Studies on the Asymmetric Synthesis of Optically Active Odorants

(光学活性な香気成分の不斉合成研究)

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THESIS

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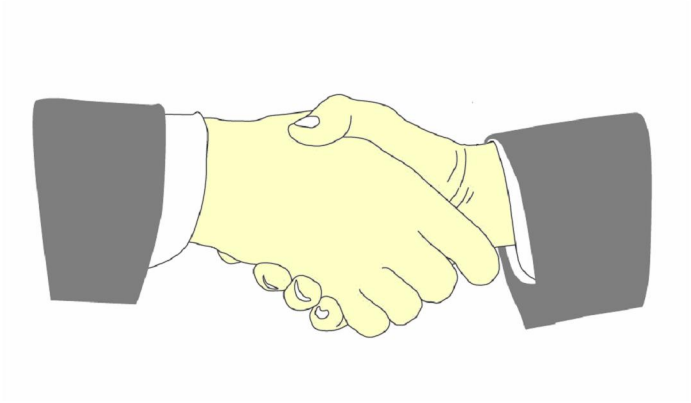
List of Abbreviations

Ac	Acetyl
Bn	Benzyl
Bz	Benzoyl
d.e.	diastereomeric excess
DME	Dimethyl Ether
DMSO	Dimethyl Sulfoxide
e.e.	enantiomeric excess
Et	Ethyl
FT	Fourier Transform
G.C.	Gas Chromatography
h	hour
HMDS	Hexamethyldisilazane
***i*-Pr**	isopropyl
LAH	Lithium Aluminum Hydride
LPS	Lipase *Pseudomonas* sp.
***m*-CPBA**	*meta*-chloroperoxybenzoic acid
Me	Methyl
MTPA	α-Methoxy-α-(trifluoromethyl)phenylacetyl
N.R.	No Reaction
***n*-Bu**	normal butyl
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Effects
P-buffer	sodium phosphate buffer
Ph	Phenyl
pH	Hydrogen ion concentration
PMB	*para*-methoxybenzyl
Py.	pyridine

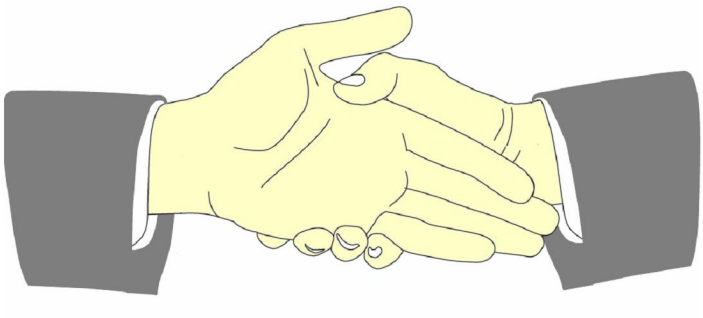
rpm	Rotations Per Minute
SMe_2	dimethyl sulfide
sp.	species
sub	substrate
r.t.	Room temperature
TBDMS	tertiary butyl dimethyl silyl
***t*-Bu**	tertiary butyl
THF	Tetrahydrofuran
TLC	Thin-Layer Chromatgraphy
TMEDA	Tetramethylethylenediamine
TMS	Tetramethylsilane
Ts	Tosyl
USD	United States Dollar
YPD	Yeast Peptone Dextrose

General Introduction

All living things in nature, including humans, have optically active molecules. The molecules in our body can recognize and interact with each other and transfer informations in a manner governed by their specific chirality (Fig. 1). The optical purity of sugars in DNA and RNA, and all amino acids in proteins is necessary for their chiral recognition to function. If the polypeptides were a racemate, i.e. if they contain a mixture of chiralities, they can then not form the specific structures necessary for enzyme production. Similarly, if even a single wrong-handed sugar were present in a DNA or RNA helix, stabilization would not be possible and they could not support life anymore.



(*R*)-(*R*) or (*S*)-(*S*) : well-matched



(*R*)-(*S*) or (*S*)-(*R*) : mismatched

Fig. 1. Chiral recognition.

Not only the sugars or amino acids, there are many chiral materials that promote or inhibit biological reactions in living systems. Because the different enantiomers or diastereomers of these materials often have different biological activities, preparing them as a single isomer is very important. It is possible to obtain them as single isomers from nature, but the extraction in a large quantity is typically very hard. Therefore chemical synthesis is indispensable. There are a lot of studies on the asymmetric synthesis of these biologically active materials, such as drugs, pheromones, and odorants.

In particular, I am interested in the synthesis of optically active odorants, since the olfactory system of humans and animals can discriminate between optical isomers. The receptors in our nose are very sensitive to distinguish both enantiomers according to their odor (Fig. 2). [1]

Fig 2. Different odor between enantiomers.

Generally, the methods for preparing a chiral product can divide into two parts. One is using bioconversion and the other is chemical synthesis. Bioconversion is a technique for producing a desired compound, using a cell, an immobilized cell or an immobilized enzyme in the chemical process (Fig. 3). Usually, this method requires lower substrate concentration and longer reaction time than a chemical conversion. But, it can be used very efficient for producing a compound which has regio-, stereo- or/and enantiospecificity.[2] Furthermore the reaction proceeds under mild conditions and the

produced byproducts have low toxicity.[3] In addition, it also allows us to produce a product in one step, which normally requires complex and difficult chemical reactions. Hence, the bioconversion process has an important part in the preparation methodology of fine chemical compounds.[4,5]

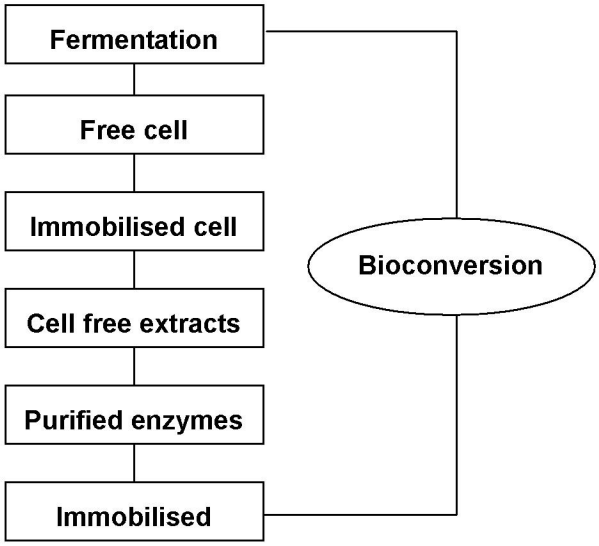


Fig. 3. Bioconversion systems

In the chemical synthesis, a lot of studies and methods for synthesis of optically active compounds have been reported. Among them, asymmetric alkylation is the most widely used process for preparing a chiral compound (Fig. 4).[6] Generally, this reaction occurs as nucleophilic substitution between a nuclophilic carbon such as ketone enolates, imines, enamines or allylic compounds and an electrophilic alkylating agent in an S_N2 or S_N2' manner. Although they are important in producing versatile organic compounds, asymmetric alkylations are still less developed, and still not well understood.[7] In particular, despite a long period of interest, S_N2' reactions are only in limited use because of their low regio- and stereoselectivities,[8] though this can be controlled by catalysts, electrophiles or leaving groups. [9]

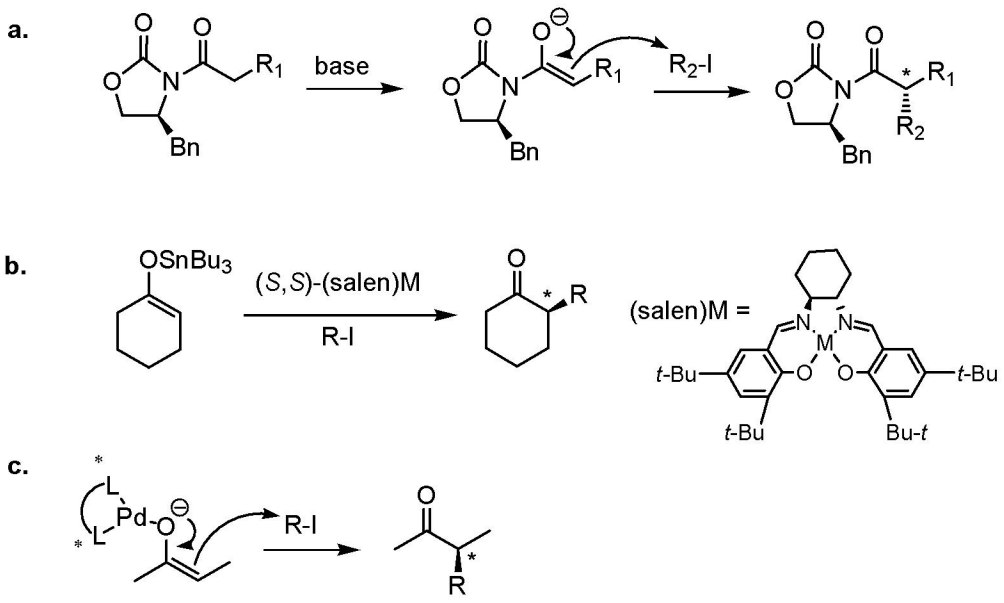


Fig. 4. Examples of asymmetric alkylation.

a. Evans, D. A. *Aldrichimica Acta*, **1982**, *15*, 23.

b. Doyle A. G., Jacobsen E. N., *J. Am. Chem. Soc.*, **2004**, *127*, 62-63.

c. Trost B. M., Xu, J., Schmidt T., *J. Am. Chem. Soc.*, **2008**, *130*, 11852-11853.

In this study, I tried to synthesize optically active odorants by using both bio-conversion and chemical synthesis. With the bioconversion method, I synthesized asymmetric alcohol with high optical purity. Then this chiral alcohol was used to synthesize the scent of an African orchid.

By chemical synthesis, a new chiral intermediate for the stereoselective S_N2' reaction was developed. And with this process, synthesis of the optically active δ-nonalactones that have sweet milky flavor was achieved with good enantioselectivity.

Chapter I-1

Chemo-biological preparation of (*R*)-4-acetoxy-2-methyl-1-butanol

1. Introduction

One of the most common process in bioconversion is the reduction of ketone (C=O) or olefin (C=C). Some highly selective asymmetric reductions of unsaturated carbon such as ketone or olefin by microbes have been reported (Fig. 5). Based on theses studies, I envisaged that these microbes are also applicable to the enantioselective hydrogenation of *exo*-olefin of asymmetric alcohol (Fig. 6).

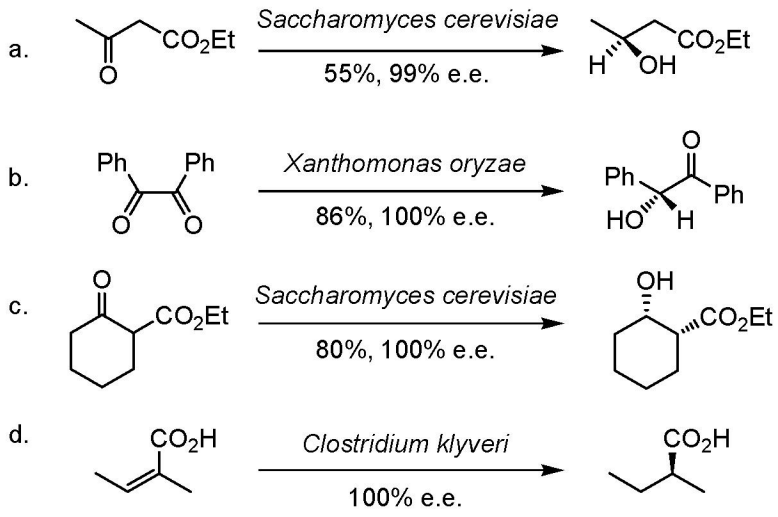


Fig. 5. Asymmetric reduction of unsaturated carbon by microbes.

a. C. Fuganti, D. Ghitinghelli, P. Grasselli, *J. Chem. Soc. Chem. Commun*, **1975**, 846.

b. J. Konishi, H. Ohta, G. Tsuchihashi, *Chem. Lett*, **1985**, 1111.

c. D. Buisson, R. Azerad. *Tetrahedron ; Asymmetry*, **1996**, *7*, 9.

d. B. Rambeck, H. Simon, *Angewandte chime*, **1974**, *86*, 675.

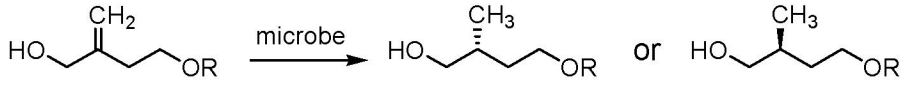


Fig. 6. Strategy of bioconversion.

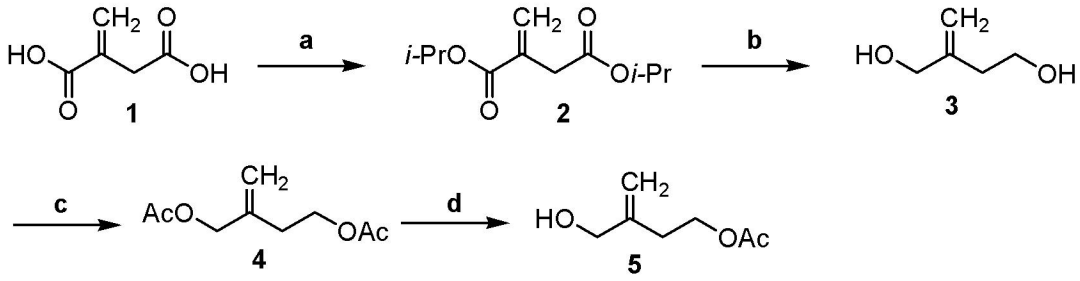
With the substrate 4-acetoxy-2-methylene-1-butanol (**5**), I screened microbes which can reduce the double bond. And I tried to use the growing cells instead of isolated enzymes, because, when enzymes are purified, some of them are unstable out of the cell system,[10] and require co-substrates or co-enzymes. However, when microbial whole cells are used, these problems are not present.[11]

The desired optically active alcohol, 4-acetoxy-2-methyl-1-butanol (**6**), is not commercially available, and a similar compound (*R*)-2-methyl-1,4-butanediol (>98.0%, G.C.) is on sale at the TCI (Tokyo Chemical Industry, Co., LTD) with a prohibitively expensive price (5 g, 162.50 USD). So, my goal was to develop the biotransformation from the cheap natural compound, itaconic acid.

2. Results and discussion

2-1. Synthesis of 4-Acetoxy-2-methylene-1-butanol (5).

Substrate **5** for the bioconversion was synthesized from itaconic acid by the sequence outlined in Scheme 1.



a) H_2SO_4, 2-propanol, reflux, 30 h, 98%, b) AlH_3, Et_2O, -30 °C, 5 min, c) Ac_2O, Py., CH_2Cl_2, 5 h, 2 steps 98%, d) LPS, P-buffer (pH 7.0), 5 min, r.t., 71%

Scheme 1. Synthesis of substrate **5**.

Itaconic acid **1** was esterified with 2-propanol in the presence of sulfuric acid to afford diisopropyl ester **2**. The reduction of the ester groups of **2** needs a suitable reducing reagent to avoid reduction of the double bond. Because it is hard to separate **3** from over-reduced product **3'**, whose contamination would be a major cause of low optical purity of the bioconversion product (**6**). However, aluminum hydride prepared from $AlCl_3$ and 3 equivalents of LAH, was formed to be a suitable reducing agent to solve this problem, while the reduction of ester **2** with LAH afforded **3'** (Fig. 7).

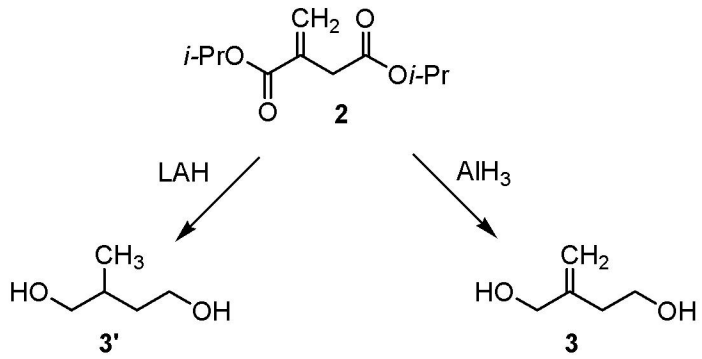


Fig. 7. Reduction of 2-methylene-succinic acid diisopropyl ester **2**.

Diol **3** was acetylated with acetic anhydride to give diacetate **4**, and the allylic acetate of **4** was selectively hydrolyzed with LPS in a sodium phosphate buffer (pH 7.0). In this reaction, over-hydrolysis to afford **3** was observed. Therefore, the reaction conditions were investigated to improve the regioselectivity (Fig. 8).

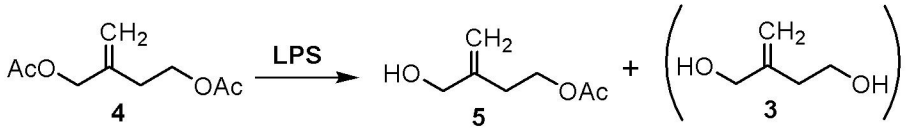


Fig. 8. Hydrolysis of **4**.

At first, the effect of the condition of LPS in the P-buffer was examined and the results are shown in Fig. 9. The best result was achieved when 200 mg (1.10 mmol) of substrate was treated with 200 mg of LPS in 10 ml of P-buffer (pH 7.0). Based on this result, the effect of the amount of substrate was also tested (Fig. 10). However, they showed very similar results regardless of the substrate concentration. Hence, this reaction has dependence only on the concentration of LPS, but not on that of the substrate in the buffer.

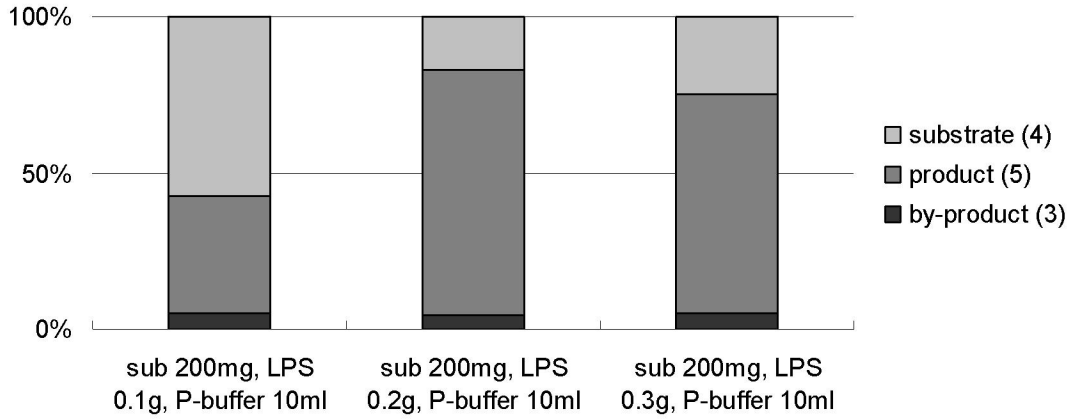


Fig. 9. Effect of LPS concentration in the P-buffer (pH 7.0)

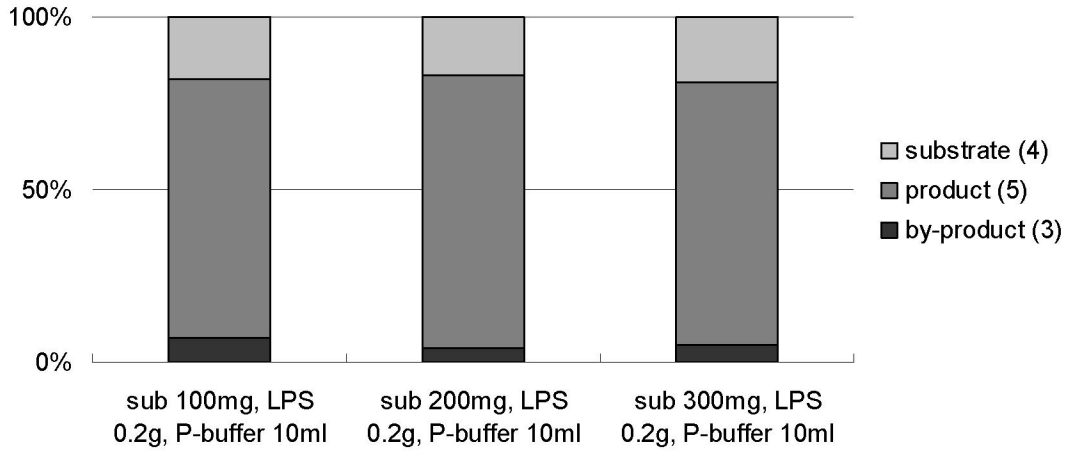


Fig. 10. Effect of substrate concentration in the P-buffer (pH 7.0).

2-2. Synthesis of (*R*)-4-Acetoxy-2-methyl-1-butanol (6).

The purpose of this study is only to reduce the double bond of **5** by microbe to get **6**. But, most microbes have hydrolase as well as reductase. Therefore, substrate **5** and product **6** are possible to be hydrolyzed to diol (Fig. 11), and it was necessary to find a microbe which reduce double bond stereoselectively without hydrolyzing acetyl group.

The screening of microbes for bioconversion of **5** was carried out with 6 kinds of cultured cells, *Candida rugosa*, *Pseudomonas putida*, *Alcaligenes* sp., *Achromobacte* sp., *Chrombacterium* sp. and *Kleuveromyces fragilis*. To each culture, substrate **5** was added and cultured at the proper temperature. After 20 h, the culture media were extracted with EtOAc and the organic phase was checked by G.C.

The results are summarized in Table 1 and *Pseudomonas putida* (entry 2) gave the best result. So this microbe was chosen for the further optimization.

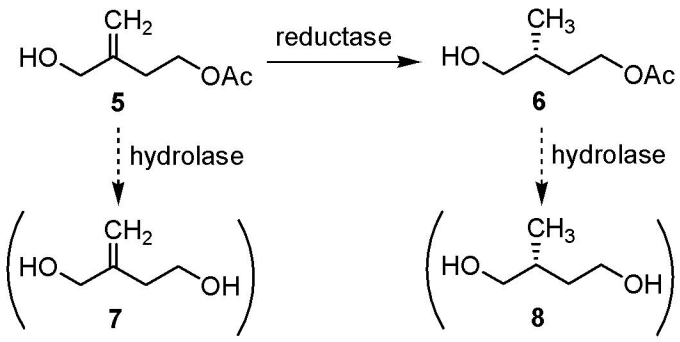


Fig. 11. Bioconversion of **5** to **6** and byproducts.

Table 1. Screening of microbes for bioconversion.

Entry	Microbe	Conversion (%)*			
		5	**6**	**7**	**8**
1	*Candida rugosa*	95	5	0	0
2	*Pseudomonas putida*	2	73	15	10
3	*Alcaligenes* sp.	93	7	0	0
4	*Achromobacter* sp.	68	31	0	0
5	*Chrombacterium* sp.	75	25	0	0
6	*Kleuveromyces fragilis*	20	65	10	5

* Determineded by G.C.

The growth curve of *P. putida* after adding seed cultured medium to YPD media (30°C, 180 rpm, 20 h) is shown in Fig. 12. To know the best time for starting the biotransformation, 4 kinds of biotransformation systems were examined (Fig. 12. **a**~**d**). The substrate (**5**) was added 0, 4, 6 and 10 h later than the addition of the seed cultured medium, and the products of all samples were checked by G.C. after 20 h. When the substrate was added at the same time of addition of seed cultured medium (Fig. 12. **a**) showed best result, and when the substrate was added after 6 h or 10 h (Fig. 12. **c**, **d**), no double bond reduction but only hydrolysis was occurred.

The effect of pH of the culturing medium was also examined. The pH was adjusted by adding 10% NaOH and the best result was achieved at pH 7.1 (Fig. 13). Time course of the biotransformation is also shown in Fig. 13. When culturing was kept after exponential phase, the hydrolysis to byproducts **7** and **8** from **5** and **6**, respectively, was observed (Figs. 11, 14). So, it was necessary to check the growing medium regularly and the process was stopped immediately when the byproducts are observed by G.C.

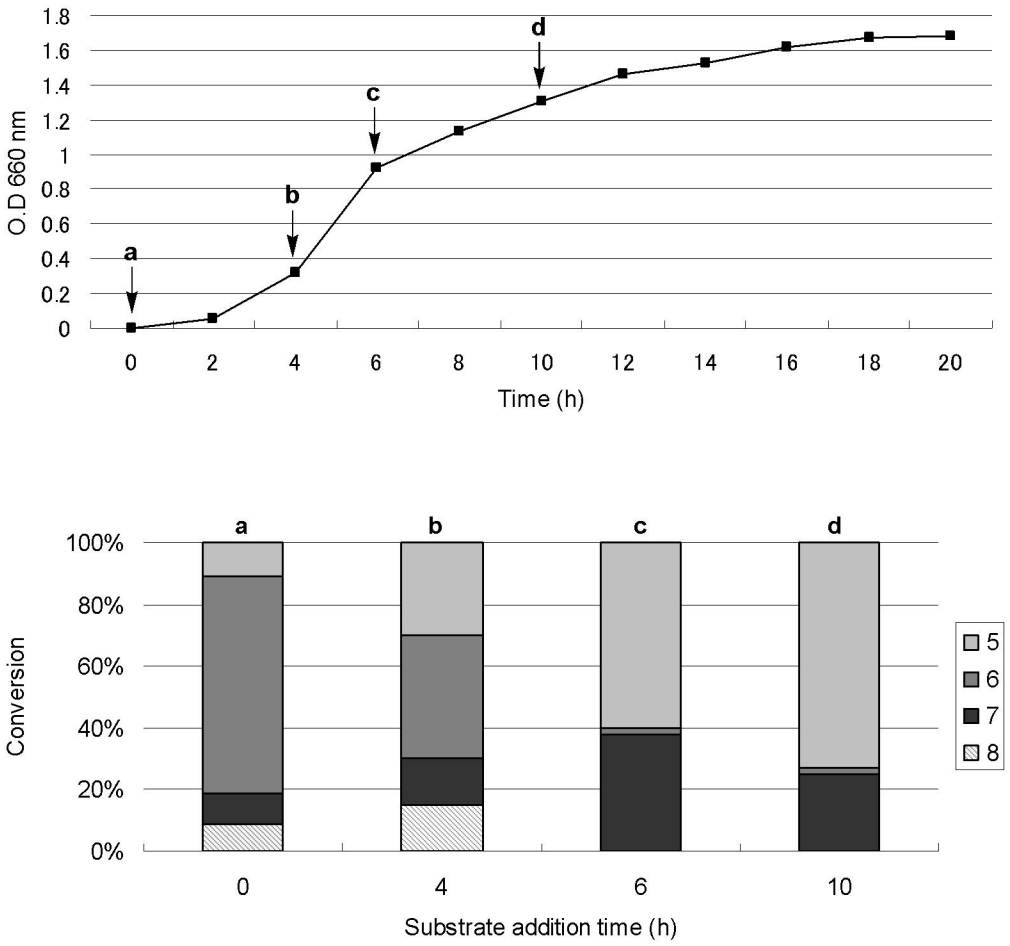


Fig. 12. Growth curve of *P. putida*. and substrate (**5**) addition time.

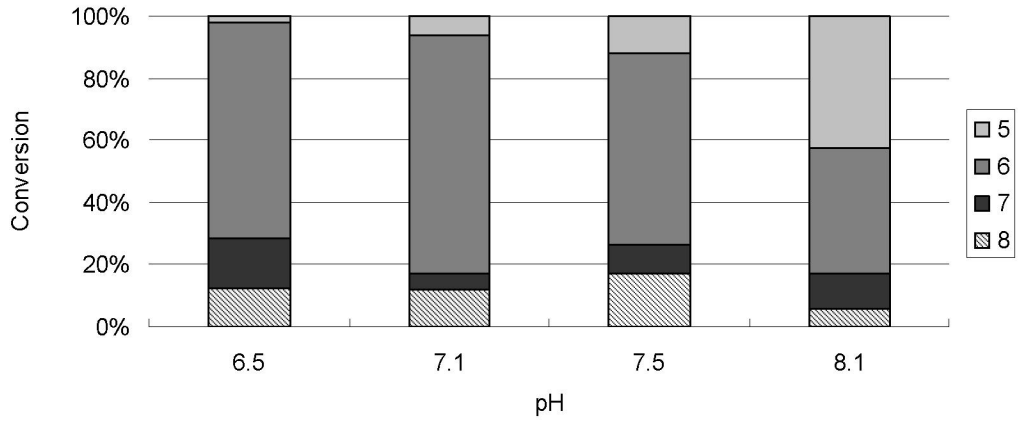


Fig. 13. Effect of pH of culturing medium.

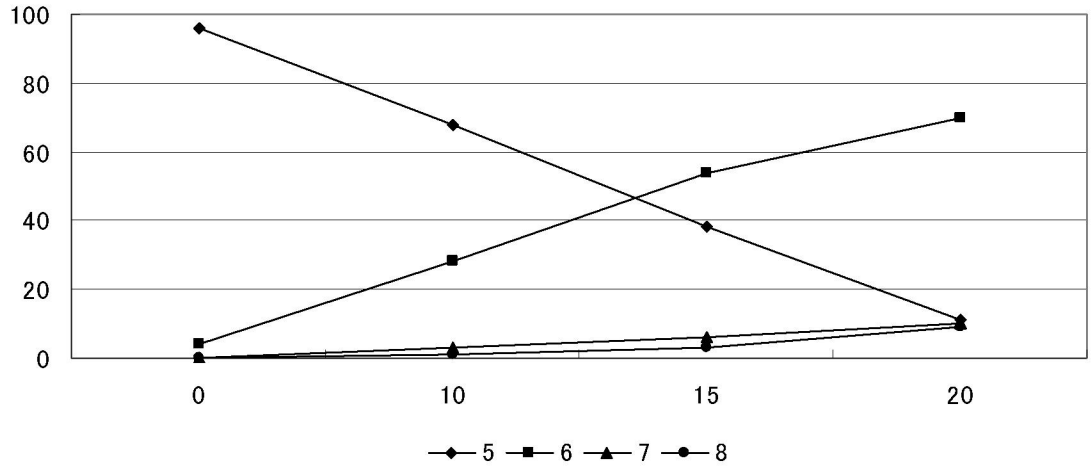
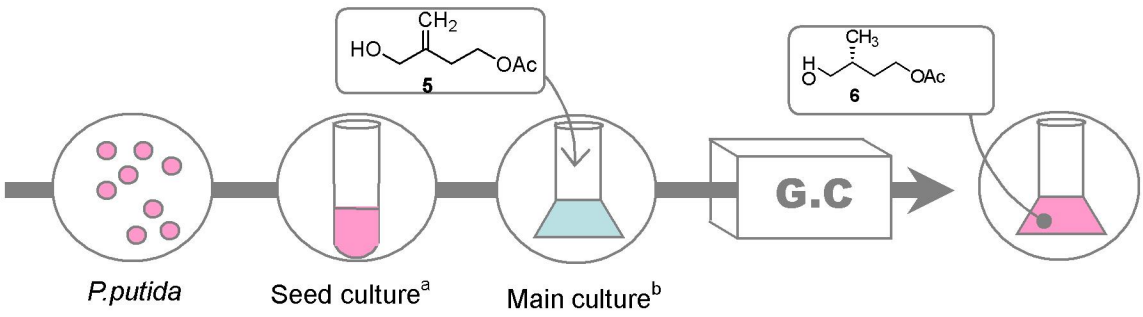


Fig. 14. Time course of bioconversion tendency of **5** by *P. putida**.

* YPD media, 30°C, 180 rpm

The best reaction condition was obtained when using a 18 h cultured seed culture medium 1.50 ml (3% of medium) with 0.10 g (0.70 mmol) of substrate in 50 ml (in 500 ml Erlenmeyer flask) of YPD media and culturing at 30°C, 180 rpm for 16 h to 20 h (Fig. 15).



a) 5 ml of YPD *, 30°C, 180 rpm, b) 100 mg of **5**, 50 ml of YPD* (final pH 7.1), 30°C, 180 rpm, 20 h,

* YPD medium; Bacto yeast extract 1%, Bacto peptone 2%, Glucose 2%

Fig. 15. The optimized bioconversion process.

Under the optimized condition, reaction occurred smoothly in 18 h, yielding optically active asymmetric alcohol (*R*)-4-acetoxy-2-methyl-1-butanol (**6**) with a high conversion rate (Fig. 16).

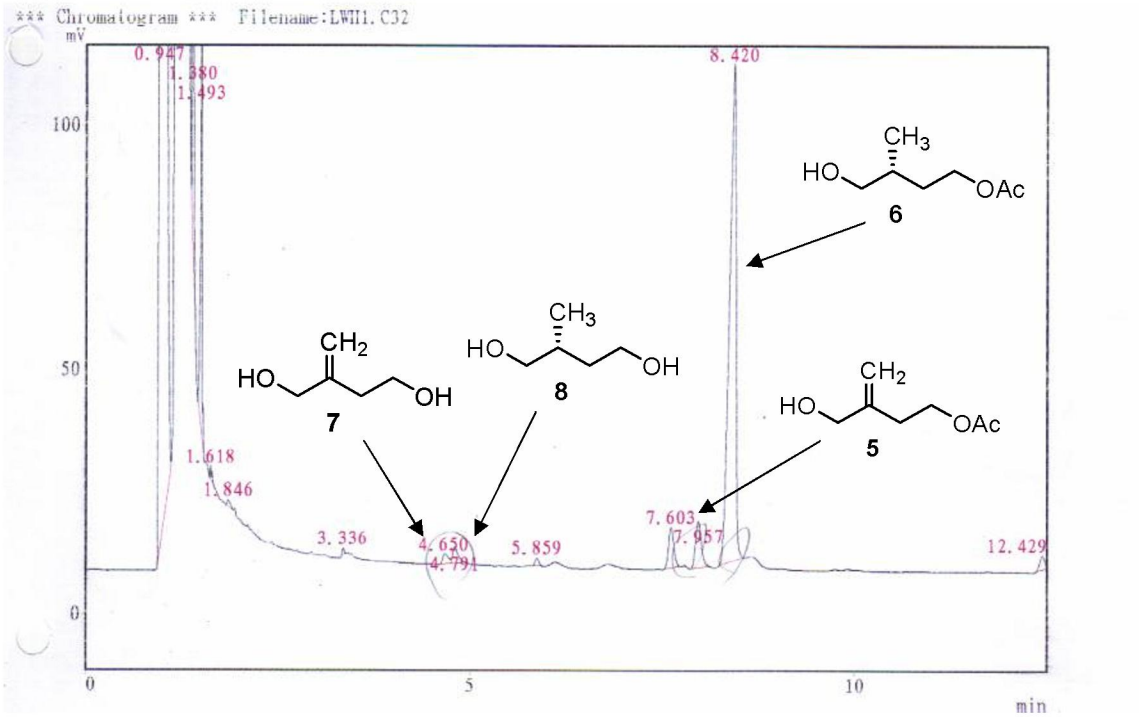


Fig. 16. Bioconversion of 5 by *P. putida* (after culturing 18-20 h).

The enantiomeric purity of **6** was checked with the corresponding (*S*)-MTPA ester of **6** using 95% e.e. of (*R*)-MTPA chloride by 400 MHz ^{1}H NMR. It showed very high e.e. as (*R*)-methyl at 0.90 ppm (d, J = 6.6 Hz) and (*S*)-methyl at 0.89 ppm (d, J = 6.7 Hz). However, an exact numerical value could not be determined, because the (*R*)-MTPA chloride was not optically pure and peaks of both stereoisomers did not separate clearly (Fig. 17).

To determine the absolute configuration of **6**, it was hydrolyzed to corresponding diol **8** and compared with the known (*R*)-2-methyl-1,4-butandiol (>98.0%, G.C.) which was obtained from TCI (Tokyo Chemical Industry Co., LTD). The specific rotation value of

diol, which was prepared from **6** was $[\alpha]_D^{27}$ = +13.1° (*c* 0.51, MeOH) and that of the purchased one was $[\alpha]_D^{27}$ = +13.2° (*c* 1.0, MeOH). Hence, it was found that that the product of bioconversion **6** possessed (*R*)-configuration with high optical purity (Fig. 18).

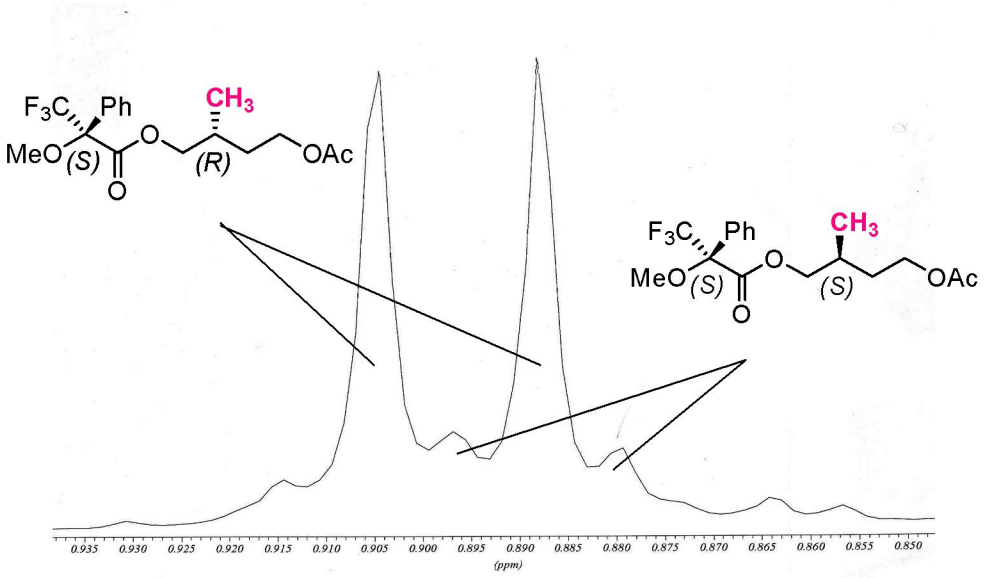
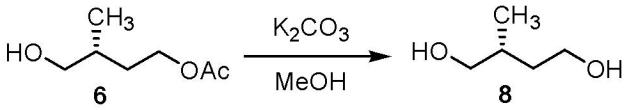


Fig. 17. ^{1}H NMR of (*S*)-MTPA* ester of **6**.

* 95% e.e. of (*R*)-MTPA chloride was used



synthesized;
$[\alpha]_D^{27}$ = +13.1° (*c* 0.5, MeOH)
purchased;
$[\alpha]_D^{27}$ = +13.2° (*c* 1.0, MeOH)
$[\alpha]_D^{27}$ = +13.6° (*c* 3.3, MeOH)
Ref.12;
$[\alpha]_D^{20}$ = +12.5° (neat)

Fig. 18. Assignment of the specific rotation values of **8**.

3. Conclusion

Starting from cheap natural compound, itaconic acid, (*R*)-4-acetoxy-2-methyl-1-butanol (**6**) with high enantiomeric purity was prepared by a chemobiological method. The bioconversion was achieved by culturing cells of *P. putida*, with relatively short reaction time (16-20 h). This bioconversion method is more convenient than the transformation using purified enzyme, immobilized cell or immobilized enzyme. In addition, the hydrogenase of *P. putida* seems to recognize the allyl alcohol as a substrate, so it should be applicable to the reduction of other more complex allylic alchols.

Chapter I-2

Synthesis of

Methyl (*R*)-and (*S*)-3-methyloctanoate

1. Introduction

Generally, chiral alcohols are very useful as intermediates for the synthesis of biologically active materials, such as drugs. As described in chapter I-1, I have developed a synthetically versatile asymmetric alcohol, (*R*)-4-acetoxy-2-methyl-1-butanol (**6**). This compound can be converted to diverse chiral products easily because of its pseudo-symmetrical structure with two oxygen functionality at the both ends.

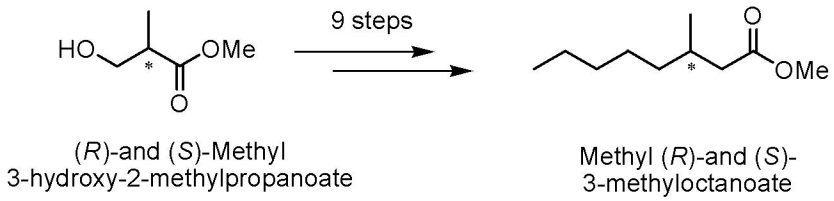


Fig. 19. Synthesis of methyl 3-methyloctanoate.[14]

The methyl 3-methyloctanoate (**12**) has been reported as the odor compound of African orchids, *Aerangis* sp., in 1992 by R. Kaiser.[13] In 1994, T. Kitahara *et.al* reported the synthesis of methyl 3-methyloctanoate from commercially available methyl 3-hydroxy-2-methylpropanoate in 9 steps (Fig. 19).[14] K. Mori and T. Kitahara showed that when their methyl ester was substituted with different alkyl groups, (*R*)-forms showed fruit-like scents, while (*S*)-forms showed flower-like.[15]

As a chiral building block, (*R*)-4-acetoxy-2-methyl-1-butanol (**6**) was thought be used for the synthesis of **12** (Fig. 20). Elongation of four carbons on the left of **6** gave (*S*)-**12** and one carbon on the left and three carbons on the right gave (*R*)-**12** via easy and simple organic steps.

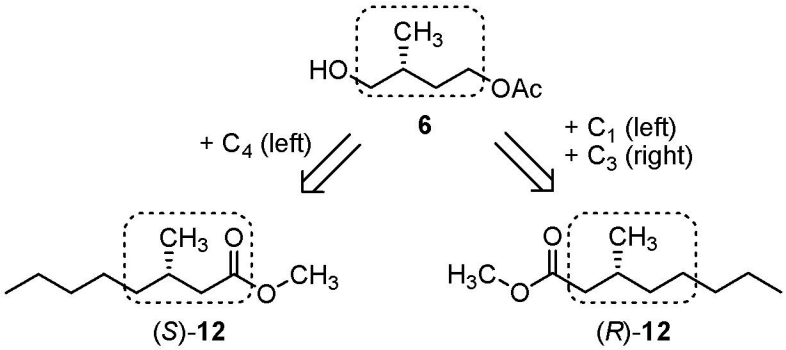
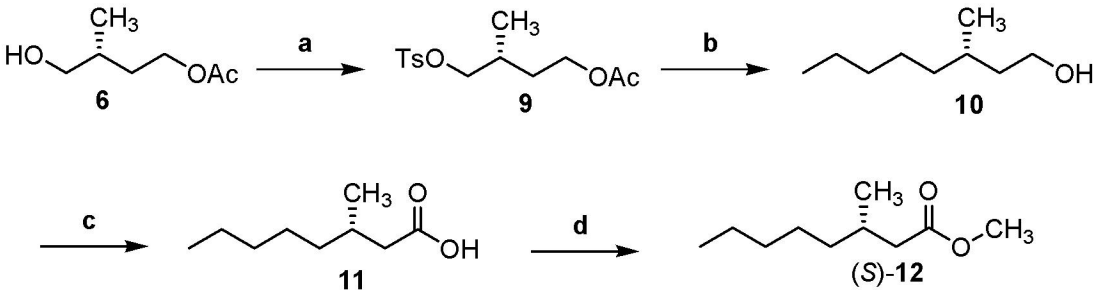


Fig. 20. Synthetic approach of **6** to methyl 3-methyloctanoate (**12**).

2. Results and discussion

2-1. Synthesis of methyl (*S*)-3-methyloctanoate (12).

Methyl (*S*)-3-methyloctanoate **12** was synthesized from **6** by 4 steps that are outlined in Scheme 2. At first, the hydroxyl group of **6** was treated with *p*-toluenesulfonyl chloride, and the carbon chain was elongated with butylmagnesium bromide and copper bromide to afford alcohol **10**. Then, the alcohol **10** was oxidized with Jones reagent and esterified with methanol to give the target compound (*S*)-**12**.



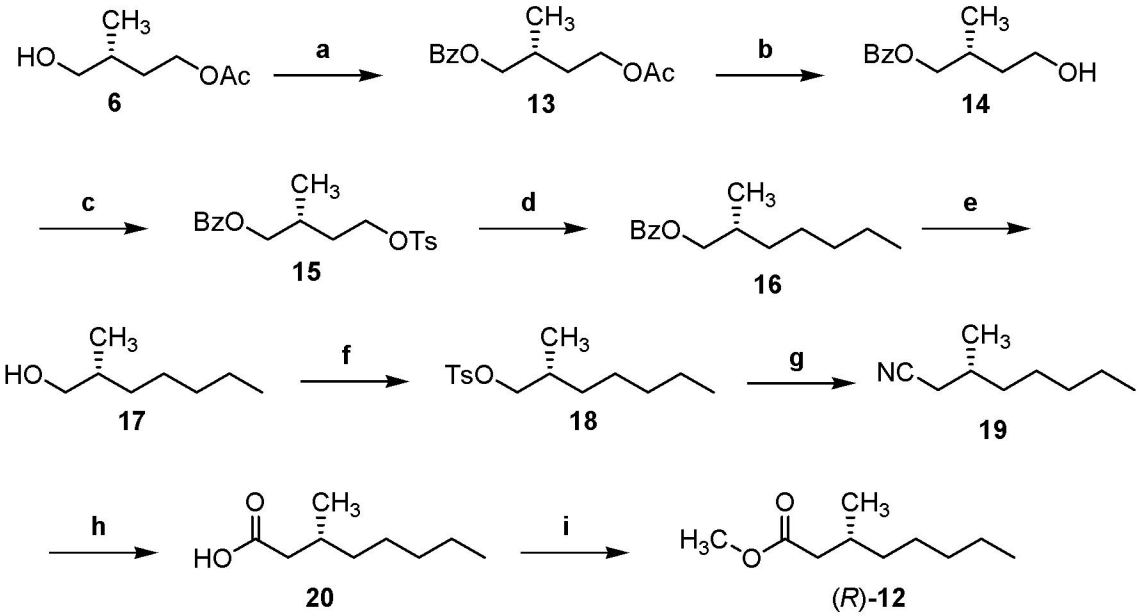
a) *p*-TsCl, Py., CH_2Cl_2, 18 h, 99%, b) CuBr, $CH_3(CH_2)_3$ MgBr, THF, -78°C to r.t., 80%

c) CrO_3, H_2SO_4, H_2O, acetone, 15 h, 98%, d) H_2SO_4, MeOH, reflux, 8 h, 77%

Scheme 2. Synthesis of methyl (*S*)-3-methyloctanoate **12**.

2-2. Synthesis of methyl (*R*)-3-methyloctanoate (12).

Methyl (*R*)-3-methyloctanoate **12** was also synthesized from **6** by 9 steps that are outlined in Scheme 3. At first, the protection of hydroxyl group of **6** with benzoyl group followed by removal of acetyl group gave alcohol **14**. Then the hydroxyl group was treated with *p*-toluenesulfonyl chloride, and the carbon chain was elongated with propylmagnesium bromide and copper (I) bromide. Thereafter the remaining benzoate was hydrolyzed, and the resulting alcohol **17** was tosylated and treated with sodium cyanide to give **19**. Then, the nitrile **19** was hydrolyzed and esterified with methanol to afford the desired compound (*R*)-**12**.



a) BzCl, py., CH_2Cl_2, 3 h, b) K_2CO_3, H_2O, MeOH, 24 h, 2 steps 86%, c) *p*-TsCl, py., CH_2Cl_2, 18 h, 88%, d) CuBr, $CH_3(CH_2)_2MgBr$, THF, -78°C to r.t., 80%, e) NaOH, H_2O, MeOH, 10 h, 2 steps 74%, f) *p*-TsCl, py., CH_2Cl_2, 10 h, 61%, g) NaCN, DMSO, 60°C, 10 h, 75%, h) EtOH, 42% NaOH, reflux, 20 h, 57%, i) H_2SO_4, MeOH, reflux, 8 h, 77%

Scheme 3. Synthesis of methyl (*R*)-3-methyloctanoate **12**.

3. Conclusion

(*R*)-4-Acetoxy-2-methyl-1-butanol **6** was efficiently used as a chiral building block for the synthesis of both enantiomers of methyl-3-methyl octanoate. Synthesis of methyl (*S*)-3-methyloctanoate **12** was achieved from **6** by 4 steps with 60% overall yield. And the methyl (*R*)-3-methyloctanoate **12** was obtained by 9 steps with 11% overall yield.

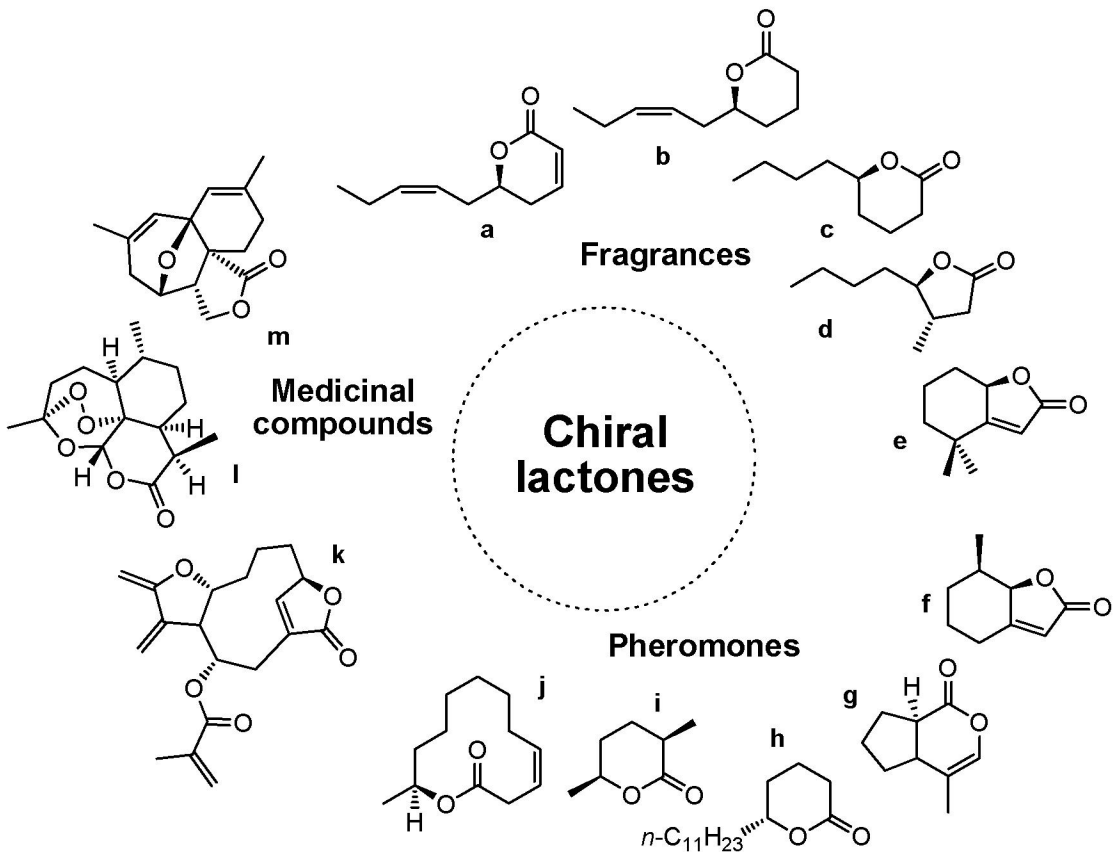
Chapter II

Sythesis of δ-nonalactone

by asymmetric S_N2' reaction

1. Introduction

Chiral lactones are commonly present in many natural products such as fragrances, pheromones or medicinal compounds.[16] They have been also used as chiral building blocks for the synthesis of other biologically active natural compounds.[17]



Fragrances: a,[18] b,[19] c,[20] d,[21] e,[22] Pheromones: f,[23] g,[24] h,[25] i,[26] j,[27]

Medicinal compounds: k,[28] l,[29] m.[30]

Fig. 21. Chiral lactones.

The alkylated δ-lactones are important ingredients for flavors in many foods such as milk, cheese, strawberry, and whisky (Fig. 21, **a**, **b**, **c**). Enantiomers of these δ-lactones

have different flavor, i.e. the (*R*)-form has a milky or creamy flavor while (*S*)-form has a peach-like flavor.[31] So, to estimate the exact difference of the flavor between enantiomers, it is often necessary to synthesize both of them in high optical purities. There are many approaches to prepare chiral 6-alkylated δ-lactones by chiral resolution or enzymatic methods. [31-33] However, synthesis of theses lactones by the direct enantioselective alkylations are less developed.

Herein, I report a stereoselective S_N2' process for preparation of optically active δ-nonalactone **21** (Fig. 21, **c**, Fig. 22) by using readily available limonene as a chiral auxiliary.

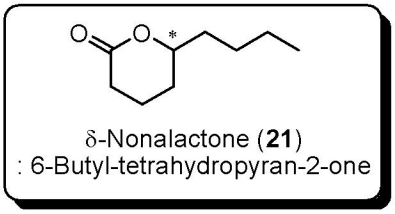


Fig. 22. δ-Nonalactone (**21**).

2. Results and discussion

I made a strategy for the synthesis of δ-nonalactone by the method shown in Fig. 23. It was found that the δ-nonalactone could be prepared from alkylidene cyclopentane by ozonolysis, followed by Baeyer-Villager oxidation. I supposed that an asymmetric introduction of butyl group could be achieved by chiral auxiliary-directed S_N2' reaction. The substrate of S_N2' reaction would be prepared easily from a chiral ketone and cyclopentenyl anion.

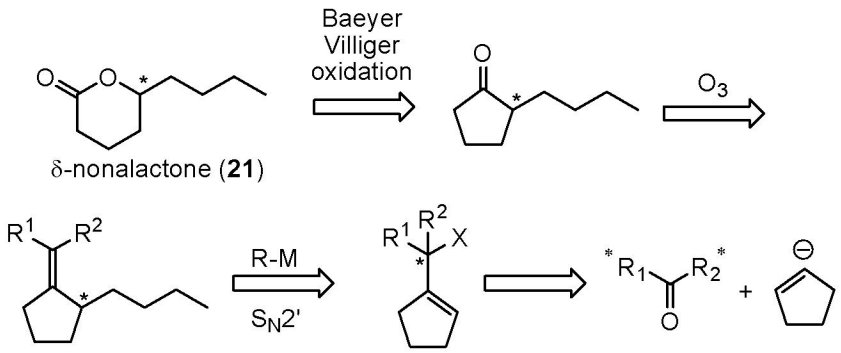
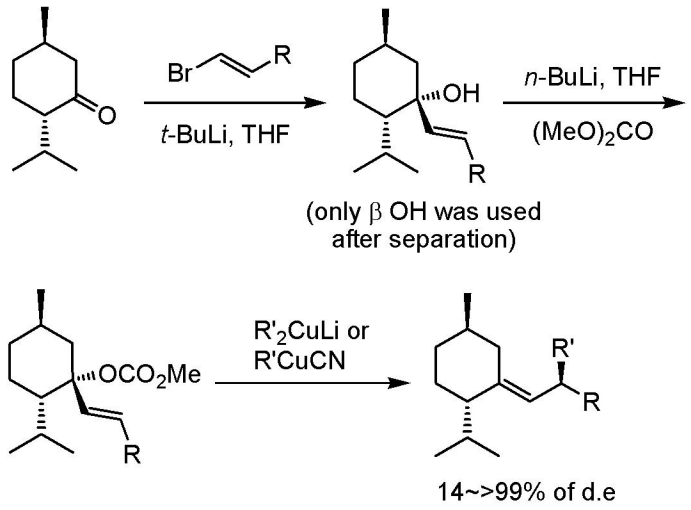


Fig. 23. Retrosynthesis of δ-nonalactone.

To realize this plan, choice of the chiral auxiliary and the leaving group was important. In 2000, C. Spino and co-workers reported a highly stereoselective S_N2' reaction using menthone as chiral auxiliary and carbonate as a leaving group.[33] Although they reported S_N2' reactions with a lot of acyclic alkenyl compounds, cycloalkenyl compounds have not been investigated (Fig. 24). So, I tried the same conditions with the cyclopentenyl compound (Scheme. 4).

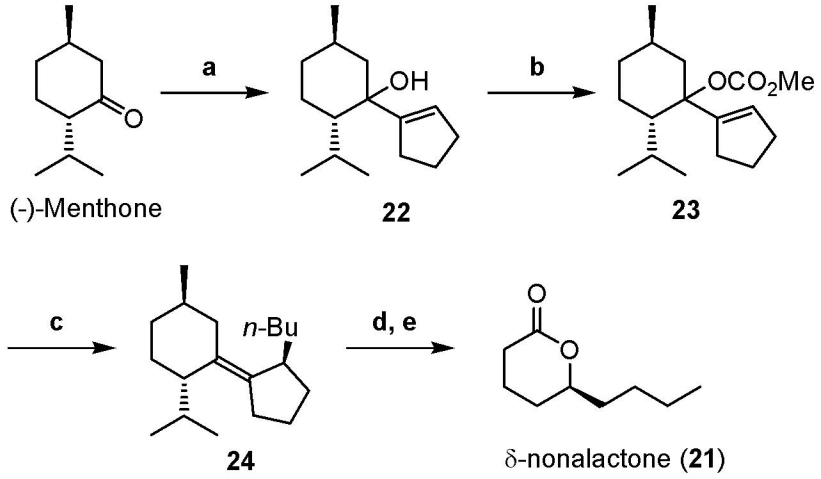


R = Me, *i*-Pr, *n*-Bu, *t*-Bu, Ph, BnO-CH_2, TBDMSO-CH_2, PMBO-$(CH_2)_2$, *p*-MeO-Ph, *m*-MeO-Ph, *p*-Cl-Ph, 6-MeO-1-Npht, 2-furanyl, 3-pyridinyl, R' = Me, *i*-Pr, *t*-Bu, Ph, *p*-Cl-Ph, $PhMe_2Si$.

Fig. 24. Reported stereoselective S_N2' reaction. [33]

The carbonyl group of (-)-menthone was cyclopentenylated to afford alcohol **22** as a single isomer (stereochemistry was not determined). The resulting hydroxyl group was acylated with methylchloroformate to give the substrate for S_N2' reaction. The unstable carbonate **23** was then treated with different kind of organic copper reagents to check the reactivity and stereoselectivity in the S_N2' reaction (Table 2).

As shown in entry 1, reaction of carbonate **23** with *n*-BuLi in the presence of CuI and LiI afforded S_N2' product **24** in a moderate yield. Because product **24** had very low polarity, it was very difficult to purify by chromatography. Its enantioselectivity was determined to be 54% e.e by chiral G.C., after conversion to δ-nonalactone **21** via ozonolysis and Baeyer-Villiger oxidation.



a) 

, MeLi, DME, 63%, b) MeLi, $ClCO_2Me$, TMEDA, THF, c) Table 2, d) O_3, CH_2Cl_2, MeOH, 0°C then Me_2S, e) *m*-CPBA, CH_2Cl_2, 2 steps, 37%

Scheme 4. Synthesis of δ-nonalactone **21** from (-)-menthone.

Table 2. S_N2' reaction of **23**.

entry	reaction condition	S_N2' product yield	Selectivity*
1	*n*-BuLi, CuI, LiI	40% (2 steps)	*S*: 77%, *R*: 23%
2	*n*-BuMgBr, CuCN	66%	*S*: 47%, *R*: 53%
3	*n*-BuMgBr, CuI	30%	*S*: 55%, *R*: 45%
4	*n*-BuLi, CuBr·SMe2	N.R.	-
5	*n*-Bu2Zn, CuCN·LiCl	N.R.	-
6	*n*-BuLi, CuCN	N.R.	-
7	Pd(PPh3)4, *n*-BuLi	Returned to alcohol	-

* Determinated by chiral G.C. after convertin in to **21**.

In the conditions as shown entry 2 and 3, S_N2' reactions proceeded in a moderate yield, but with poor enantioselectivity. In other cases (entry 4-7), S_N2' reactions did not proceed. Having these results, use of menthone as chiral auxiliary seemed not suitable to achieve higher enantioselectivity (Fig. 25).

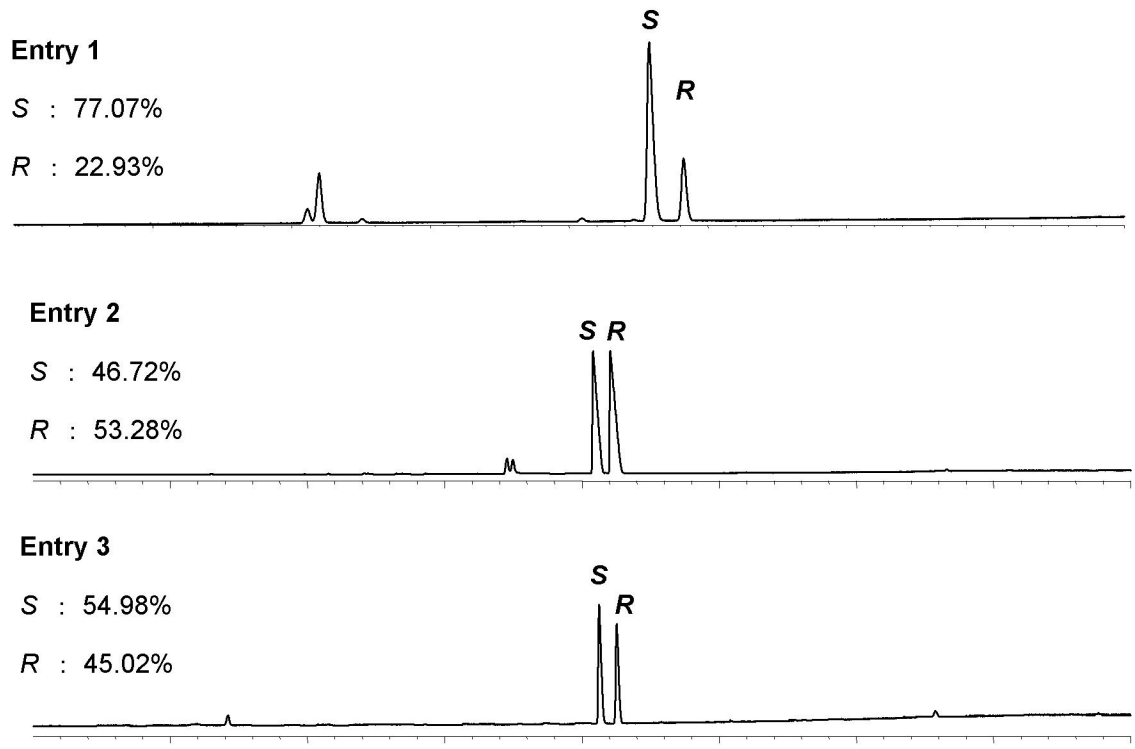


Fig. 25. G.C. results of S_N2' reactions of **23**.

G.C. Condition – Instrument: Agilent 6890N, Column: CIRAMIX (0.25 mmI.D×30 mL df = 0.25 μm), Column Temp. : 40°C to 180°C (0.7°C /min), Carrier gas: Nitrogen, Column Flow rate: 0.7 ml/min, Detector: FID, Injection Temp.: 230°C, Detector Temp.: 250°C.

Improved synthetic strategy is shown in Fig. 26. A cyclic carbonate compound (**I**) was selected as a substrate for the S_N2' reaction. Compared with menthone, the use of **I** has several advantages: 1) **I** has non-epimerizable chiral centers during the reaction with cyclopentyllithium. 2) Direct formation of cyclic carbonate (**II**), which can act as a good leaving group, is expected. 3) After the S_N2' reaction, the product (**III**) will be more polar alcohol, and may be purified more easily than the menthone-derivative. 4) Most of all, the higher steric effect of neighboring quaternary center is expected to improve the stereoselectivity of the S_N2' reaction.

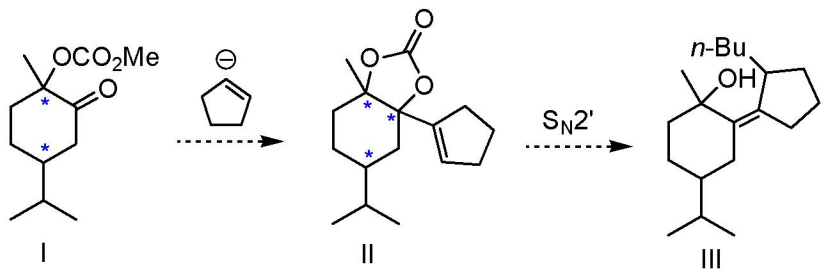
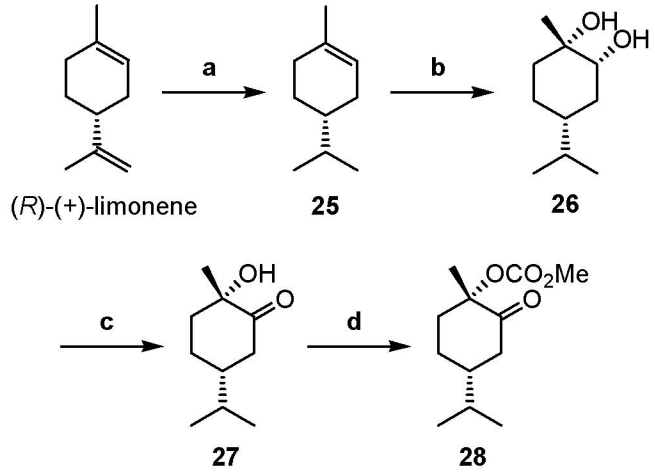


Fig. 26. Revised synthetic strategy of δ-nonalactone.

(*R*)-(+)-Limonene was used as starting material in the new approach(Scheme 5). The double bond of isopropenyl group was hydrogenated in the presence of platinum dioxide, and the remaining double bond was dihydroxylated with AD-mix-β to afford diol **26** according to the reported manner.[35] Only the secondary alcohol of **26** was oxidized by Swern oxidation to give α-hydroxyketone **27**. The remaining hydroxyl group of **27** was acylated with methylchloroformate and the desired keto carbonate **28** was obtained.

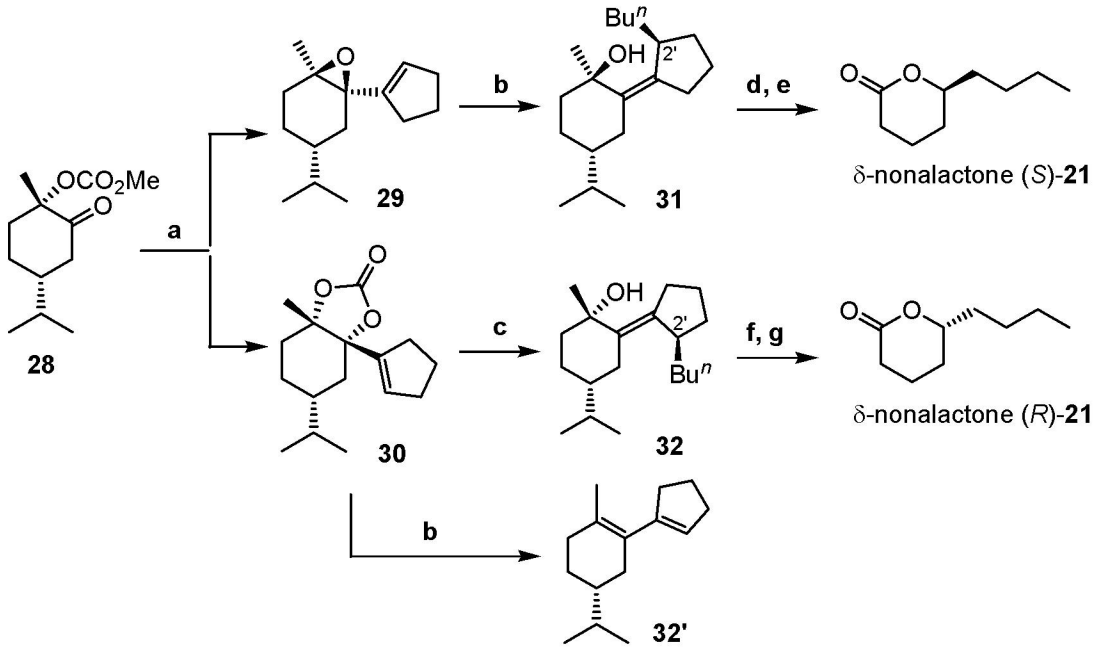


a) H_2, PtO_2, MeOH, 85%, b) AD-mix-β, $CH_3SO_2NH_2$, *t*-BuOH, H_2O, 2 days, then Na_2SO_3, 72%, c) DMSO, oxalyl chloride, CH_2Cl_2, Et_3N, -78°C, 74%, d) LiHMDS, MeOCOCl, THF, -78°C to r.t., 73%

Scheme 5. Synthesis of keto carbonate **28**.

Cyclopentenylation of the carbonate **28** was examined. Interestingly, two main products were obtained in this reaction (Scheme 6). One was a cyclic carbonate **30** (46%) as expected, and the other was an unexpected epoxide **29** (38%). Both of the cyclic carbonate **30** and epoxide **29** would be good substrate for the S_N2' reaction. So, they were subjected to the next step, after separation (Scheme 6).

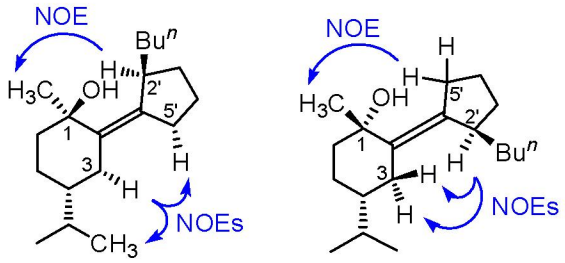
For the S_N2' reaction, both of them were treated with lithium di-*n*-butylcuprate. While the epoxide **29** gave the S_N2' products **31** in a good yield, the cyclic carbonate gave decarbonylated compound **32'**. On the other hand, treatment of the cyclic carbonate **30** with monobutylcyano cuprate afforded the S_N2' product **32** in a good yield, while the epoxide **29** showed no reactivity.



a) Cyclopentenyl iodide, *t*-BuLi, THF, -78°C, 30 min; -78°C to r.t., 10 h, 38% of **29** and 46% of **30**, b) CuI, LiI, *n*-BuLi, Et_2O, -78°C to r.t., over night, 65%, c) *n*-BuMgBr, CuCN, THF, -78°C; -20°C, 2-4 h, 75%, d) O_3, CH_2Cl_2, MeOH, 0°C ; Me_2S, e) *m*-CPBA, CH_2Cl_2, 20 h, 2 steps, 13%, f) O_3, CH_2Cl_2, MeOH, 0°C ; Me_2S, e) *m*-CPBA, CH_2Cl_2, 20 h, 2 steps, 28%

Scheme 6. Synthesis of (*R*)- and (*S*)-δ-nonalactone **21**.

From **29** and **30**, the formation of (*E*)- and/or (*Z*)- products are possible. To determine the (*E*)/(*Z*)-configuration, nuclear Overhauser effects (NOEs) in the ^{1}H NMR spectra of **31** and **32** were observed (Fig. 27).



(*Z*)-**31** (*E*)-**32**

Fig. 27. ^{1}H NMR NOEs of **31** and **32**.

In the case of **31**, the NOEs were observed between a proton of C2' and protons of C1-CH_3, and between a proton of C3 and protons of C5' and CH_3 of isopropyl group. So the structure of **31** was defined as (*Z*)-form. In the case of **32**, on the other hand, the NOEs were observed between a proton of C2' and protons of C3, and between a proton of C5' and protons of C1-CH_3. So the structure of **32** was defined as (*E*)-form.

The absolute configurations of butyl group (at C2') of **31** and **32** were determined by the specific rotation values after conversion to δ-nonalactone **21** via ozonolysis and Baeyer-Villiger oxidation. The specific rotation value of **21**, which was derived from **31**, was $[\alpha]_D^{24}$ = -39. 6° (*c* = 0.85, $CHCl_3$) [Ref. 32: $[\alpha]_D^{20}$ = -48° (*c* = 0.83, $CHCl_3$), Ref. 33: $[\alpha]_D^{20}$ = -52.5° (*c* = 1.0, $CHCl_3$)], while that from **32** was $[\alpha]_D^{18}$ = +49.7° (*c* = 0.5, $CHCl_3$) [Ref. 33: $[\alpha]_D^{20}$ = +50.6° (*c* = 1.0, $CHCl_3$)]. These results indicated that the absolute configurations at C2' of **31** and **32** were *S* and *R*, respectively.

As a side-product of the ozonolysis of **32**, hydroxyketone **27** was recorvered. However, the ozonolysis of **31** afforded the stereoisomer **27'** (Fig. 28). Hence, the stereochemistries of epoxide **29** and carbonate in **30** were established as depicted in

Scheme 6. Enantiomeric purities of (*S*)- and (*R*)-**21** were determined to be 89% e.e and 90% e.e by analysis with chiral G.C. Thus, I could succeed in the synthesis of both enantiomers of δ-nonalactone via enantioselective S_N2' reaction.

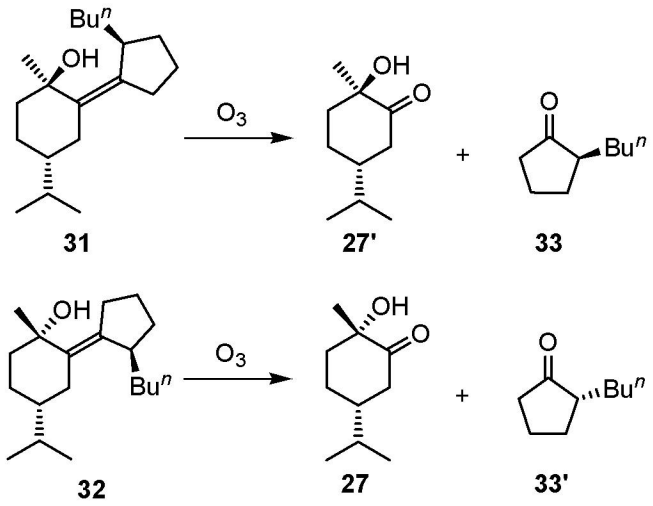


Fig. 28. Ozonolysis of **32** and **33**.

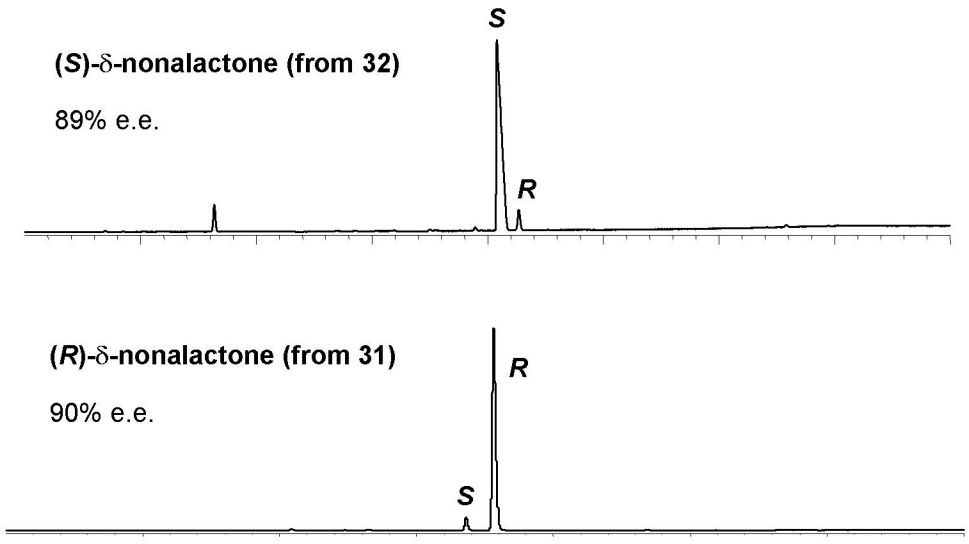


Fig. 29. G.C. analysis of δ-nonalactone **21**.

G.C. Condition - Instrument: Agilent 6890N, Column: CIRAMIX (0.25 mmI.D×30 mL df = 0.25 μm), Column Temp. : 40°C to 180°C (0.7°C /min), Carrier gas: Helium, Column Flow rate: 0.7 ml/min, Detector: FID, Injection Temp.: 230°C, Detector Temp.: 250°C.

Though both enantiomers of δ-nonalactone had been estabilished, the yield of ozonolysis and Baeyer-Villiger oxidation were quite low, so conditions of both reaction were investigated.

Because of their steric hindrance, tetra-substituted olefin is difficult to convert to ozonide **c** from intermediates **a** and **b** by ozonolysis. If the intermediate **a** makes a stable peroxide dimmer **d** or peroxide polymer **e,** it is getting difficult to be a common ozonolyzed product. However, in existence of the alcohol solvent, **a** will produce an alkoxy hydroperoxide **f** and it would be decomposed by the reducing agent to the usual ozonolyzed product (Fig. 30).

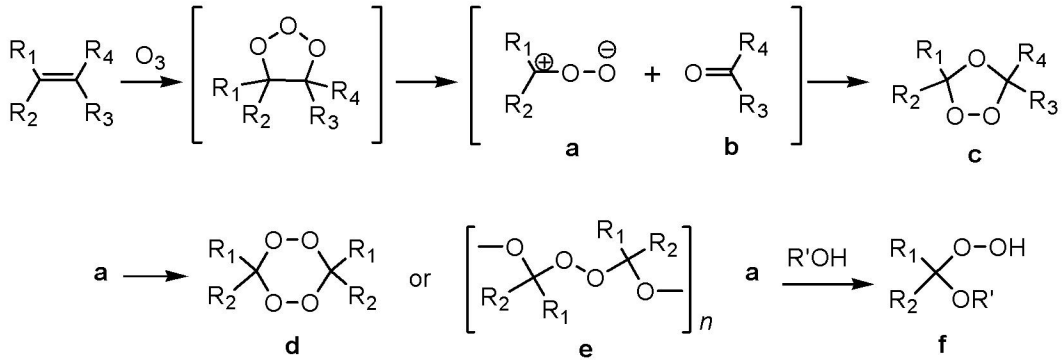
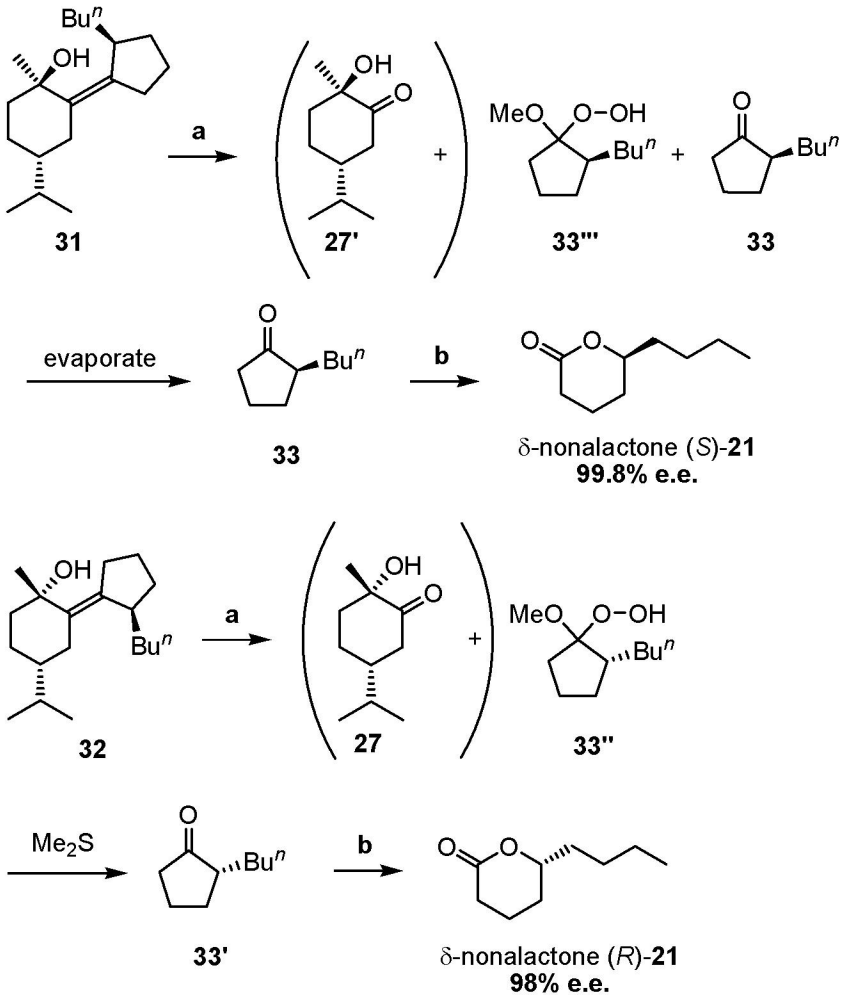


Fig. 30. Ozonolysis of the 4 substituted olefins*

* A. Suzuki, *新実験化学講座 15 酸化と還元 I-2*, 564-565,日本化学会, 1980.

So, I tried the ozonolysis of **31** and **32** in the only MeOH as a sole solvent at -78°C. From **31**, methoxy hydroperoxide **33'''** was produced along with cyclopentanone **33** and hydroxyl ketone **27'**. Treating with Me_2S, **33'''** was converted to **33**. And from **32**, hydroxyl ketone **27** and only methoxy hydroperoxide **33''** was produced. And **33''** was also converted to **33'** by Me_2S. Baeyer-Villiger oxidation of **33** and **33'** gave (*R*)-and (*S*)-δ-nonalactone (**21**) with good yields and high e.e. (98% e.e. of (*R*)-**21**, 2 steps 72% from **32**, 99.8% e.e. of (*S*)-**21**, 2 steps 73% from **31**, Scheme 7, Fig. 31).



Scheme 7. Synthesis of δ-nonalactone from **32** and **31** (improved method).

a) O_3, MeOH, -78°C ; Me_2S, b) *m*-CPBA, CH_2Cl_2, 20 h

72% (2 steps from **32**), 73% (2 steps from **31**)

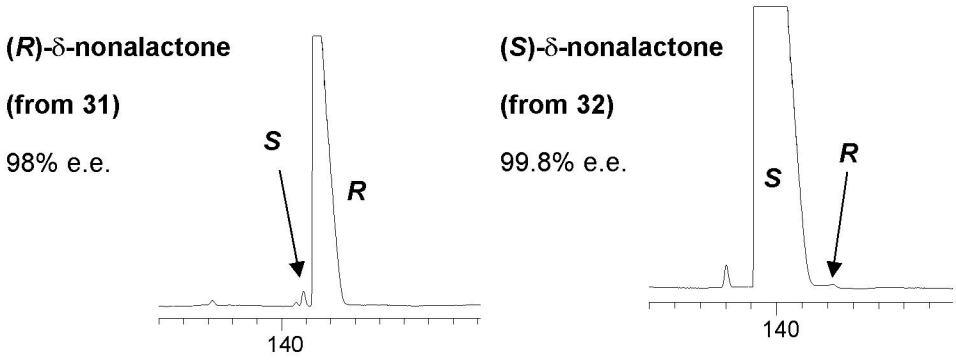


Fig. 31. Enantiopurity of (*R*)-and (*S*)-**21**

As stereochemistry of **29**, **30** and **31**, **32** had been estabilished, mechanisms of cyclopentenylation and S_N2' reaction are discussed here.

The reaction mechanism of the cyclopentenylation to **28** could be suggested to operate according to Fig. 32. When the cyclopentenyl nuclophile attacks the carbonyl carbon from the axial side, the resultant oxide anion is placed near to the carbonyl carbon of carbonate. Hence it can easily convert to the cyclic carbonate **30**. On the other hand, when the addition occurs from equatorial side, the resulting oxide anion is far off from the carbonyl group and should form an unstable *trans*-fused five membered carbonate. So methoxycarbonyloxy group acted as an eliminating group to form the epoxide **29**.

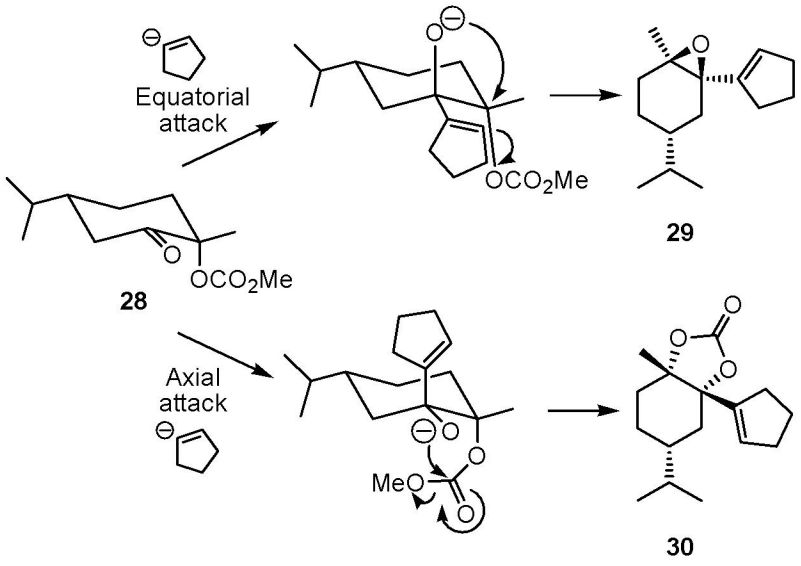


Fig. 32. The reaction mechanism of formation of epoxide (**29**) and cyclic carbonate (**30**).

From cyclic carbonate **30**, (*E, R*)-product (**32**) was obtained. Among the 4 possible conformations, **A** ~ **D**, **A** can cause the *anti*-S_N2' reaction with the least steric repulsion between the nucleophile (R-Cu, Fig. 33).

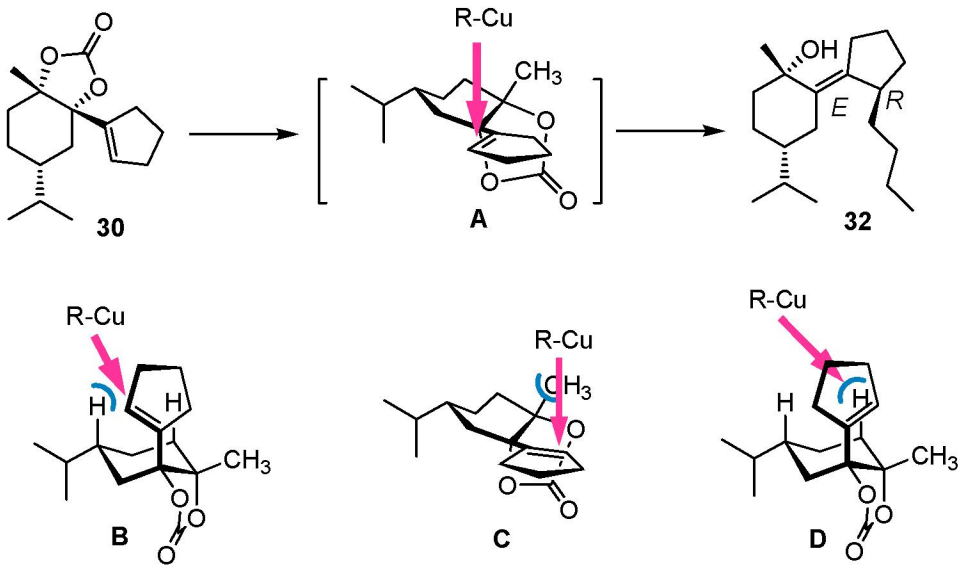


Fig. 33. Stereochemistry of S_N2' reaction of cyclic carbonate **30**.

On the other hand, S_N2' reaction of epoxide **29** is more complicated and the mechanism is unclear. If the reaction takes place in *anti*-S_N2' manner, the product should be isomeric **31'** or **31"** via the conformer **E** or **F**, respectively. However, **31** was obtained unexpectedly. This result can be explained if the epoxide is once opened by iodide and the intermediate (**G** or **H**) is substituted with *n*-butyl nucleophile (Fig. 34).

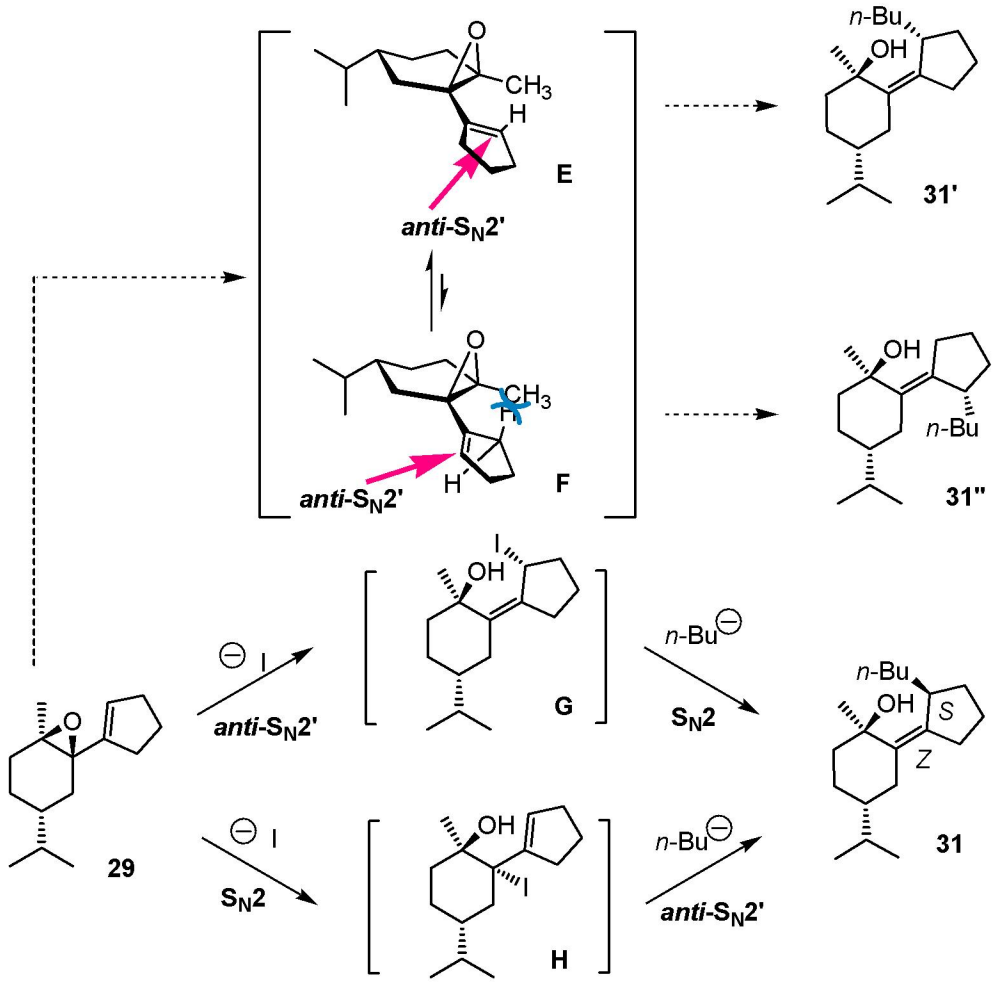


Fig. 34. Unusual *syn*-S_N2' reaction of epoxide **29**.

3. Conclusion

Starting from (*R*)-(+)-limonene, both (*R*)-and (*S*)-δ-nonalactone **21** have been synthesized enantioselectively. In this study, the asymmetric S_N2' reaction was effectively used as a chemical synthetic method.

(*R*)-(+)-limonene was converted to keto carbonate **28** in 4 steps and it was subjected to addition of cyclopentenyl nucleophile. It produced two optically active intermediates, the epoxide (**29**) and the cyclic carbonate (**30**), as substrates of S_N2' reaction. Both of them produced different S_N2' product with different manner. From the epoxide **29**, (*Z, S*)-product **31** was produced and it was converted to (*S*)-δ-nonalactone (89% e.e) by ozonolysis and Baeyer-Villiger oxidation.

On the other hand, from the cyclic carbonate **30**, (*E, R*)-product **32** was obtained and it was converted to (*R*)-δ-nonalactone (90% e.e) by oxidation steps. These results suggest that, if the stereoselectivity of vinyl group attack to the keto carbonate can be controlled, it would be possible to make only cyclic carbonate or epoxide, which enables us to synthesize only the desired stereoisomer.

It is also expected that the cyclic carbonate and epoxide could be used for synthesis of many other optically active products, by changing their alkenyl group or alkyl nucleophile. So, this area is under research now.

Final conclusion

In this study, I synthesized optically active odorants, methyl (*R*)- and (*S*)-3-methyloctanoate (**12**), and (*R*)- and (*S*)-δ-nonalactone (**21**) by using both bioconversion and chemical conversion.

In chapter I-1, I synthesized (*R*)-4-acetoxy-2-methyl-1-butanol (**6**) by bioconversion with high optical purity. Starting from the cheap natural compound, itaconic acid, I have synthesized 4-acetoxy-2-methylene-1-butanol (**5**), in 4 steps. The bioconversion of **5** was achieved by culturing cells of *P. putida*, with relatively short reaction time (16-18 h) and high enantioselectivity (≥98%, Fig. 34). This bioconversion method is thought to be applicable to other more complex allyl alchol substrates.

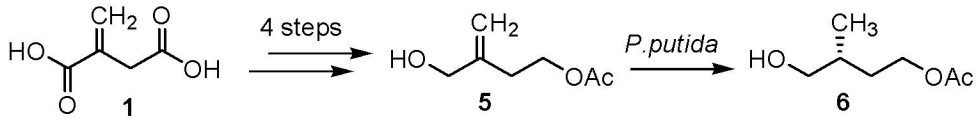


Fig. 34. Synthesis of (*R*)-4-acetoxy-2-methyl-1-butanol (**6**).

In chapter I-2, chiral alcohol (**6**) was used for synthesis of methyl (*R*)- and (*S*)-3-methyloctanoate. The synthesis of methyl (*S*)-3-methyloctanoate **12** was achieved from **6** by 4 steps with 60% overall yield. And the (*R*)-methyl-3-methyloctanoate **12** was synthesized by 9 steps with 11% overall yield (Fig. 35).

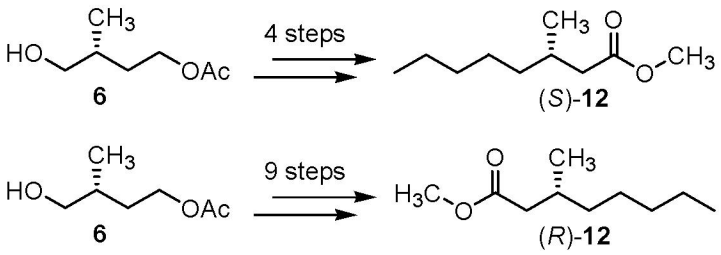


Fig. 35. Synthesis of methyl (*S*)- and (*R*)-3-methyloctanoate.

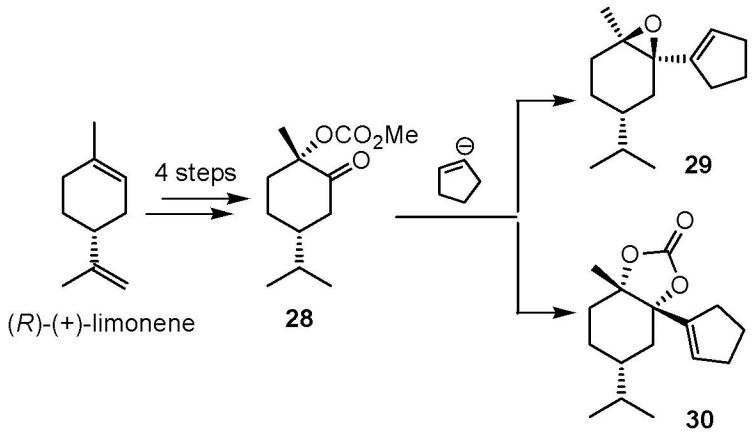


Fig. 36. Synthesis of substrates of S_N2' reaction.

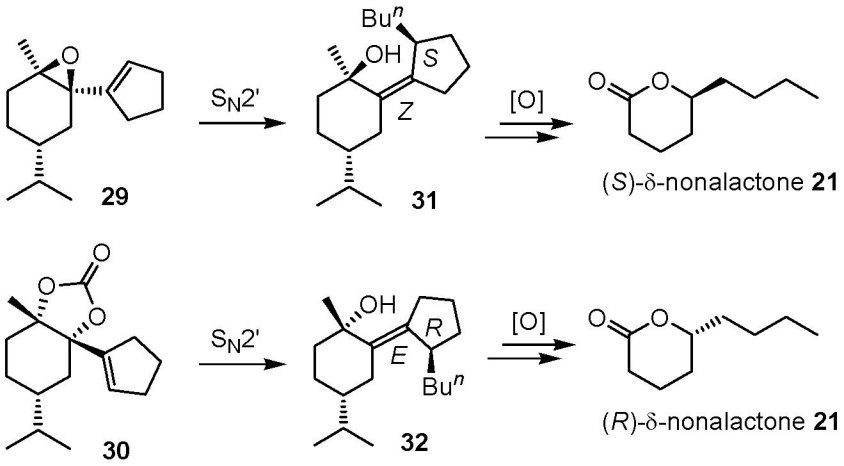


Fig. 37. Synthesis of (*R*)-and (*S*)-δ-nonalactone.

In chapter II, I synthesized (*R*)-and (*S*)-δ-nonalactone **21** by enantioselective chemical conversion. Starting from (*R*)-(+)-limonene, keto carbonate **28** was prepared in 4 steps. The cyclopentenyl addition to **28** produced two optically active intermediates, the epoxide **29** and the cyclic carbonate **30** (Fig. 36). Both of them are used as substrates for S_N2' reaction and they produced different S_N2' product with different manner. From epoxide **29**, (*Z,S*)-product **31** was obtained and it was converted to (*S*)-δ-nonalactone (99.8% e.e.) by ozonolysis and Baeyer-Villiger oxidation. On the other hand, from the cyclic carbonate **30**, (*E,R*)-product **32** was obtained and it was converted to (*R*)-δ-nonalactone (98% e.e.) by oxidation steps (Fig. 37). By changing their alkenyl group or alkyl nucleophile, the cyclic carbonate and epoxide would be used for synthesis of many other optically active products.

In this study, I synthesized both enantiomer of methyl (*R*)- and (*S*)-3-methyloctanoate and (*R*)- and (*S*)-δ-nonalactone by using both bioconversion and chemicalconversion. Methyl (*R*)-3-methyloctanoate showed pineapple like odor while (*S*)-3-methyloctanoate showed lily like odor, as expected.

And (*R*)- δ-nonalactone showed sweet coconut like odor while (*S*)- δ-nonalactone showed sweet peach like odor. These compounds are important as good flavoring agents. And those methods, enantioselective reduction by *P. putida* and stereoselective S_N2' reaction are applicable for synthesis of other optically active compounds.