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Selected abstracts from the 6th Japanese Symposium on the Chemistry of Biocatalysis

Yasuhisa Asano [a,*], Kaoru Nakamura [b]

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Introduction

The symposium on the chemistry of biocatalysis was held on 12–13 December 2002 in Nara. Approximately 140 researchers from industry and academic world and 60 students participated to the symposium. We had the following program:

Plenary lecture: Prof. H. Griengl (University of Technical, Graz, Austria); "The interrelation between academia and industry in the area of biocatalysis in Europe".

Progress Research on Biocatalysis in USA and Europe: Dr. H.H. Groeger (Degussa AG, Germany) "Biocatalysis in the fine chemicals field in Germany—current applications and future trends", Dr. S. Yamazaki (Merck, USA) "Biocatalysis in Pharmaceutics: Challenges and Trends at Merck/USA".

Biocatalysis in venture business: Dr. Moon-Hee Sung (Laboratory of Microbial Functions, KRIBB, Taejon, Korea) "Venture business in Korea", Prof. Y. Okahata (Tokyo Institute of Technology) "Venture business growing from university in Japan".

Biocatalysis in industry in Japan: Dr. T. Fujio (Kyowa Hakkou Kogyo Co.) "Application of Genome Science to Bioprocess", Dr. H. Nakazawa (Ajinomoto Co.) "What I learnt from the development of enzymatic synthesis of amino acids and nucleic acids", Dr. J. Hasegawa (Kaneka Co.) "Industrial production of chiral compounds". Discussion for "Biocatalysis in industry: present and future". This section was coordinated by Dr. Y. Kobayashi.

Panel discussion: Organized by Dr. Y. Kobayashi (Daicel Chemical Industries Ltd.) "Future Impact of Biocatalysis in Industry and University".

Poster presentation (64 posters): We enjoyed lectures and poster presentations as well as discussions.

Symposium organizer: Kaoru Nakamura

We thank Prof. T. Ito (Tottori University) for his help in summarizing this abstracts.

Yasuhisa Asano, Editor

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Poster Presentations

Lipase-catalyzed regio- and enantioselective reactions of *p*-hydroxytrifluoroethylphenols

Katsuya Kato*, Roxana Irimescu, Takao Saito, Yoshiyuki Yokogawa

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Lipase LIP from *Pseudomonas aeruginosa* catalyzed the enantioselective alcoholysis of racemic 4-(1-acetoxy-2,2,2-trifluoroethyl)phenyl acetate with *n*-butanol, affording (*S*)-4-(1-hydroxy-2,2,2-trifluoroethyl)phenol at >99% e.e. (E ≥ 100).

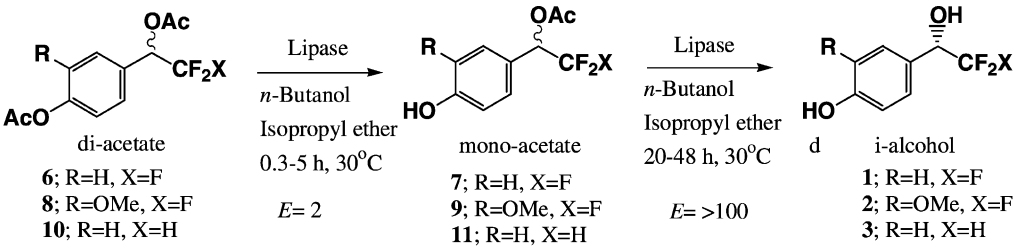


Fig. 1. Lipase-catalyzed kinetic resolution of 4-(1-hydroxy-2,2,2-trifluoroethyl)phenol, **1**, and its related compounds **2** and **3** by enantioselective alcoholysis of their acetates. The two-enzymatic deacetylation steps of the di-acetates, **6**, **8** and **10**.

High-level expression of phenylacetaldoxime dehydratase of *Bacillus* sp. strain OxB-1, in heterologous hosts and application for the enzymatic nitrile synthesis

Yasuo Kato*, Sheng-Xue Xie, Yasuhisa Asano

Biotechnology Research Center, Faculty of Engineering, Toyama Prefectural University, 5180 Kurokawa, Kosugi, Toyama 939-0398, Japan. E-mail: yasu@pu-toyama.ac.jp

Conditions overexpressing novel heme-containing FMN-dependent lyase, phenylacetaldoxime dehydratase of *Bacillus* sp. strain OxB-1, in heterologous hosts were examined and the recombinant cells were applied for the high-yield synthesis of various ary-, alkly-, and arylalkyl nitriles from their corresponding aldoximes.

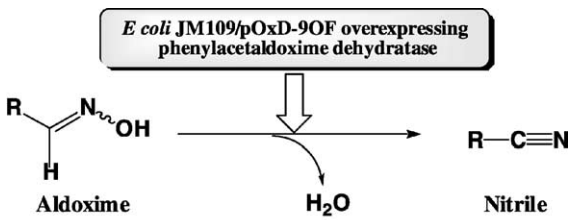


Fig. 2. Enzymatic synthesis of nitriles from aldoximes by the recombinant *E. coli*.

Oxidation of both termini of *p*- and *m*-xylene by *Escherichia coli* transformed with xylene monooxygenase gene

Takahiro Maruyama[a], Hiroshi Iida[a], Hitoshi Kakidani[a,b,*]

[a]Sagami Chemical Research Center, Japan

[b]Tosoh Corporation, Japan. E-mail: kakidani@sagami.or.jp

Escherichia coli strains transformed with xylene monooxygenase (XMO) gene from *Pseudomonas putida* were found to oxidize both termini of *p*- and *m*-xylene, giving rise to various oxidized compounds, including *p*- and *m*-xylyleneglycol.

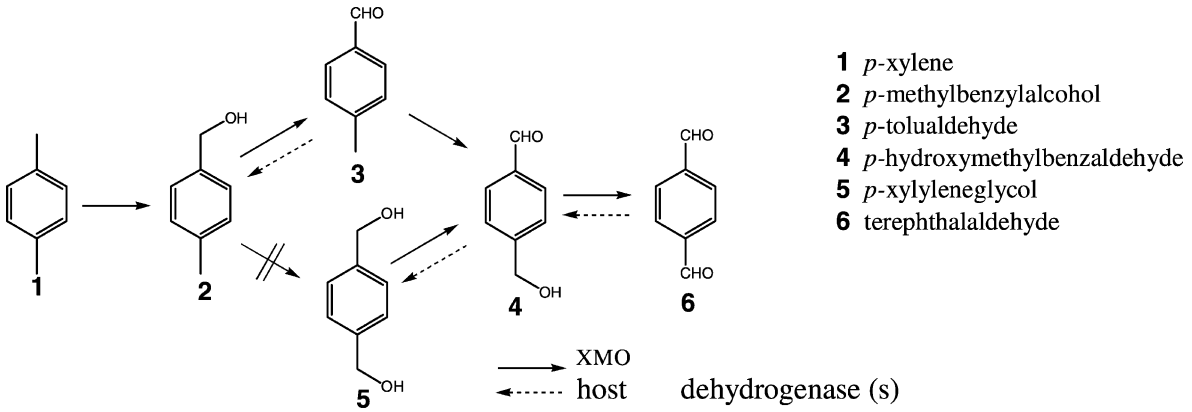


Fig. 3. Proposed oxidation route of *p*-xylene and related compounds.

Synthesis and evaluation of lipases encapsulated within sol–gel derived materials

Katsuya Kato*, Junko Tachibana, Roxana Irimescu, Takao Saito, Yoshiyuki Yokogawa

National Institute of Advanced Industrial Science and Technology (AIST), 2266-98 Anagahora, Simoshidami, Moriyama-ku, Nagoya 463-8560, Japan. E-mail: katsuya-kato@aist.go.jp

Four kinds of lipases (from *Candida antarctica*, *Pseudomonas cepacia*, *Pseudomonas fluorescens*, and *Pseudomonas aeruginosa*) were encapsulated in inorganic matrices by the sol–gel method in order to synthesize chiral compounds by kinetic resolution.

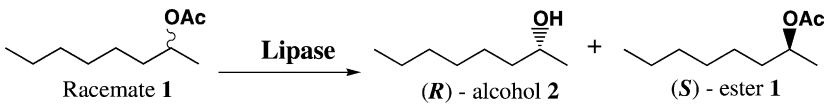


Fig. 4. Encapsulation of lipase SP525 in sol–gel derived materials.

The synthesis of both enantiomers of secondary alcohols by reduction with a single microbe

Mikio Fujii[a], Yoshiteru Ida[a], Kaoru Nakamura[b,*]

[a]School of Pharmaceutical Science, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan

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

E-mail: nakamura@scl.kyoto-u.ac.jp

The reduction with single microbe, *G. candidum* IFO 5767, afforded both enantiomers of aromatic secondary alcohol in excellent e.e. respectively by changing reaction conditions.

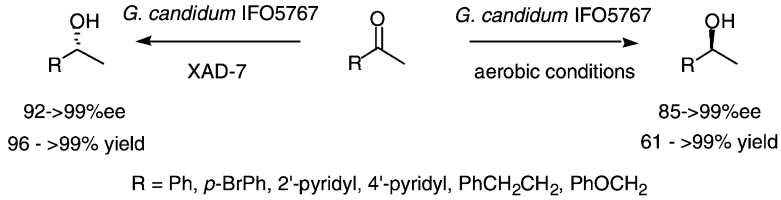


Fig. 5. Synthesis of (*S*)- and (*R*)-secondary alcohols by asymmetric reduction with *Geotrichum candidum*.

Direct enzymatic esterification with water removal under vacuum: a simple and efficient method for kinetic resolution of secondary alcohols

Roxana Irimescu*, Katsuya Kato, Takao Saito

National Institute of Advanced Industrial Science and Technology (AIST), 2266-98 Anagahora, Simoshidami, Moriyama-ku, Nagoya 463-8560, Japan. E-mail: roxana.irimescu@aist.go.jp

Kinetic resolution of some chiral secondary alcohols with high enantioselectivity ($E > 300$) was achieved by direct esterification with free fatty acids catalyzed by immobilized *Candida antarctica* B lipase.

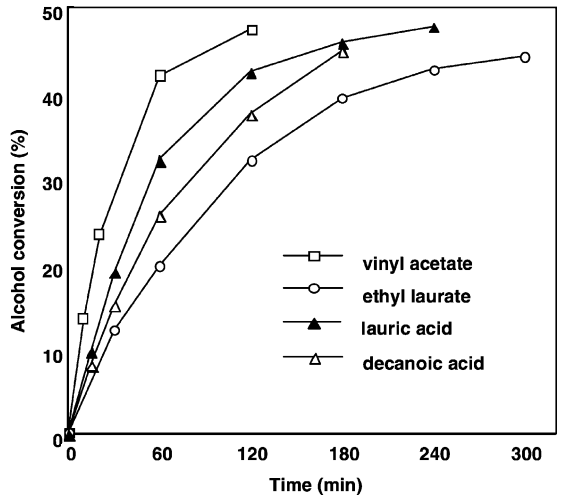


Fig. 6. Kinetic resolution of 2-octanol with different acyl donors.

Identification of genes involved in the formation of a novel extendar unit for PKase and application for the engineered biosynthesis of novel 6-deoxyerythronolides

Yasuo Kato[a,*], Linquan Bai[b], Tin-Wein Yu[b], Heinz G. Floss[b]

[a]Biotechnology Research Center, Faculty of Engineering, Toyama Prefectural University, 5180 Kurokawa, Kosugi, Toyama 939-0398, Japan

[b]Department of Chemistry, University of Washington, P.O. Box 351700, Seattle, Washington 98195-1700, USA. E-mail: yasu@pu-toyama.ac.jp

By analyzing the biosynthetic gene cluster of Ansamitocin, a set of genes involved in the formation of the novel "methoxymalonyl-ACP" extendar unit for PKase were identified and applied for the engineered biosynthesis of novel 6-deoxyerythronolides.

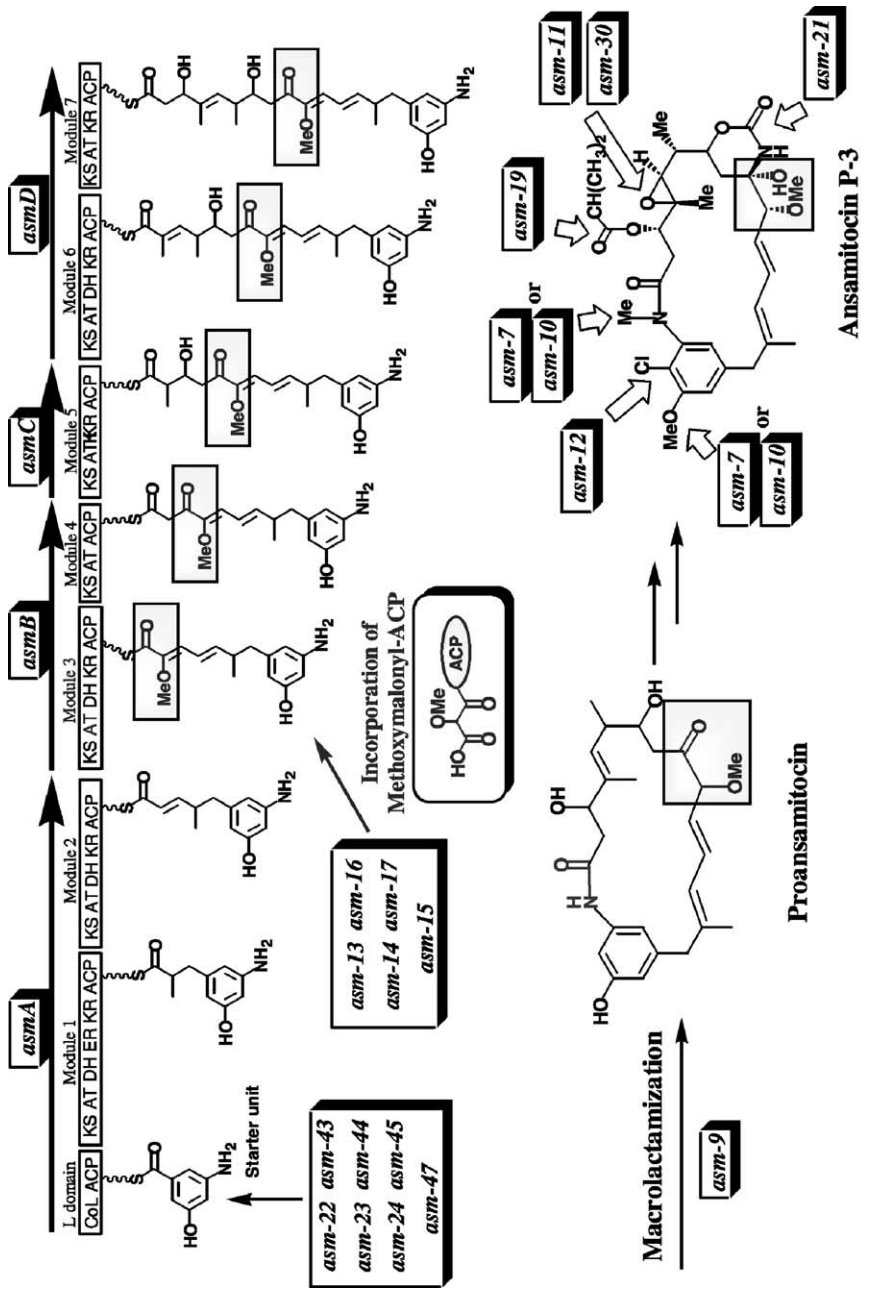
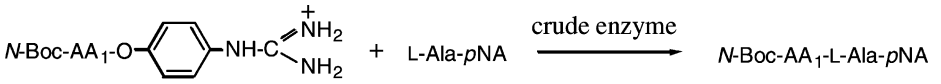


Fig. 7. Proposed biosynthetic pathway of Ansamitocin.

Peptide synthesis catalyzed by crude enzyme from mid-gut gland of an Ezo giant scallop

Haruo Sekizaki*, Kunihiko Itoh, Eiko Toyota, Kazutaka Tanizawa
Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Japan.
E-mail: sekizaki@hoku-iryo-u.ac.jp

A crude enzyme was isolated from mid-gut gland of an Ezo giant scallop, and this enzyme was effective as catalyst for the peptide bond formation using “inverse substrate” as acyl donor component.



AA_1 = L-Ala; D-Ala; L-Leu; D-Leu etc.

Fig. 8. Enzymatic peptide synthesis using inverse substraters.

Lipase-catalyzed enantioselective acylation in a halogen-free ionic liquid solvent

Toshiyuki Itoh*, Nozomi Ouchi, Yoshihito Nishimura
Department of Materials Science, Faculty of Engineering, Tottori University, 4-101 Koyama-cho Minami, Tottori 680-8552, Japan. E-mail: titoh@chem.tottori-u.ac.jp

Various types of imidazolium alkyloxysulfonates were prepared and evaluated their use as solvent for transesterification of secondary alcohols by lipase-catalyzed reaction.

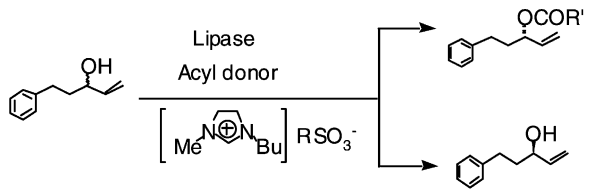


Fig. 9. Lipase-catalyzed enantioselective acylation of secondary alcohols in a halogen-free ionic liquid solvent.

Synthesis of optically active 2,3-dihydorxybenzofuran derivatives through lipase-catalyzed enantioselective acylation

Toshiyuki Itoh*, Kimio Kawai
Department of Materials Science, Faculty of Engineering, Tottori University, 4-101 Koyama-cho Minami, Tottori 680-8552, Japan. E-mail: titoh@chem.tottori-u.ac.jp

Synthesis of optically active 2,3-dihydrobenzofuran derivatives was accomplished by a combination strategy of ferric ion catalyzed cycloaddition of styrene derivatives with quinone and subsequent lipase-catalyzed enantioselective transesterification.

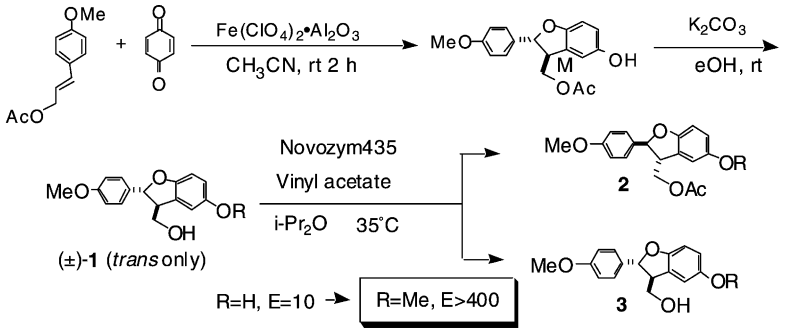


Fig. 10. Synthesis of optically active 2,3-dihydrobenzofuran derivatives through Fe^{3+}-catalyzed cycloaddition reaction and lipase-catalyzed reaction.

Substrate specificity of the thermostable FPP synthases from *Bacillus stearothermophilus*—substrate analogs having sulfur atom in their prenyl chain

Y. Gotoh[a], Y. Sasaki[a], M. Tsuchimoto[a], N. Ohya[a], T. Koyama[b], T. Nishino[c], M. Nagaki[d], Y. Maki[a]
[a]Department of Materials and Biol. Chem., Yamagata University, Japan
[b]IMRAM, Tohoku University, Japan
[c]Department of Biochemistry and Bioengineering, Tohoku University, Japan
[d]Department of Materials Science and Technology, Hirosaki University, Japan.
E-mail: maki@sci.kj.yamagata-u.ac.jp

The substrate specificities of the wild and the mutated FPSs (W.T, Y81S, Y81R, Y81D) from *Bacillus stearothermophilus* were studied by using DMAPP and GPP analogs having sulfur atom in their prenyl chain.

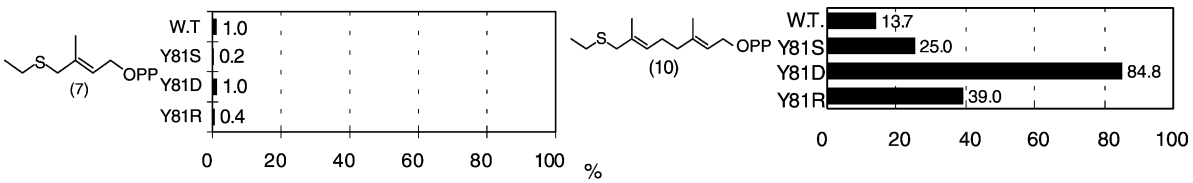


Fig. 11. Reactivities of analogs studied (reactivity of GPP for the wild-type FPS = 100%).

Synthetic study on optically active α-(fluoromethyl)alanines

Masayuki Kirihara[a,*], Ryu Takizawa[a], Akihiko Hatano[a], Masashi Kawasaki[b]
[a]Shizuoka Institute of Science and Technology, Japan
[b]Tosyama Prefectural University, Japan

Although, all our attempts to get the chiral monoacetates of 2-(fluoromethyl)-2-methylpropane-1,3-diol using lipase-catalyzed reaction were failed, we could synthesize chiral monoacetates of 2-benzoyloxycarbonylamino-2-methyl-1,3-diol which is the starting material for the synthesis of chiral α-(fluoromethyl)alanines.

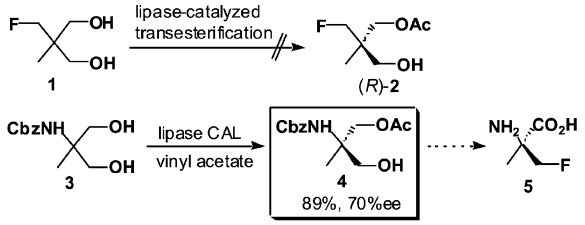


Fig. 12. Synthetic study on optically active α-(fluoromethyl)alanine.

Preparation of optically active 2,6-bis(1-aminoethyl)pyridines

Jun'ichi Uenishi*, Taro Takami, Sachiko Aburatani, Yoshihiko Ito
Kyoto Pharmaceutical University, Yamashina, Kyoto, 607-8412, Japan. E-mail: juenishi@mb.kyoto-phu.ac.jp

Lipase catalyzed kinetic acetylation of 2,6-bis(1-hydroxyethyl)pyridine with vinyl acetate gave (*R*,*R*)-diacetate, (*R*)-monoacetate, and (*S*,*S*)-diol in 1:2:1 ratio with an excellent conversion. Stereospecific substitution of the two chiral hydroxylcarbon centers with amines via methanesulfonate occurred to give optically pure chiral triamine ligands.

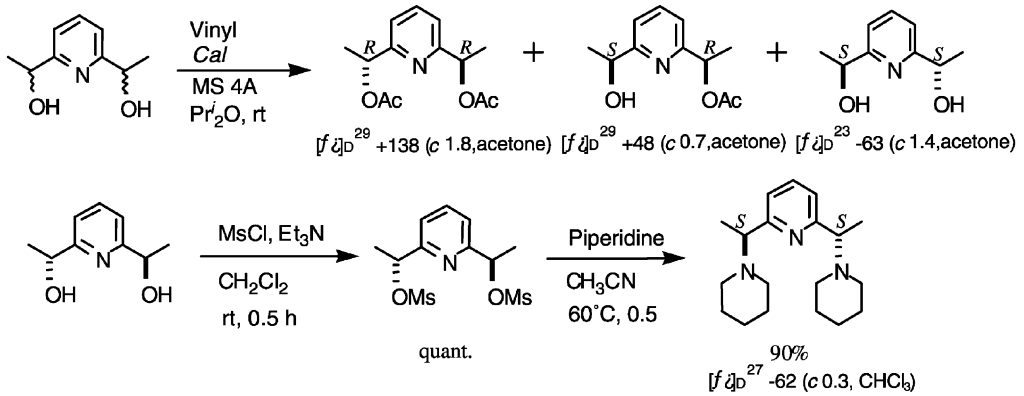


Fig. 13. Lipase catalyzed kinetic resolution of 2,6-bis(1-aminoethyl)pyridine and stereospecific substitution with piperidine.

Synthesis of double-chiral compound by two-step enzymatic asymmetric reduction

Masaru Wada[a,*], Ayumi Yoshizumi[a], Yumiko Noda[a], Hiroshi Takagi[a], Michihiko Kataoka[b], Sakayu Shimizu[b], Shigeru Nakamori[a]
[a]Department of Bioscience, Fukui Prefectural University, Japan
[b]Graduate School of Agriculture, Kyoto University, Japan. E-mail: masaru@fpu.ac.jp

A practical enzymatic synthesis of a double-chiral key compound, (4*R*,6*R*)-4-hydroxy-2,2,6-trimethylcyclohexanone, starting from the readily available 2,6,6-trimethyl-2-cyclohexen-1,4-dione is described.

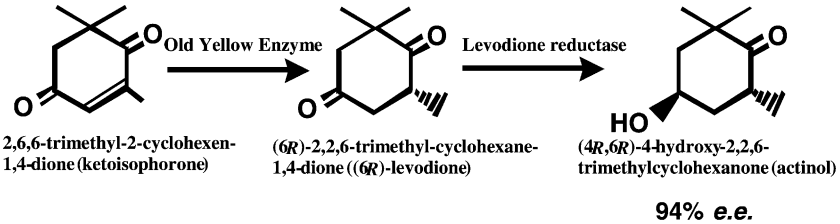


Fig. 14. Production of (4*R*,6*R*)-4-hydroxy-2,2,6-trimethylcyclohexanone by two-step enzymatic asymmetric reduction.

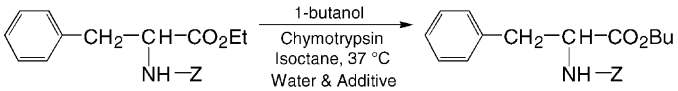
Relationship between enzymatic activity and conformational flexibility of enzymes brought about by additive effects for transesterification in organic media

Hisashi Yasuoka[a], Keiichi Watanabe[b], Takashi Okamoto[b], Shin-ichi Ueji[a,b,*]
[a]Graduate School of Cultural Studies and Human Science, Kobe University, Kobe 657-8501, Japan
[b]Graduate School of Science and Technology, Kobe University, Kobe 657-8501, Japan. E-mail: ueji@kobe-u.ac.jp.

The mechanistic details for the additive-induced high enzymatic activity for chymotrypsin-catalyzed esterification in organic solvents were investigated on the basis of the discussion of the kinetic study and the enzyme's flexibility estimated from the mobility of the spin-label bound to the enzyme by the ESR measurement.

Relationship between the Hi/(Ha + Hi) value as a direct measure of chymotrypsin flexibility and the initial rate in the chymotrypsin-catalyzed transesterification of Z-L-Phe-OEt with 1-butanol in isooctane containing the additives

Table 1



Additive (vol.%)		Initial rate (nmol/h)	Flexibility Hi/(Ha + Hi)
Water	DMSO		
0	0	0	0.22
0.6	0	2.8	0.68
0.6	0.3	53	0.48

The Hi/(Ha + Hi) value was estimated from the ESR measurement of the spin-labeled enzyme (see Poster Number P-41).

Development of methodology for transmembrane movement studies of isoprenoid compounds

Koichi Koseki, Seiji Takahashi, Tanetoshi Koyama*
Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, Japan.
E-mail: koyama@tagen.tohoku.ac.jp

For the purpose of investigating the transmembrane movement of isoprenoid compounds (undecaprenyl phosphate, geranylgeranyl phosphate and farnesyl phosphate), we have synthesized some fluorescent probe compounds and determined their UV and fluorescence characteristics.

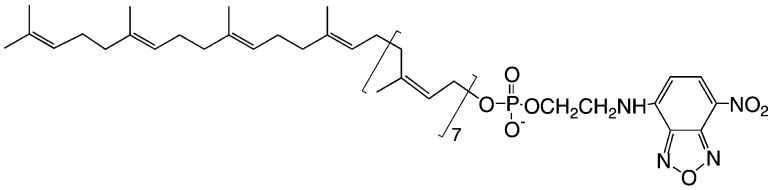


Fig. 15. Fluorescent probe compound.

The direct glycosylation by plant suspension cells

Syogo Ohiwa[a], Hiroki Hamada[a,*], Tsutomu Furuya[a], Kohji Ishihara[b], Nobuyoshi Nakajima[c]

[a]Department of Applied Science, Faculty of Science, Okayama University of Science, 1-1 Ridai-cho, Okayama 700-0005, Japan

[b]Department of Chemistry, Kyoto University of Education, Fushimi-ku, Kyoto 612-8522, Japan

[c]Department of Nutritional Science, Okayama Prefectural University, Soja, Okayama 719-1197, Japan. E-mail: hamada@das.ous.ac.jp

We have investigated the biotransformation of organic compound by plant suspension cells. We study the biotransformation of capsaicin and hinokitiol by plant suspension cells and it was found that plant suspension cells glycosylate the hydroxyl group of capsaicin and hinokitiol.

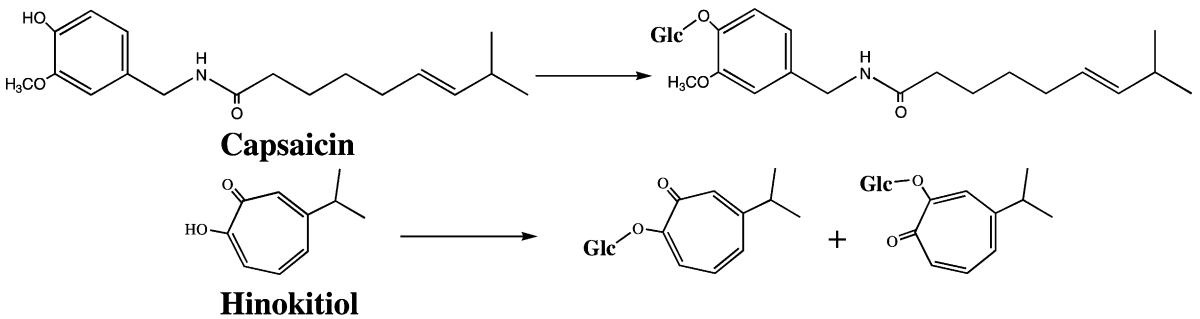


Fig. 16. The direct glycosylation of capsaicin and hinokitiol by plant suspension cells.

Asymmetric synthesis of 2-substituted 4-chromanones: synthesis of chiral intermediate through lipase-catalyzed reaction

Masashi Kawasaki[a,*], Michimasa Goto[b], Tadashi Kometani[b]

[a]Faculty of Engineering, Toyama Prefectural University, 5108 Kurokawa, Kosugi-Machi, Toyama 939-0398, Japan

[b]Toyama National College of Technology, 13 Hongo, Toyama 939-8630, Japan.
E-mail: kawasaki@pu-toyama.ac.jp

(*R*)- and (*S*)-2-methyl-4-chromanone were synthesized from the chiral intermediates which were obtained by lipase-catalyzed enantioselective esterification.

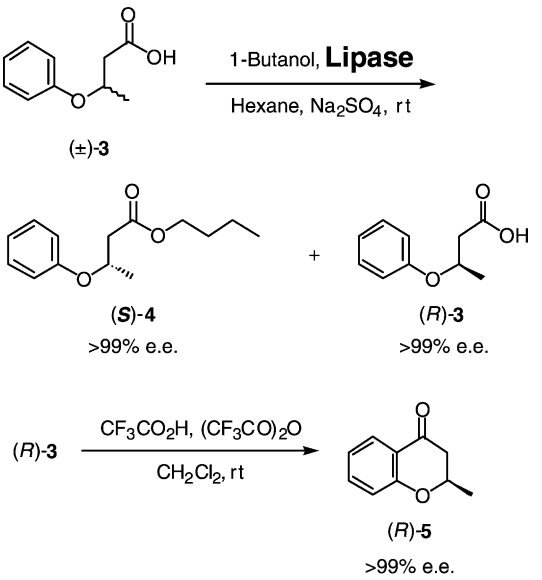


Fig. 17. Synthesis of (*R*)-2-methyl-4-chromanone.

Lipase-catalyzed dynamic kinetic resolution of hemiaminals

Mohd. Sharfuddin[a], H. Kaga[a,*], Y. Iwai[b], S. Yamada[b]
[a]National Institute of Advanced Industrial Science and Technology (AIST), Japan
[b]Ochanomizu University, Japan. E-mail: h.kaga@aist.go.jp

Lipase PS, lipase AK and lipase QL were found to catalyze enzymatic dynamic kinetic resolution of *racemic N*-acylhemiaminals in the presence of isopropenyl acetate to afford the corresponding enantiomerically rich acetates in quantitative yield.

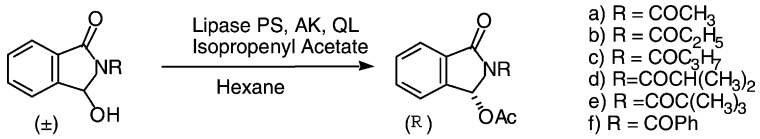


Fig. 18. Dynamic kinetic resolution of *racemic N*-acylhemiaminals.

Stereoselective hydrolysis of acetates of primary alcohol enantiomers catalyzed by *Pseudomonas cepacia* lipase (PCL): a study of rate-determining step

Tomoaki Yokota, Hideto Kimura, Yoshinori Inoue, Hideo Hirohara*
Department of Materials Science, The University of Shiga Prefecture, 2500 Hassaka, Hikone 522-8533, Japan. E-mail: hirohara@mat.usp.ac.jp

From the kinetic and thermodynamic studies for PCL-catalyzed hydrolysis of acetates of both enantiomers of various primary alcohols and we have observed that substrates having benzyl group in the molecule have a quite different rate-determining and stereoselective step from that of the substrates having phenoxy group.

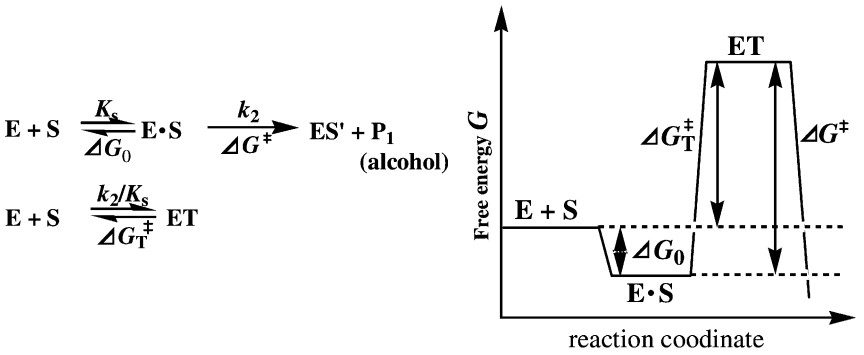


Fig. 19. Energy diagram of the reaction.

Synthetic study for chiral 2*H*-chromenes having a long side-chain

Seiji Yamaguchi, Yohei Murayama, Masaru Ishibashi, Masahiro Miyazawa, Yoshiro Hirai
Department of Chemistry, Faculty of Science, Toyama University, Gofuku, Toyama 930-8555, Japan

For effective preparation of teretifolione B (**2a**) and deoxyteretifolione B (**2b**), and connocurvone (**1**), a pyranotetralone acetate (**3c**), prepared by regioselective thermal cyclization of corresponding propargyl ether followed by deprotection-acetylation, was subjected to asymmetric hydrolysis using Lipase AK (Amano) and gave chiral alcohol (90% e.e.), which might be a chiral building block for **2a**, **2b**, and **1**.

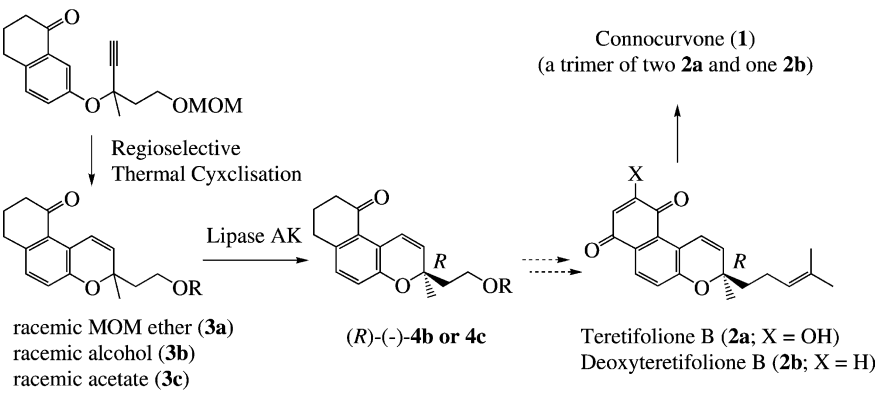


Fig. 20. Asymmetric hydrolysis of acetates of chromene derivatives.

Enantioselective oxidation and reduction of acyclic compounds by a yeast

Kazutsugu Matsumoto, Key Hashimoto, Jyunichi Tatsuta, Yuto Nagai
Meisei University. E-mail: mkazu@chem.meisei-u.ac.jp

The oxidation of *dl*-**1** using *Yamadazyma farinosa* proceeded with high enantioselectivity to afford the optically pure (*R*)-**1** and **2**. On the other hand, the asymmetric reduction of **2** by the yeast also proceeded to give an optically active (*R*)-**3**.

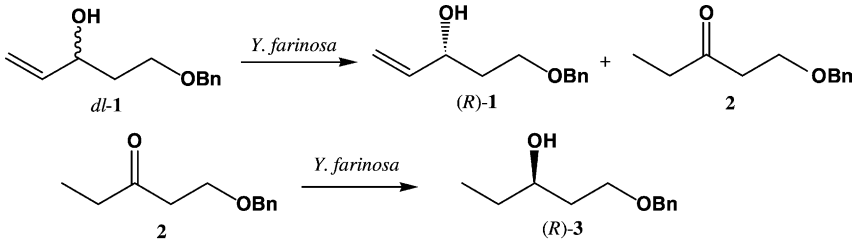


Fig. 21. Enantioselective oxidation of secondary alcohol by *Yamadazyma farinose*.

Syntheses of several optically active alcohols using recombinant *E. coli* cells

Momoko Ueda*, Hiroaki Yamamoto, Masatake Kudoh, Norihiro Kimoto, Akinobu Matsuyama, Yoshinori Kobayashi

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We have developed practical bioconversion processes to the synthesis of chiral alcohols in both configurations starting from ketones or racemic alcohols using whole-cell biocatalysts.

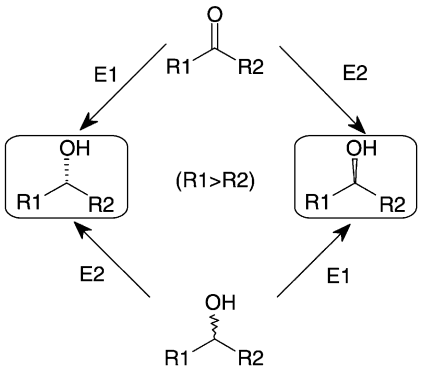

Fig. 22. Syntheses of optically active alcohols by asymmetric reduction or enantioselective oxidation.

Kinetic resolution of racemic hetero-atom containing compounds by enantioselective redox reaction using rat liver S-9 fraction

Koji Uwai, Harumi Nagashima, Tomoko Oshima, Naoko Sasaki, Mitsuhiro Takeshita*

2nd Department of Pharmaceutical Sciences, Tohoku Pharmaceutical University, 4-4-1 Komatsushima, Aoba-ku, 981-8558 Sendai, Japan. E-mail: mtake@tohoku-pharm.ac.jp

The enantioselective redox reaction of nitrogen or sulfur containing compounds by rat liver S-9 fraction gave optically active compounds.

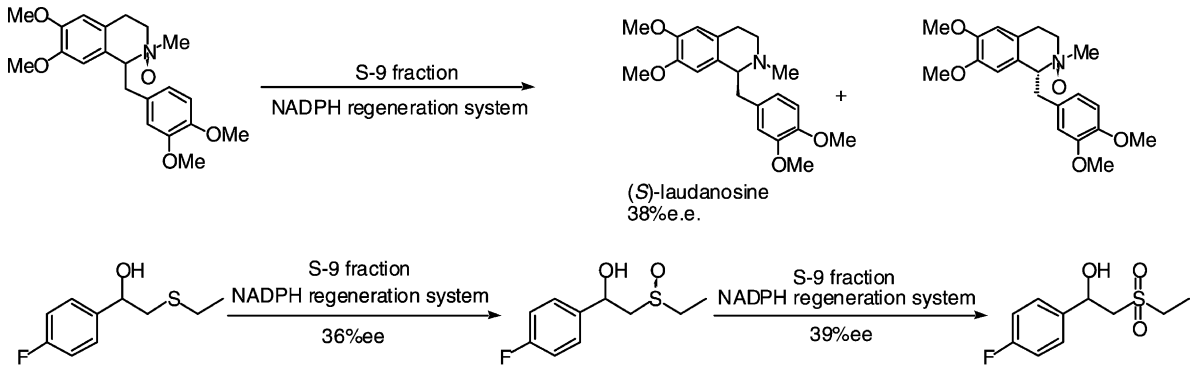

Fig. 23. Enantioselective redox reaction of nitrogen or sulfur containing compounds by rat liver S-9 fraction. Each steps are started with racemic compounds.

Mechanism of stereoselective catalysis of *Candida antarctica* lipase B (CALB): studies on acylation step

Hideto Kimura, Tomoaki Yokota, Yoshinori Inoue, Hideo Hirohara*

Department of Materials Science, The University of Shiga Prefecture, 2500 Hassaka, Hikone 522-8533, Japan. E-mail: hirohara@mat.usp.ac.jp

We have investigated kinetics and thermodynamic studies for CALB-catalyzed hydrolysis of the acetates of primary and secondary alcohol enantiomers and we have obtained the results that substrates having benzyl group in the molecule have a quite different rate-determining and enantioselective step from that of the substrates having phenoxy group.

Table 2
Thermodynamic activation parameters for CALB-catalyzed hydrolysis

		ΔG^a (kcal/mol)	ΔH^a (kcal/mo1)	$T\Delta S^a$ (kcal/mol)	ΔS^a (cal/(mo1 T))
2-Benzyl-1-butylaoetate					
k_{cat}	*R*	18.9	11.4	−7.5	−23
	S	17.6	14.3	−3.3	−10.5
R–S		$\Delta\Delta G = 130$	$\Delta\Delta H = -2.9$	$T\Delta\Delta S = -4.2$	$\Delta\Delta S = -12.5$
2-Phenoxy-1-butylacetate					
k_{cat}	*R*	16.5	7.7	−8.8	−28
	S	18.2	9.6	−8.6	−27
R–S		$\Delta\Delta G = -1.70$	$\Delta\Delta H = -1.90$	$T\Delta\Delta S = -0.20$	$\Delta\Delta S = -1.00$

[a] pH = 7.0; temperature = 40 °C.

Lipase-catalyzed enantioselective desymmetrization of prochiral 3,3-bis(hydroxymethyl)oxindoles

Shuji Akai, Toshiaki Tsujino, Emi Akiyama, Kouichi Tanimoto, Yasuyuki Kita

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The oxindoles **4** having a chiral, non-racemic quaternary carbon center at the C-3 position were prepared from the readily available oxindoles **2**, in which the enantioselective desymmetrization of the prochiral 1,3-diols **3** using a *Candida rugosa* lipase (Meito OF) and 1-ethoxyvinyl-2-furoate **1** was employed as the key step.

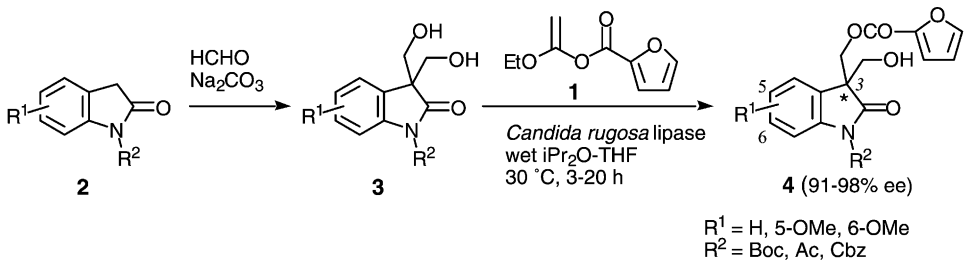

Fig. 24. Preparation of oxindoles having a chiral quaternary carbon center at the C-3 position via lipase-catalyzed desymmetrization.

Lipase-catalyzed domino kinetic resolution/rntramolecular Diels–Alder reactions

Shuji Akai, Kouichi Tanimoto, Sohei Omura, Tadaatu Naka, Yasuyuki Kita

Graduate School of Pharmaceutical Sciences, Osaka University, Yamadaoka, Suita, Osaka 565-0871, Japan. E-mail: kita@phs.osaka-u.ac.jp

The first lipase-catalyzed domino reaction was developed in which the acyl moiety installed during the enzymatic kinetic resolution was utilized as a part of the constituent structure for the subsequent Diels–Alder reaction. Thereby, the optically active 7-oxabicyclo[2.2.1]heptene derivatives **3** having five chiral, non-racemic carbon centers were prepared from achiral β-substituted acrylic acids **1** and racemic furfuryl alcohols **2** in a one-pot operation.

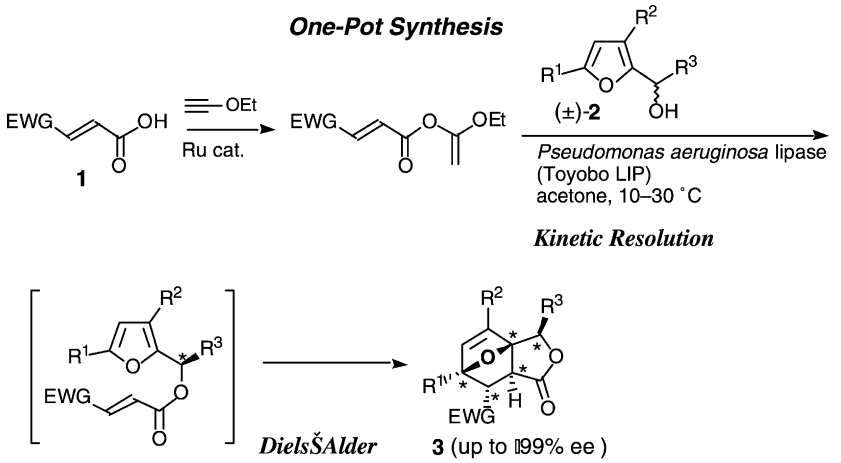

Fig. 25. Lipase-catalyzed domino kinetic resolution (Diels–Alder reactions).

Synthesis of rotaxane by acylative end-capping catalyzed by lipases

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In order to develop a novel method for synthesis of rotaxanes, lipase catalyzed acylative end-capping of pseudorotaxane **3** in equilibrium with dibenzo-24-crown-8 (**2**) and dibenzylammonium salt **1**, that possesses dimethylphenyl and hydroxy groups at each terminus, was investigated.

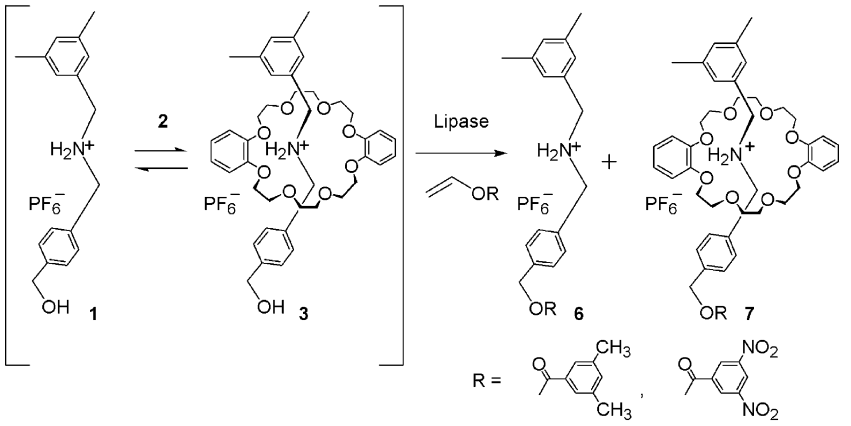

Fig. 26. Acylative end-capping of **1** and **3** catalyzed by a lipase.

Correlation between the origin of the enzyme's enantioselectivity and the conformational flexibility induced by the effects of solvents or additives for lipase-catalyzed esterification in organic solvents

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The variation of the enantioselectivity (*E* value) for lipase-catalyzed esterification in organic solvents was found to be successfully correlated with the lipase's flexibility (Hi/(Ha + Hi)) brought about by the effects of solvents or additives, the flexibility of which was estimated from the mobility of the spin-label bound to lipase by the ESR measurement.

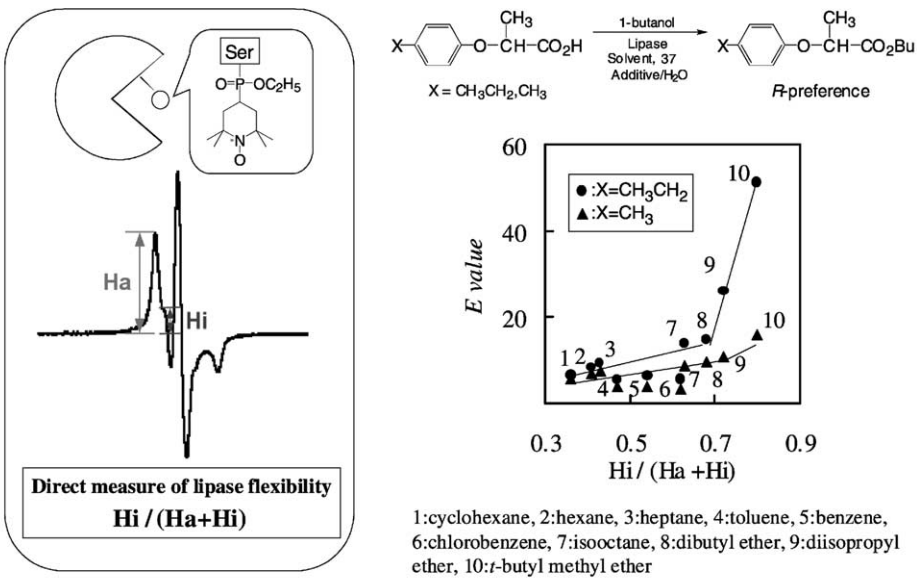

Fig. 27. Correlation between the Hi/(Ha + Hi) value as a direct measure of lipase flexibility and the *E* value in the lipase-catalyzed esterification of 2-(4-substituted phenoxy)propionic acids with 1-butanol in organic solvents.

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

Yoshihito Nishimura, Nozomi Ouchi, Takaaki Fukuba, Han Shi Hui, Toshiyuki Itoh*

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The transesterification of alcohols took place smoothly under reduced pressure when 0.5 rq. of methyl phenylthioacetate was used as acyl donor in [bmim]PF_6, and we succeeded in obtaining the corresponding acylated compound in optically pure form; this makes it possible to use lipase repeatedly because there was no drop in the reaction rate despite three repetitions of the process.

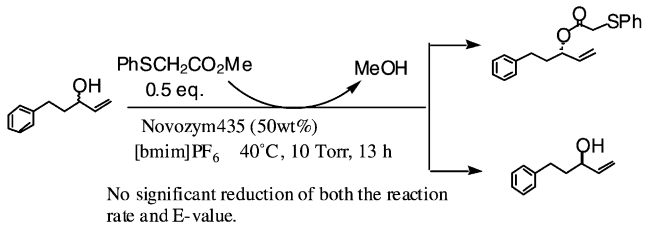

Fig. 28. Enantioselective acylation of alcohols under reduced pressure conditions using an ionic liquid solvent system.

Microbial deracemization of α-substituted carboxylic acids —expansion of substrate specificity and mechanistic investigation

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

[a]Department of Biosciences and Informatics, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223-8522, Japan

[b]Sumitomo Chemical Co. Ltd., 4-2-1 Takatsukasa, Takarazuka 665-8555, Japan. E-mail: hohta@bio.keio.ac.jp

We could establish the new reaction conditions to suppress the degradation reactions of two types of compound, 2-phenylthiopropanoic acid and 2-methyl-3-phenylpropanoic acid, and succeeded in the deracemization reaction in high efficiency.

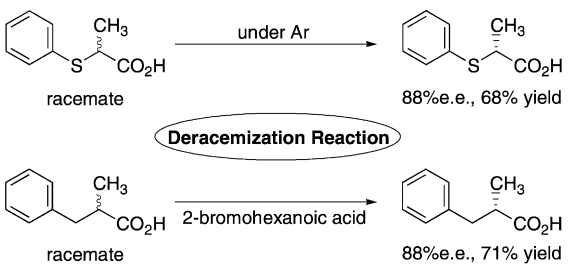

Fig. 29. New reaction conditions to suppress the degradation reactions using the resting cell system of *Nocardia diaphanozonaria*.

Polyester synthesis by enzyme-catalyzed ring-opening polymerization of lactones

Yoichi Suzuki[a,*], Seiichi Taguchi[b], Shuichi Matsumura[a], Yoshiharu Doi[b]

[a]Department of Biosciences and Informatics, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223-8522, Japan

[b]Polymer Chemistry Laboratory, RIKEN Institute, 2-1 Hirosawa, Wako-shi, Saitama 351-0198, Japan. E-mail: suzuki@bio.keio.ac.jp

Involvement of catalytic triad and characteristic reactivities in enzyme-catalyzed ring-opening polymerization of lactones could be demonstrated using three kinds of site-specific mutants and tertiary structural homology model.

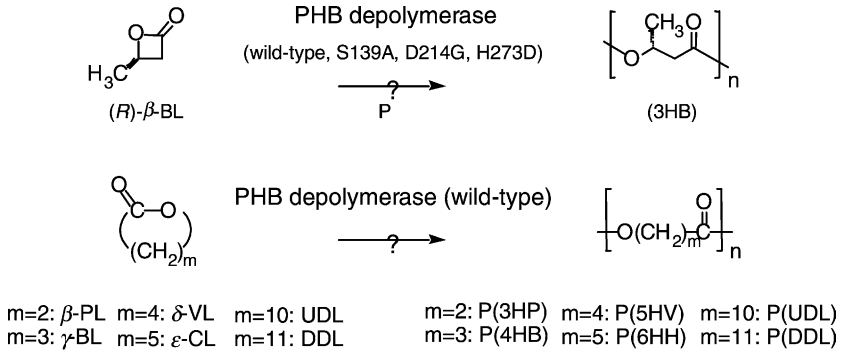

Fig. 30. Polyester synthesis by enzyme-catalyzed ring-opening polymerization of lactones.

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

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

We tried the inversion of the enantioselectivity of the decarboxylation reaction by using S71C/C188S, L72C/C188S, M73C/C188S, G74C/C188S, T75C/C188S, and S76C/C188S double mutant AMDase, of which best result is shown below.

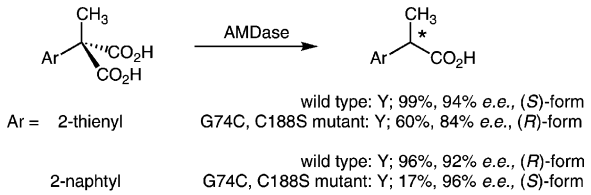

Fig. 31. Wild type and mutant AMDase reaction.

Search for the docking conformations between a lipase enzyme and xenobiotics using biomolecular modeling calculations

Yoshinobu Naoshima[a,*], Masanori Koura[a], Yoshihiro Mori[b], Takatomo Kimura[c], Makoto Kamezawa[c], Hojun Tachibana[c], Takehiko Ohtani[c]
[a]Faculty of Informatics, Okayama University of Science, 1-1 Ridai-cho, Okayama 700-0005, Japan
[b]Venture Business Laboratory, Graduate School of Okayama, Okayama, Japan
[c]Konan Chemical Industry Co. Ltd., 5-21 Nakagawa-cho, Takatsuki-shi, Osaka 569-0066, Japan.
E-mail: naoshima@sp.ous.ac.jp

Using biomolecular modeling and simulation for *Candida antarctica* lipase type B (CALB), we have undertaken a mechanistic study on the binding specificity and the enantioselectivity toward foreign chiral substrates shown by lipase enzymes.

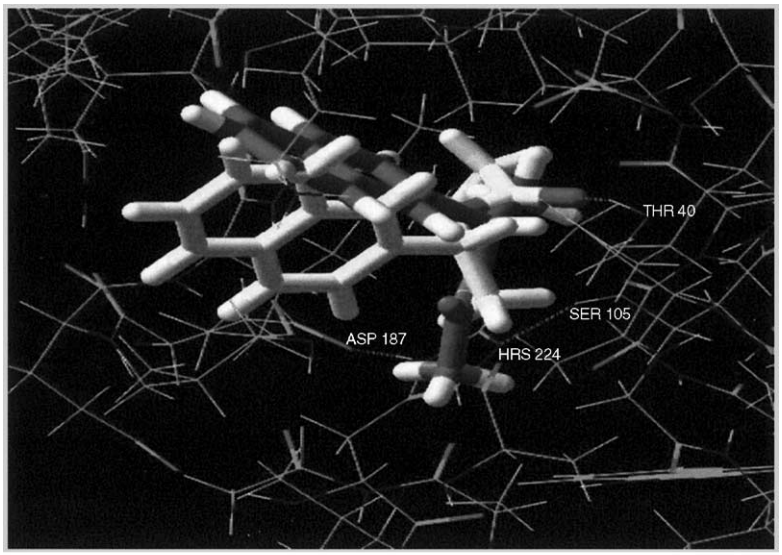

Fig. 32. Docking simulations of CALB and chiral esters.

Simple preparation of optically pure trifluoromethylalkanol through lipase catalyzed reaction

Yumiko Takagi[a,*], Fumi Yamana[a], Toshiyuki Itoh[b]

[a]Department of Chemistry, Faculty of Education, Kagawa University, 1-1 Saiwai-cho, Takamatsu, Kagawa 760-8522, Japan

[b]Department of Materials 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 diacetates of bis(trifluoromethyl)alkanediols and synthesis of novel liquid crystalmolecules which possesses chiral bis(trifluoromethyl)alkanol moieties and aromatic core structure at the center of the molecular flame.

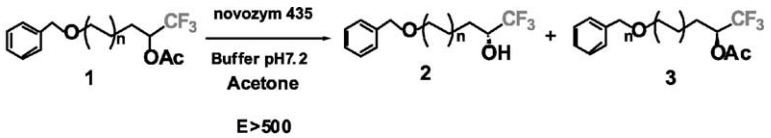

Fig. 33. Optical resolution of 1,1,1-trifluoromethyl-2-alkanol by CAL-catalyzed enantioselective hydrolysis.

Mechanism-based enzymatic method for reliable determination of absolute configuration of secondary alcohols

Tadashi Ema*, Masataka Yoshii, Toshinobu Korenaga, Takashi Sakai

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

The absolute configurations of six 1-substituted ethanols were determined by the mechanism-based enzymatic method combined with the MTPA method, and a conformational similarity between the transition-state model (left) and the MTPA ester (right) has been discussed.

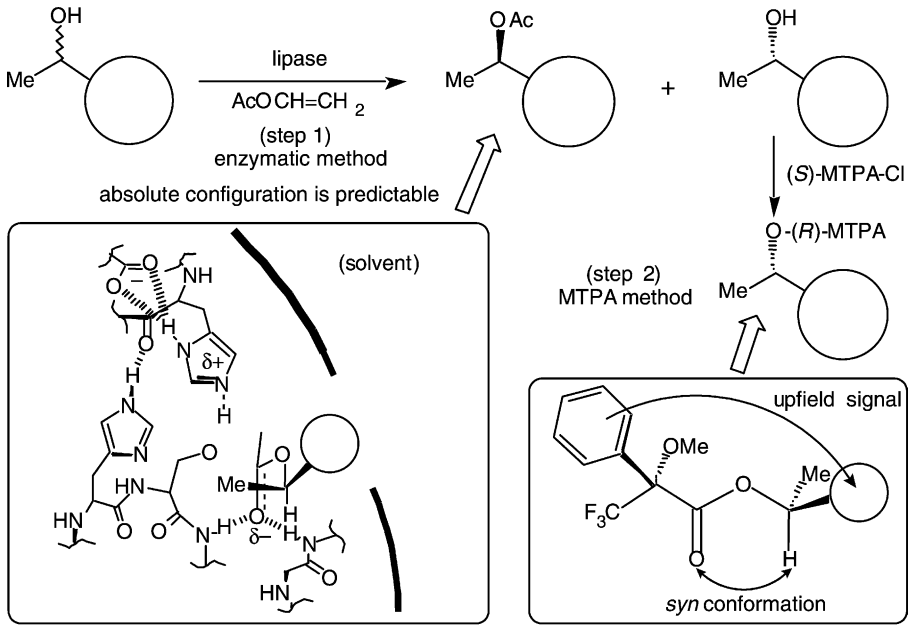

Fig. 34. Two-steps reliable determination of absolute configurations using lipase and MTPA.

Synthesis of dehydrogenated cyclic dipeptides by actinomycetous dehydrogenases

Banri Ikeda, Atsushi Morimoto, Teruhiko Nitoda, Hiroshi Kanzaki*
Faculty of Agriculture, Okayama University, Japan. E-mail: hkanzaki@cc.okayama-u.ac.jp

Novel dehydro cyclic dipeptides, cyclo(ΔMet–ΔMet) and cyclo(ΔPhe–ΔPro), were effectively synthesized from the corresponding cyclic dipeptides by an actinomycetous enzyme system involved in albonoursin biosynthesis, indicating that the enzyme system showed broad substrate specificity for cyclic dipeptides, and thus, was useful for the preparation of various dehydro cyclic dipeptides.

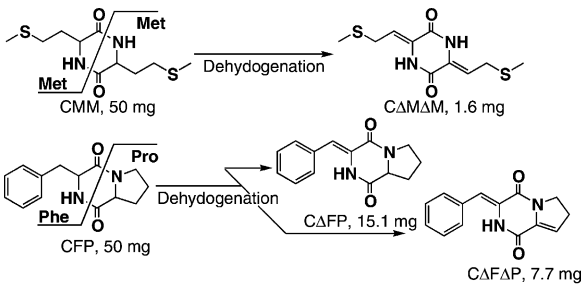

Fig. 35. Dehydogenation of CMM and CFP.

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

Ken-ichi Fuhshuku, Mina Tomita, Takeshi Sugai*
Department of Chemistry, Keio University, Hiyoshi, Yokohama 223-8522, Japan. E-mail: sugai@chem.keio.ac.jp

Substrate specificity of *Torulaspora delblueckii* IFO10921 on the carbonyl compounds as well as the possibility of air-dried cell preparation as the long-term preservation reagents were studied.

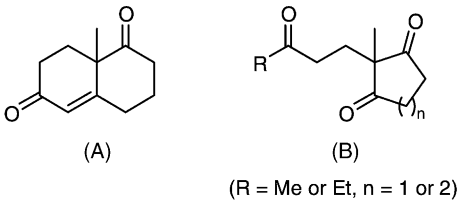

Fig. 36. Good substrates for the reduction of carbonyl groups mediated by *Thrulaspora delblueckii* 1F010921.

Synthesis of enantiomers of proline-related compounds via enzyme-catalyzed kinetic resolution

Masayuki Kurokawa[a], Takeyuki Shindo[a], Masumi Suzuki[a], Nobuyoshi Nakajima[b], Kohji Ishihara[c], Takeshi Sugai[a,*]
[a]Department of Chemistry, Keio University, Hiyoshi, Yokohama 223-8522, Japan
[b]Department of Nutritional Science, Okayama Prefectural University, Soja, Okayama 719-1112, Japan
[c]Department of Chemistry, Kyoto University of Education, Japan. E-mail: sugai@chem.keio.ac.jp

C. antarctica lipase B (Chirazyme L-2) was effective for the kinetic resolution by means of the hydrolysis of *N*-Boc and *N*-Cbz proline esters with E > 100.

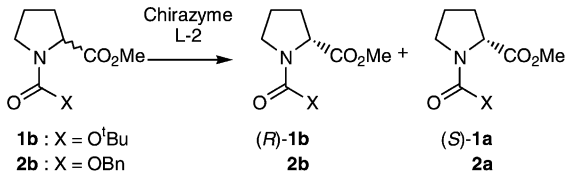

Fig. 37. Kinetic resolution by the chirazyme L-2 mediated hydrolysis of racemic forms of *N*-protected proline esters.

Light-mediated regulation of asymmetric reduction of ketones by a cyanobacterium

Rio Yamanaka, Kaoru Nakamura*
Institute for Chemical Research, Kyoto University. E-mail: nakamura@scl.kyoto-u.ac.jp

We find that the chemical yield and/or enantioselectivity of the reduction of ketones with *Synechococcus elongatus* PCC 7942 increases as a result of illumination with fluorescent light. Furthermore, DCMU, an inhibitor of photosynthesis, affects the stereoselectivity of the reduction.

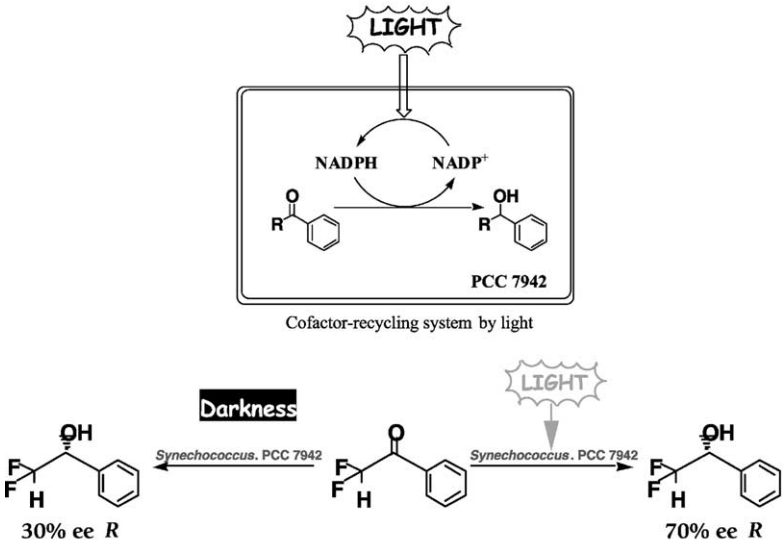

Fig. 38. Reduction of α,α-difluoroacetophenone by *S. elongatus* PCC 7942.

Analysis of hydrolytic reaction by glucoamylase using a quartz-crystal microbalance

Hidekazu Nishino*, Takanori Nihira, Toshiaki Mori, Yoshio Okahata
Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Japan.
E-mail: hnishino@bio.titech.ac.jp

The amylopectin immobilized 27 MHz quartz-crystal microbalance (QCM) is a useful tool to detect directly and quantitatively each step of the amylopectin hydrolytic reactions of glucoamylase in the aqueous solution.

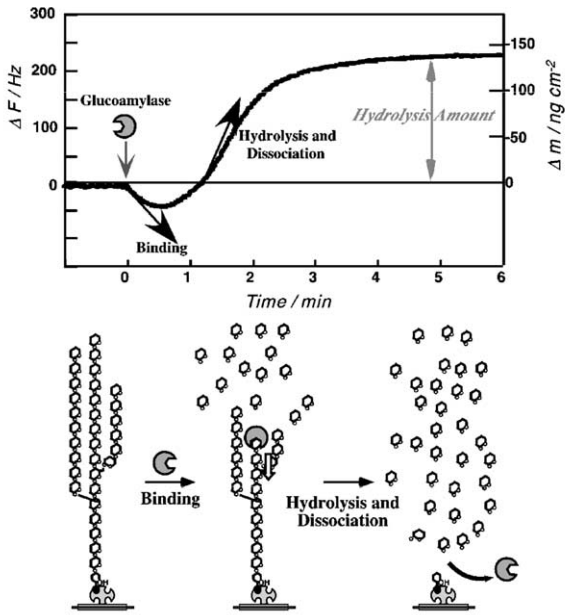

Fig. 39. Monitoring of three steps (binding, hydrolysis and dissociation) of glucoamylase reactions.

The enhancement of the enantioselectivity of enzyme-catalyzed reactions by addition of several protein denaturants

Shuichi Mori[a], Keiichi Watanabe[b], Miyuki Nishimura[a], Rina Matsumi[a], Miki Tachibana[c], Hiromi Yumoto[c], Shin-ichi Ueji[a,b,c,*]

[a]Graduate School of Cultural Studies and Human Science, Kobe University, Nada, Kobe 657-8501, Japan

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E-mail: ueji@kobe-u.ac.jp

The enantioselectivity of the lipase-catalyzed esterification of 2-(4-substituted phenoxy) propanoic acids (**1–9**) was found to be dramatically enhanced by addition of several protein denaturants such as SDS, urea, and guanidine HCl, as a new type of additive to the reaction medium, the enantioselectivity enhancement of which can be attributed to the lipase flexibility estimated from the result of the ESR spectra.

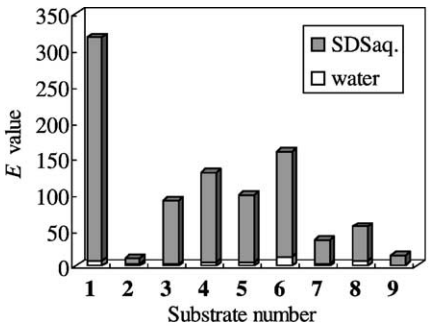

Fig. 40. Enhancement of the enantioselectivity (*E* value) by addition of aqueous SDS (0.7 M, 1.2 vol.%) for the lipase-catalyzed esterification of **1**–**9** in isopropyl ether: **1** = Et; **2** = H; **3** = Me; **4** = *n*-Pr; **5** = *n*-Bu; **6** = Ome; **7** = Cl; **8** = CF3; **9** = *t*-Bu.

Chiral alcohol production by β-ketoester reductase from *Penicillium citrinum* coupled with regeneration system of NADPH

Nobuya Itoh[a,*], Kiminori Banno[a], Hiroyuki Asako[b], Ryuhei Wakita[b], Masatoshi Shimizu[b]

[a]Biotechnology Research Center, Toyama Prefectural University, Kurokawa 5180, Kosugi, Toyama 939-0398, Japan

[b]Organic Synthesis Research Laboratory, Sumitomo Chemical Co. Ltd., Tsukahara 2-10-1, Takatsuki, Osaka 569-1093, Japan. E-mail: itoh@pu-toyama.ac.jp

NADPH-dependent β-keto esters reductase (KER) was used to produce methyl (*S*)-4-bromo-3-hydroxybutyrate coupled with NADPH regeneration system comprising glucose dehydrogenase.

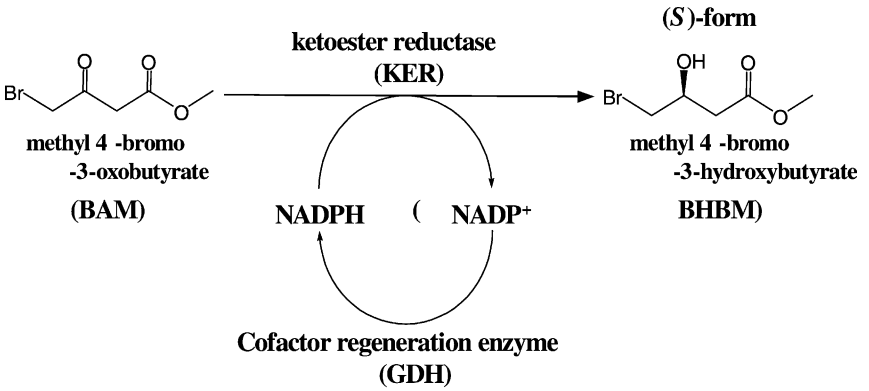

Fig. 41. Asymmetric reduction process by KER coupled with NADPH regenerating system.

Syntheses of oxidatively modified glycerophospholipid and cholesteryl esters via enzymatic oxidation and hydrolysis reactions

Arnold N. Onyango[a], Shuhei Nakajima[a], Sakayu Shimizu[b], Naomichi Baba[a]
[a]Department of Bioresources Chemistry, Faculty of Agriculture, Okayama University, Japan
[b]Division of Applied Life Science, Graduate School of Agriculture, Kyoto University, Japan.
E-mail: babanaom@cc.okayama-u.ac.jp

Phosphatidylcholine and cholesteryl esters bearing polyunsaturated fatty acid hydroperoxides or unsaturated ω-oxo fatty acids were prepared via soybean lipoxygenase-catalysed oxidation of the fatty acids and lipase-catalysed hydrolysis of the methyl esters of protected fatty acid hydroperoxides or unprotected ω-oxo fatty acids.

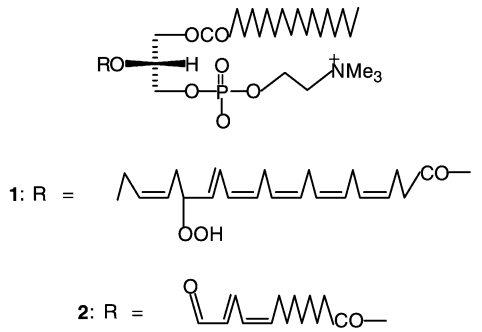

Fig. 42. Lipase-catalysed hydrolysis of the methyl esters of protected fatty acid hydroperoxides or unprotected ω-oxo fatty acids.

Regio- and stereoselective hydroxylation of natural resource rosin by microorganisms

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The hydroxylation of rosin by *Mucor* sp. and *Mortierella* sp. was performed to give products have the hydroxyl group at C-2 position.

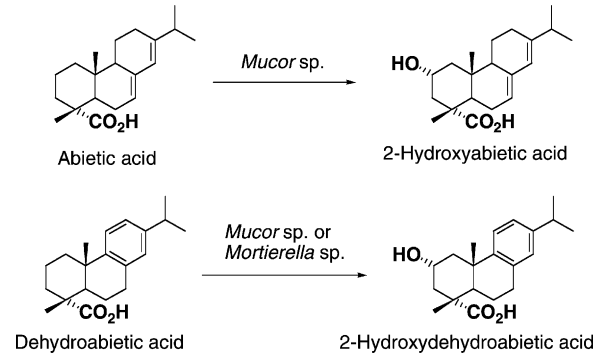

Fig. 43. Microbial hydroxylation of abietic acid and dehydroabietic acid.

Enzymatic synthesis and applications of dendrimer–catechin conjugates

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This study deals with enzymatic synthesis of poly(amidoamine) dendrimer–cataechin conjugates and evaluation of their antioxidant capacity. Antioxidant activity of catechin was greatly amplified by the enzymatic conjugation to external functional groups of the dendrimer surface.

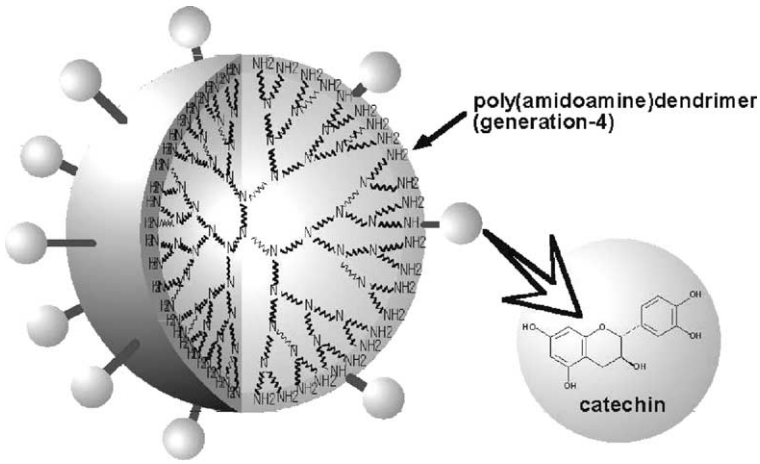

Fig. 44. Dendrimer–catechin conjugate showing high antioxidant activity.

Asymmetric transfer hydrogenation process by phenylacetaldehyde reductase and its application

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We have established a practical asymmetric hydrogen transfer process using 2-propanol as the hydrogen donor by phenylacetaldehyde reductase (PAR) expressed in *E. coli* cells.

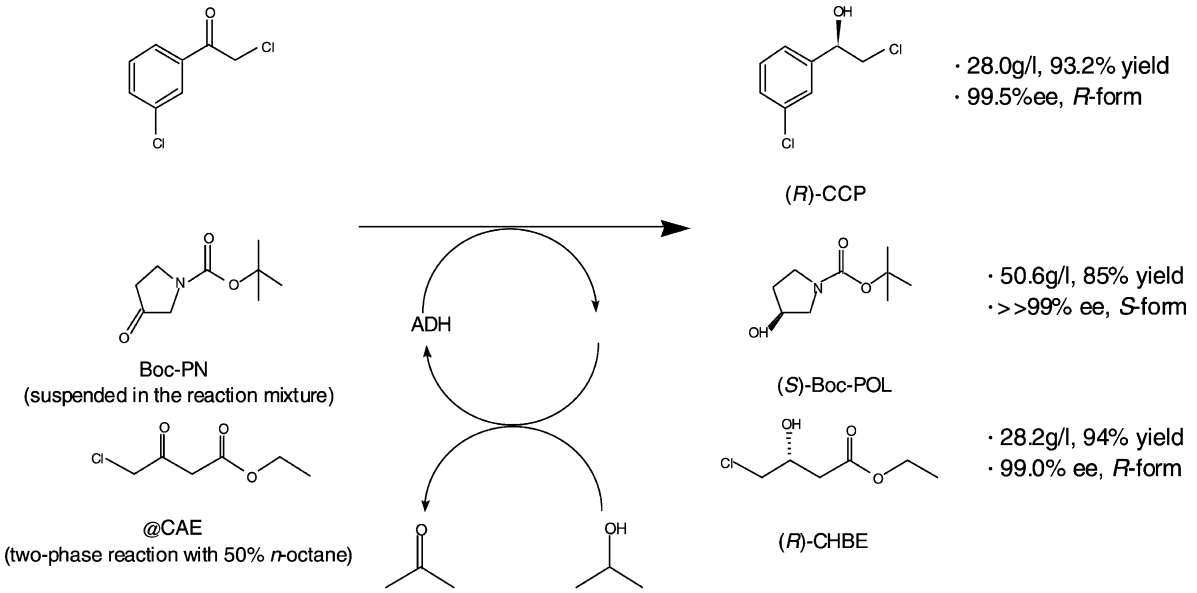

Fig. 45. Asymmetric transfer hydrogenation process by PAR.