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Abstracts

Selected abstracts from the 5th Japanese Symposium on the Chemistry of Biocatalysis

Yasuhisa Asano [a,*], Toshiyuki Ito [b]

[a] *Faculty of Engineering, Biotechnology Research Center, Toyama Prefectural University, 5180 Kurokawa, Kosugi, Toyama 930-0398, Japan*
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Introduction

The 5th Japanese Symposium on the Chemistry of Biocatalysis was held in Okayama, Japan, on 13–14 December 2001, organized by Professor Takashi Sakai of Okayama University. Shown below are the selected short abstract (59 titles) of the presentation. Thanks are due to those who gladly sent the abstracts to us.

Yasuhisa Asano, Editor

Plenary Lectures

Discovering and creating enantioselective hydrolases for organic synthesis

Romas J. Kazlauskas

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Although mutations far from the active site can moderately increase enantioselectivity, mutations within the active site show much larger increases in enantioselectivity. For example, wild-type esterase from *Pseudomonas fluorescens* catalyzes moderately enantioselective hydrolysis of the chiral intermediate methyl 3-bromo-2-methylpropanoate ($E = 12$). A mutation far from the active site (Thr230Ile) moderately increased enantioselectivity ($E = 19$), but a mutation within the active site (Trp29Leu) dramatically increased enantioselectivity ($E = 58$) (Table 1).

Screening for new enzymes and their fine-tuning to synthetic applications

Yasuhisa Asano

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Directed molecular evolution technique was successfully used to tune up some properties of newly discovered microbial enzymes, D-amino acid amidase and nucleoside pyrophosphate phosphotransferase, and the enzymes were efficiently used as catalysts for synthetic purposes (Fig. 1).

Oral Presentations

How can the conformational flexibility of enzyme affect the discrimination between enantiomers for enzyme-catalyzed reactions of its natural substrate or non-natural one in organic solvent?

Keiichi Watanabe[a], Takashi Yoshida[b], Shin-ichi Ueji[a,b,*]

[a]Graduate School of Science and Technology, Kobe University, Japan. [b]Graduate School of Cultural Studies and Human Science, Kobe University, Japan. E-mail: ueji@kobe-u.ac.jp

* Corresponding author. Tel.: +81-766-56-7500x530; fax: +81-766-56-2498.
E-mail address: asano@pu-toyama.ac.jp (Y. Asano).

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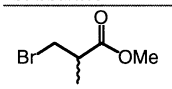
PII: S1381-1177(02)00046-2

Table 1. Increase of enantioselectivity by mutant esterases.

substrate	PFE mutant	E	Location
	Wild type	12 (S)	-
	Thr230Ile	19 (S)	~10 Å from substrate
	Trp29Leu	58 (S)	Direct contact with substrate

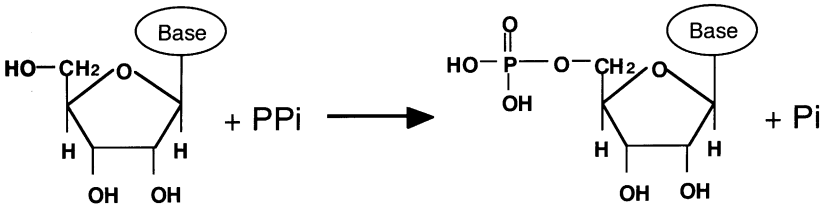


Fig. 1. Nucleoside phosphotransferase reaction with pyrophosphate as a substrate.

The difference in the enzyme's enantiorecognition between its natural substrate and non-natural one is proposed by the discussion based on the conformational flexibility of subtilisin estimated from the ESR spectra and the Michaelis–Menten kinetics for each enantiomer used (Fig. 2).

Efficient synthesis of optically active 2-phenylpropionic acid through epimerase-involving reaction

Koichi Mitsukura, Toyokazu Yoshida, Toru Nagasawa*

Department of Biomolecular Science, Faculty of Engineering, Gifu University, Japan. E-mail: kmitsu@biomol.gifu-u.ac.jp

Efficient synthesis of optically active 2-phenylpropionic acid through isomerization reaction with *Nocardia diaphanozonaria* JCM3208 resting cells has been demonstrated (Fig. 3).

Industrially feasible technology in the synthesis of single-enantiomer compounds using hydrolytic enzymes

Hideo Hirohara

Department of Materials Science, University of Shiga Prefecture, Hikone 522-8533, Japan. E-mail: hirohara@mat.usp.ac.jp

A number of industrial processes for the production of single-enantiomer chiral compounds by hydrolytic enzymes were reviewed with the emphasis of the feasibility being primarily dependent upon the total use of starting racemic compounds with racemization or inversion of the useless enantiomers (Table 2).

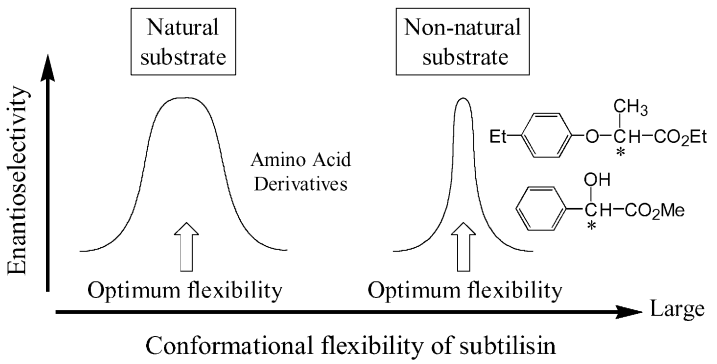


Fig. 2. Conformational flexibility of subtilisin on enantiorecognition.

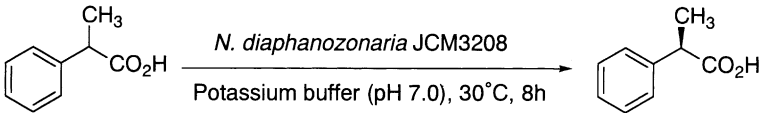


Fig. 3.. Synthesis of R-(-)-2-Phenylpropionic acid by *N. diaphanozonaria* resting cells.

Table 2. Feasibility study for synthesis of single-enantiomer compounds in industry.

Feasibility study for synthesis of single-enantiomer compounds in industry	
Hydrolase (or substrate)	Condition
Stereoselectivity	E > several 100
Starting substrate	Total conversion to desired single-enantiomer (through racemization or inversion of undesired enantiomer
Activity	Completion of reaction of [S]/[E] > 100 in 20 h
Productivity	[P]/[E] > several 100 (preferably > 1000)

Effect of weak ultrasonic wave on the enzyme activity: β-*N*-acetylglucosaminidase

Takayoshi Kawasaki, Hideyuki Mitomo, Yu Hoshino, Yoshio Okahata*

Department of Biomolecular Engineering, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Nagatsuta 4259, Midori-ku, Yokohama 226-8501, Japan. E-mail: tkawasak@bio.titech.ac.jp

Reactions of two kinds of β-*N*-acetylglucosaminidase were controlled by weak ultrasound irradiation (Fig. 4).

Posters

Effect of the conformational flexibility of the enzyme on the enantioselectivity enhancement for enzyme-catalyzed reactions in organic solvents

Takashi Yoshida[a], Keiichi Watanabe[b], Junko Yoshikawa[b], Hitoshi Ohta[b,c], Shinichi Ueji[a,b]

[a]Graduate School of Cultural Studies and Human Science, Kobe University, Japan. [b]Graduate School of Science and Technology, Kobe University, Japan. [c]Venture Business Laboratory, Kobe University, Japan. E-mail: ueji@kobe-u.ac.jp

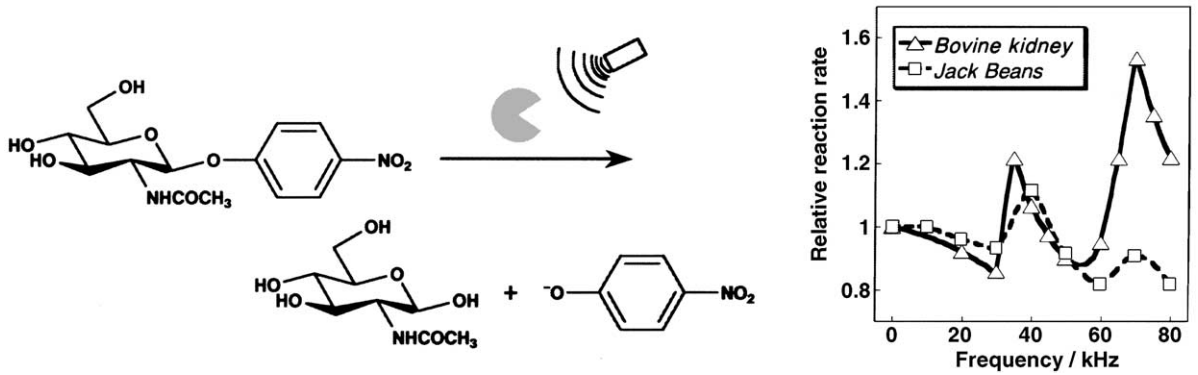


Fig. 4. Schematic representation of the reaction and ultrasound frequency dependence of activity of *β*-*N*-acetylglucosaminidases.

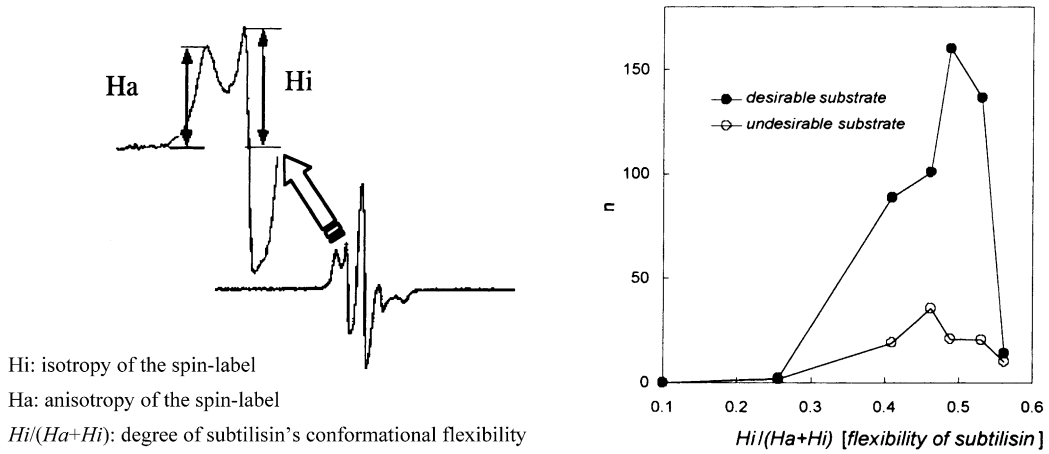


Fig. 5. Conformational flexibility of subtilisin.

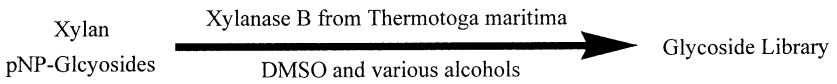


Fig. 6.

The relationship between the enantioselectivity and the conformational flexibility of subtilisin estimated from the ESR spectroscopic study provides the first evidence that the enzyme has the optimum flexibility to produce the maximal enantioselectivity toward the given substrates (Fig. 5).

Analysis of transglycosylation catalyzed by xylanase B from hyperthermophilic bacteria *Thermotoga maritima*

Atsushi Kobayashi, Motomitsu Kitaoka, Kiyoshi Hayashi

National Food Research Institute.
E-mail: akobayas@nfri.affrc.go.jp

Xylanase B (XynB) from *Thermotoga maritima* was stable in water-miscible organic solvents and was able to catalyzed transglycosylation reaction from various donors to aliphatic alcohols (Fig. 6).

Microbial deracemization of α-substituted carboxylic acids

Dai-ichiro Kato[a], Satoshi Mitsuda[b], Hiromichi Ohta[a,*]

[a]Department of Chemistry, Keio University, Japan.
[b]Sumitomo Chemical Co. Ltd., Japan.
E-mail: hohta@chem.keio.ac.jp

An enzyme system of *Nocardia diaphanozonaria* catalyzes the inversion of the chirality of various

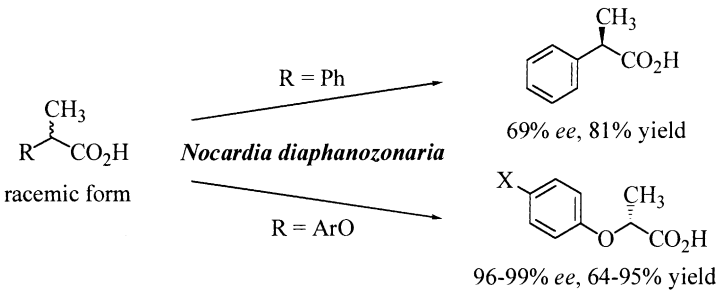


Fig. 7. Microbial deracemization of 2-substituted propanoic acids.

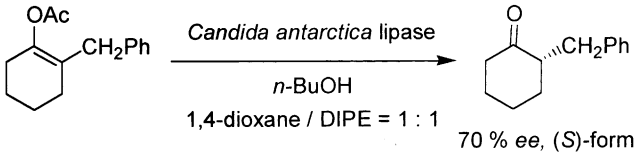


Fig. 8. Enzymatic asymmetric protonation of end esters in organic solvents.

α-substituted carboxylic acids, such as 2-phenylpropanoic acid and 2-phenoxypropanoic acid derivatives, via a novel deracemization reaction (Fig. 7).

Enzymatic asymmetric protonation of enol esters in organic solvents

Hiroki Tokoro, Dai-ichiro Kato, Hiromichi Ohta*
Department of Chemistry, Keio University, Japan. E-mail: hohta@chem.keio.ac.jp

Optically active α-substituted ketones were prepared via enzyme-catalyzed enantioselective protonation of enol esters in organic media, using butanol as the proton donor (Fig. 8).

Synthesis of (*R*)-flurbiprofen via enzymatic asymmetric decarboxylation

Yosuke Terao[a], Yoichiro Ijima[a], Hitoshi Kakidani[b], Hiromichi Ohta[a,*]
[a]Department of Chemistry, Keio University, Japan. [b]Tosoh Co. Ltd., Japan. E-mail: hohta@chem.keio.ac.jp

Malonic acid derivative **1** prepared from D, L-flurbiprofen was enzymatically decarboxylated by arylmalonete decarboxylase (AMDase) to give (*R*)-flurbiprofen **2** with high enantiomeric excess, which has the anticancer activity (Fig. 11).

Inversion of enantioselectivity of arylmalonate decarboxylase (AMDase) by point mutation

Yoichiro Ijima[a,*], Kaori Matoishi[a], Nobuhide Doi[b], Hiroshi Yanagawa[b], Hiromichi Ohta[a]
[a]Department of Chemistry, Keio University, Japan. [b]Department of Applied Chemistry, Keio University, Japan. E-mail: hohta@chem.keio.ac.jp

We tried the inversion of enantioselectivity of the decarboxylation reaction by using G74C, C188S double mutant AMDase, which gave opposite enantiomer with those of obtained via wild type enzyme (Fig. 10).

Synthesis of novel *gem*-difluorocyclopropane analogues

Toshiyuki Itoh[a,*], Nanae Ishida[b], Kunihiko Tanimoto[b], Fumiko Yamauchi[b]
[a]Department of Material Science, Faculty of Engineering, Tottori University, Japan. [b]Department of Science Education, Graduate School of Education, Okayama University, Japan.
E-mail: titoh@chem.tottori-u.ac.jp

Synthesis of several types of novel *gem*-difluorocyclopropane derivatives has been accomplished starting from chiral 1,3-bishydroxymethyl-2,2-difluorocyclopropane or 1,6-bishydroxymethyl-2,2,5,5-tetrafluoro-

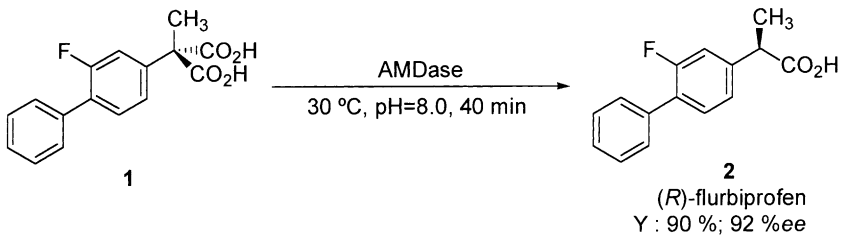


Fig. 9. Synthesis of (*R*)-flurbiprofen via enzymatic asymmetric decarboxylation.

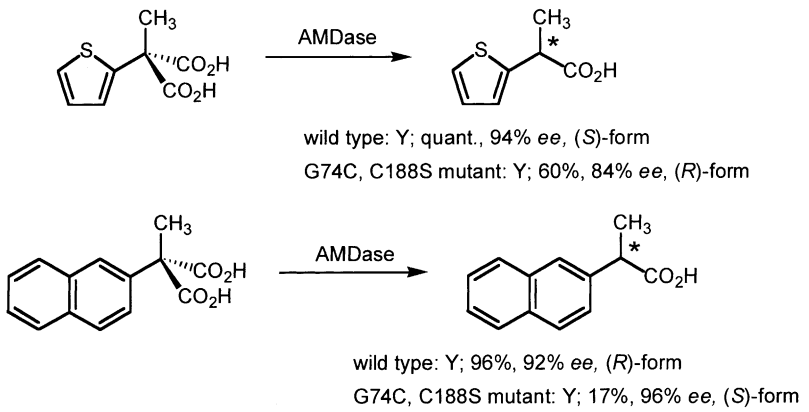


Fig. 10. Inversion of the enantioselectivity of arylmalonate decarboxylase by point mutation.

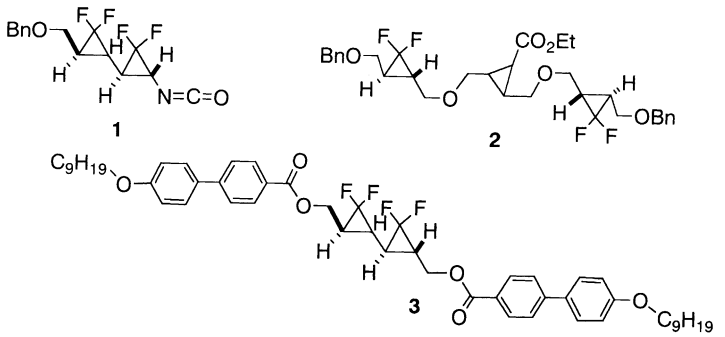


Fig. 11. Novel compounds which possess gem-difluorocyclopropane moieties.

bicycopropane which were prepared via lipase-catalyzed reaction (Fig. 11).

Lipase-catalyzed reaction in an ionic solvent system

Toshiyuki Itoh[a,*], Eri Akasaki[b], Yoshihito Nishimura[b]

[a]Department of Material Science, Faculty of Engineering, Tottori University, Japan. [b]Department of Science Education, Graduate School of Education, Okayama University, Japan. E-mail: titoh@chem.toffori-u.ac.jp

Lipase-catalyzed transesterification was demonstrated using methyl esters as acyl donors under reduced pressure in an ionic liquid ([bmim]PF_6) solvent system (Fig. 12).

Suitable supporting materials for lipase-catalyzed enantioselective acylation of secondary alcohols in an ionic liquid solvent system

Toshiyuki Itoh[a,*], Yoshihito Nishimura[b], Eri Akasaki[b], Masaya Kashiwagi[c], Makoto Onaka[c]

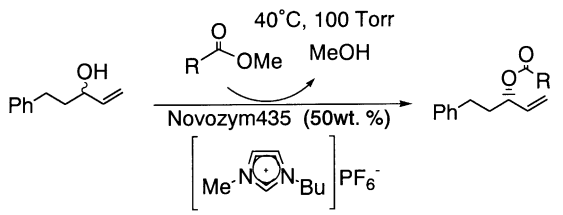


Fig. 12. Lipase-catalyzed enantioselective acylation under reduced pressure conditions in an ionic liquid solvent system.

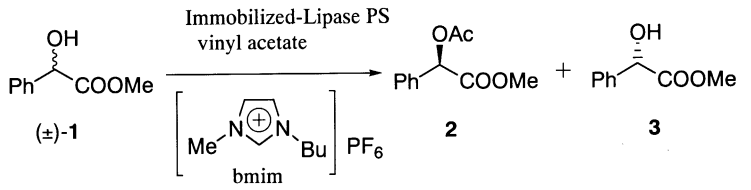


Fig. 13. Enantioselective acylation of mandelic acid methyl ester catalyzed by immobilized–lipase PS in an ionic liquid solvent system.

[a]Department of Material Science, Faculty of Engineering, Tottori University, Japan. [b]Department of Science Education, Graduate School of Education, Okayama University, Japan. [c]Graduate School of Arts and Sciences, The University of Tokyo, Japan. E-mail: titoh@chem.tottori-u.ac.jp

Lipase-catalyzed enantioselective transesterification was demonstrated in an ionic liquid solvent ([bmim]PF_6) system using several types of immobilized lipase PS (Fig. 13).

Stereochemical behaviors of cyclohexanols in lipase-mediated acetylations

Rikuhei Tanikaga*, Yoshimasa Matsumoto

Department of Bioscience and Biotechnology, Faculty of Science and Engineering, Ritsumeikan University, Nojihigashi 1-1-1, Kusatsu, Shiga 525-8577, Japan. E-mail: tanikaga@se.ritsumei.ac.jp

Acetylations of *trans*-2-substituted cyclohexanols using vinyl acetate and lipase PS gave the corresponding acetates in very high *E* values, while the *cis*-isomers containing a large substituent or an alkyl group were very slow to react, and these findings suggest that the stabilization by π electrons in the transition state seems to promote the reactions with high stereoselectivty (Table 3).

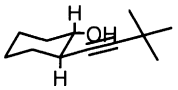
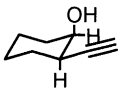
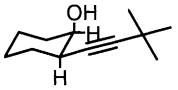
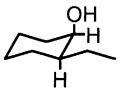
substrate	time (h)	E
	72	>200
	6	>200
	458	16
	46	111

Table 3. LPS–catalyzed acetylations of 2-substituted cyclohexanols.

Enzymatic synthesis and application of amino acid oligomers

Hiroshi Uyama, Shiro Kobayashi

Department of Materials Chemistry, Graduate School of Engineering, Kyoto University, Japan. E-mail: uyama@mat.polym.kyoto-u.ac.jp

Protease-catalyzed oligomerization of L-glutamic acid diethyl ester hydrochloride regioselectively proceeded in a buffer of high concentration, leading to the exclusive formation of the oligo(a-peptide) (Fig. 14).

Reduction of ketones by cyanobacteria

Rio Yamanaka, Kaoru Nakamura

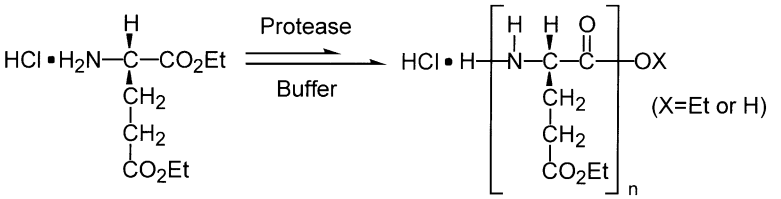


Fig. 14. Protease–catalyzed regioselective polymerization of diethyl L–glutamate hydrochloride.

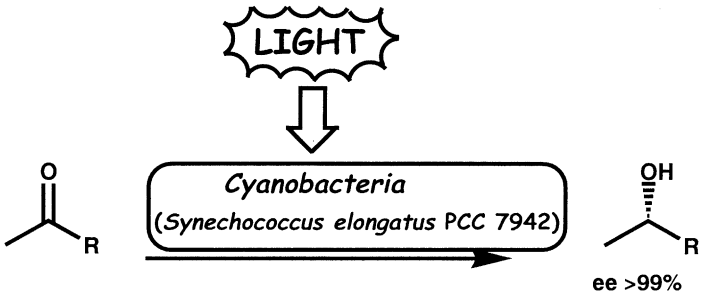


Fig. 15. Reduction of Ketones with *Cyanobacteria*.

Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan. E-mail: rio@boc.kuicr.kyoto-u.ac.jp

Methyl ketones were reduced to the corresponding (*S*)-alcohols in excellent enantioselectivities (>99% ee) with *Synechococcus elongatus* PCC 7942 (Fig. 15).

Carboxylation of pyrrole by cells of *B. megaterium* in supercritical CO_2

Tomoko Matsuda[a,*], Yoichi Ohashi[a], Tadao Harada[a], Reiko Yangihara[b], Toru Nagasawa[b], Kaoru Nakamura[c]

[a]Department of Materials Chemistry, Faculty of Science and Technology, Ryukoku University, Otsu, Shiga 520-2194, Japan. [b]Department of Biomolecular Science, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan. [c]Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan. E-mail: matsuda@rins.ryukoku.ac.jp

Pyrrole was converted to pyrrole-2-carboxylate in supercritical CO_2 using cell of *Bacillus megaterium* PYR 2910, and the yield of the carboxylation reaction in supercritical CO_2 was 12 times higher than that under atmospheric pressure (Fig. 16).

The exploitation of 'P6C world' using biotransformation

Tadashi Fujii, Hitosi Agematu, Kunio Isshiki

Mercian Corp., Bioresource Laboratories, Japan. E-mail: fujii-td@mercian.co.jp

Δ^1-Piperideine-6-carboxylate (P6C) is chemically unstable, which prevented the characterization of enzymes that convert P6C to other useful chemicals, nevertheless, we are exploiting 'P6C world', the collection of the chemicals derived from P6C using biotransformation, such as L-α-aminoadipic acid or L-pipecolic acid (Fig. 17).

Purification and characterization of glucosyltransferase from the cultured cells of *Catharanthus roseus*

Shin-ya Yamane, Kohtaro Watanabe, Kei Shimoda, Toshifumi Hjrata*

Department of Mathematical and Life Sciences, Graduate School of Science, Hiroshima University, Japan. E-mail: thirata@sci.hiroshima-u.ac.jp

Forty-one kilo-Dalton glucosyltransferase which specifically catalyze the glucosylation of the 5-hydroxyl group of gentisic acid (2,5-dihydroxybenzoic acid) was isolated from the cultured cells of *Catharanthus roseus* (Fig. 18).

Trypsin-catalyzed synthesis of oligopeptide esters with inverse substrates as acyl donor component

Haruo Sekizaki*, Kunihiko Itoh, Eiko Toyota, Kazutaka Tanizawa

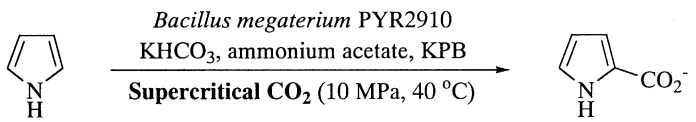


Fig. 16. Carboxylation of pyrrole in supercritical CO_2.

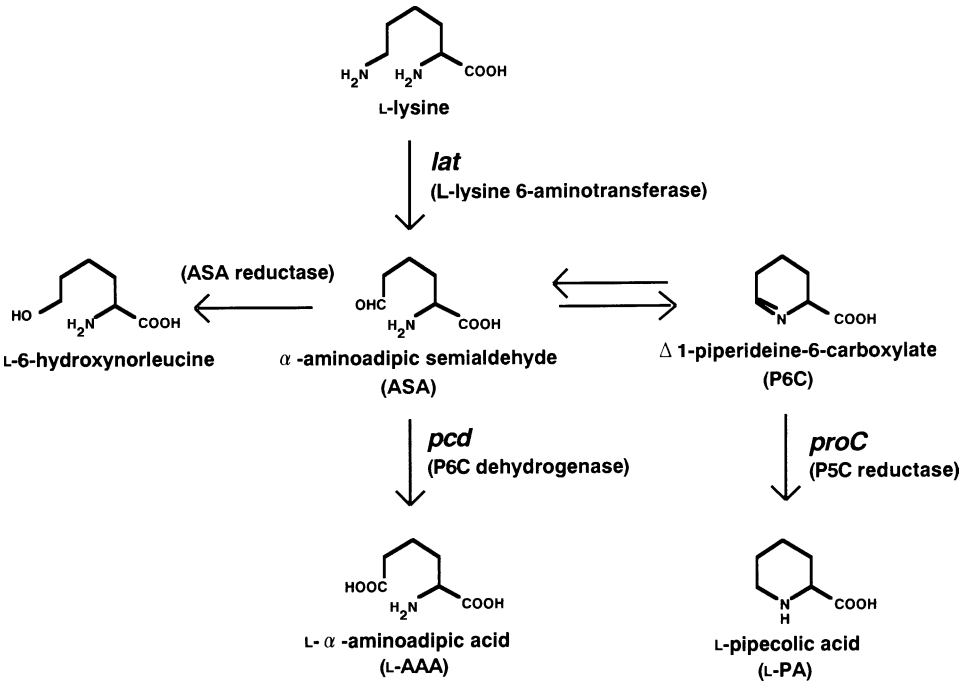


Fig. 17. The explanation of 'P6C world' using biotransformation.

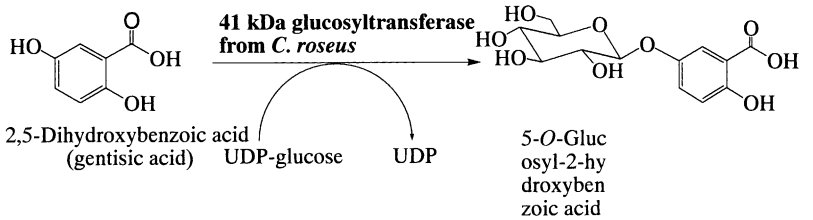


Fig. 18. Glucosylation of gentisic acid with a glucosyltransferase from *C. roseus*.

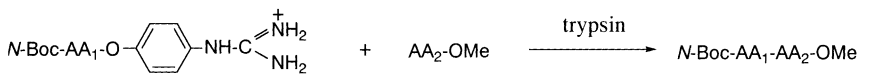
Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Japan. E-mail: sekizaki@hoku-iryo-u.ac.jp

Trypsin-catalyzed synthesis of the oligopeptide ester was demonstrated using inverse substrates as acyl donor with D-amino acid containing dipeptide esters as acyl acceptors (Fig. 19).

Simple preparation of optically pure trifluoromethylalkanol through lipase catalyzed reaction

Yumiko Takagi[a,*], Yousuke Sumino[a], Kouzo Inoue[a], Toshiyuki Itoh[b]

[a]Department of chemistry, Faculty of Education, Kagawa University, Japan. [b]Department of Material



AA_1 = L-Ala; D-Ala; L-Ala-L-Ala; D-Ala-D-Ala
AA_2 = L-Ala-D-Ala; L-Ala-D-Ala; D-Ala-D-Ala

Fig. 19. Enzymatic coupling of inverse substrates with dipeptide esters.

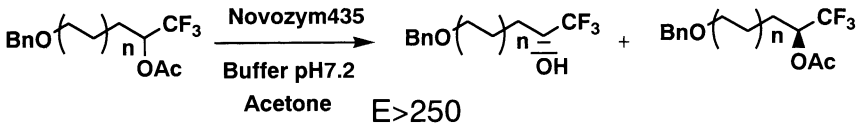


Fig. 20. Lipase-catalyzed trans esterification of 1,1,1-trifluoro-2-alkanoles.

Science, Faculty of Engineering, Tottori University, Japan. E-mail: ytakagi@ed.kagawa-u.ac.jp

We report the results of lipase-catalyzed hydrolysis reaction of various types of diacetates of bis(trifluoromethyl)alkanediols and synthesis of novel liquid crystal molecules which possesses bis(trifluoromethyl)akanol moieties and aromatic core structure at the center of the molecular flame (Fig. 20).

Oxidative modification of tryptophan-43 in the heme vicinity of the F43W/H64L myoglobin mutant

Shin-ichi Ozaki[a], Isao Hara[b], Takahumi Ueno[b], Shinobu Ito[c], Ken-ichi Lee[d], Norikazu Ueyama[d], Yoshihito Watanabe[b]

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Tryptophan-43 in the F43W/H64L myoglobin mutant (F43W/H64L Mb) is oxidatively modified in the reaction with *m*-chloroperbenzoic acid (*m*CPBA; Fig. 21).

Novel serine protease from earthworm. Part II. Characterization and application

Nobuyoshi Nakajima[a,*], Kohji Ishihara[b], Takashi Nakahara[a], Manabu Sugimoto[c]

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Novel serine proteases purified from earthworm were very stable and strongly resistant to organic solvents, and degraded various proteins, peptides and ester compounds.

Enzymatic conversion of bioactive compounds. Part IV. Stabilization and functionalization of naturally occurring plant pigments

Kohji Ishihara[a,*], Yoshihito Nishimura[b], Nobuyoshi Nakajima[c]

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Regioselective acylation of flavonoid glucosides was achieved by lipase-catalyzed transesterification in dry organic solvent. The participation of the acyl

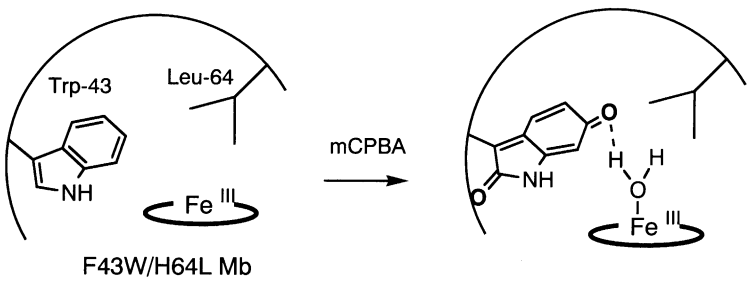


Fig. 21. The oxidative modification of Top-43 in the mutant.

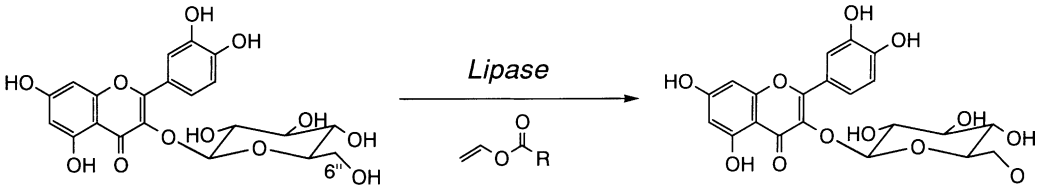


Fig. 22. The reduction of α-keto esters by SCKER.

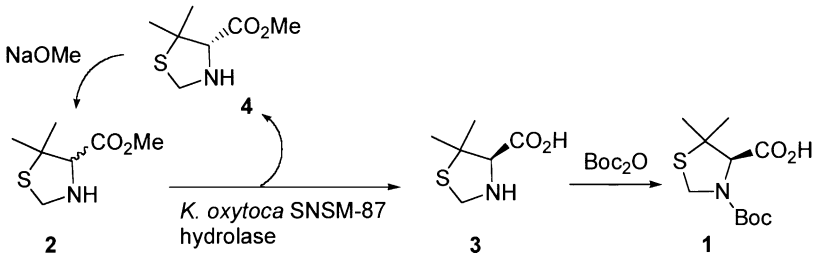


Fig. 23. Chemoenzymatic synthesis of 1.

group in flavonoid glucoside molecules resulted in increasing of the physiological function of the acylated flavonoid glucosides (Fig. 22).

An enantioselective synthesis of (*R*)-3-*tert*-butoxycarbonyl-5,5-dimethyl-1,3-thia-zolidine-4-carboxylic acid using a *Klebsiella oxytoca* SNSM-87 hydrolase

Yukifumi Nishimoto*, Toru Inoue, Masaya Ikunaka
Research and Development Center, Nagase & Co. Ltd., 2-2-3 Murotani, Nishi-ku Kobe 651-2241, Japan.
E-mail: yukifumi.nishimoto@nagase.co.jp

(*R*)-3-*tert*-Butoxycarbonyl-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid **1** is synthesized via enantioselective hydrolysis of methyl (±)-5,5-dimethyl-1,3-thiazolidine-4-carboxylate **2** with a *Klebsiella oxytoca* SNSM-87 hydrolase, which is now available in quantities from the *Escherichia coli* strain transformed to overexpress it (Fig. 23).

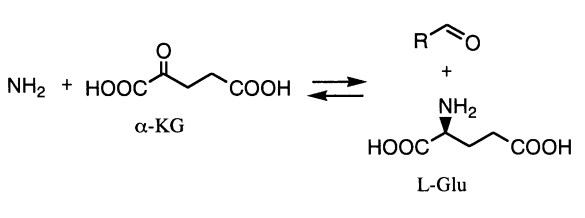
A novel hyperthermostable ω-aminotransferase from *Pyrococcus furiosus*

Seigo Oe, Satoshi Hanzawa, Hitoshi Kakidani
Tokyo Research Center, Tosoh Corporation, Japan.
E-mail: kakidani@tosoh.co.jp

A novel hyperthermostable ω-aminotransferase (referred to as Pfω-III) expressed in *E. coli* exhibited a unique broad substrate specificity, most preferably toward L-ornithine as amino donor and α-ketoglutarate as amino acceptor (Fig. 24).

Substrate specificity and phylogenetic analysis of three aminotransferases from hyperthermophilic archaea

Satoshi Hanzawa*, Seigo Oe, Kenji Tokuhisa, Kazuhisa Kawano, Hitoshi Kakidani
Tokyo Research Center, Tosoh Corporation, Japan.
E-mail: hanzawa@tosoh.co.jp



L-Ornithine	100	(%)
L-Lysine	53	
D-Ornithine	6	
D-Lysine	32	
5-Aminovalerate	33	
4-Aminobutyrate	6	
1,4-Diaminobutane	0.6	

(α-KG as amino acceptor, measured at 70℃)

Fig. 24. Substrate specificity of Pf_w–III toward various amino donors.

Table 4
Comparison of MsATs with an aminotransferase belonging to subfamily Iγ

Origin	Enzyme	Amino acid substrate	Subfamily	Sequence around K258
Thermococcus profundus	MsAT	Aliphatic and aromatic	Novel	TFS**K**ILA**P**–GF**R**I**G**WV
Pyrococcus furiosus	MsAT	Aliphatic and aromatic	Novel	TFS**K**ILA**P**–GF**R**L**G**WI
Aeropyrum pernix	MsAT	Aliphatic and aromatic	Novel	TFS**K**ILA**P**–GL**R**L**G**LT
P. horikoshii	Aromatic AT	Aromatic	Iγ	GFS**K**TFSMTGW**R**L**G**YI

From wide substrate range and unique sequence around active site lysine of the three aminotransferases (multi-substrate aminotransferases, MsATs), we proposed a novel group of AT, close to but distinct from subfamily Iγ (Table 4).

Mechanism of stereoselective action of lipase from *Candida antarctica* (CAL-B) (1): stereoselectivity of acetate of primary and secondary aryl or aryloxy alcohols

Hideto Kimura*, Seiji Shinohara, Yoshinori Inoue, Hideo Hirohara

Department of Materials Science, The University of Shiga Prefecture, Hassaka 522-8533, Japan. E-mail: v21hkimura@ec.usp.ac.jp

CAL-B-catalyzed hydrolysis of the single enantiomers of primary and secondary aryl or aryloxy alcohol esters was investigated kinetically aiming at elucidating the mechanism of stereoselective action of the enzyme (Table 5).

Preparation of modified ceramics supports "Toyonites" with silane coupling agents and the characteristics of the supports in enzyme immobilization

Masanobu Kamori[a], Yoshitaka Yamashita[a], Yoshinobu Naoshima[b,*]

[a]Toyedenkakogyo Co. Ltd., 2-2-25 Hagi-machi, Kochi 780-8525, Japan. [b]Faculty of Informatics, Okayama University of Science, 1-1 Ridai-cho, Okayama 700-0005, Japan. E-mail: naoshima@sp.ous.ac.jp

Modified ceramics supports Toyonite②-M and Toyonite②-P possessing methacryloyloxy and phenylamino functions on each porous surface showed excellent selectivities toward lipases PS, OF, and CHIRAZYMES compared with the groups-free Toyonite support (Fig. 25).

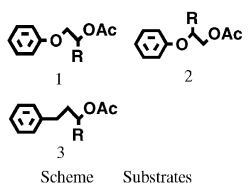
The substrate specificities of the wild and the mutated FPP synthases from *Bacillus stearothermophilus* (3)

T. Ikeda[a], M. Tsuchimoto[a], M. Komabayashi[a], N. Ohya[a], H. Hemmi[b], T. Nishino[b], T. Koyama[c], Y. Maki[a]

[a]Department of Chemistry, Yamagata University, Japan. [b]Department of Biochemistry Bioengineering, Tohoku University, Japan. [c]IMRAM, Tohoku University, Japan. E-mail: maki@sci.kj.yamagata-u.ac.jp

The substrate specificities of the wild and the mutated FPSs from the thermostable bacteria were studied by using DMAPP analogs and GPP analogs having the chains with a various length and sulfur atom or phenyl group in their prenyl chain (Fig. 26).

Table 5. Stereoselectivity to acetates of primary and secondary aryl or aryloxy alcohol by *Candida antarctica* lipase



Scheme Substrates

Table E values : $(k_{cat}/K_M)_R/(k_{cat}/K_M)_S$

R	1	2	3
CH_3	>1000	1.3	>1000
CH_2CH_3	∞	13	>1000
$CH(CH_3)_2$	∞	57	∞
$CH_2(CH_2)_3CH_3$	∞	20	∞

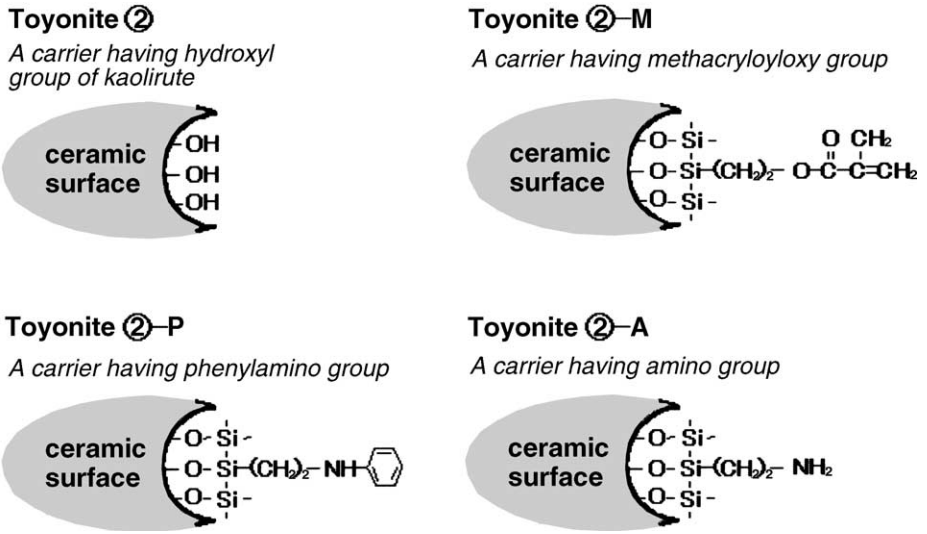


Fig. 25. Preparation of 'Toyonites' with Silane coupling agents.

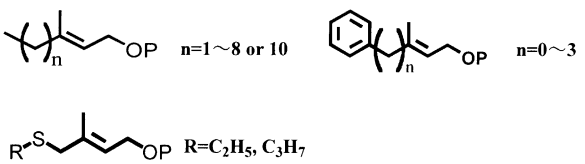


Fig. 26. Substrate analogs studied in this work.

Syntheses of chiral epoxyalcohols by use of a thermostable FPP synthase

Masahiko Nagaki[a,*], Kazuhiro Miyata[a], Yuji Maki[b], Tokuzo Nishino[c], Tanetoshi Koyama[d]

[a]Department of Materials Science and Technology, Faculty of Science and Technology, Bunkyo-cho Hirosaki Aomori 036-8561, Japan. [b]Department of Biological and Material Chemistry, Faculty of Science, Yamagata University, Koshirakawa-cho, Yamagata 990-8560, Japan. [c]Department of Biochemistry and Engineering, Faculty of Engineering, Tohoku University, Aobaku, Sendai, Japan. [d]Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, Aobaku, Sendai, Japan. E-mail: nagaki@cc.hirosaki-u.ac.jp

In order to synthesize some chiral epoxyalcohols, we have examined the condensations between 6,7-epoxygeranyl diphosphate and some 3-alkyl homologs of IPP by use of FPP-synthase (FPS) as well as a mutant FPS, Y81R, which shows different substrate specificities from the wild-type (Fig. 27).

Synthesis of biologically active compounds from Darzens condensation products by using biocatalysts

Komiyama Takuzou, Hamamoto Hiromi, Ashraful Alam, Mamedov Vakhid A., Tsuboi Sadao

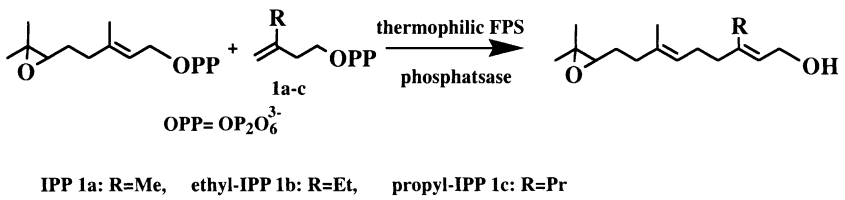


Fig. 27. FPS reaction of epoxygeranyl diphosphate with IPP–homologs.

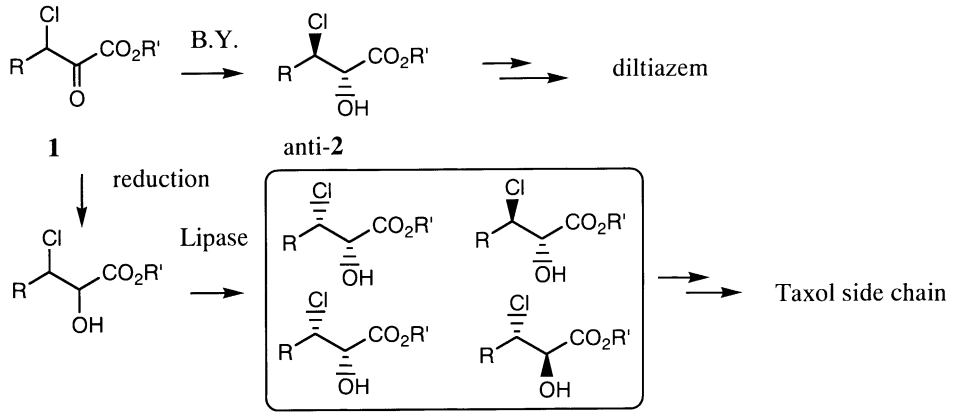


Fig. 28. Synthesis of biologically active compounds.

Faculty of Environmental Science and Technology, Okayama University, Okayama 700-8530, Japan

Chemoenzymatic syntheses of the C-13 side chain of taxol and diltiazem with lipase and baker's yeast were investigated from the starting material, α-aryl-α-chloropyruvate, which was obtained by Darzens condensation of aldehydes with dichloroacetates (Fig. 28).

Leucylglycine hydrolases from cyclo(Gly-Leu)-assimilating bacterium

Kazuyuki Miyoshi, Teruhiko Nitoda, Hiroshi Kanzaki

Faculty of Agriculture, Okayama University, Japan. E-mail: hkanzaki@cc.okayama-u.ac.jp

Leucylglycine hydrolases from *Agrobacterium radiobacter* NM5-3 that participate in the metabolism of cyclo(Gly-Leu), one of the bioactive diketopiperazines, were purified and characterized (Fig. 29).

Novel actinomycetous dehydrogenases useful for production of bioactive dehydrogenated cyclic dipeptides

Atsushi Morimoto, Banri Ikeda, Teruhiko Nitoda, Hiroshi Kanzaki

Faculty of Agriculture, Okayama University, Japan. E-mail: hkanzaki@cc.okayama-u.ac.jp

The purified PMS-dependent enzyme involved in albonoursin biosynthesis of *Streptomyces albulus* KO23 was found to catalyze the conversion of cyclo(Leu-Phe) to cyclo(Leu-ΔPhe), not to cyclo(ΔLeu-Phe) or albonoursin (Fig. 30).

A search for insect chitinase inhibitors of fungal origin

Teruhiko Nitoda, Hirokazu Usuki, Hiroshi Kanzaki

Faculty of Agriculture, Okayama University, Japan. E-mail: nitoda@cc.okayama-u.ac.jp

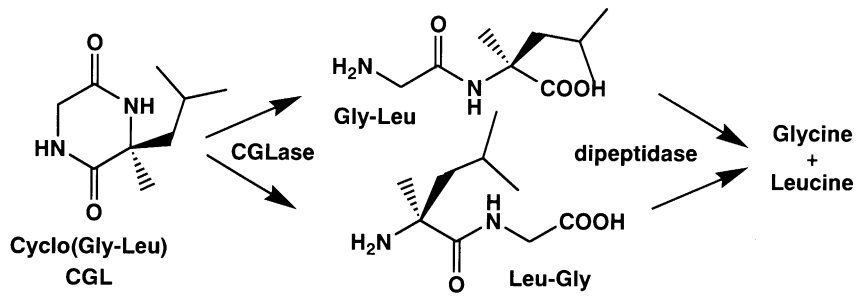


Fig. 29. Cyclo(Gly–Leu) hydrolysis by *Agrobacterium radiobacter* NM5-3.

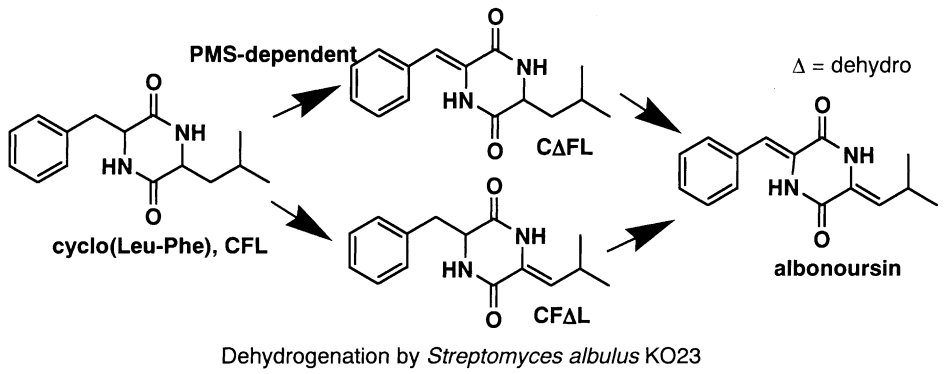


Fig. 30. Cyclo(Leu-Phe) dehydrogenation by *Streptomyces albulus* KO23.

Table 6
The characters of chitinase inhibitors from 5 fungal strains

Strain	Molecular weight[a]	Thermostability (100 °C, 10 min)	Ionic character
TNPT116-Cz	≒80000	Stable	Non-ionic
F76	30000–100000	Stable	Non-ionic
F77	30000–100000	Unstable	Non-ionic
AKF46	3000–10000	Stable	Non-ionic
HUF45	30000–100000	Stable	Non-ionic

[a] As a globular protein.

The partial characterization of insect chitinase inhibitors from five strains obtained by screening of 776 fungal strains revealed that these strains produced at least four distinct compounds which were different from known chitinase inhibitors (Table 6).

Kinetic resolution of 2,2-difluorohomoallylalcohols through lipase-catalyzed reaction

Masayuki Kirihara[a,*], Masashi Kawasaki[b], Hirotaka Katsumata[a]

[a]Department of Materials Science, Shizuoka Institute of Science and Technology, Japan. [b]Faculty of Engineering, Toyama Prefectural University, Japan. E-mail: kirihara@sist.ac.jp

The synthesis of optically active 2,2-difluorohomoallylalcohols has been accomplished through the lipase-catalyzed transesterification (Fig. 31).

Biotransformation of organic compound by plant suspension cells

Hiroki Hamada*, Shuhei Nakagawa, Tsutomu Furuya

Department of Applied Science, Faculty of Science, Okayama University of Science, 1-1 Ridai-cho, Okayama 700-0005, Japan. E-mail: hamada@das.ous.ac.jp

The eucalyptus suspension cells glycosylate the hydroxyl group of phenolic compounds, such as kojic

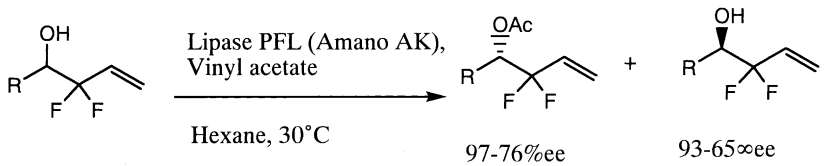


Fig. 31. Kinetic resolution of 2,3-difluorohomoalylalcohols.

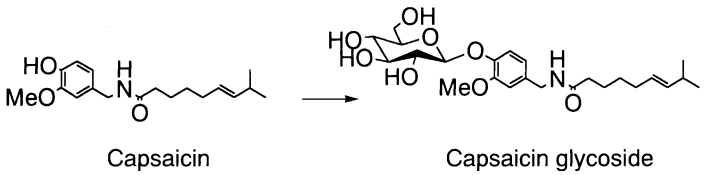


Fig. 32. The direct glycosylation of capsaicin by *Eucalyptus* cultured suspension cells.

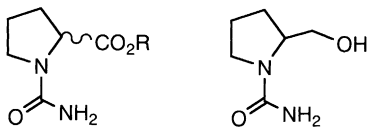


Fig. 33. Enzyme–catalyzed resolution of racemic prolines and prolinols.

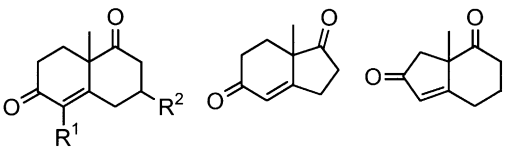


Fig. 34. Substrate specificity of *Torulaspora delbrueckii*—mediated reduction on bicyclic substrates.

acid and capsaicin. The other plant cells have the conversion abilities; enantioselective oxidation, regioselective hydroxylation and stereoselective reduction (Fig. 32).

Enzyme-catalyzed enantiomeric resolution of *N*-carbamylproline derivatives

Masayuki Kurokawa, Takeyuki Shindo, Masumi Suzuki, Takeshi Sugai

Department of Chemistry, Keio University, Japan. E-mail: sugai@chem.keio.ac.jp

Toward the preparation of enantiomerically enriched forms of 1-amino-2-methoxypyrrolidine, enzyme-catalyzed kinetic resolution of *N*-carbamylproline esters and *N*-carbamylprolinol esters was examined (Fig. 33).

Application of *Torulaspora delbrueckii*-mediated reduction in organic synthesis

Mina Tomita, Ken-ichi Fuhshuku, Takeshi Sugai

Department of Chemistry, Keio University, Japan. E-mail: sugai@chem.keio.ac.jp

Substrate specificity of the reduction of carbonyl compounds with *Yamadazyma farinosa* IFO10921 and long-term preservation of the yeast cells were examined (Fig. 34).

Enantioselective synthesis of the fish deterrent, sporochnols

Atsuhito Kuboki*, Taisuke Hasegawa, Maki Nomura, Eri Ogasawara, Takato Kikuchi, Tatsuhiko Kutsukake, Susumu Ohira

Department of Biochemistry, Okayama University of Science, 1-1 Ridai-cho, Okayama 700-0005, Japan. E-mail: kuboki@dbc.ous.ac.jp

Sporochnols, fish deterrent, were synthesized using enantioselective hydrolysis with poricine pancreas lipase and the C–H insertion of alkylidenecarbene, which was generated from lithiotrimethylsilyldiazomethane and ketone, as the key steps (Fig. 35).

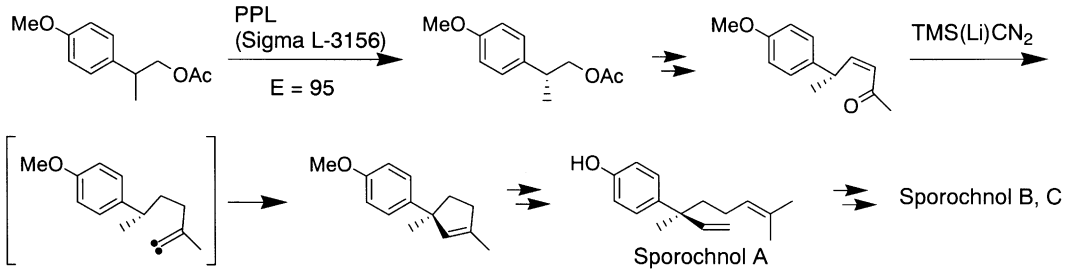


Fig. 35. Synthetic route to Sporochnols.

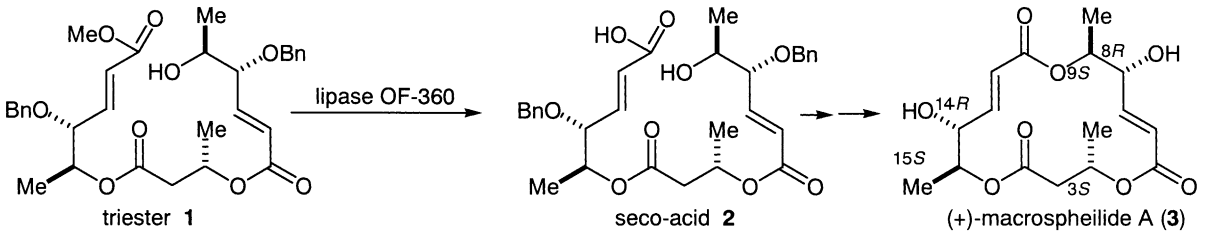


Fig. 36. Synthesis of Macrosphelide A based on enzymatic hydrolysis of triester.

Synthetic study of macrosphelide A based on regioselective hydrolysis using lipase

Machiko Ono*, Hiroshi Nakamura, Hiroyuki Akita
School of Pharmaceutical Science, Toho University, 2-2-1, Miyama, Funabashi, Chiba 274-8510, Japan. E-mail: machiko@phar.toho-u.ac.jp

Regioselective hydrolysis of triester **1** using lipase OF-360 from *Candida rugosa* gave a seco-acid **2**, which was subjected to chemical macrolactonization followed by deprotection to afford the 16-membered ring antibiotic, macrosphelide A (**3**) (Fig. 36).

Synthetic study of (+)-ambrein

Naoko Fujiwara*, Masako Kinoshita, Hiroyuki Akita
School of Pharmaceutical Science, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan. E-mail: akita@phar.toho-u.ac.jp

(+)-Ambrein (**1**), a triterpene alcohol obtained from ambergris, can be disconnected into chiral-decalin part (8a*S*)-**4** and chiral-cyclohexane part (1*S*, 2*S*)-**5**, which were prepared based on lipase-catalyzed kinetic resolution of (±)-**4** and (±)-**5**, respectively (Fig. 37).

Conversion of 4-benzyloxy-5-hydroxy-(2*E*)-hexenoate into osmundalactone and digitoxose

Xiying Zhao*, Machiko Ono, Hiroyuki Akita
School of Pharmaceutical Science, Toho University, 2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan. E-mail: akita@phar.toho-u.ac.jp

Both enantiomers (4*R*, 5*S*)-4-benzyloxy-5-hydroxy-(2*E*)-hexenoate (**1**) and (4*S*, 5*R*)-**1** prepared based on enzymatic hydrolysis of an acetate of (±)-**1** using lipase Amano PS from *Pseudomonas* sp. were converted into (−)-osmundalactone (**2**) possessing anti-feeding activity for the yellow butterfly and 5-hydroxy-2-hexen-4-olide (**3**), and methyl D-digitoxoside (**4**), respectively (Fig. 38).

Characterization of nitroalkene reductases

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Reduction of a trisubstituted nitroalkene by novel nitroalkene reductases afforded the corresponding

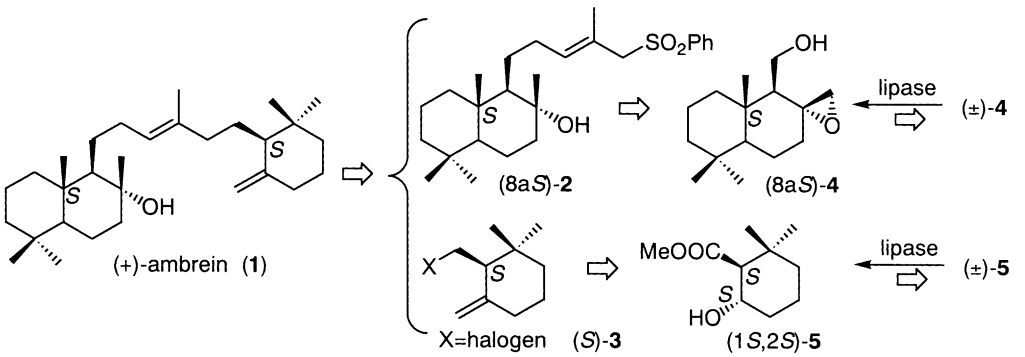

Fig. 37. Synthesis of (+)-ambrein based on the coupling of enzymatic reaction products.

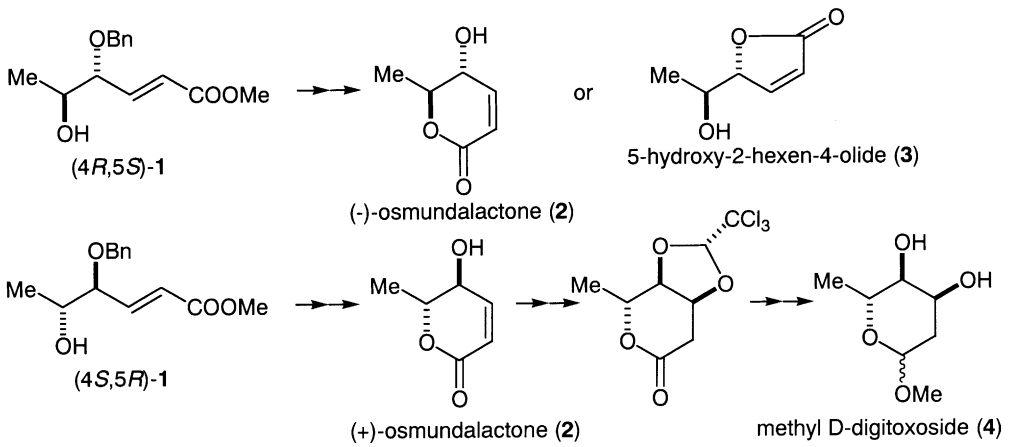

Fig. 38. Lactonization along with trans-cis isomerization from enzymatic reaction products.

nitroalkane with excellent enantioselectivity, moderate diastereoselectivity, and in good yield (Fig. 39).

Temperature effect of the lipase-catalyzed reactions at very low temperatures

Takashi Sakai, Yuichi Kumashiro, Toshinobu Korenaga, Tadashi Ema

Department of Applied Chemistry, Faculty of Engineering, Okayama University, Tsushima, Okayama 700-8530, Japan. E-mail: tsakai@cc.okayama-u.ac.jp

The enantioselectivity in the lipase-catalyzed kinetic resolutions was found to be increased markedly with decreasing the reaction temperature, and the Eyring plots of $\ln E$ versus 1/T generally consist of two straight lines, intersecting at a point defining a temperature called the inversion temperature (Fig. 40).

Optimization of the organic bridge for Toyonite-immobilized lipase

Takashi Sakai, Fumika Yano, Megumi Ino, Toshinobu Korenaga, Tadashi Ema

Department of Applied Chemistry, Faculty of Engineering, Okayama University, Tsushima, Okayama 700-8530, Japan. E-mail: tsakai@cc.okayama-u.ac.jp

Some types of organic bridges for immobilization of lipases to Toyonite were synthesized, and their potentialities were examined from a viewpoint of low-temperature reactions (Fig. 41).

Synthesis and selective functional group transformation of optically active azirines

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The optically active azirinemethanol which was prepared by the lipase-catalyzed resolution was transformed into substituted aziridinemethanol and amino alcohol derivatives diastereoselectively (Fig. 42).

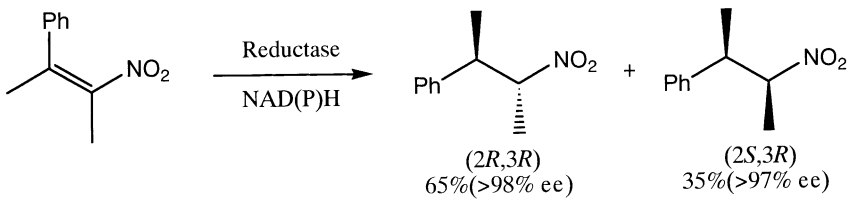

Fig. 39. Asymmetric reduction of a nitroalkene by novel nitroalkane reductases.

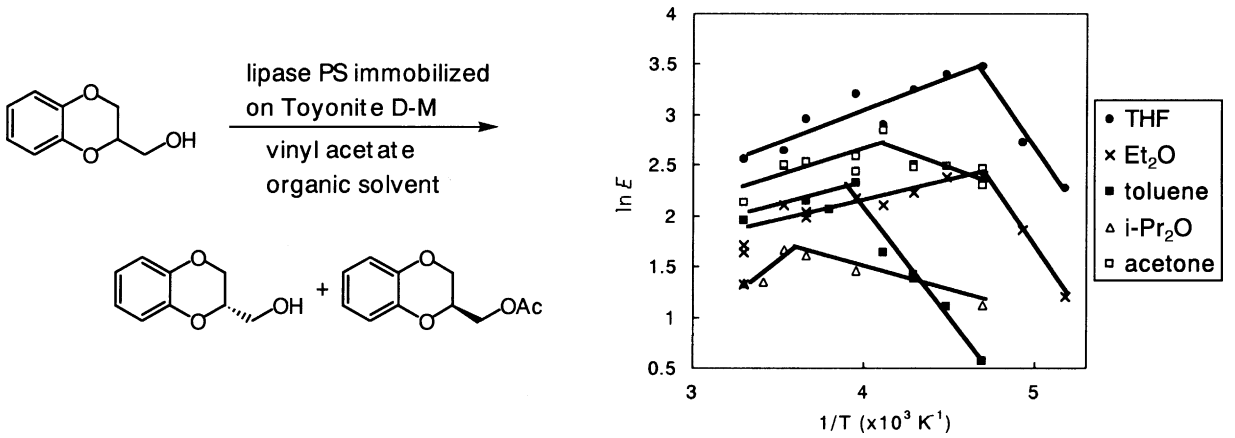

Fig. 40. Inversion temperatures in the lipase–catalyzed kinetic resolutions.

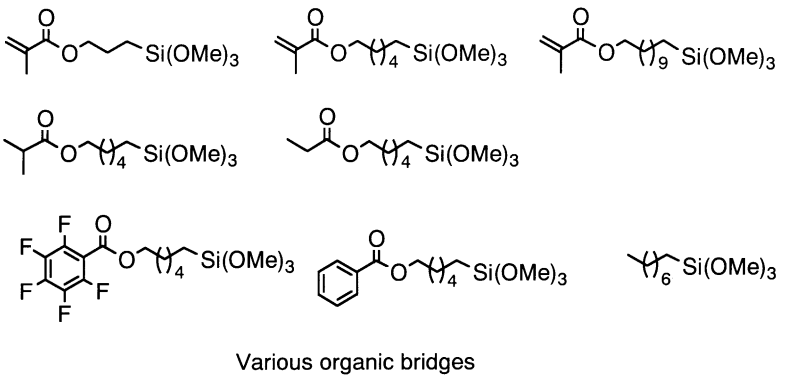

Fig. 41. Organic bridges used for immobilization of a lipase.

CGTase-catalyzed selective glucosidation of chiral alcohol

Takashi Sakai, Nono Oga, Nobuaki Tanaka, Makoto Takahashi, Masahide Miura, Tadashi Ema, Toshinobu Korenaga

Department of Applied Chemistry, Faculty of Engineering, Okayama University, Tsushima, Okayama 700-8530, Japan. E-mail: tsakai@cc.okayama-u.ac.jp

Regio- and enantioselectivities in the CGTase-catalyzed glucosidations of chiral 1,2-diols were investigated (Fig. 43).

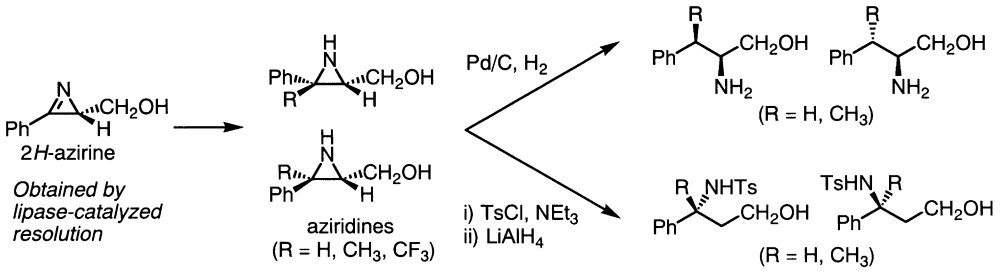

Fig. 42. Optically active azirines as the Chiral building block.

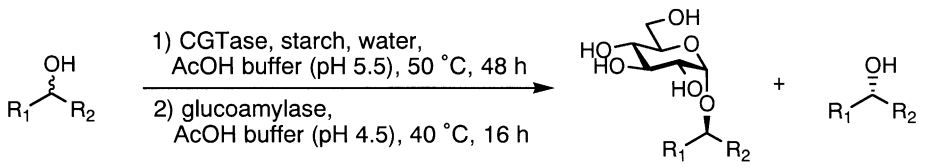

Fig. 43. Enantioselective and regioselective glucosidation of 1,2-diols by CGTase.

Characteristics and molecular mechanism of versatile enzymes

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Mechanistic aspects of versatile enzymes, such as lipases, subtilisins, and a reductase isolated from bakers' yeast, showing broad substrate specificity and high enantioselectivity simultaneously, have been reported and discussed (Fig. 44).

Directed evolution of sialic acid aldolase

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Using in vitro evolution, *E. coli* sialic acid aldolase has been converted with altered substrate specificity and stereoselectivity (Fig. 45).

Oxidative polymerization of phenol catalyzed by crude enzyme from horseradish

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The polymerization of phenol in hydrophilic organic solvent—phosphate buffer solution, catalyzed by desalting powder of ammonium sulfate precipitation of horseradish, was examined, and it was found that obtained polymers show different characteristics according to the kind of organic solvents (Fig. 46).

Lipase immobilization onto mesoporous silica

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[a]Ceramics Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Japan. [b]Toyota Central R&D Laboratory, Japan. E-mail: katsuya-kato@aist.go.jp

Lipases SP 525, AK, and PS were immobilized onto three kinds of mesoporous silica (FMS-16, PESO,

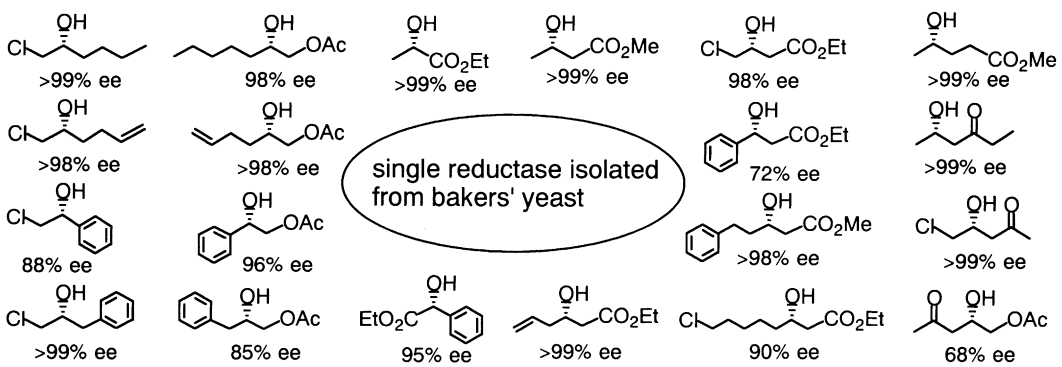

Fig. 44. Asymmetric reduction of ketones using a reductase isolated from bakers' yeast.

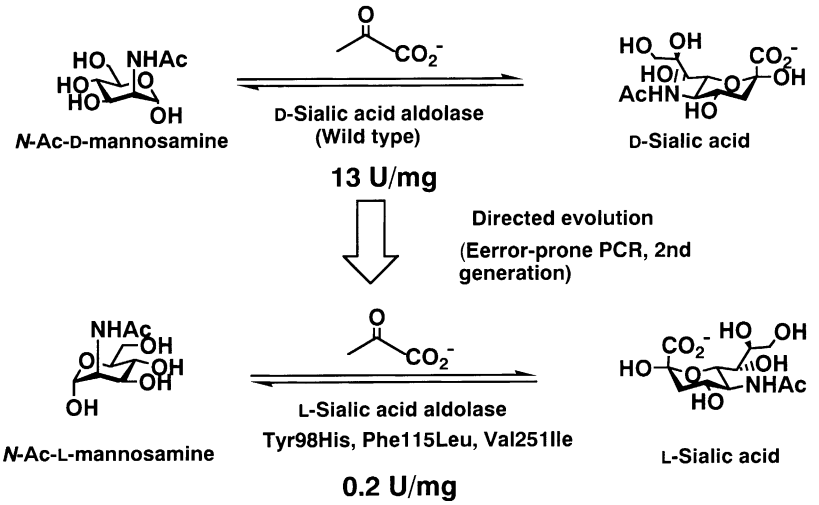

Fig. 45. In vitro evolution of *E. coli* sialic acid aldolase.

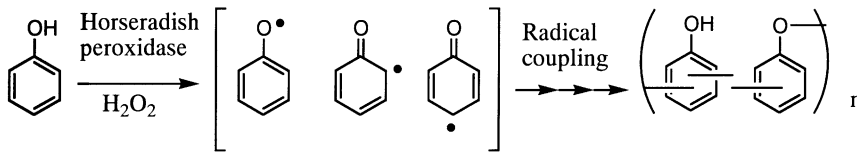

Fig. 46. Polymerization of phenol catalyzed by crude peroxidase from horseradish.

SBA-15) with various types of diameters from 27 to 92 Å (Fig. 47).

Dynamic kinetic resolution of hemiaminals

Mohd. Sharfuddin[a], K. Miyazawa[b], S. Yamada[b], H. Kaga[a]

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Dynamic kinetic resolution of *racemic N*-acylhemiaminals using lipase PS and vinyl acetate at 70 °C afforded enantiomerically rich acetates in quantitative yield (Fig. 48).

Bio-catalyzed resolution of indandiols

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Bio-catalyzed kinetic resolution of *cis*- and *trans*-indandiol diacetate mixture furnished enan-

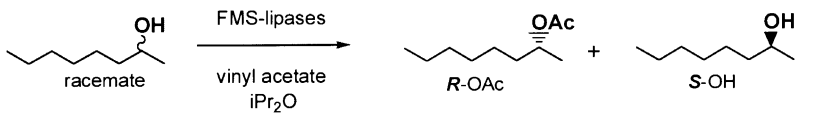

Fig. 47. Enantioselective acetylation of 2-octanol by lipases immobilized on mesoporous silica FMS.

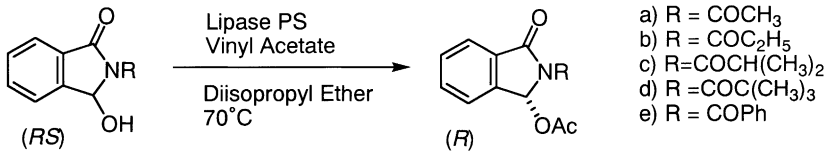

Fig. 48. Dynamic kinetic resolution of hemiaminals using Lipase PS.

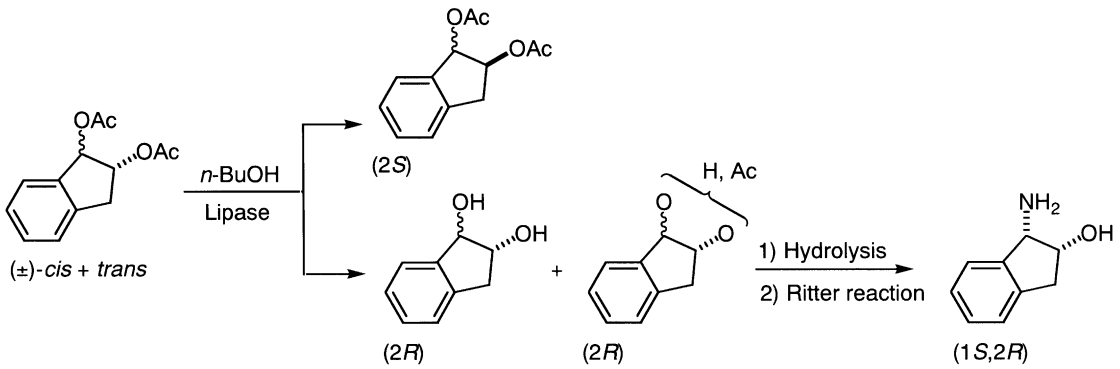

Fig. 49. Bio-catalyzed resolution of cis- and trans-indandiol diacetate mixture.

tiomerically pure *cis*- and *trans*-indandiol mixture, which provided an efficient synthetic route to optically pure 1-amino-2-indanol (Fig. 49).

Enantioselective esterification with lipase in $scCO_2$

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The enantioselectivity of the esterification with lipase can be tuned continuously from $E = 10$ to 50 by adjusting pressure of CO_2. The effect of the solvent was examined without changing the kind of solvent (Fig. 50).

Purification and characterization of α-keto ester reductase from *Streptomyces coelicolor* A3(2)

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[a]Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan. [b]Department of Nutri-

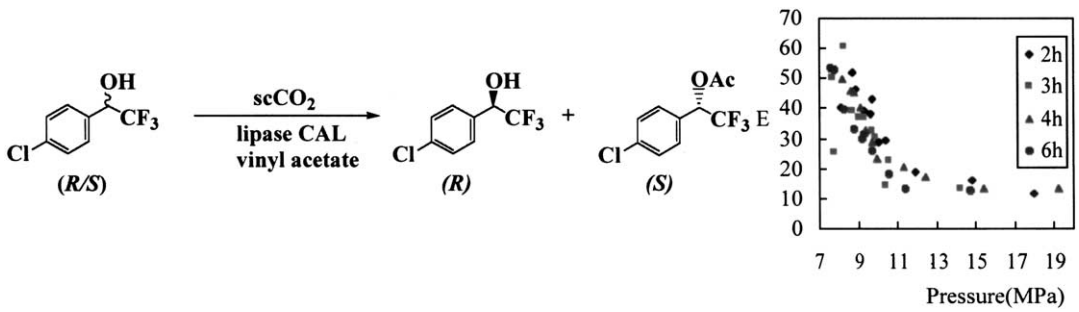

Fig. 50. Enantioselective esterification in supercritical CO_2.

Table 7. The reduction of α-keto esters by SCKER.

R / R'	ee (%)	Config.	R / R'	ee (%)	Config.
Me / Et	14	*S*	*n*-Pen / Et	95	*R*
Et / Et	38	*R*	*i*-Pr / Et	25	*R*
n-Pr / Et	62	*R*	Ph / Et	35	*R*
n-Bu / Et	85	*R*	Ph / Me	72	*R*

tional Science, Okayama Prefectural University, Soja, Okayama 719-1197, Japan. [c]Department of Chemistry, Kyoto University of Education, Fushimi-ku, Kyoto 612-8522, Japan. E-mail: kishi@kyokyo-u.ac.jp

An α-keto ester reductase from *Streptomyces coelicolor* A3(2) (SCKER) was purified and characterized. SCKER required zinc ion for the reducing activity and reduced various α-keto esters and α-keto acids using NADH as a coenzyme (Table 7).

Preparation of poly(siloxane) catalyzed by a lipid-coated enzyme

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Lipid-coated lipases were found to catalyze the enzymatic polymerization of diethoxydimethylsilane (DEDMS) in organic solvents (Fig. 51).

Resolution and synthesis of optical active alcohols by stereoselective oxidation with immobilized food protein as new bio-catalysts

Hiroyuki Nagaoka[a], Hiroshi Kayahara[b]

[a]Department of Research & Development, Sanyo Shokuhin Co. Ltd., Japan. [b]Department of Sci-

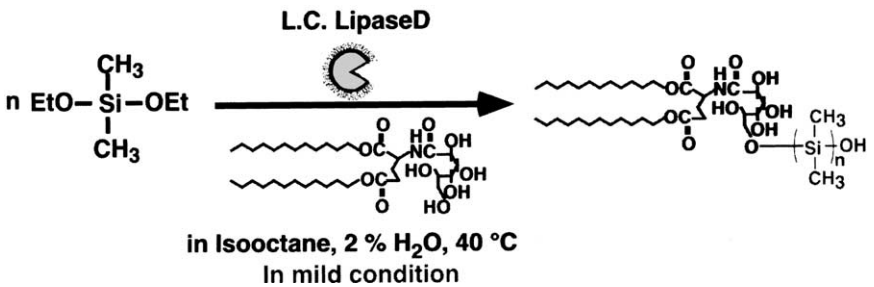

Fig. 51. Lipase–catalyzed polymerization of diethoxydimethylsilane.

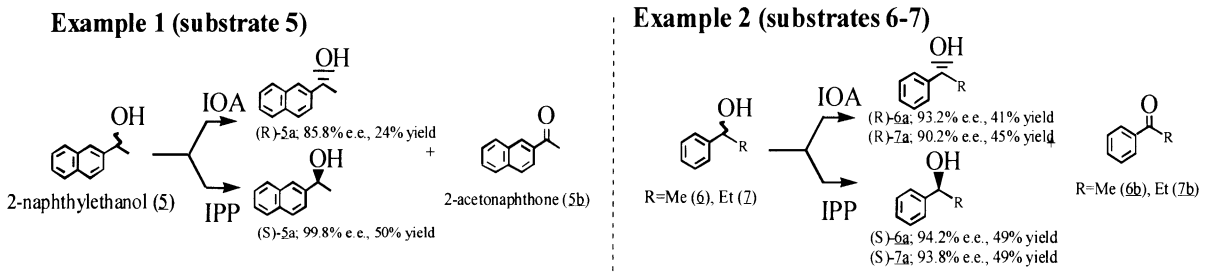

Fig. 52. A specific use for each enantiomer with food proteins.

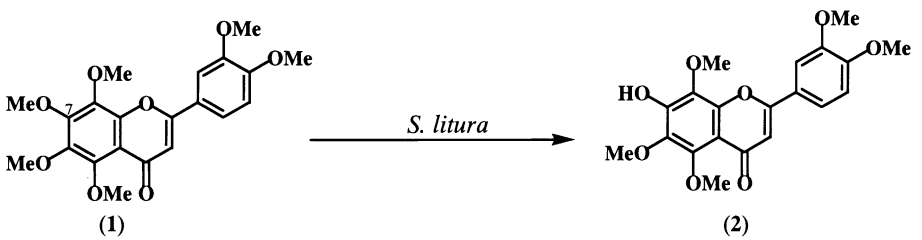

Fig. 53. Biotransformation of Nobiletin (1) by *S. litura*.

ences of Functional Foods, Graduate School of Agriculture, Shinshu University, Japan. E-mail: hnagaoka@sanyofoods.co.jp

It was found that a novel synthesis method which comprises preparing a powdery crude protein fraction from cereal, bean and egg tissues, treating substrate racemic alcohols with these fraction, and thus stereoselectively oxidizing one of the enantiomers to thereby resolve optically active alcohols with high optical purity (Fig. 52).

Biotransformation of polymethoxyflavonoid (nobiletin) by the larvae of common cutworm (*Spodoptera litura*) as a biocatalyst

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Department of Applied Chemistry, Faculty of Science and Engineering, Kinki University, Japan. E-mail: miyazawa@apch.kindai.ac.jp

Polymethoxyflavonoid (nobiletin (**1**)) was biotransformation to 7-hydroxy-5,6,8,3′,4′-pentamethoxyflavon (**2**) by the larvae of common cutworm (*Spodoptera litura*; Fig. 53).

A first synthesis of a phosphatidylcholine bearing docosahexaenoic and tetracosahexaenoic acids

Yoshihiro Mori[a], Md. Khorshed Alam[a], Syed S. Haider[a], Masatoshi Tanaka[a], Shuhei Nakajima[a], Sakayu Shimizu[b], Naomichi Baba[a]

[a]Department of Bioresources Chemistry, Faculty of Agriculture, Okayama University, Tsushima, Okyama

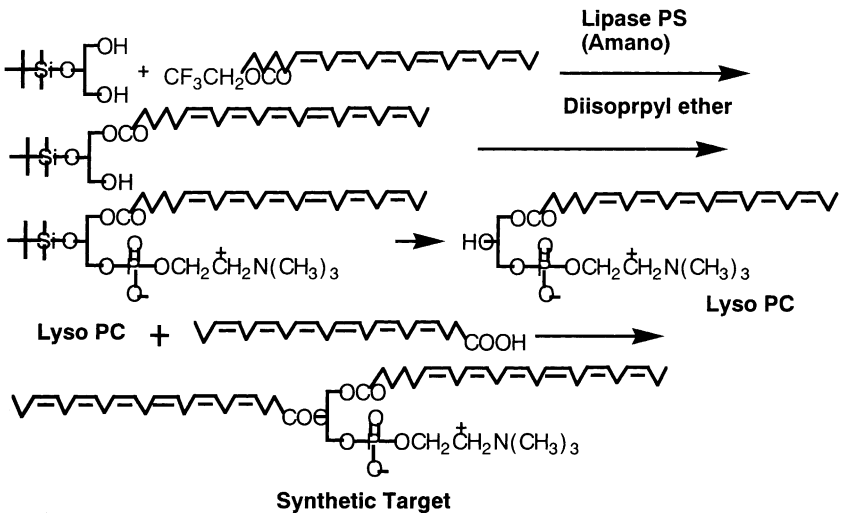

Fig. 54. Synthetic route to 2-docosahexaenoyl-1-tetracosahexaenoyl-sn glycerophosphochdine.

700-8530, Japan, [b]Division of Applied Life Science, Graduate School of Agriculture, Kyoto University, Kitashirakawa, Sakyo-ku, Kyoto 606-8052, Japan

By the aid of lipase-catalyzed stereoselective acylation, an optically active glycerophospholipid having tetracosahexaenoic and docosahexaenoic acids was synthesized for the first time and the stereochemistry of the chiral center was determined (Fig. 54).