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A Synthesis Of (*R*)-Recifeiolide By The Aid Of Biochemical Reaction As The Key-Step†

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Abstract—(*R*)-Recifeiolide, a naturally occurring macrolactone, was synthesized in optically pure form by the aid of biocatalysts. Lipase-catalyzed lactonization of the racemic precursor afforded the desired compound with a concomitant kinetic resolution. The optically active acyclic precursor could be synthesized by the reduction of corresponding ketone with *Pichia farinosa* IAM 4682. The yeast reduction proceeded with the anti-Prelog rule, selecting *si*-face attack on the carbonyl group to give (*R*)-alcohol with >95% e.e.

Introduction

Recifeiolide **1**, a naturally occurring 12-membered lactone (Fig. 1) has been isolated as a metabolite of a fungus *Cephalosporium recifei.*[1] Because of its simple structure which has only one chiral center and *trans* double bond, it serves as a target toward the development of synthetic methodology for medium-sized ring compounds, as demonstrated by a number of chemical syntheses.[2]

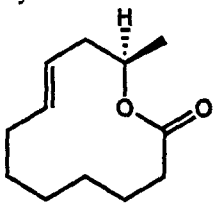
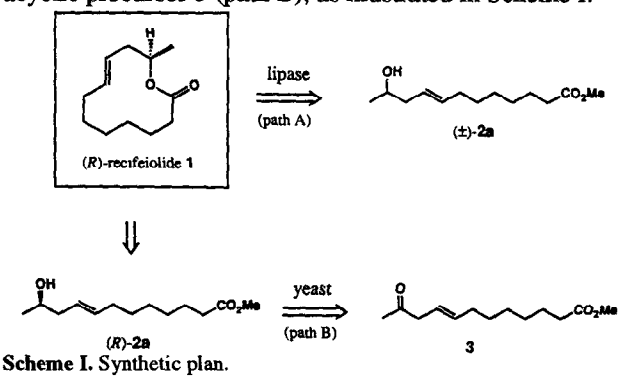


Figure 1. (*R*)-recifeiolide **1**

We have currently been interested in the biocatalysts-aided synthesis of the characteristic structure [$CH_3C(OH)CH_2$-], which is widespread in natural products. Here we disclose our two approaches toward this compound; (1) lipase-catalyzed macrolactonization of the racemic acyclic precursor **2a** (path A), (2) the yeast-mediated reduction of acyclic precursor **3** (path B), as illustrated in Scheme I.



Scheme I. Synthetic plan.

† Preparation of enantiomerically enriched compounds by using Enzymes, Part 18. For Part 17 Watanabe, N.; Sugai, T.; Ohta, H. (1992) *Chem. Lett.*, 667.

‡ On leave from applied technology research laboratory, Asahi Breweries, Ltd. (1991–1993).

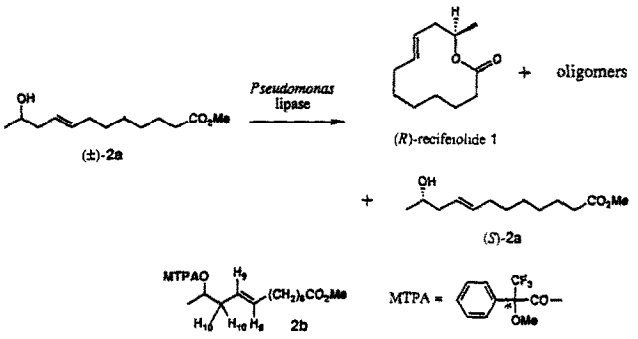
Results and Discussion

Lipase-catalyzed lactonization of racemic precursor

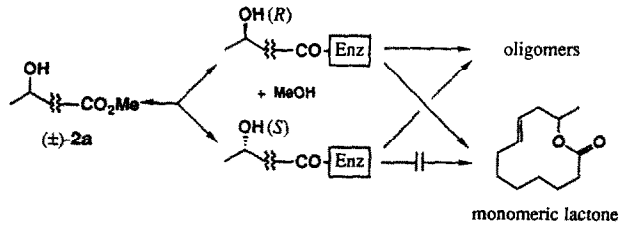
Lipase-catalyzed formation of medium- and large-membered ring lactone in organic solvent has a growing interest.[3] The remarkable advantage of this reaction is the concomitant kinetic resolution to afford the optically active product even when the racemate is used as the starting material, because of the chiral recognition by the enzyme. One of the eventual goals is the application to the synthesis of natural products. A recent report[4] in this field has prompted us to report our own results on the synthesis of natural enantiomer of recifeiolide.

In the present synthesis, the established conditions[3d] for lipase-catalyzed lactonization of racemic corresponding hydroxy ester was applied. Thus, (±)-**2a**[2b] was treated with lipase PS (Amano, from *Pseudomonas* sp.) in isooctane at 65°C for 120 h. The reaction resulted in a mixture of desired monomeric lactone, undesired oligomeric lactones and open chain oligomers, similar to our previous result. The desired monomeric lactone could be isolated and proved to be (*R*)-(+)-recifeiolide **1** (>95%e.e.) $[\alpha]_D^{21}$ +66° (c 0.69, $CHCl_3$) [lit.[2a] $[\alpha]_D$ +70° (c 1, $CHCl_3$)]. The yields of (*R*)-monomeric lactone and recovered starting material [(*S*), 49%e.e.] were 14 and 30%, respectively (Scheme II). Their enantiomeric excess (e.e.) was determined by the ^1H NMR measurement (see experimental) of MTPA ester derivative **2b**. The MTPA ester **2b** from (*R*)-**1**, through alkaline hydrolysis, followed by methylation and MTPA esterification, was a single diastereomer. On the other hand, the diastereomeric excess of **2b** from the recovered **2a** as above was 49%.

Our combined results[3d] indicate that only the substrate–enzyme complex that is derived from (*R*)-enantiomer is allowed to cyclize into monomeric lactone, resulting in the high e.e. of the desired product **1** (Scheme III). This lipase-catalyzed reaction was, however, essentially oriented to the oligomeric products formation. Accordingly, as it was supposed to be difficult to achieve a higher yield, we turned our attention to a different approach.



Scheme II. Lipase-catalyzed macrolactonization.



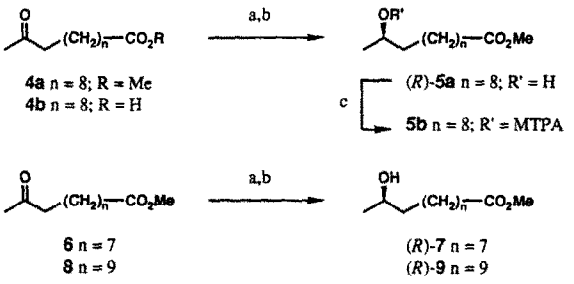
Scheme III. Proposed stereochemical course of the macrolactonization.

Reduction of alkyl methyl ketone by Pichia farinosa *IAM 4682*

Among a number of examples concerning the biocatalyst-mediated reduction[5] of ketones, the stereochemistry of the reduction generally follows the "Prelog"-rule,[6] i.e. the nucleophilic attack occurs from the *re*-face of the carbonyl group, resulting in the formation of the (*S*)-alcohol. As this is also the case for the enzyme-mediated reduction of aliphatic ω-keto esters,[11] these methods cannot be applied directly to the synthesis of natural recifeiolide **1**. However, recently several kinds of microorganism such as *Pseudomonas* sp.,[7] *Lactobacillus kefir*,[8] *Pichia farinosa* IAM 4682[9] and *Geotrichum* sp.,[10] whose facial stereoselectivity is the reverse of the normal one, have been found. In the following experiments, we tried the reduction using *Pichia farinosa*, expecting the desired (*R*)-configuration at the newly created chiral center.

First, the experimental conditions were examined by using a readily accessible saturated substrate, methyl 11-oxododecanoate **4a**, instead of the precursor for recifeiolide. The reduction of **4a** proceeded to give a hydroxy acid with the concomitant hydrolysis of the methyl ester. The hydroxy ester **5a** was obtained in 14% yield after methylation with diazomethane. The product was, to our disappointment, completely racemic, which was confirmed by the ^{1}H NMR analysis of MTPA ester **5b**. After extensive examination of the incubation conditions, an anaerobic condition was found to be effective for an enhancement of the selectivity. The desired (*R*)-**5a** was obtained, although neither the yield nor e.e. was satisfactory. The use of analogous substrates **6** and **8** brought about almost the same results.

Table 1. The microbial reduction of ω-keto esters



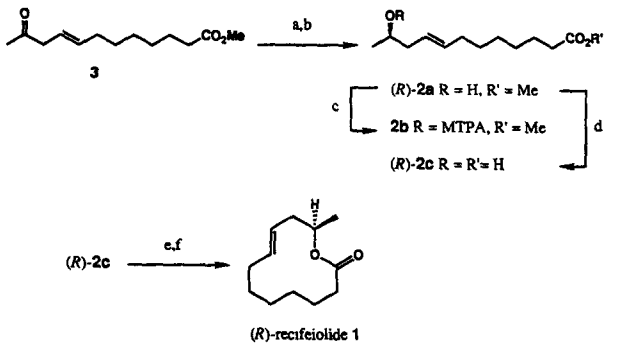
Substrate	Condition	Incubation (h)	Yield (%)	%e.e.
4a	aerobic	48	14	0
4a	anaerobic	48	16	47
6	anaerobic	48	28	33
8	anaerobic	48	24	44
4a	anaerobic	12	54	61
8	anaerobic	12	50	65
4b	anaerobic	12	48	68

a) *Pichia farinosa* IAM 4682.
b) CH_2N_2.
c) MTPA-Cl/pyridine.

Both the yield and the e.e. of the product were enhanced to 54% and 61%e.e. respectively, simply by shortening the incubation period from 48 to 12 h. The modification was also effective when homolog **8** was subjected as the substrate. In all cases so far, the methyl ester is easily cleaved by hydrolytic enzyme in yeast prior to the reduction.[14] In the controlling experiment, the corresponding keto acid **4b** was converted to a product with similar e.e. These results suggest that the direct substrate in the reduction was keto acid **4b**, even when keto ester **4a** was used as the substrate. The prolonged incubation, especially under the aerobic condition might cause the degradation of the desired (*R*)-hydroxy acid.

Application to the acyclic precursor and the synthesis of (R)-*recifeiolide*

Based on the incubation condition examined so far, the yeast-mediated reduction was applied to the synthesis of acyclic precursor (*R*)-**2a**. The reduction of **3** smoothly proceeded to give (*R*)-**2a** $[\alpha]_D^{21}$ -7.9° (c 1.06, $CHCl_3$) [lit.[2a] $[\alpha]_D$ -9° (c 1, $CHCl_3$)] in 48% yield. The higher e.e. (>95%) of the product indicates that the selectivity is affected by a β,γ-unsaturated bond in the substrate. Similar results have also been observed in the case of related compounds.[9] Starting from (*R*)-**2a**, the corresponding hydroxy acid **2c**,was chemically cyclized into the eventual target molecule, (*R*)-recifeiolide **1** via Yamaguchi's lactonization[12] in 72% yield; $[\alpha]_D^{18}$+67°(c 0.60, $CHCl_3$) (Scheme IV).



Scheme IV. Reduction of acyclic precursor and the synthesis of (*R*)-recifeiolide.

Experimental

All b.p.s were uncorrected. IR spectra were measured as films on a Jasco IRA-202 spectrometer. ^{1}H NMR spectra were measured in $CDCl_3$ with TMS as the internal standard at 90 MHz on a JEOL JNM FX-90 spectrometer, at 270 MHz on a JEOL JNM EX-270, or at 400 MHz on a JEOL JNM GX-400 spectrometer. Optical rotations were recorded on a Jasco DIP 360 polarimeter. Mass spectra were recorded on a Hitachi M-80 spectrometer at 70 eV. Silica gel 60 K070-WH (70–230 mesh) of the Katayama Chemical Co. was used for column chromatography.

Lipase-catalyzed lactonization of methyl (E)-(±)-*11-hydroxy-8-dodecenoate* **2a**

To a solution of (±)-**2a** (238 mg, 1.04 mmol) in dry isooctane (1000 mL) was added *Pseudomonas* lipase (Amano, lipase PS, 1 g) and powdered molecular sieves 4A (2 g). The suspension was stirred at 65°C for 5 days. The enzyme and molecular sieves were filtered off, and the filtrate was concentrated *in vacuo*. The residue was repeatedly chromatographed on silica gel to give pure (*R*)-**1** and (*S*)-**2a.**

(*R*)-**1** (29 mg, 14%): b.p. 130°C/3 Torr (bulb-to-bulb distillation), $[\alpha]_D^{21}$ +66° (c 0.69, $CHCl_3$) [lit.[4a] $[\alpha]_D$ +70° (c 1, $CHCl_3$)]; IR νmax 1720, 1435, 1360, 1215, 1150, 965 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.0–1.7 (8H, br), 1.22 (3H, d, J = 6.6 Hz), 1.7–2.5 (6H, br), 4.95–5.55 (3H, br); MS: m/z 196.1481. Calc. for $C_{12}H_{20}O_2$ (M^+): 196.1463. Its IR and NMR spectra were in good accordance with those reported previously.[2a] The *e.e.* was estimated to be >95% by the NMR analysis of **2b**.

2b from (±)-**2a**: δ (400 MHz, $CDCl_3$, H_{10} irradiation) 5.51 (0.5H, d, $J_{8\text{-}9}$ =15.2 Hz, H_9), 5.43 (0.5H, d, $J_{8\text{-}9}$ =15.2 Hz, H_9). **2b** from (*R*)-**1** through alkaline hydrolysis followed by methylation and MTPA esterification: The signal at δ 5.43 was not detected.

(*S*)-**2a** (72 mg, 30%): $[\alpha]_D^{21}$+4.6° (c 3.03, $CHCl_3$) [lit.[2a] $[\alpha]_D$ +7.5° (c 1, $CHCl_3$)]. Its IR and ^{1}H NMR spectra were identical with those of the starting material. MS: m/z 210.1646. Calc. for $C_{13}H_{22}O_2$ (M^+–H_2O): 210.1620. ^{1}H NMR analysis of **2b** from (*S*)-**2a**; δ (400 MHz, $CDCl_3$, H_{10} irradiation) 5.51 (0.24H, d, $J_{8\text{-}9}$ =15.2 Hz, H9), 5.43 (0.76H, d, $J_{8\text{-}9}$ =15.2 Hz, H9).

Reduction of ω-keto esters and acids with Pichia farinosa *IAM 4682*

Pichia farinosa IAM 4682 was incubated according to the reported procedure.[9a] The washed wet cells (4 g) were suspended in phosphate buffer solution (pH 6.5, 0.1M, 12.5 mL). To this was added glucose (2 g) and the mixture was shaken for 30 min (150 cpm) at 30°C. Then **4a** (100 mg, 0.44 mmol) was added and the mixture was further shaken for 12 h at 30°C under Ar. After acidification, the mixture was centrifuged (3000 rpm) and the supernatant was extracted with ether after saturating with NaCl. The precipitated cells were sonicated in acetone and filtered. The filtrate was concentrated *in vacuo* and the residue was extracted with ether. Solid material on the filter was further extracted with ether by applying sonication. The organic extracts were combined and washed with water and brine, dried over Na_2SO_4 and concentrated *in vacuo*. After the treatment with etherial solution of diazomethane, the residue was purified by SiO_2 flash column chromatography (10 g). Elution with hexane/EtOAc (3/1) afforded **5a** (54 mg, 54%); $[\alpha]_D^{22}$ -4.7° (c 1.2, $CHCl_3$); IR νmax 3400, 1735, 1190, 1170 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.18 (3H, d, J = 6.3 Hz), 1.20–1.70 (16H, br), 2.30 (2H, t, J = 7.6 Hz), 3.67 (3H, s), 3.78 (lH, m); MS: m/z 213.1850. Calc. for $C_{13}H_{25}O_2$ (M^+–OH): 213.1855. The e.e. was estimated to be 61% by the ^{1}H NMR analysis of **5b**: δ (400 MHz, $CDCl_3$) 3.55 (2.42H, broad s, MTPA–OCH_3), 3.57 (0.58H, broad s, MTPA–OCH_3). An authentic sample of (*S*)-**5a** was prepared by the catalytic hydrogenation of (*S*)-**2a** obtained from the recovery of lipase-catalyzed lactonization as above; $[\alpha]_D^{20}$ +2.5° (c 1.4, $CHCl_3$).

5a from **4b**.(52 mg, 48%): $[\alpha]_D^{27}$ -4.9° (c 1.56, $CHCl_3$). ^{1}H NMR analysis of **5b**: δ (400 MHz, $CDCl_3$) 3.55 (2.52H), 3.57 (0.48H); 68%e.e.

Other reactions were carried out according to the conditions listed in Table 1; **7** (28 mg, 28%). $[\alpha]_D^{18}$ -1.9° (c 0.96, $CHCl_3$); IR νmax 3400, 1735, 1195, 1170 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.18 (3H, d, J = 6.3 Hz), 1.20–1.70 (14H, br), 2.30 (2H, t, J =7.4 Hz), 3.67 (3H, s), 3.78 (lH, m); MS: m/z 199.1667. Calc for $C_{12}H_{23}O_2$ (M^+–OH): 199.1699. ^{1}H NMR analysis of the corresponding MTPA ester: δ (400 MHz, $CDCl_3$) 3.55 (2.00H, broad s, MTPA–OCH_3), 3.57 (1.00H, broad s, MTPA–OCH_3); 33°% e.e.

9 (50 mg, 50%). $[\alpha]_D^{20}$ -4.3° (c 1.38, $CHCl_3$); IR νmax 3350, 1740, 1190, 1170 cm^{-1}; ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.19 (3H, d, J = 6.3 Hz), 1.20–1.75 (18H, br), 2.30 (2H, t, J = 7.6 Hz), 3.66 (3H, s), 3.78 (lH, m); MS: m/z 227.1988. Calc. for $C_{14}H_{27}O_2$ (M^+–OH): 227.2012.

This was converted to (*R*)-(–)-tridecane-1,12-diol by the reduction with $LiAlH_4$, $[\alpha]_D^{22}$ -3.2° (c 1.49, MeOH) [lit.[15] $[\alpha]_D^{20}$ -6.2° (c 0.939, MeOH)]; IR νmax 3300, 1120, 1040 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) δ 1.18 (3H, d, J = 6.3 Hz), 1.20–1.80 (22H, br), 3.55 (2H, t, J = 7.2 Hz), 3.70 (lH, m). Its IR and NMR spectra were in good accordance with those reported previously.[15] ^1H NMR analysis of the corresponding MTPA ester of **9**: δ (400 MHz, $CDCl_3$) 3.55 (2.48H, broad s, MTPA–OCH_3), 3.57 (0.53H, broad s, MTPA–OCH_3); 65%e.e. The configuration of the newly created chiral center of **7** was concluded to be (*R*), because of the minus sign of optical rotation, as well as the fact that the area of the signal at high field is larger than that of the signal at low field which was consistent with (*R*)-**5a** and (*R*)-**9**.

Methyl (R)*-(–)-11-hydroxy-8-dodecenoate* ***2a***

Ketone **3** [250 mg, 1.11mmol, prepared by the Swern oxidation of (±)-**2a**] was incubated with *Pichia farinosa* IAM 4682 for 18 h in a similar manner to that described above. (*R*)-**2a** (120 mg, 48%): $[\alpha]_D^{21}$ -7.4° (c 1.06, $CHCl_3$) [lit.[2a] $[\alpha]_D$ -9° (c 1, $CHCl_3$)]. Its IR and ^1H NMR spectra were identical with those of (±)-**2a**. MS: m/z 228. 1750. Calc. for $C_{13}H_{24}O_3$ (M^+): 228.1752. ^1H NMR analysis of **2b** from (*R*)-**2a**; δ (400 MHz, $CDCl_3$, H_{10} irradiation) 5.51 (lH, d, $J_{8\text{-}9}$ =15.2 Hz, H_9, single signal); >95%e.e.

(R)*-Recifeiolide* ***1*** *from* (R)*-**2a***

To a stirred solution of (*R*)-**2a** (110 mg, 0.48 mmol) in methanol (2 mL) was added a solution of NaOH (3N, 2 mL) and the mixture was stirred under reflux for 2 h. After acidification, the mixture was extracted three times with ether. The organic layer was washed twice with water, dried over Na_2SO_4 and concentrated *in vacuo* to give crude **2c** (98 mg, 95%); IR νmax 3400, 1710, 970 cm^{-1}; ^1H NMR (270 MHz, $CDCl_3$) δ 1.19 (3H, d, J = 5.9 Hz), 1.20–1.70 (8H, br), 1.90–2.30 (4H, br), 2.34 (2H, t, J= 7.4 Hz), 3.80 (lH, tq, J = 5.9, 6.4 Hz), 5.42 (lH, dt, J= 15.1, 6.9 Hz), 5.53 (lH, dt, J = 15.1, 6.4 Hz). This was employed for the next step without further purification.

A mixture of **2c** (50 mg, 0.23 mmol), 2,4,6-trichlorobenzoyl chloride (62 mg, 0.26 mmol), triethylamine (28.3 mg) and THF (4 mL) was stirred for 2h at room temperature. The mixture was filtered to remove triethylamine hydrochloride, and the filtrate was diluted with toluene (100 mL). This solution was added dropwise to a solution of 4-(*N,N*-dimethylamino)pyridine (124 mg) in toluene (25 mL) over a period of 1.5 h. The mixture was concentrated *in vacuo*, and the residue was diluted with water and extracted with ether. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel (20 g). Elution with hexane/EtOAc (10/1) afforded (*R*)-**l** [35 mg, 76% from (*R*)-**2a**]: b.p. 130°C/3 Torr (bulb-to-bulb distillation), $[\alpha]_D^{21}$ +67.0° (c 0.60, $CHCl_3$) [lit.[2a] $[\alpha]_D$ +70° (c 1, $CHCl_3$)]. Its IR and ^1H NMR were identical with those obtained by the lipase-catalyzed lactonization. MS: m/z 196.1466. Calc. for $C_{12}H_{20}O_2$ (M^+): 196.1463. The e.e. was estimated to be >95% by the NMR analysis of **2b**.

Acknowledgements

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References

1. Vesonder, R. F.; Stodola, F. H.; Wickerham, L. J.; Ellis, J. J.; Rohwedder, W. K. (1971) *Can. J. Chem.* **49**, 2029.

2. (a) Gerlach, H.; Oertle, K.; Thalman, A. (1976) *Helv. Chim. Acta* **59**, 755; (b) Narasaka, K.; Yamaguchi, M.; Mukaiyama, T. (1977) *Chem. Lett.* 959; (c) Utimoto, K.; Uchida, K.; Yamaya, M.; Nozaki, H. (1977) *Tetrahedron Lett.* 3641; (d) Schreiber, S. L. (1980) *J. Am. Chem. Soc.* **102**, 6163; (e) Kaino, M.; Naruse, Y.; Ishihara, K.; Yamamoto, H. (1990) *J. Org,. Chem.* **55**, 5814. For other syntheses, see a review: Boeckman, Jr, R. K.; Goldstein, S. W. The total synthesis of macrocyclic lactones, In *The Total Synthesis of Natural Products*, Vol. 7, pp. 1–139, Ed. J. ApSimon, Wiley, New York (1988).

3. (a) Makita, A.; Nihira, T.; Yamada, Y. (1987) *Tetrahedron Lett.* **28**, 805; (b) Guo, Z.-W.; Ngooi, T. K.; Scilimati, A.; Fülling, G.; Sih, C. J. (1988) *Tetrahedron Lett.* **29**, 5583; (c) Guo, Z.-W.; Sih, C. J. (1988) *J. Am. Chem. Soc.* **110**, 1999; (d) Yamada, H.; Ohsawa, S.; Sugai, T.; Ohta, H.; Yoshikawa, S. (1989) *Chem. Lett.* 1775. Recent reviews, see: Chen, C.-S.; Sih, C. J. (1989) *Angew. Chem., Int Ed. Engl.* **28**, 695.

4. Mori, K.; Tomioka, H. (1992) *Liebigs Ann. Chem.* 1011. Lipase-catalyzed macrolactonization was applied to the syntheses of several insect pheromones.

5. (a) Servi, S. (1990) *Synthesis* 1; (b) Csuk, R.; Glänzer, B. I. (1991) *Chem. Rev.* **91**, 49

6. (a) Prelog, V. (1964) *Pure Appl. Chem.* **9**, 119; (b) MacLeod, R.; Prosser, H.; Fikentscher, L.; Lanyi, J.; Mosher, H. S. (1964) *Biochemistry* **3**, 838; see also (c) Shieh, W.-R.; Gopalan, A. S.; Sih, C. J. (1985) *J. Am. Chem. Soc.* **107**, 2993, and the references cited therein.

7. (a) Shen, G.-J.; Wang, Y.-F.; Bradshaw, C. W.; Wong, C.-H. (1990) *J. Chem. Soc., Chem. Commun.* 677; (b) Bradshaw, C. W.; Fu, H.; Shen, G.-J.; Wong, C.-H. (1992) *J. Org. Chem.* **57**, 1526.

8. Bradshaw, C. W.; Hummel, W.; Wong, C.-H. (1992) *J. Org. Chem.* **57**, 1532.

9. (a) Sugai, T.; Ohta, H. (1990) *Agric. Biol. Chem.* **54**, 1577; (b) Sugai, T.; Yokochi, T.; Watanabe, N.; Ohta. H. (1991) *Tetrahedron* **47**, 7227.

10. Gu, J.-X.; Li, Z.-Y.; Lin, G.-Q. (1992) *Tetrahedron:Asymmetry* **3**, 1523.

11. (a) Keinan, E.; Seth, K. K.; Lamed, R.; Ghirlando, R.; Singh, S. P. (1990) *Biocatalysis* **3**, 57; (b) Keinan, E.;

Sinha, S. C.; Sinha-Bagchi, A. (1991) *J. Chem. Soc., Perkin I* 3333; (c) Naoshima, Y.; Hasegawa, H.; Nishiyama, T.; Nakamura, A. (1989) *Bull. Chem. Soc. Jpn.* **62**, 608.

12. Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. (1979) *Bull. Chem. Soc. Jpn.* **52**, 1989.

13. Matsumoto, K.; Tsutsumi, S.; Ihori, T.; Ohta, H. (1990) *J. Am. Chem. Soc.* **112**, 9614, and the references cited therein.

14. Sugai, T.; Sakuma, D.; Kobayashi, N.; Ohta, H. (1991) *Tetrahedron* **47**, 7237.

15. Quinkert, G.; Heim, N.; Bats, J. W.; Oschkinat, H.; Kessler, H. (1985) *Angew. Chem., Int. Ed. Engl.* **24**, 987.