ELSEVIER

Journal of Molecular Catalysis B: Enzymatic 2 (1997) 257–269

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

Review

Application of lipase-catalyzed transformations for the synthesis of insect pheromones and related compounds

Eiichiro Fukusaki [a,*], Shiro Satoda [b]

[a] *Department of Biotechnology, Faculty of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka, 565, Japan*
[b] *Product Development Center, Medical Products Sector, Nitto Denko Corporation, 1-1-2 Shimohozumi, Ibaraki, Osaka, 567, Japan*

Received 15 August 1996; revised 27 September 1996; accepted 27 September 1996

Abstract

This review describes efficient means of preparing optically pure insect pheromones and related compounds via lipase-catalyzed enantioselective reaction on a large scale. (1) A new synthesis of the Japanese beetle pheromone, (*R,Z*)-(−)-5-(1-decenyl)oxacyclopentan-2-one, established by a combination of two lipase-catalyzed transformation was demonstrated. (2) A chemico-enzymatic procedure for the syntheses of both enantiomers of cupreous chafer beetle pheromone, (*R,Z*)- and (*S,Z*)-5-(1-octenyl)oxacyclopentan-2-one, was described. (3) An optical resolution of (±)-2,3-epoxy-8-methyl-1-nonanol, the key intermediate of the synthesis of gypsy moth pheromone, was demonstrated. (4) A practical chemico-enzymatic synthesis of (+)-disparlure in large scale was demonstrated. (5) A facile synthesis of carboxyalkyl acrylate, which is special monomers in the synthesis of the new polymers, by two lipase-catalyzed regioselective reactions was described.

Keywords: Lipase; Enzyme; Optical resolution; Enantioselective; Acylation; Transesterification; Sex pheromone

1. Introduction

Enzymes have been widely used as catalysts in organic synthesis. Among the enzymes, lipases have been widely used for the optical resolution of various racemic alcohols, because they have a remarkable ability to assume a variety of conformations to accommodate substrates of varying sizes and shapes [1–4].

Lipases usually do not require any cofactor regeneration system. These are very strong points for their application. In addition, these enzymes are readily available commercially in large quantities and at relatively low cost. Moreover, lipases are uniquely stable in nonpolar organic solvents. This trait of lipases is very important for their application because water is not the ideal reaction medium for most process in organic syntheses. For preparative organic syntheses, it would be better to carry out enzymatic conversions in organic solvents rather than water for several reasons: 1) many organic substrates dissolve better in organic solvents than they do in water; 2) enzymes can be easily recovered and reused because enzymes are insoluble in organic solvents; 3) compounds that are unstable in water, such as acid anhydrides could be used as substrates; 4) thermodynamic

* Corresponding author.

PII S1381-1177(96)00027-6

equilibria of many processes are unfavorable in water – examples include syntheses of esters from carboxylic acids and alcohols; 5) product recovery from aqueous solutions is often difficult and expensive. For these reasons, lipase-catalyzed transformation is becoming increasingly important in organic synthesis. Studies have compared the utility of the lipase-catalyzed optical resolution of racemic alcohol, as a way of obtaining chiral alcohol, with asymmetric reduction of the corresponding ketone by a chemical catalyst [5–12]. Although the chemical method is efficient at the laboratory scale, it has some disadvantages and is thereby not practical on a large scale. The disadvantages of the chemical method include: 1) the method requires expensive asymmetric reducing agents; 2) in many cases, the reaction temperature should be kept very low – below −50°C; and 3) it requires dangerous reagents such as lithium aluminum hydride. For the above reasons, in industrial application, lipase-catalyzed transformation is expected to become a new powerful procedure that is safe and inexpensive.

This review describes the utility of lipase-catalyzed transformations for preparing valuable compounds on a comparatively large scale. The target compounds are sex pheromones of pests (including the Japanese beetle (*Popillia japonica* Newman) [13], the cupreous chafer beetle (*Anomala cupurea* Hope) [14], and the gypsy moth (*Lymantria dispar* L.) [15]), that devastate a variety of trees and crops. Also investigated is carboxyalkyl acrylate, which is a new material for the polymer industry.

2. Insect sex pheromones

Insect sex pheromones are expected to become a new type of pesticides that work by disrupting mating or mass trapping [16–19]. All these target pheromones are optically active molecules whose chiral centers are critical to their activities. In some cases, unnatural enantiomers that correspond to natural ones strongly inhibit a pheromone's male response [20]. Therefore extremely high optical purity is essential for practical use of pheromone. Because the utility of pheromone is obvious, efforts of many groups have been devoted to developing efficient routes for large scale preparation of these compounds. This review describes an application of the lipase-catalyzed enantioselective transformations for their preparation.

3. Synthesis of Japanese beetle pheromone

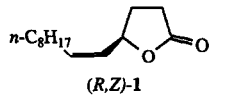
3.1. Japanese beetle

The Japanese beetle, *Popillia japonica* Newman, is a devastating pest of a variety of trees and crops in the United States. Tumlinson et al. have isolated its pheromone from virgin females and identified it as (*R,Z*)-(−)-5-(1-decenyl)oxacyclopentan-2-one ((*R,Z*)-**1**, Fig. 1) [13].

This pheromone has a unique feature whereby a small amount of unnatural (*S,Z*)-isomer strongly inhibits the male response to it. Indeed, 2% contamination of (*S,Z*)-isomer causes the mixture to be three times less active than optically pure pheromone [13]. Therefore, extremely high optical purity is essential for the practical use of this pheromone.

In the original synthesis of Doolittle et al. (*R*)-(−)-glutamic acid was used as its starting material [21]. Since then, a number of other syntheses have been reported [5,6,9] [22–29]. In several of these studies, the acetylenic lactone (*R*)-**2** has been established as the key intermediate [5,30].

Among them, a synthesis involving the lipase-catalyze enantioselective lactonization of (*RS*)-**3** to yield (*R*)-**2** as the key reaction (Fig. 2) has been reported by Sugai et al. [31].



(*R,Z*)-**1**

Fig. 1. Japanese beetle sex pheromone.

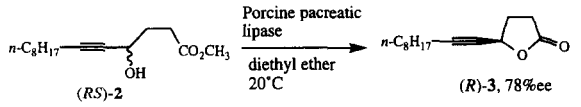


Fig. 2. Lipase-catalyzed enantioselective lactonization of (*RS*)-**2**.

In Sugai's synthesis, optically active lactone (*R*)-**2** of 97% ee was prepared by repetitions of the above reaction. The repetition of lipase-catalyzed reaction could not raise the optical purity sufficiently high for practical use because the enantioselectivity of the above reaction was not so high ($E = 20$) [32].

We show a practical chemicoenzymatic synthesis of Japanese beetle pheromone (*R,Z*)-**1** of over 99% ee which involves two lipase-catalyzed crucial steps: enantioselective acylation of γ-hydroxy ester **3** and the subsequent enantioselective hydrolysis of γ-acyloxy ester **4**.

3.2. Synthesis of Japanese beetle sex pheromone by lipase-catalyzed enantioselective transformation [33]

First, several commercially available lipases were surveyed for the acylation of (*RS*)-**3** in organic solvent. Lipase PS from *Pseudomonas* sp. (Amano Pharm., Japan) was found to catalyze the enantioselective acylation of (*RS*)-**3** with several anhydrides [34] as acylating reagents in diisopropyl ether (Fig. 3).

The best result was obtained when succinic anhydride was used as the acylating reagent ($E = 115$). On the other hand, glutaric anhydride and phthalic anhydride provided very poor yields (data not shown). Among acyclic anhydrides, *n*-butyric anhydride proved to be the best acylating reagent ($E = 94$). Results are summarized in Table 1. As for solvents, toluene and isooctane, which are recognized as suitable for enzymatic reactions in non-aqueous media [35–37], were also examined as reaction solvents. Diisopropyl ether was selected as the best solvent because the reaction was faster than toluene, and lactone **3** was formed as a by-product in isooctane.

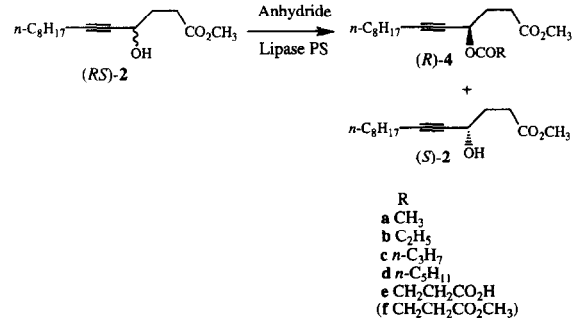


Fig. 3. Lipase-catalyzed enantioselective acylation of (*RS*)-**2** with anhydrides as acyl donors.

Table 1
Acylation of (*RS*)-**2** by Lipase PS

Entry	Anhydride	Conv. (%)	Time (h)	Product, ee (%)		E [a]
1	acetic	51	18	(*R*)-**4a**, 85	(*S*)-**2**, 90	3
2	popionic	51	18	(*R*)-**4b**, 86	(*S*)-**2**, 91	40
3	n-butyric	50	18	(*R*)-**4c**, 93	(*S*)-**2**, 93	94
4	n-caproic	50	18	(*R*)-**4d**, 92	(*S*)-**2**, 92	79
5	succinic	50	24	(*R*)-**4e**, 94	(*S*)-**2**, 94	115

[a] $E = \{\ln[1 - c[1 + \mathrm{ee(P)}]\}/\{\ln[1 - c(1 - \mathrm{ee(P)}]\}$.

To enrich further the ee of the desired (*R*)-enantiomer, asymmetric hydrolysis of the butyrate of the secondary alcohol moiety of **4** was attempted. First, hydrolysis of butanoate **4c** was examined. After surveying several commercially available lipases, Palatase A, a lipase from Aspergillus niger (Novo Ind., Denmark), was found to catalyze the hydrolysis of **4c** (Fig. 4). It hydrolyzed (*RS*)-**4c** in 0.1 M phosphate buffer (pH 7) to give (*R*)-**2** (60% ee) and (S)-**4c** (59% ee). The reaction was moderately stereoselective ($E = 7$).

These results, however, were unsatisfactory for preparing the extremely pure target lactone (R)-**3**.

A higher stereoselectivity was expected by

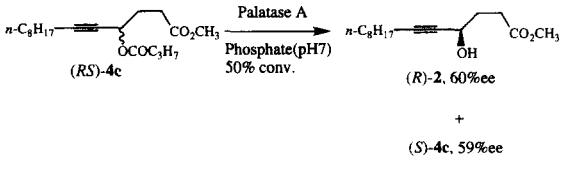


Fig. 4. Lipase-catalyzed enatioselective hydrolysis of (*RS*)-**4c**.

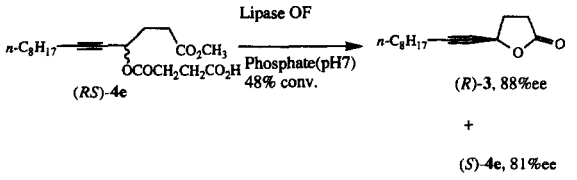


Fig. 5. Lipase-catalyzed enantioselective lactonization of (*RS*)-**4e**.

using succinoate **4e** as the substrate. Although the enzymatic hydrolysis of **4e** was unsuccessful, we found that lipase OF from *Candida cylindracea* (Meito Sangyo, Japan) catalyzed a hydrolysis and the subsequent spontaneous lactonization of **4e** to yield (*R*)-**3** (88% ee) (Fig. 5). The *E* value in this case was 39.

Lipase catalyzed lactonization of hydroxy esters in organic solvents have been previously reported [38–43]. Inspired by those reports, the direct lactonization of (*RS*)-**4c** or (*RS*)-**4e** was tried in organic solvents such as toluene, isooctane, diethyl ether and diisopropyl ether. Those attempts from racemic compounds, however, were not successful (data not shown). Anyway, as it turned out that (*R*)-enantiomer lactonized further than the (*S*)-enantiomer, in order to refine the methodology to obtain highly optically pure lactone (*R*)-**3**, this lipase OF-catalyzed reaction was applied to an already enantiomericaly enriched form of (*R*)-**4e**, which was prepared in 94% ee with Lipase PS (Fig. 6). The reaction was stopped after 23 h and (*R*)-**3** was isolated with over 99% ee (83% yield).

Semihydrogenation of obtained (*R*)-**2** by a previously reported procedure [9] gave the Japanese beetle pheromone (*R,Z*)-**1** (Fig. 7).

In conclusion, a new effective synthesis of

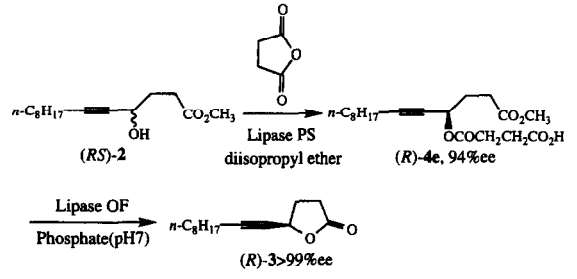


Fig. 6. Synthesis of (RS)-3 by a combination of lipase-catalyzed enatioselective acylation and lactonization.

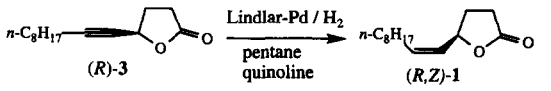


Fig. 7. Synthesis of Japanese beetle sex pheromone by semihydrogenation of (*R*)-**3**.

the Japanese beetle pheromone (R,Z)-**1** was established by combining enzymatic and chemical methods without using any chiral auxiliaries.

4. Synthesis of cupreous chafer beetle sex pheromone

4.1. Cupreous chafer beetle

The cupreous chafer beetle, *Anomala cuprea* Hope, is a devastating pest to a variety of crops in Japan. Leal has isolated its pheromone from field-captured female beetles and identified it as (*R,Z*)-(−)-5-(1-octenyl)oxacyclopentan-2-one ((*R,Z*)-**5**, Fig. 8) [14].

Its structure closely resembles sex pheromone (*R,Z*)-**1** of the Japanese beetle, *Popillia japonica* Newman [13]. The sex pheromone of the Japanese beetle has the unique feature whereby a small amount of the unnatural (*S,Z*)-isomer strongly inhibits the male response to it. We synthesized both optically pure enantiomers of **5** in order to evaluate the male response to them [44]. Later some other syntheses were reported [45–47].

4.2. Synthesis of enantiomers of a sex pheromone of cupreous chafer beetle, anomala cuprea hope [47]

This synthesis was according to the same strategy for the preparation of Japanese beetle sex pheromone. The key reaction of this synthesis was a lipase-catalyzed enantioselective acylation of (*RS*) methyl-4-hydroxy-5-dodecynoate

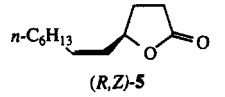


Fig. 8. Cupreous chafer beetle sex pheromone.

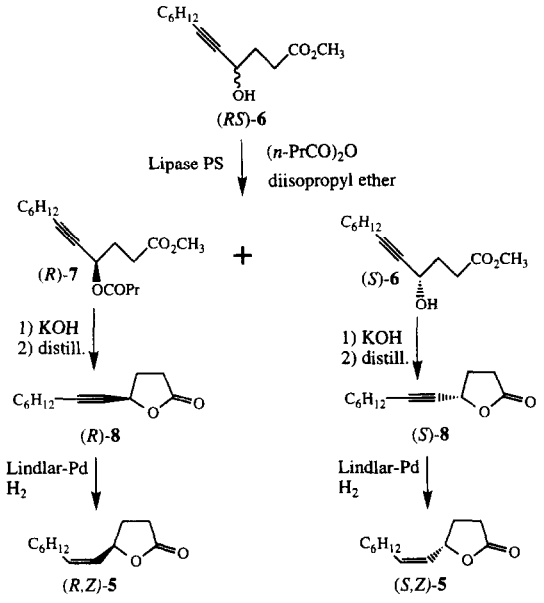


Fig. 9. synthesis of both enantiomers of cupreous chafer beetle sex pheromone ((*R*,*Z*)-**5** and (*S*,*Z*)-**5**) by lipase-catalyzed enantioselective acylation.

((*RS*)-**6**). Hydroxy ester (*RS*)-**6** was subjected to a lipase-catalyzed enatioselective acylation with *n*-butyric anhydride as an acylating agent in diisopropyl ether to yield methyl (*R*)-4-butanoyloxy-5-dodecynoate) ((*R*)-**7**(93% ee)) and methyl (*S*)-4-hydroxy-5-dodecynoate ((*S*)-**6**(73% ee)). The product obtained by primary enzymatic reaction was subjected to the second enzymatic reactions independently to yield optically pure products, (*R*)-**7** and (*S*)-**6**, which were converted to (*R*)- and (*S*)-(−)-5-(1-octynyl)oxacyclopentan-2-one ((*R*)-**8** and (*S*)-**8**), respectively. Both enantiomers of the cupreous chafer beetle pheromone, (*R*,*Z*)- and (*S*,*Z*)-(−)-5-(1-octenyl)oxacyclopentan-2-one ((*R*,*Z*)-**5** and (*S*,*Z*)-**5**), were synthesized in one step from these optically active lactones (Fig. 9).

5. Synthesis of gypsy moth sex pheromone

5.1. Gypsy moth

(+)-Disparlure, *cis*-(7*R*,8*S*)-7,8-epoxy-2-methyloctadecane (**9**, Fig. 10) was identified as

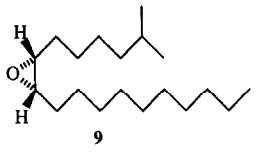


Fig. 10. Gypsy moth sex pheromone.

the sex pheromone for gypsy moth, *Lymantria dispar* L., which is a harmful forest pest [15,48].

Since then, a number of syntheses have been reported [49–72].

Among them, Mori et al. reported an interesting synthesis of (+)-disparlure that was derived from the optically active epoxy alcohol, (2*S*,3*R*)-2,3-epoxy-8-methyl-1-nonanol ((2*S*,3*R*)-**10**) prepared by Sharpless asymmetric epoxidation [73]. (Fig. 11)

A synthesis involving the lipase-catalyzed enantioselective lactonization of (*RS*)-**3** to yield (*R*)-**2** as the key reaction (Fig. 2) has been reported by Sugai et al.

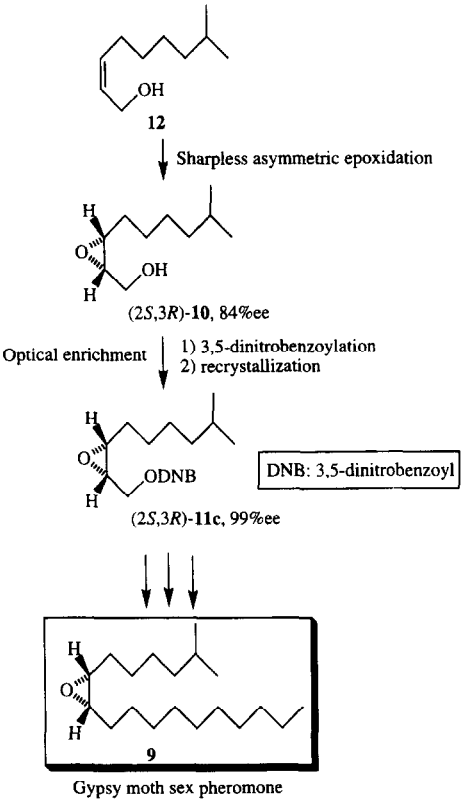


Fig. 11. Synthesis of gypsy moth sex pheromone by Mori and Eabata.

Recently, an enzymatic approach to the optically active form of **10** by an enantioselective transesterification of its racemate with ethyl acetate as acyl donor and as reaction medium was reported [63] (Fig. 12).

By this method, it was very difficult to control the conversion of the reaction around 50% that theoretically would give a high optical yield of the target enantiomer, because of the following reason. Under the condition of large excess amount of acyl donor, the conversion readily exceeded 50% resulting in relatively lower optical purity of desired enantiomer. To overcome this problem, the use of acid anhydride [34] was decided to be an acyl donor in lipase-catalyzed enantioselective acylation of (**10**); the conversion of the reaction would be controlled by a molar equivalence of anhydride. A lipase-catalyzed enantioselective alcoholysis of an epoxy ester in organic solvent was also attempted.

5.2. An optical resolution of racemic epoxyalcohol (10) by lipase-catalyzed enantioselective reactions [74]

Initially, several commercially available lipases were surveyed for the acylation of (±)-**10** to yield corresponding epoxy esters (**11**) with one equivalent of several anhydrides, such as acetic, propionic, *n*-butyric, *n*-caproic and *i*-butyric anhydride. Pancreatin F (Amano Pharm., Japan), a lipase from porcine pancreas, was found to catalyze the enantioselective acylation

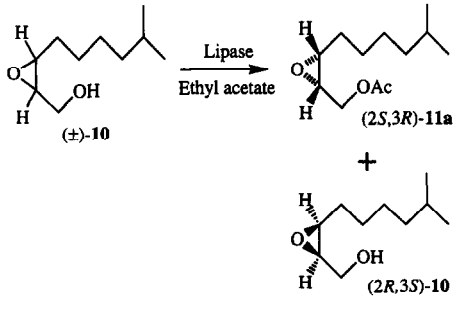


Fig. 12. Lipase-catalyzed enantioselective transesterification of (±)-**10** with ethyl acetate by Bianch et al.

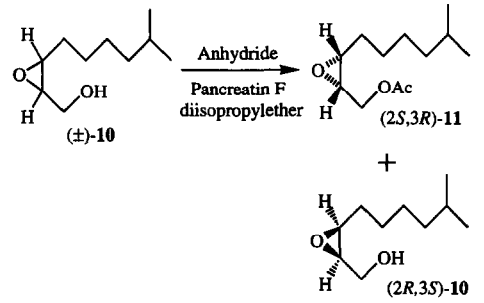


Fig. 13. Lipase-catalyzed enantioselective acylation of (±)-**10** with acid anhydride as acyl donors.

of (±)-**10** with several anhydrides as acylating agent in a most enantioselective manner (Fig. 13).

In all cases, half equivalents of anhydrides were used and the reactions were carried out at 30°C in diisopropyl ether. Results are summarized in Table 2.

The highest enantioselectivities were obtained when acetic anhydride was used as acylating agent ($E = 13$). Other acyclic anhydrides, such as propionic, *n*-butyric and *n*-caproic anhydride, had almost teh same properties as well as acetic anhydride, however, the enantioselectivities of the reactions with them were slightly lower than that of the reactions with acetic anhydride. The anhydrides that had a branch at the α-position, such as *i*-butyric anhydride and

Table 2
Lipase-catalyzed enatioselective acylation of (±)-**10** with acid anhydrides as acyl donors

Anhydride	Conv. (%) [a]	Time (h)	ee (%), recov. (%)		E [b]
			ester	alcohol	
acetic	57	7	66, 49	86, 44	13
popionic	56	7	59, 48	76, 44	9
n-butyric	56	7	61, 46	78, 48	10
n-caproic	55	7	65, 47	80, 45	11
i-butyric	33	50	72, 34	35, 64	8
benzoic	28	200	83, 25	33, 70	11
succinic	33	24	66, 11	33, 56	7
phthalic	nd	200	nd	nd	nd [c]

[a] Calculated on the basis of ee(S) and ee(P): conv. = ee(S)/[ee(S) + ee(P)].
[b] $E = \{\ln[1 - c[1 + ee(P)]]/\{\ln[1 - c(1 - ee(P)]\}$.
[c] No product was detected.

benzoic anhydride, showed very low reactivities.

Cyclic anhydrides such as succinic and phthalic anhydride were also examined. However, in neither case was a good result obtained. For example, phthalic anhydride provided a very poor yield (data not shown).

The results showed that acetic anhydride was the best acylating agent, however, in this case, the optical purity of the formed ester (66% ee) was lower than that of remaining alcohol (86% ee). Even in the case that only half equivalent of acetic anhydride was added as acylating agent, the reaction proceeded over 50% conversion. We speculated that the free acetic acid that formed from acetic anhydride as a by-product might be involved in further acetylation. To solve the above problem, removal of free acetic acid was attempted by addition of weak bases [75,76]. The addition of potassium bicarbonate and 2,6-lutidine were examined. The amount of base added was two equivalents to acetic anhydride and other conditions were kept to those of above reaction. Results were summarized in Table 3.

Addition of bases was effective to supress the reaction exceeding 50% conversion (Table 3; run 1 and 2). As another way to solve the above problem, enzyme adsorbed on celite [34,75] was examined. The use of Pancreatin F that was adsorbed to celite was also effective (Table 3; entry 4), and in this case, the enantioselectivity

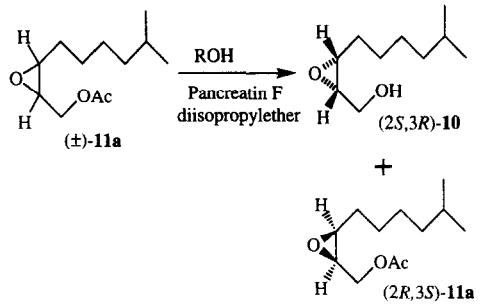


Fig. 14. Lipase-catalyzed enantioselective alcoholysis of (±)-**11a**.

was higher ($E = 16$) than that of control (Table 3; run 3 ($E = 12$)). To obtain higher enantioselectivity, the reaction at lower temperature [77] was examined. The reaction by Panceatin F adsorbed on celite at 5°C gave a higher enantioselectivity (Table 3; run 5, ($E = 23$)).

For further enrichment of the optical purity of the desired (2*S*,3*R*)-enantiomer, enantioselective alcoholysis of epoxy acetate (**11a**) in organic solvent was attempted. Through surveying several commercially available lipases, Pancreatin F (Amano Pharm., Japan), the same enzyme as in the above reaction, was found to catalyze the alcoholysis of (±)-**11a** with several alcohols (Fig. 14).

The results are summarized in Table 4. The reactions were carried out in diisopropyl ether with several alcohols, such as methanol, ethanol, *n*-propyl alcohol and *i*-propyl alcohol at 30°C for the indicated times. When linear alcohols

Table 3
Effects of base addition and enzyme immobilization on lipase-catalyzed enantioselictive acylation of (±)-**10**

Run [a]	Base [b]	Enzyme	Temp (°C)	Time (h)	Conv. (%) [c]	ee (%), recov. (%)		E [d]
						Ester	Alcohol	
1	KHCO3	free	30	6	51	70, 50	73, 46	12
2	2,6-lutidine	free	30	6	40	74, 41	62, 46	12
3	none	free	30	6	57	66, 49	86, 44	13
4	none	on celite	30	6	53	73, 49	81, 47	16
5	none	on celite	5	14	50	79, 47	79, 46	23

[a] In all reactions, acetic anhydride was used as an acyl donor.
[b] Added in equal equivalent to substrate.
[c] Calculated on the basis of ee(S) and ee(P) [conv. = ee(S)/(ee(S) + ee(P))].
[d] $E = \{\ln[1 - c(1 + ee(P))]\}/\{\ln[1 - c(1 - ee(P))]\}$.

Table 4
Lipase-catalyzed enantioselective alcoholysis of (±)-**11a**

R	Temp (°C)	Time (h)	Conv. (%) [a]	ee (%), recov. (%)		E [b]
				alcohol	ester	
methyl	30	64	49	72, 49	69, 48	13
ehtyl	30	85	41	75, 40	51, 57	12
n-propyl	30	90	41	78, 38	55, 58	14
i-propyl	30	140	19	47, 19	11, 72	1
methyl	5	110	35	85, 34	45, 65	20

[a] Calculated on the basis of ee(S) and ee(P): conv. = ee(S)/[ee(S) + ee(P)].
[b] $E = \{\ln[1 - c[1 + ee(P)]]/\{\ln[1 - c(1 - ee(P)]]$.

were used as the acceptors, an increase of the chain length of the alcohol made the reactivities lower, however, it didn't affect the enantioselecty of the reaction. *i*-Propyl alcohol that had a branch at α-position gave very low enantioselectivity ($E = 1$). This reaction at lower temperature (5°C) gave an enhanced enantioselectivity ($E = 20$).

In order to refine the methodology to obtain optically very pure epoxy alcohol (2*S*,3*R*)-**10**, two enzymatic reactions were combined. (2*S*,3*R*)-**11a** was prepared in 79% ee by the first reaction, lipase-catalyzed enantioselective acylation. Also, an obtained (2*S*,3*R*)-**11a** was applied to the second reaction, lipase-catalyzed enantioselective alcoholysis. The reaction was stopped after 90 h and (2*S*,3*R*)-**10** was isolated with 95% ee (Fig. 15).

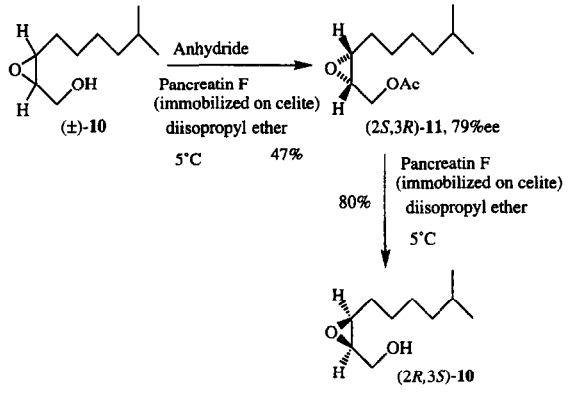


Fig. 15. Synthesis of (2*S*,3*R*)-**10** by a combination of lipase-catalyzed enantioselective acylation and alcoholysis.

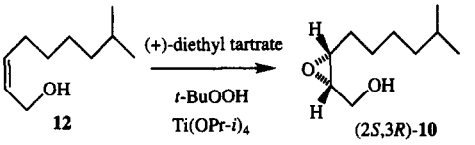


Fig. 16. Sharpless asymmetric epoxidation of allyl alcohol.

5.3. Large-scale preparation of (+)-disparlure, the gypsy moth sex pheromone, by a practical chemico-enzymatic procedure [78]

In the previous section, we described a method for a preparation of the optically active epoxyalcohol ((2*S*, 3*R*)-**10**) by lipase-catalyzed optical resolution methods. A major problem encountered in this method is that it is seldom possible to use an undesired (2*R*, 3*S*)-enantiomer formed as a by-product. In this section, a practical method for a preparation of (2*S*, 3*R*)-**10** by a combination of Sharpless' asymmetric epoxidation [79] and lipase-catalyzed enantioselective acylation is described.

The key reaction of the Mori's synthesis [60] (Fig. 11) described above is Sharpless' asymmetric epoxidation of 8-methyl-2-nonen-1-ol (**12**) to yield an optically active epoxy alcohol ((2*S*,3*R*)-**10**) (Fig. 16). We tried to reproduce this result (Fig. 16) on a large scale. It was possible to obtain (2*S*,3*R*)-**10** at over 80% ee of optical purity, although a long reaction time (over 7 days) and low temperature (below −20°C) were essentially required (Table 5). This reaction was very efficient on a laboratory scale, but it is very costly on a large scale.

Table 5
Effects of reaction temperature on Sharpless asymmetric epoxidation of allyl alcohol (**12**)

Temp (°C)	Reaction time (h)	ee of product
−20	170	82
5	40	65
15	15	52

Reaction conditions: allyl alcohol (**12**) (42 g, 0.27 mol), (+)-diethyl tartrate (38 g, 0.19 mol), $Ti(OPr\text{-}i)_4$ (46 g, 0.16 mol), t-BuOOH (3.0 M, 134 ml, 0.40 mol), molecular sieves 3A (55 g), dichloromethane (5 l).

Especially the necessity of long reaction times over 7 days was a problem for industrial use. The reaction at higher temperature could reduce the required time but it would cause a remarkable drop in stereoselectivity. The reaction at 5°C required 40 h to be completed and the optical purity of the obtained (2*S*,3*R*)-**10** was 65% ee. The reaction at 15°C required only 15 h to be completed and the optical purity of the obtained (2*S*,3*R*)-**10** was 52% ee (Table 5). These optical purities were too low to perform the optical enrichment by recrystallization of corresponding 3,5-dinitrobenzoate in sufficient recovery yield. In a preliminary experiment, we found that over 80% ee of optical purity was necessary for good recovery over 60%. To obtain higher optical purity, over 80% ee, the application of lipase-catalyzed kinetic resolution as was described above to (2*S*,3*R*)-**10** with low optical purity (< 60% ee) prepared by Sharpless epoxidation at room temperature was examined.

An allylic alcohol (**12**) was submitted to the modified Sharpless epoxidation which used molecular sieves 3A [80]. L-(+)-Diethyl tartrate was employed as chiral auxiliary. The reaction took 18 h to complete and gave optically active epoxyalcohol (2*S*,3*R*)-**10**, whose optical purity was determined to be as low as 52% ee. Next, the epoxyalcohol thus obtained with an optical purity of 52% ee was submitted to the lipase-catalyzed enantioselective acylation to obtain an optically purer one. The enzymatic reaction was carried out in diisopropyl ether with *n*-butyric anhydride as acylating agent at room temperature for 5 h to give epoxyester **11b** of 85% ee.

To further enrich the optical purity of the desired (2*S*,3*R*)-enantiomer, epoxyester obtained by the above reaction was converted to the corresponding 3,5-dinitorobenzoate **11c** [60] and recrystallization of **11c** was performed to yield the optically pure **11c**. The 3,5-dinitro benzoate **11c** was converted to tosylate **11d** by hydrolysis and subsequent tosylation. Finally, the tosylate **11d** was treated with (n-$C_9H1_9)_2$CuLi in ether and toluene according to the reported procedure [73] to give gypsy moth sex pheromone **9** (Fig. 17). This method makes

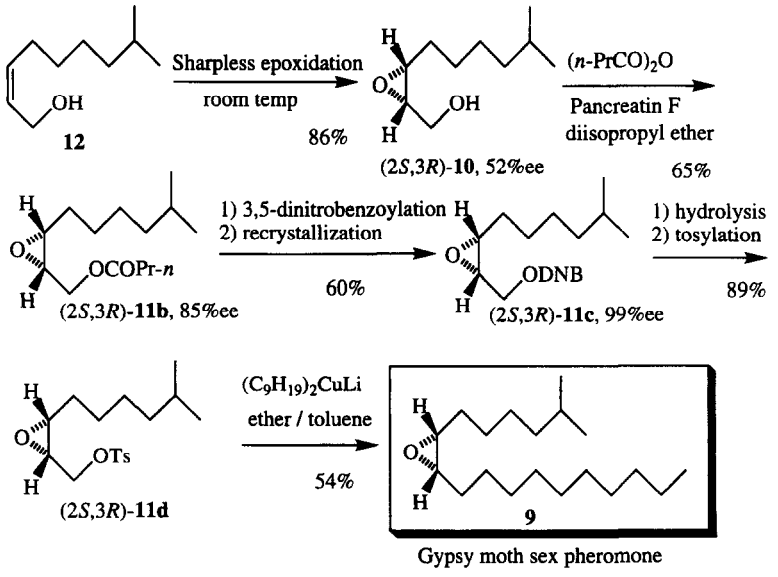


Fig. 17. Synthesis of gypsy moth sex pheromone by a combination of Sharpless asymmetric epoxidation and enzymatic reaction.

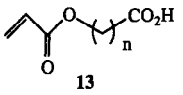


Fig. 18. Carboxyalkyl acrylate.

it possible to produce gypsy moth sex pheromone in kilogram squantities.

6. Synthesis of carboxyalkyl acryrate

6.1. Carboxyalkyl acrylate

Polymers resulting from polymerization of acrylate have been widely used in a large number of important industrial applications [81–84]. These polymers are normally produced from the lower acrylates such as methyl, ethyl or butyl acrylate. Recently, an acrylate bearing carboxyl group, namely carboxyalkyl acrylate (Fig. 18) has been noted as a special monomer. This monomer would provide sites for further chemical modification or ionic effects in resulted polymers. Therefore, these polymers with carboxyl pendants are expected to be new materials, such as bio-materials, novel membrane and functional polymer material [85–88].

A direct chemically catalyzed transesterification method [89] cannot, however, serve as an efficient route for the preparation of carboxyalkyl acrylate (**13**) since it would yield a complex mixture of unreacted alcohol and partially and fully substituted ester products. (Fig. 19)

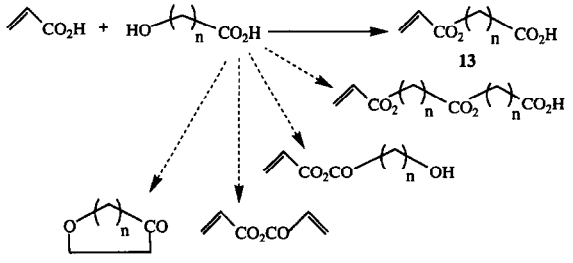


Fig. 19. By-products of chemical procedure on the synthesis of carboxyalkyl acrylate(**13**).

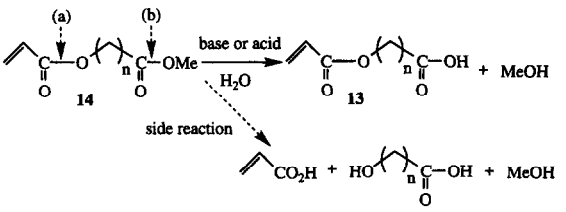


Fig. 20. Side reaction of chemical hydrolysis of **14**.

Chemical hydrolysis of methoxycarbonylalkyl acrylate (**14**), which includes two ester bonds, is a possible route for preparation of **13**. However, this would not be efficient because of its low regioselectivity. In this procedure, a mixture of **14** and acrylic acid would be obtained (Fig. 20).

In this section, we present a regioselective hydrolysis of methoxycarbonylalkyl acrylate (**14**) by lipase to yield carboxyalkyl acrylate (**13**).

6.2. Synthesis of carboxyalkyl acrylate [90]

The starting materials, methoxycarbonylalkyl acrylates (**14**) were readily prepared from hydroxycarboxylic acids methyl ester and acryloyl chloride in the usual manner (Fig. 21).

Several commercially available lipases were surveyed for the hydrolysis of methoxycarbonylslkyl acrylate (**14**) in phosphate buffer. Lipase OF, a lipase from *Candida cylindracea*

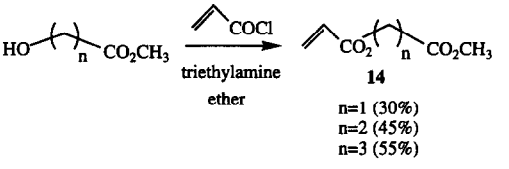


Fig. 21. Syntheses of methoxycarbonylalkyl acrylates(**14**).

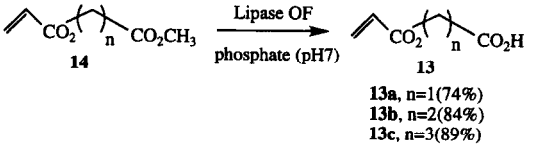


Fig. 22. Synthesis of carboxyalkyl acrylate (**13**) by lipase-catalyzed regioselective hydrolysis.

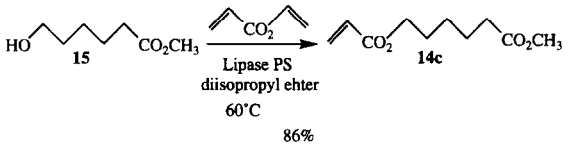


Fig. 23. Synthesis of methoxycarbonylpentyl acrylate (**14c**) by lipase-catalyzed regioselective transesterification.

(Meito Sangyo, Japan), was found to specially recognize site (b). Methoxycarbonylalkyl acrylate (**14**) was regioselectively hydrolyzed by lipase OF to yield carboxyalkyl acrylate (**13**) without any by-product (Fig. 22).

Among these acrylates, carboxypentyl acrylate (**13c**; $n = 5$) is especially in demand as a monomer for the synthesis of pressure sensitive adhesive polymers [91–93]. If a large scale production of **13c** is required according to this new developed method (Fig. 22), the current method for preparation of the substrate (**14c**) (shown in Fig. 21) also needs to be improved, because of its low yield (55%).

As we mentioned above, the transestrerification activity of lipase on the acrylate ester was lower, compared with that on the normal fatty acid ester. Only a few examples have been reported for the lipase-mediated synthesis of acrylate esters [94,95].

In this context, the possibility of lipase-catalyzed transesterification was tested for preparing of **14c**. After surveying several commercially available lipases, Lipase PS (a lipase from *Pseudomonas* sp., Amano Pharm., Japan) was found to catalyze the transesterification between vinyl acrylate and methyl 6-hydroxyhexanoate in diisopropyl ether to give **14c** (86% yield, Fig. 23).

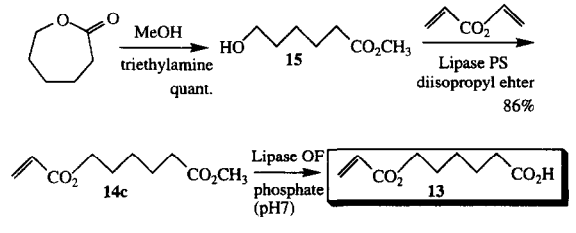


Fig. 24. Synthesis of carboxypentyl acrylate(**13c**) by a combination of lipase-catalyzed regioselective transesterification and hydrolysis.

In conclusion, a convenient route for preparation of carboxyalkyl acrylate was established by a combined use of enzymatic procedures (Fig. 24).

7. Conclusion

In this review, we have described the utility of lipase-catalyzed transformations for preparations of valuable compounds. These enzymatic reactions are inexpensive and available in large scale. In the production of valuable compounds, enzymatic transformations and chemical syntheses compete with each other. The best solution to a large-scale indutrial synthesis is obtained through a combination of the advantages of both methods, demonstrated by our present examples. In the near future, enzymatic procedures will be powerful tools for large scale preparation of valuable compounds, for example drugs and pesticides.

References

[1] A.M. Klibanov, Acc. Chem. Res., 23 (1990) 114–120.
[2] C. Wong, Science, 244 (1990) 1145–1152.
[3] C. Wong and G.M. Whitesides, Use of hydrolytic enzymes: amidases, proteases, esterases, lipases, nitriases, phosphatases, epoxide hydrolases, in: Enzymes in Synthetic Organic Chemistry, 70–107, 1994.
[4] Y. Kageyama, T. Nihira and Y. Yamada, Enzyme Engineering 10, 613 (1990) 681–685.
[5] M. Nishizawa, M. Yamada and R. Noyori, Tetrahedron Lett., 22 (1981) 247–250.
[6] M.M. Midland and A. Tramontano, Tetrahedron Lett., 21 (1980) 3549–3552.
[7] S. Yamaguchi and H.S. Mosher, J. Org. Chem., 38 (1973) 1870–1877.
[8] R.S. Brinkmeyer and V.M. Kapoor, J. Am. Chem. Soc., 99 (1977) 8339–8341.
[9] S. Senda and K. Mori, Agric. Biol. Chem., 47 (1983) 2595–1598.
[10] K.A. Parker, J. Org. Chem., 61 (1996) 3214–3217.
[11] E.J. Corey and C.J. Helal, Tetrahedron Lett., 37 (1996) 4837–4840.
[12] S. Hashiguchi, A. Fujii, J. Takehara, T. Ikariya and R. Noyori, J. Am. Chem. Soc., 117 (1995) 7562–7563.
[13] J.H. Tumlinson, M.G. Kleinn, R. Doolittle, T.T. Ladd and A.T. Proveaux, Science, 197 (1977) 789–792.
[14] W.S. Leal, Naturwissenschaften, 78 (1991) 521–523.

[15] B.A. Bierl, M. Beroza and C.W. Collier, Science, 170 (1970) 87–89.
[16] Behavior-modifying chemicals for insect management, 1990.
[17] P.J. Shea, ACS Symp. Ser., 595 (1995) 272–283.
[18] J. Vrkoc, Chem. Scr., 29 (1989) 407–410.
[19] Y. Tamaki, Natl. Inst. Agric. Sci., Japan, 37 (1980) 22–25.
[20] K. Mori, The synthesis of pheromones, in: The total synthesis of natural products, 9, 1992, pp. 1–534.
[21] R.E. Doolittle, J.H. Tumlinson, A.T. Proveaux and R.R. Heath, J. Chem. Ecol., 6 (1980) 473–485.
[22] W.H. Pirkle and P.E. Adams, J. Org. Chem., 44 (1979) 2169–2175.
[23] K. Sato, T. Nakayama and K. Mori, Agric. Biol. Chem., 43 (1979) 1571–1575.
[24] M.M. Midland and N.H. Nguyen, J. Org. Chem., 46 (1981) 4107–4108.
[25] R. Baker and V.B. Rao, J. Chem. Soc. Perkin Trans I, (1982) 69–71.
[26] Y. Nishida, M. Konno, H. Hori, H. Ohrui and H. Meguro, Agric. Biol. Chem., 51 (1987) 635–640.
[27] H. Nemoto, H. Ishibashi, M. Mori, S. Fujita and K. Fukumoto, J. Chem. Soc. Perkin Trans I, (1990) 2835–2840.
[28] S. Ramaswamy and A.C. Oehlschlager, Tetrahedron, 47 (1991) 1112–1114.
[29] T. Ebata, H. Kqwakami, K. Koseki, K. Matsumoto and H. Matsushita, Biosci. Biotechnol. Biochem., 56 (1992) 818–819.
[30] K. Sato, T. Nakayama and K. Mori, Agric. Biol. Chem., 43 (1979) 1571–1575.
[31] T. Sugai, S. Ohsawa, H. Yamada and T. Ohta, Synthesis, (1991) 1112–1114.
[32] C. Chen, Y. Fujimoto, G. Girdankas and C.J. Sih, J. Am. Chem. Soc., 104 (1982) 7294–7299.
[33] E. Fukusaki, S. Senda, Y. Nakazono and T. Omata, Tetrahedron, 47 (1991) 6223–6230.
[34] D. Bianch, P. Cesti and E. Battistel, J. Org. Chem., 53 (1988) 5531–5534.
[35] C. Chen and C.J. Sih, Angew. Chem. Int. Ed. Engl., 28 (1989) 695–707.
[36] K. Nakamura, M. Kinoshita and O.A., Tetrahedron, 51 (1995).
[37] A. Bosetti, D. Bianchi, P. Cesti and P. Golini, Biocatalysis, 9 (1994) 71–77.
[38] A. Makita, T. Nihira and Y. Yamada, Tetrahedron Lett., 28 (1987) 805–808.
[39] A.L. Gutman, K. Zuobi and T. Bravdo, J. Org. Chem., 55 (1990) 3546–3552.
[40] T. Sugai, O. Katoh and H. Ohta, Tetrahedron, 51 (1995) 11987–119998.
[41] B. Henkel, A. Kunath and H. Schick, Liebigs Ann. Chem., (1995) 921–923.
[42] S.K. Taylor, R.F. Arkinson, F. Richard, E.P. Almli, M.D. Carr and H. Van, Tetrahedron Asymmetry, 6 (1995) 157–164.
[43] M. Lobell and M.P. Schneider, Tetrahedron Asymmetry, 4 (1993) 1027–1030.
[44] E. Fukusaki, M. Konno, S. Senda and T. Omata, Jpn. Kokai Tokkyo Koho, (1990) JP 90-305719.
[45] B.S. Leal, Jpn. Kokai Tokkyo Koho, (1991) JP 91-245990.
[46] K. Koseki, H. Kawakami, T. Ebata, H. Matsushita and M. Ono, Jpn. Kokai Tokkyo Koho, (1991) JP 91-77376.
[47] E. Fukusaki, S. Senda, Y. Nakazono and T. Omata, Biosci. Biotech. Biochem., 56 (1992) 1160–1161.
[48] S. Iwaki, S. Maruo, T. Saito, M. Yamada and K. Katagiri, J. Am. Chem. Soc., 96 (1974) 7842–7844.
[49] K. Mori, T. Takigawa and M.M., Tetrahedoron Lett., (1976) 3953–3956.
[50] D.G. Farnum, T. Veysoglu, A.M. Carde, B. Duhl-Emswiler, T.A. Pancoast, T.J. Reitz and R.T. Carde, Tetrahedron Lett., (1977) 4009–4012.
[51] W.H. Pirkle and P.L. Rinaldi, J. Org. Chem., 44 (1979) 1025–1028.
[52] K. Okada, K. Mori and Hirai, Agric. Biol. Chem., 41 (1977) 2485–2486.
[53] D.G. Farnum, T. Veysoglu, A.M. Garde, B. Duhl-Emswier, P. Barbara, A. Taylor, T.J. Reitz and R.T. Garde, Tetrahedron Lett., (1977) 4009–4012.
[54] K. Mori and T. Ebata, Tetrahedron Lett., 22 (1981) 4281–4282.
[55] J.H. Markgraf, S.I. Lusskin, E.C. McDonald and B.D. Volpp, J. Chem. Ecol., 9 (1983) 211–218.
[56] H.C. Brown and D. Basavaiah, Synthesis, (1983) 283–284.
[57] K.K. Wang and K.H. Chu, J. Org. Chem., 49 (1984) 5175–5178.
[58] O.J. Achmatowicz and A. Sadownik, J. Carbohydr. Chem., 4 (1985) 435–440.
[59] V.B. Jigajinni and R.H. Wightman, Carbohydr. Res., 147 (1986) 145–148.
[60] K. Mori and T. Ebata, Tetrahedron, 42 (1986) 3471–3478.
[61] S. Pilul, M. Kozlowska and J. Jurczak, Tetrahedron Lett., 28 (1987) 2627–2628.
[62] S. Tsuboi, H. furutani, A. Takeda, K. Kawazoe and S. Sato, Bull. Chem. Soc. Jpn, 60 (1987) 2475–2479.
[63] D. Bianch, W. Cabri, P. Cesti, F. Flancalanci and F. Rama, Tetrahedron Lett., 29 (1988) 2455–2358.
[64] T. Sato, T. Itoh and T. Fujisawa, Tetrahedron Lett., 28 (1987) 5677–5680.
[65] T. Kametani, M. Tsubuki and T. Honda, Chem. Pharm. Bull., 36 (1988) 3706–3709.
[66] S. Marczak, M. Masnyk and J. Wicha, Liebigs Ann. Chem., (1990) 345–347.
[67] T. Kametani, M. Tsubuki, Y. Tatsuzaki and T. Honda, J. Chem. Soc. Perkin Trans I, (1990) 639–646.
[68] S.K. Kang, Y.S. Kim, J.S. Lim, K.S. Kim and S.G. Kim, Tetrahedron Lett., 32 (1991) 353–366.
[69] J.L. Brevet and K. Mori, Synthesis, (1992) 1007–1012.
[70] E. Keinan, S.C. Sinha, A. Sinha-Bagchi, Z.M. Wang, X.L. Zhang and K.B. Sharpless, Tetrahedron Lett., 33 (1992) 6411–6414.
[71] S.Y. Ko, Tetrahedron Lett., 35 (1994) 3601–3604.
[72] A. Sinha-Bagchi, S.C. Sinha and E. Keinan, Tetrahedron Asymmetry, 6 (1995) 2889–2892.
[73] K. Mori and T. Ebata, Tetrahedron, 42 (1986) 3471–3478.
[74] E. Fukusaki, S. Senda, Y. Nakazono, H. Yuasa and T. Omata, J. Ferment. Biotech., 73 (1992) 280–283.
[75] C. Berger, C.G. Rabiller, K. Koningsberger, K. Faber and H. Griengl, Tetrahedron Asymmetry, 1 (1990) 541–546.

[76] F. Theil, H. Schick, G. Winter and G. Reck, Tetrahedron, 47 (1991) 7569–7582.
[77] L.K.P. Lam, R.A.H. Hui and J.B. Jones, J. Org. Chem., 51 (1986) 2047–2050.
[78] E. Fukusaki, S. Senda, Y. Nakazono, H. Yuasa and T. Omata, J. Ferment. Biotech., 73 (1992) 284–286.
[79] P. Besse and P. Vescheambre, Tetrahedron, 50 (1994) 8885–8927.
[80] Y. Gao, R.M. Hanson, J.M. Klunder, S.Y. Ko, H. Masamune and K.B. Sharpless, J. Am. Chem. Soc., 109 (1987) 5765–5780.
[81] B.B. Kine and R.W. Novak, Kirk–Othmer Encyclopedia of Chemical Technology, Vol. 1, 1978, pp. 386–480.
[82] T. Matunaga and S. Kono, Jpn. Kokai Tokkyo Koho, (1993).
[83] H. Hasebe, K. Takeuchi and H. Takatsu, Jpn. Kokai Tokkyo Koho, (1993) JP93-129008.
[84] U. Behrens and H.-Z. Kizerow, Polym. Adv. Technol., 5 (1994) 433–437.
[85] T. Budtova and P. Navard, Macromolecules, 29 (1996) 3931–3936.
[86] S. Aranganathan and J.C.T. Kwak, Langmuir, 12 (1996) 1381–1390.
[87] A. Matsuda, J. Sato, H. Yasunaga and Y. Osada, Macromolecules, 27 (1994) 7695–7698.
[88] M. Bryjak, Angew. Makromol. Chem., 215 (1994) 129–138.
[89] J.W. Nemec and W. Bauer, Kirk–Othmer Encyclopedia of Chemical Technology, Vol. 1, 1978, pp. 330–354.
[90] E. Fukusaki, S. Senda, Y. Nakazono, H. Yuasa and T. Omata, Bioorg. Med. Chem. Lett., 2 (1992) 411–414.
[91] T. Kinoshita, H. Akemi, S. Otsuka and Y. Hosaka, Jpn. Kokai Tokkyo Koho, (1990) JP 90-2767998.
[92] A. Yamanaka, S. Otsuka, Y. Hosaka, Eur. Pat. Appl. (1991) EP 91-105933.
[93] H. Onishi and T. Yatagai, Jpn. Kokai Tokkyo Koho (1988) JP 88-101143.
[94] A.L. Margolin, P.A. Fitzpatrick, P.L. Dubin and A.M. Klibanov, J. Am. Chem. Soc., 113 (1991) 4693–4694.
[95] I. Ikeda, J. Tanaka and K. Suzuki, Tetrahedron Lett., 32 (1991) 6865–6866.