Sakai, T.; Utaka, M. *J. Org. Chem.* **1996**, *61*, 8610.
11. The *E* values for the reactions using isopropenyl acetate and methyl 3-phenylpropanoate are 2 and 6, respectively. The procedures of each reaction are the same as those of entry 10 described in the Experimental section using isopropenyl acetate or methyl 3-phenylpropanoate as an acyl donor.
12. Kawasaki, M.; Goto, M.; Kawabata, S.; Kodama, T.; Kometani, T. *Tetrahedron Lett.* **1999**, *10*, 5223.
13. As examples of the use of chiral acyl donors: for the resolution of chiral secondary alcohol and secondary chiral amine, see: Pozo, M.; Gotor, V. *Tetrahedron: Asymmetry* **1995**, *6*, 2797; for hydrolysis catalyzed by *Candisa rugosa* lipase, see: Berglund, P.; Holmquist, M.; Hult, K. *J. Mol. Catalysis B-Enzymatic* **1998**, *5*, 283.
14. Naemura, K.; Murata, M.; Tanaka, R.; Yano, M.; Hirose, K.; Tobe, Y. *Tetrahedron: Asymmetry* **1996**, *7*, 3285.
15. Yamamoto, K.; Ikeda, K.; Yin, L. K. *J. Organomet. Chem.* **1989**, *370*, 319.
16. Swern, D.; Billen, G. N.; Knight, H. B. *J. Am. Chem. Soc.* **1947**, *69*, 2439.
17. Levene, P. A.; Rothen, A. *J. Org. Chem.* **1936**, *1*, 76. Jaouen, G.; Meyer, A. *J. Am. Chem. Soc.* **1975**, *97*, 466.
18. Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. *J. Am. Chem. Soc.* **1982**, *104*, 7294.
19. Burgess, K.; Jennings, L. D. *J. Am. Chem. Soc.* **1991**, *113*, 6129.
20. Hæffner, F.; Norin, T. *Chem. Pharm. Bull.* **1999**, *47*, 591.