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Selected abstracts from the 3rd Japanese Symposium on the Chemistry of Biocatalysis

Yasuhisa Asano*

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Introduction

The 3rd Japanese Symposium on the Chemistry of Biocatalysis was held in Atami, Shizuoka, Japan, on January 20–21, 2000, organized by Professor Yoshio Okahata of Tokyo Institute of Technology. Shown below are the selected short abstracts (59 titles) of the presentation. Thanks are due to those who gladly sent the abstracts to me.

Yasuhisa Asano, Editor

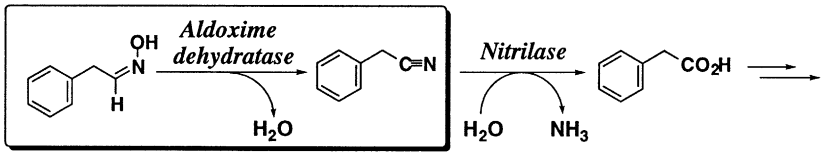
Plenary Lectures

A novel aldoxime dehydratase from phenylacetaldoxime-degrading bacterium, *Bacillus* sp. strain OxB-1

Yasuo Kato, Haruhiko Sakiyama, Yasuhisa Asano*

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

We purified and characterized a novel dehydratase that catalyzes the stoichiometric dehydration of *Z*-phenylacetaldoxime to phenylacetonitrile from a cell-free extract of *Bacillus* sp. strain OxB-1, and its gene was cloned, sequenced, and overexpressed.



Asymmetric reduction of ketones by *Geotrichumcandidum*

Tomoko Matsuda[a]*, Tadao Harada[a], Nobuyoshi Nakajima[b], Rio Yamanaka[c], Kaoru Nakamura[c]*

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[b]Department of Nutritional Science, Faculty of Health and Welfare, Okayama Prefectural University, Soja, Okayama 719-1112, Japan

[c]Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan. E-mail: matsuda@rins.ryukoku.ac.jp

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1381-1177/01/$ – see front matter
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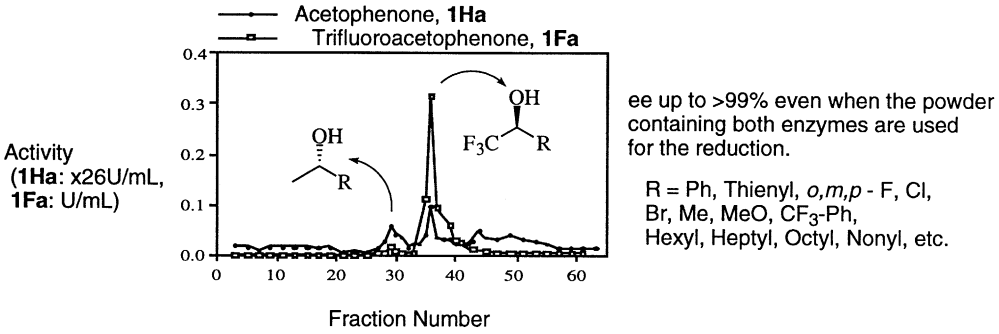
Reduction of trifluoromethyl ketones by acetone powder of *Geotrichum candidum* IFO 4597 affords (*S*)-trifluoromethyl carbinols in excellent ee, whereas the reduction of methyl ketones gives the corresponding alcohols of the opposite configuration in excellent ee; this powder contains several enzymes with different stereoselectivities, but one enzyme selectively catalyzes the reduction of methyl ketone and another catalyzes the reduction of trifluoromethyl ketones.

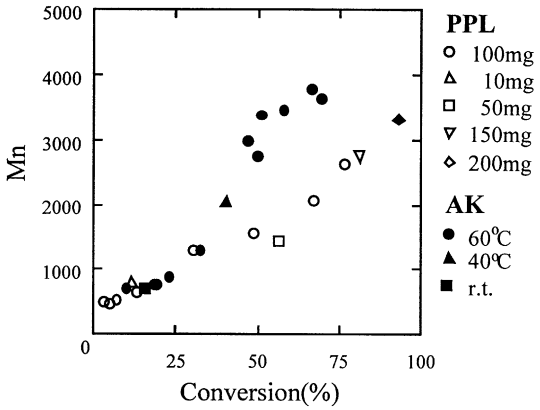


Does lipase catalyze the polymerization in ring-opening bulk polymerization of lactones?

Shigeru Kunugi*, Naoki Serata

Department of Polymer Science and Engineering, Kyoto Institute of Technology, Matsugasaki, Sakyo, Kyoto 606-8585, Japan. E-mail: kunugi@ipc.kit.ac.jp

The ring-opening polymerization of lactones in the presence of lipases (*Pseudomonas fluorescens* (AK), *Pseudomonas cepacia* (PS, AH), *Candida rugosa* (AY), *Mucor javanicus* (M); from Amano, and porcine pancreatic lipase (PPL); from Sigma) and a pig liver esterase (PLE) were tested, and AK and PPL were found to give the better results in both conversion and polymerization, the results of which showed an apparent linear correlation between the conversion and Mn, implying a simple ring-opening polymerization mechanism; the enzyme not catalytically participating in the propagation of the polymer chain.

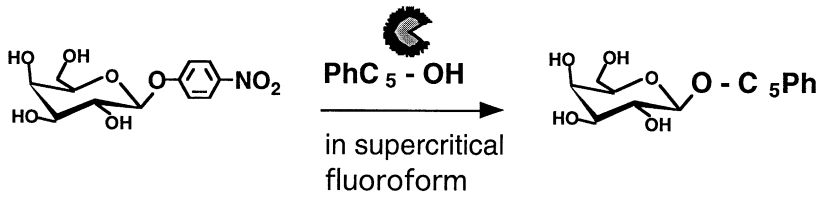


Enzymatic reactions catalyzed by a lipid-coated glycoside hydrolase in supercritical fluids

Toshiaki Mori, Yoshio Okahata*

Department of Biomolecular Engineering, Tokyo Institute of Technology, 4259 Nagatsuda, Midori-ku, Yokohama 226-8501, Japan. E-mail: tmori@bio.titech.ac.jp

The lipid-coated glycoside hydrolase was soluble in supercritical fluids such as a carbon dioxide or fluoroform, and showed a higher transgalactosylation (25 times) in supercritical fluoroform (30°C/60 atm) than in organic solvent for the reaction of 5-phenylpentanol with p-nitrophenyl-beta-D-galactopyranoside.

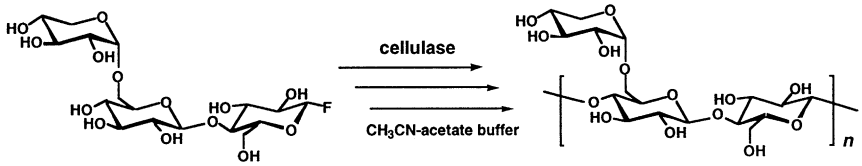


Glyco-chemistry cycles: material production utilizing complementarity of biocatalysts

Shin-ichiro Shoda*, Masaya Fujita

Department of Materials Chemistry, Graduate School of Engineering, Tohoku University, Aoba, Sendai 980-8579, Japan. E-mail: shoda@poly.che.tohoku.ac.jp

Regio- and stereo-selective synthesis of a novel xyloglucan has been achieved by the cellulase-catalyzed polycondensation of 6′-*O*-a-xylopyranosyl-cellobiosyl fluoride.

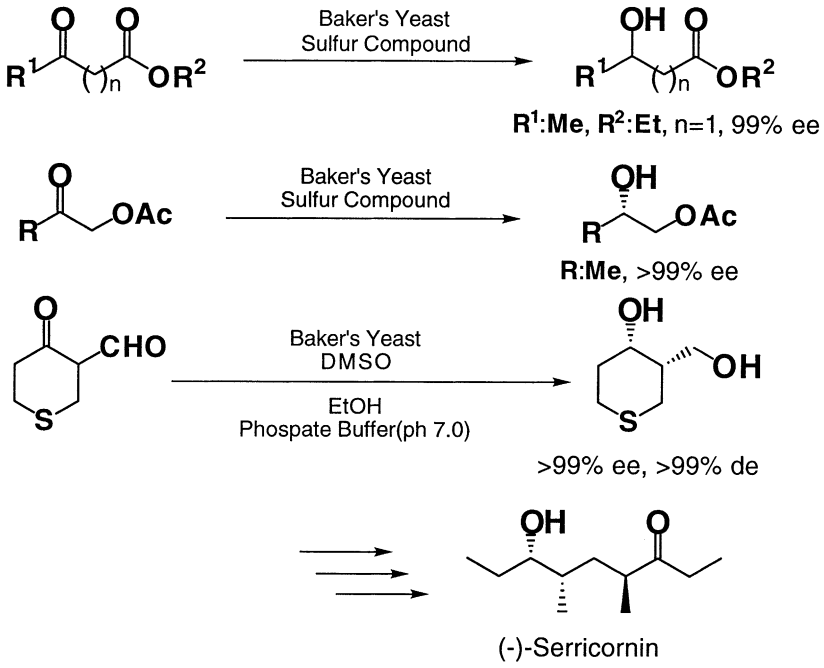


The baker's yeast reduction in the presence of a sulfur compound

Ryuuichirou Hayakawa, Makoto Shimizu*

Department of Chemistry for Materials, Mie University, Tsu, Mie 514-8507, Japan. E-mail: mshimizu@chem.-mie-u.ac.jp

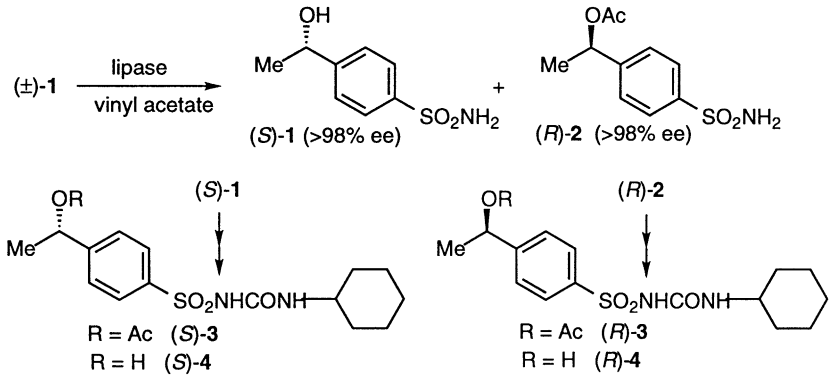
Enhancement of reactivity and improvement of the selectivities were achieved using a sulfur compound as an additive in the baker's yeast reduction of α- and β-keto esters, α-acetoxy ketones, and β-keto aldehydes.



Synthesis of chiral sulfonylurea characterized by short lasting hypoglycemic effect based on the enzymatic function

Hiroyuki Akita[a]*, Shigeo Yamamura[a], Katsumi Kurashima[b], Kazuko Sanai[b], Kenji Seri[b], Yorishige Imamura[c]
[a]School of Pharmaceutical Science, Toho University, 2-2-1, Miyama, Funabashi, Chiba 274-8510, Japan
[b]Central Research Laboratory, Godo Shusei Co., Ltd., 250, Nakahara, Kamihongo, Matsudo, Chiba 271-0064, Japan
[c]Faculty of Pharmaceutical Sciences, Kumamoto University, 5-1, Oe-honmachi, Kumamoto 862-0973, Japan. E-mail: akita@phar.toho-u.ac.jp

Enzymatic acetylation of (±)-**1** gave stereoselectively (*S*)-**1** and (*R*)-**2** in high yield, which were converted to the chiral sulfonylureas ((*S*)-**3**, (*S*)-**4**, (*R*)-**3** and (*R*)-**4**) characterized by short lasting hypoglycemic effect.



Posters

The structure of allyl alcohol dehydrogenase from the cultured cells of *Nicotianatabacum*

Naoyuki Yokobatake, Kei Shimoda, Yoshiyuki Ashida, Toshifumi Hirata*
Department of Mathematical and Life Sciences, Graduate School of Science, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima 739-8526, Japan. E-mail: thirata@sci.hiroshima-u.ac.jp

The primary structure of the allyl alcohol dehydrogenase (allyl-ADH) from the cultured cells of *N. tabacum* was determined; the amino acid sequence was similar to the oxidoreductases that belong to a ζ-crystalline subfamily of medium-chain dehydroganase/reductase super family.

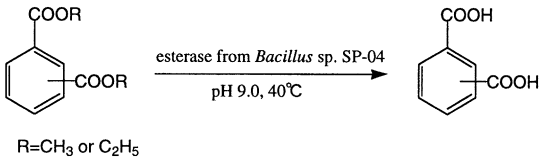
Table 1. Comparison of allyl-ADH with the other reductases

Origin	Protein	Length (a.a.)	Homology (%)
Nicotiana tabacum	allyl-ADH	343	——
Arabidopsis thaliana	probable NADP-dependent oxidoreductase P2	342	72.4
Arabidopsis thaliana	NADPH oxidoreductase:ISOTYPE=P2	342	72.1
Arabidopsis thaliana	probable NADP-dependent oxidoreductase P1	345	71.3

Partial characterization of an aromatic carboxylesterase from *Bacillus* sp. SP-04

Munenori Takehara*, Akinori Saiki, Yoshinori Inoue, Hideo Hirohara
Department of Materials Science, The University of Shiga Prefecture, Hassaka, Hikone 522-8533, Japan. E-mail: takehara@mat.usp.ac.jp

An esterase from newly isolated *Bacillus* sp. SP-04 was partially characterized, and was found to hydrolyze aromatic dicarboxylic acid alkyl esters as well as benzoic acid and benzyl acid esters.



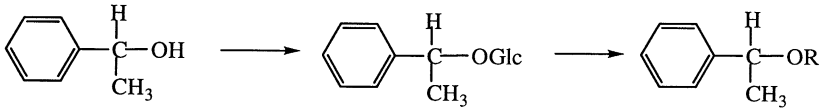
Asymmetric glycosylation by the cultured cells of *Catharanthusroseus*

Takeshi Fujino[a], Shin-ya Yamane[a], Kei Shimoda[a], Shinji Ohta[b], Toshifumi Hirata[a]*

[a]Department of Mathematical and Life Sciences, Graduate School of Science, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima 739-8526, Japan

[b]Instrument Center for Chemical Analysis, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima 739-8526, Japan. E-mail: thirata@sci.hiroshima-u.ac.jp

The cultured cells of *Catharanthus roseus* were found to be able to transform diastereoselectively 1-phenylethanol into the glycosides with disaccharides, such as vicianose, primeverose and gentiobiose.



1: R=Glc^{6}-^{1}Ara
2: R=Glc^{6}-^{1}Xyl
3: R=Glc^{6}-^{1}Glc

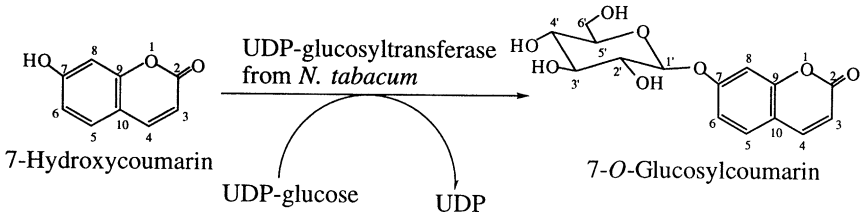
UDP-Glucosyltransferase from the cultured cells of *Nicotianatabacum*

Shin-ya Yamane[a], Takeshi Fujino[a], Kei Shimoda[a], Shinji Ohta[b], Toshifumi Hirata[a]*

[a]Department of Mathematical and Life Sciences, Graduate School of Science, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima 739-8526, Japan

[b]Instrument Center for Chemical Analysis, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima 739-8526, Japan. E-mail: thirata@sci.hiroshima-u.ac.jp

Glucosyltransferase from the cultured cell of *Nicotiana tabacum* catalyzed the transformation of hydroxycoumarins into their corresponding monoglucosides.



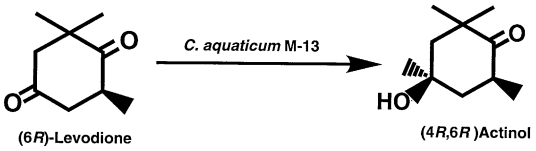
Screening of cyclic diketone reducing microbial enzymes

Masaru Wada[a]*, Ayumi Yoshizumi[a], Shigeru Nakamori[a], Sakayu Shimizu[b]

[a]Department of Bioscience, Fukui Prefectural University, 4-1-1 Kenjyojima, Matsuoka-cho, Fukui 910-1195, Japan.

[b]Division of Applied Life Science, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan. E-mail: masaru@fpu.ac.jp

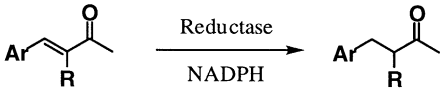
(6*R*)-2,2,6-trimethyl-1,4-cyclohexanedione (levodione) reducing microorganisms were screened, and the soil isolate bacterium *Corynebacterium aquaticum* M-13 was found to be the best producer of the enzyme which catalyzed regio- and stereoselective reduction of levodione to (4*R*,6*R*)-4-hydroxy-2,2,6-trimethylcyclohexanone (actinol).



Purification and characterization of a carbon–carbon double bond reductase from baker's yeast

Yasushi Kawai*, Motoko Hayashi
Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan. E-mail: kawai@scl.kyoto-u.ac.jp

In the reduction of enones with a carbon–carbon double bond reductase from baker's yeast, ketones having a chiral center at the alpha position are obtained stereoselectively.

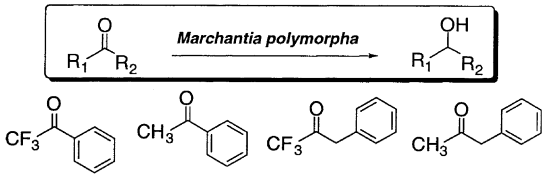


Ar = Ph, substituted Ph, Py, *N*-O-Pyridyl, *N*-Me-Pyridinium^{+}
R = Me, Et

Reduction of fluorinated ketones with plant cell cultures

Kaoru Nakamura[a]*, Rio Yamanaka[a], Tomoko Matsuda[b], Hiroki Hamada[c]
[a]Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-001, Japan
[b]Ryukoku University, Seta, Otsu 520-2194, Japan
[c]Okayama University of Science, Ridaicho 1-1, Okayama 700-0005, Japan. E-mail: nakamura@scl.kyoto-u.ac.jp

Effect of fluorination on the reactivity and selectivity of the reduction with suspension cultured cells has been investigated. Fluorinated ketones showed higher reactivity than the corresponding unfluorinated ketones.



Enhancement of efficiency in the kinetic resolution using porous ceramics-immobilized lipases at low temperature

Takashi Sakai*, Kyoko Hayashi, Mika Takahara, Tadashi Ema, Masanori Utaka
Department of Applied Chemistry, Faculty of Engineering, Okayama University, Tsushima, Okayama 700-8530, Japan. E-mail: tsakai@cc.okayama-u.ac.jp

Immobilized lipases in porous ceramics supports (Toyonite) was found to markedly accelerate the reaction rate in transesterification 77 times at best and made the low-temperature method (rt to −40°) practical for the enhancement of the enantioselectivity.

		E		TTN/h[a)]	
		30 ℃	−40 ℃	30 ℃	−40 ℃
PS	*Celite*	6.8	15	6400	22
	Toyonite	6.8	15	110000	1700
AK	*Celite*	9	55	11000	110
	Toyonite	3.2	21	53000	1400

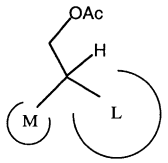
a) TTN : Total Turnover Number

Mechanism of stereoselective catalysis of lipases: enhancement of selectivity in primary alcohol esters

Seiji Shinohara*, Yoshinori Inoue, Munenori Takehara, Hideo Hirohara

Department of Materials Science, The University of Shiga Prefecture, Hassaka, Hikone 522-8533, Japan. E-mail: i21sshinohara@ec.usp.ac.jp

In the lipase-catalyzed hydrolysis of the acetates of primary alcohols, the *E* value has been enhanced by the increase of the size of medium-sized substituent M, e.g., from CH_3 to C_2H_5 or C_3H_7, and then lowered by the further increase, e.g., to C_5H_{11}.



M	CH_3	C_2H_5 , C_3H_7	C_5H_{11}
E value	small	large	small

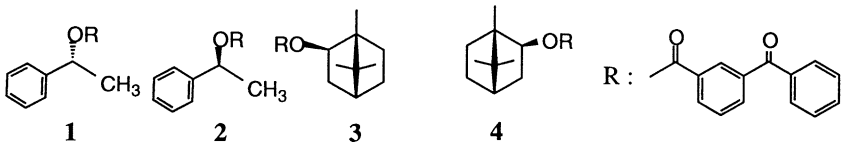
L : large-sized substituent, e.g., phenoxy

MALDI-TOF MASS analysis of enantioselectivity in the hydrolysis by lipase

Ryoichi Utsumi, Shunsuke Izumi, Toshifumi Hirata*

Department of Mathematical and Life Sciences, Graduate School of Science, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima 739-8526, Japan. E-mail: thirata@sci.hiroshima-u.ac.jp

To clarify the mechanism of occurrence of the enantioselectivity in the hydrolysis by lipase, photoaffinity reaction of lipase with the ligand, **1**–**4**, was monitored by MALDI-TOF MASS.



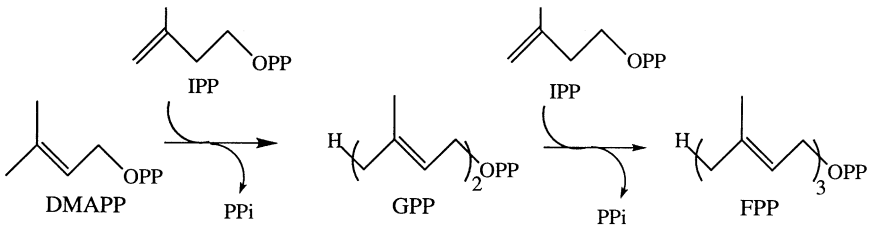
Studies on substrate binding and catalytic reaction of farnesyl diphosphate synthase with QCM

Hongtao Liu[a], Yuan-Wei Zhang[b], Toshishige Suzuki[a], Tanetoshi Koyama[b]*

[a]Tohoku National Industrial Research Institute, 4-2-1, Nigatake, Miyagino-ku, Sendai 983-8551, Japan

[b]Institute for Chemical Reaction Science, Tohoku University, 2-1-1, Katahira, Aoba-ku, Sendai 980-8577, Japan. E-mail: koyama@icrs.tohoku.ac.jp

Using the electrode coated by farnesyl diphosphate synthase of *Bacillus stearothermophilus*, the following enzyme reaction was studied.

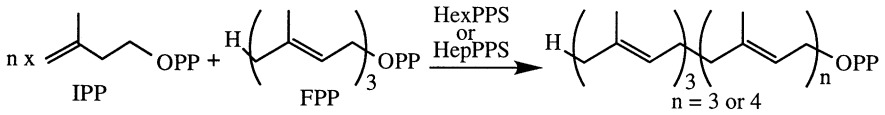


Site-directed mutagenesis of medium-chain (*E*)-prenyl diphosphate synthases

Yuan-Wei Zhang, Xiao-Yuan Li, Tanetoshi Koyama*

Institute for Chemical Reaction Science, Tohoku University, Katahira 2-1-1, Aoba-ku, Sendai 980-8577, Japan. E-mail: koyama@icrs.tohoku.ac.jp

Several site-directed mutations were introduced into each subunit of medium-chain prenyl diphosphate synthases, which are composed of two different heteromeric subunits, and their effects on substrate specificity and chain length of the enzymatic reaction product were investigated.



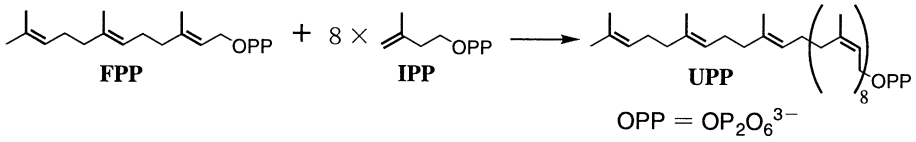
Identification of substrate binding sites of undecaprenyl diphosphate synthase

Keitaro Fujikura[a], Yuan-Wei Zhang[a], Yuji Maki[b], Tanetoshi Koyama[a]*

[a]Institute for Chemical Reaction Science, Tohoku University, Katahira 2-1-1, Aoba-ku, Sendai 980-8577, Japan

[b]Department of Material and Biological Chemistry, Yamagata University, Kojirakawa 1-4-12, Yamagata 990-8560, Japan. E-mail: koyama@icrs.tohoku.ac.jp

Several amino acid residues of undecaprenyl diphosphate (UPP) synthase from *Micrococcus luteus* B-P 26, which catalyzes the condensation of isopentenyl diphosphate (IPP) with (*E*,*E*)-farnesyl diphosphate (FPP) to form a C_{55}-prenyl diphosphate with *E*,*Z*-mixed stereochemistry, was identified to be involved in binding of substrate by site-directed mutagenesis.

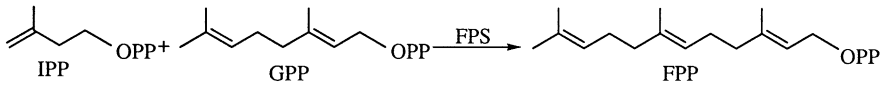


Selenomethionyl farnesyl diphosphate synthase of *Bacillusstearothermophilus*: overproduction, purification, and characterization

Yugesh Kharel, Yuan-Wei Zhang, Tanetoshi Koyama*

Institute for Chemical Reaction Science, Tohoku University, Katahira 2-1-1, Aoba-ku, Sendai 980-8577, Japan. E-mail: koyama@icrs.tohoku.ac.jp

Selenomethionyl farnesyl diphosphate synthase was overproduced, purified to homogeneity, and its substrate specificity, thermostability, kinetic parameters and immunochemical properties were compared to those of wild-type enzyme.



Engineering of catalytic functions to the myoglobin active site framework

Shin-ichi Ozaki[a,b]*, Toshitaka Matsui[b], Mark P. Roach[b], Yoshio Goto[b], Hui-Jun Yang[b], Isao Hara[b], Yoshihito Watanabe[b]

[a]Faculty of Education, Yamagata University, Kojirakawa, Yamagata 990-8560, Japan

[b]Institute for Molecular Science, Myodaiji, Okazaki 444-8585, Japan. E-mail: ozaki@ke-sci.kj.yamagata-u.ac.jp

The sperm whale myoglobin active site mutants (L29H/H64L and F43H/H64L Mb) have shown to catalyze the asymmetric oxidation of sulfides to sulfoxides and olefins to epoxides, respectively.

substrate	wild type		L29H/H64L Mb		F43H/H64L Mb	
	rate[a]	ee(%)	rate[a]	ee(%)	rate[a]	ee(%)
thioanisole	0.25	25 (*R*)	5.5	97 (*R*)	47	85 (*R*)
trans-β-methylstyrene	0.076	39 (1*R*, 2*R*)	0.29	83 (1*R*, 2*R*)	16	96 (1*R*, 2*R*)
cis-β-methylstyrene	0.0026	3 (1*R*, 2*S*)	0.12	99 (1*R*, 2*R*)	0.15	45 (1*R*, 2*R*)

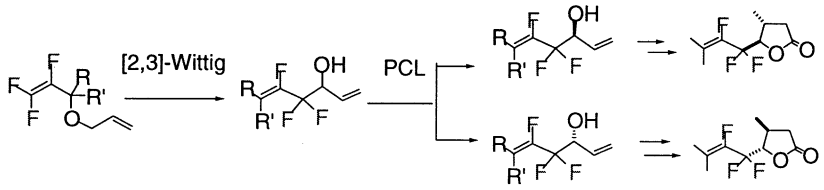
[a]turnover number/min

Synthesis of partially fluorinated-pheromone using chemo-enzymatic reaction strategy

Toshiyuki Itoh*, Kazutoshi Kudo, Naoko Tanaka

Department of Chemistry, Faculty of Education. Okayama University, Okayama 700-8530, Japan. E-mail: ytakagi@sci.hyogo-u.ac.jp

[2,3]-Wittig rearrangement of 1,1,2-trifluoroallylic ethers gave several novel 4,4,5-trifluroalk-1,5-dien-3-ols. The rearrangement reaction gave the alcohols with perfect (*E*)-selection over the new created olefin bond for several substrates. Both enantiomers of partially fluorinated eldanolide have been synthesized through the lipase-catalyzed optical resolution of 6-ethyl-4,4,5-trifluorooct-1,5-dien-3-ol and subsequent radical cyclization chemistry successfully.

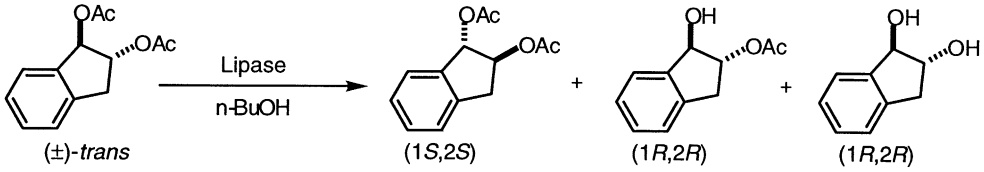


Lipase-mediated alcoholysis of indan-1,2-diol diacetate

Kunio Hirosawa, Tomiki Takahashi, Kouhei Goto, Harumi Kaga*

Hokkaido National Industrial Research Institute, Sapporo 062-8517, Japan. E-mail: kaga@hniri.go.jp

Resolution of racemic *cis*- and *trans*-indan-1,2-diol was effectively performed by an enantioselective lipase-catalyzed alcoholysis of the corresponding diacetates, respectively.

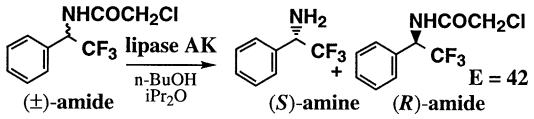


Lipase-catalyzed optical resolution of 2,2,2-trifluoro-1-phenylethylamine

Katsuya Kato*, Yuefa Gong, Satoko Tanaka, Masato Katayama, Hiroshi Kimoto

Department of Chemistry, National Industrial Research Institute of Nagoya, 1-1 Hirate, Kita, Nagoya 462-8510, Japan. E-mail: ktykato@nirin.go.jp

Optical resolution of 2,2,2-trifluoro-1-phenylethylamine was achieved by enantioselective alcoholysis of its chloroacetamide with *Pseudomonas fluorescens* lipase.

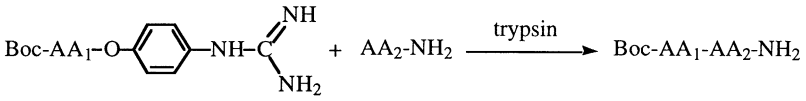


Trypsin-catalyzed oligopeptide synthesis using inverse substrates: application to synthesis of enkephalin analogs

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

Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Ishikari-Tobetsu, Hokkaido 061-0293, Japan. E-mail: sekizaki@hoku-iryo-u.ac.jp

Comparative study of trypsins from different origin (bovine, *streptomyces griseus*, and chum salmon) revealed that chum salmon trypsin was the most efficient catalyst enkephalin analogs synthesis.



AA_1 = Tyr; Tyr-Gly; Tyr-Gly-Gly; Tyr-Gly-Gly-Phe
AA_2 = Gly-Gly-Phe-Leu; Gly-Phe-Leu; Phe-Leu; Leu
AA_1-AA_2 = Tyr-Gly-Gly-Phe-Leu

Studies toward the synthesis of chiral azulene alcohols with lipases: first synthesis and application of chiral azulene alcohols possessing a trifluoromethyl group

Takatomo Kimura[a], Makoto Kamezawa[a], Hojun Tachibana[a], Kazuko Kohara[a], Takehiko Ohtani[a], Yoshinobu Naoshima[b]*

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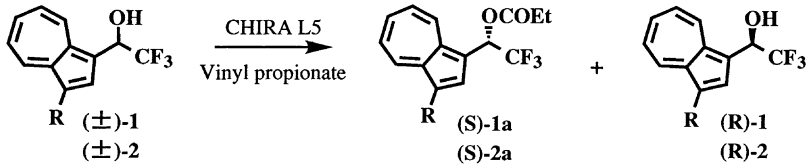
[b]Faculty of Informatics, Okayama University of Science, 1-1 Ridai-cho, Okayama 700-0005, Japan. E-mail: naoshima@sp.ous.ac.jp

Chiral azulene alcohols (1,2) possessing a trifluoromethyl group were first synthesized in highly enantiomerically pure forms by lipase-catalyzed biotransformations with CHIRAZYME L5 (*Candida antarctica*).



Substrate	Time (h)	Conv. (%)	Yield(%)/ee(%) Ester	Alcohol	*E*
(±)-1 R=H	4	47	40/94	40/84	86
(±)-2 R=Cl	4	48	40/98	40/92	>300

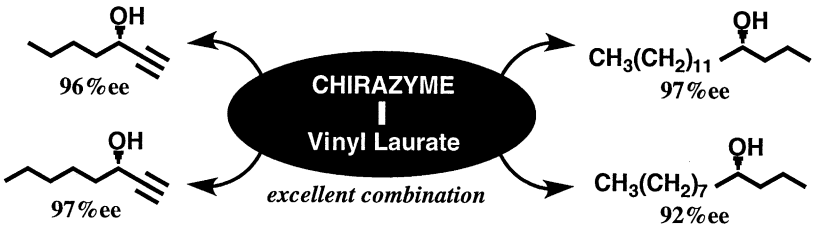
Asymmetric synthesis of chiral building blocks possessing an alkanol skeleton by lipase-catalyzed biotransformations

Makoto Watanabe[a], Makoto Kamezawa[b], Hojun Tachibana[b], Takehiko Ohtani[b], Yoshinobu Naoshima[a]*

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

[b]Konan Chemical Industry Co. Ltd., 5-21, Nakagawa-cho, Takatsuki-shi, Osaka 569-0066, Japan. E-mail: naoshima@sp.ous.ac.jp

A convenient and highly enantioselective preparation of chiral building blocks including 1-alkyn-3-ols and 4-alkanols for the synthesis of biologically active compounds has been accomplished by enzymatic acylations with a combination of lipase CHIRAZYME (*Candida antarctica*) and vinyl laurate.



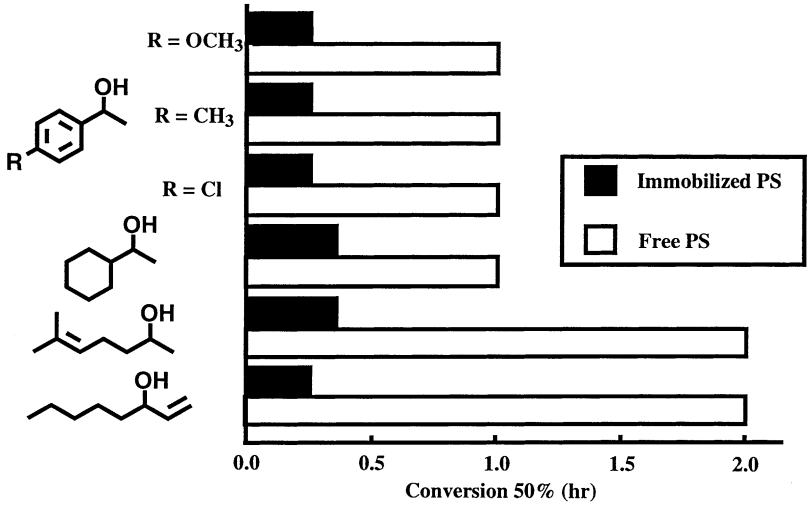
Characteristic of immobilized lipase supported on ceramics carrier Toyonite

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

[a]Toyodenkakogyo Co. Ltd., 2-2-25, Hagi-cho, 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

A new inorganic ceramics support, Toyonite, for the immobilization of lipases was prepared hydrothermally from the minerals of kaolinite, and lipase PS (*Pseudomonas cepacia*) immobilized on Toyonite showed much higher stability and reactivity than the free crude enzyme.

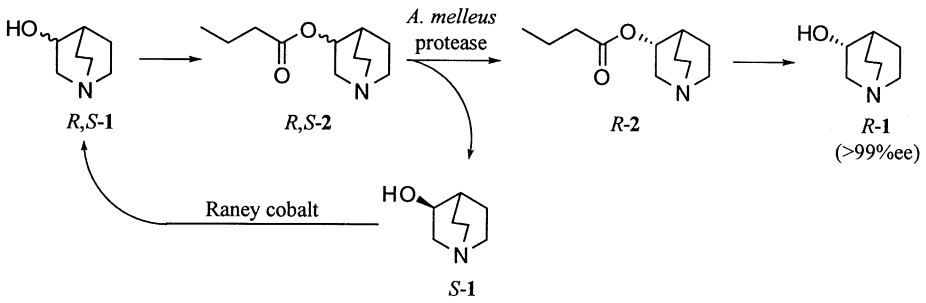


Total process for the production of (*R*)-3-quinuclidinol using biocatalyst

Fumiki Nomoto*, Yukifumi Nishimoto, Yoshihiko Hirayama, Koutaro Ohtsuka

Nagase & Co., Research and Development Center, 2-3, Murotani 2-chome, Nishi-ku, Kobe 651-2241, Japan. E-mail: fumiki.nomoto@nagase.co.jp

The method containing two key steps (i, asymmetric hydrolysis of *R*,*S*-**2** by *Aspergillus melleus* protease; ii, racemization of *S*-**1** by raney cobalt) was established for the production of (*R*)-3-quinuclidinol (*R*-**1**).

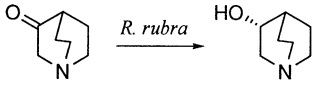


Preparation of (*R*)-3-quinuclidinol using biocatalyst

Yukifumi Nishimoto*, Fumiki Nomoto, Akiko Kuramura, Koutaro Ohtsuka

Nagase & Co., Research and Development Center, 2-3, Murotani 2-chome, Nishi-ku, Kobe 651-2241, Japan. E-mail: yukifumi.nishimoto@nagase.co.jp

3-Quinuclidinone was reduced to (*R*)-3-quinuclidinol using *Rhodotorula rubra* JCM3782 with high yield in excellent enantioselectivity (>99% e.e.).

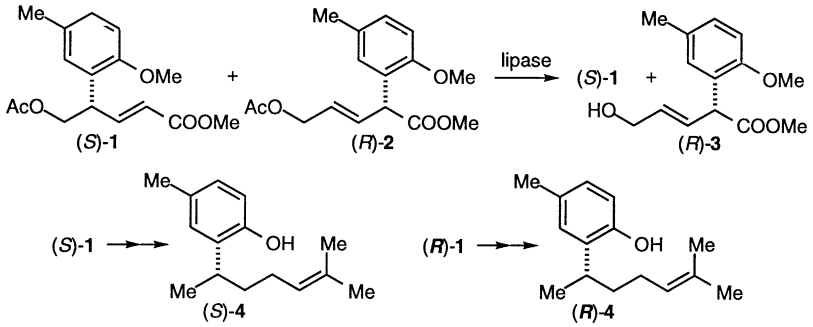


Syntheses of (+) and (−)-elvirol based on separation of structural isomers by lipase

Machiko Ono*, Keiko Suzuki, 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

Total synthesis of (*S*)-(+)-elvirol **4** was achieved based on separation of inseparable mixture of acetates (*S*)-**1** and (*R*)-**2** using lipase.

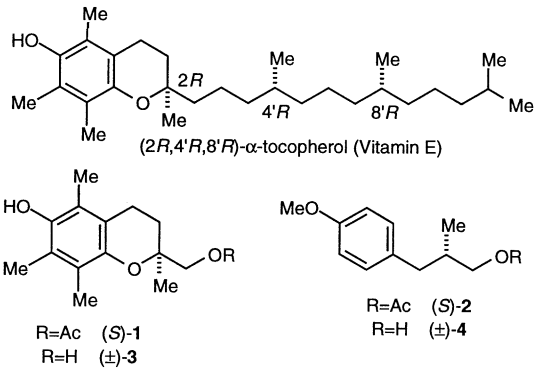


Total synthesis of vitamin E based on enzymatic function

Masako Nozawa*, Keiko Takahashi, Keisuke Kato, Hiroyuki Akita

School of Pharmaceutical Science, Toho University, 2-2-1, Miyama, Funabashi, Chiba 274-8510, Japan. E-mail: masako@phar.toho-u.ac.jp

Total synthesis of vitamin E was achieved based on using the chiral acetates (*S*)-**1** and (*S*)-**2**, which were obtained by the enantioselective acetylation of the corresponding racemic alcohols (±)-**3** and (±)-**4**, respectively.



Stereoselective reduction of keto esters with marine algae

Kohji Ishihara[a]*, Nobuyoshi Nakajima[b], Hitomi Yamaguchi[c], Kaoru Nakamura[c], Yu-ushi Uchimura[d]

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

[b]Department of Nutritional Science, Okayama Prefectural University, Soja, Okayama 719-1112, Japan

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

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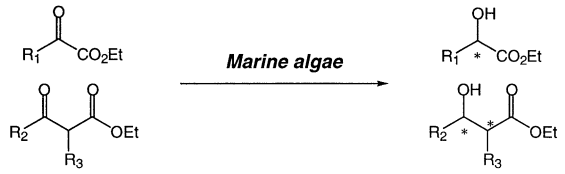
Various α- and β-keto esters were reduced stereoselectively to the corresponding hydroxy esters by marine algae such as *Chaetoceros*, *Nannochloropsis*, and *Pavlova*.



Stereoselective reduction of α-keto esters with thermophilic actinomycete

Kohji Ishihara[a]*, Hitomi Yamaguchi[b], Kaoru Nakamura[b], Nobuyoshi Nakajima[c]

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

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

[c]Department of Nutritional Science, Okayama Prefectural University, Soja, Okayama 719-1112, Japan. E-mail: kishi@wsml.kyokyo-u.ac.jp

An α-keto ester reductase, which catalyses the reduction of ethyl 3-methyl-2-oxobutanoate to the corresponding (*R*)-hydroxy ester, was purified from a thermopilic actinomycete, *Streptomyces thermocyaneoviolaceus* IFO 14271, and the enzyme has different properties from (*S*)-hydroxy ester producing enzyme from the same microorganism in substrate specificity and thermostability.

Table. α-Keto ester reduction by (*S*)-enzyme and (*R*)-enzyme

	(*S*)-enzyme			(*R*)-enzyme		
RCOCO$_2$Et	relative activity (%)	e.e. (%)	confign.	relative activity (%)	e.e. (%)	confign.
CH_3	100	>99	*S*	9	40	*R*
n-C_5H_{11}	82	>99	*S*	170	46	*S*
$CH(CH_3)_2$	9	>99	*S*	100	>99	*R*

Enzymatic conversion of bioactive compounds by glucosylation and esterification: Part III. Stabilization and functionalization of naturally occurring plant pigments by the enzymatic acylation

Nobuyoshi Nakajima[a]*, Kohji Ishihara[b], Sei-ichiro Kawabe[c], Kaoru Nakamura[d], Hiroki Hamada[e], Tsutomu Furuya[e]

[a]Department of Nutritional Science, Okayama Prefectural University, Soja, Okayama 719-1112, Japan

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

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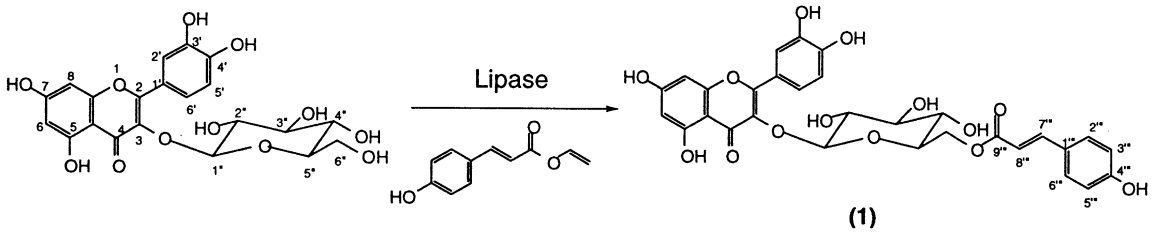
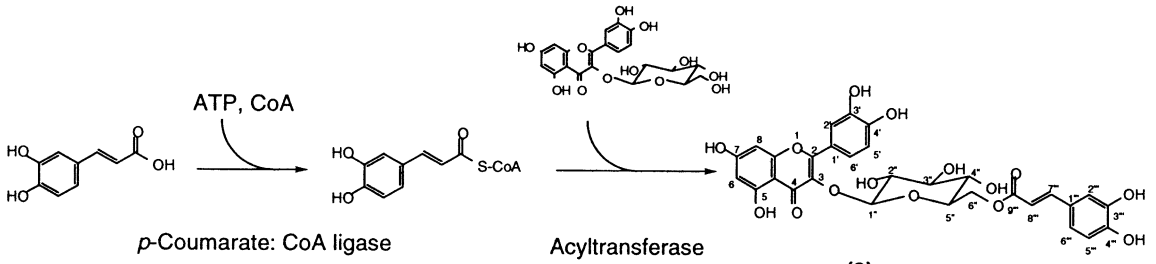
[d]Institute for Chemical Research, Kyoto University, Uji, Kyoto 612-0011, Japan

[e]Department of Applied Science, Okayama University of Science, Ridai-cho, Okayama 711-0005, Japan. E-mail: nakajima@fhw.oka-pu.ac.jp

Stable plant pigments (acylated flavonoid glucosides) were synthesized effectively by esterification with aromatic acids through the two different enzymatic methods: (**I**) Synthesis of isoquercitrin *p*-coumarate (**1**) by trans-esterification with bacterial lipases [N. Nakajima et al., JBB, 87 (1999) 105–107; K. Ishihara et al., JMC B: Enz., 7 (1999) 307–310].



(1)



(2)

Enantioselective reduction of aryl ketones with highly active yeasts: high productivity by addition of vegetable oil

Akinori Hanatani*, Yoshinori Inoue, Munenori Takehara, Hideo Hirohara

Department of Materials Science, The University of Shiga Prefecture, Hassaka, Hikone 522-8533, Japan. E-mail: i23ahanatani@ec.usp.ac.jp

The addition of a vegetable oil to the reaction system increases the productivity greatly in highly active yeast's whole cell-mediated reduction of aryl ketones.

	Conversion (%)	
Yeast	No addition of oil	Addition of 5% oil
Candida saitoana	0.8	60.7
Rhodotorula rubra	1.6	62.9
Yarrowia lipolytica	3.9	57.2
Baker's yeast	0.1	14.4

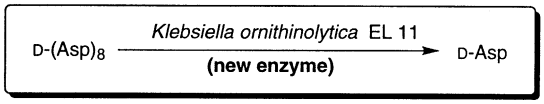
acetophenone 43mM, temp. 30℃, reaction time 72h

Isolation of microorganisms utilizing acidic D-amino acid oligomers

Yasuhisa Asano*, Makiko Umezaki, Yong-Fu Li, Shuichirou Tsubota, Tina L. Lübbehüsen

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

Microorganisms utilizing acidic D-peptide octamer were isolated from soil samples, and D-aspartic acid formation from D-aspartic acid octamer was confirmed by the cell reaction of *Klebsiella ornithinolytica* EL 11.

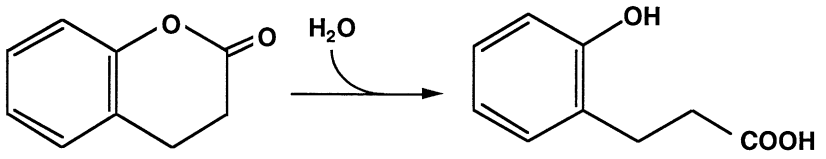


A novel dihydrocoumarin hydrolase with haloperoxidase activity

Michihiko Kataoka*, Kohsuke Honda, Sakayu Shimizu

Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan. E-mail: kataoka@kais.kyoto-u.ac.jp

A novel dihydrocoumarin hydrolase, which exhibits brominating activity of monochlorodimedon in the presence of H_2O_2, and dihydrocoumarin or organic acid, was purified from *Acinetobacter calcoaceticus* F46.



Dihydrocoumarin 3-(2-Hydroxyphenyl)propionic acid

A single mutation of tyrosine-81 in farnesyl diphosphate synthase from *Bacillusstearothermophilus* affects the substrate specificities

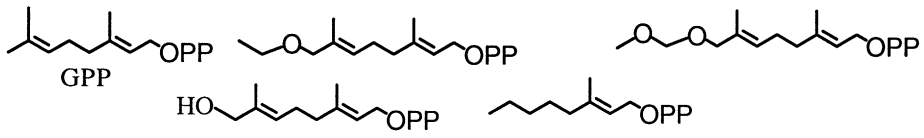
Mariko Komabayashi[a]*, Shoko Kikuchi[a], Hiroshi Sakadume[a], Norimasa Ohya[a], Yuji Maki[a], Hisashi Hemmi[b], Tokuzo Nishino[b], Tanetoshi Koyama[c]

[a]Department of Material and Biological Chemistry, Yamagata University, Yamagata 990-8560, Japan.

[b]Department of Biochemistry and Engineering, Graduate School of Engineering, Tohoku University, Sendai 980-8577, Japan

[c]Institute for Chemical Reaction Science, Tohoku University, Sendai 980-8577, Japan. E-mail: maki@sci.kj.yamagata-u.ac.jp

The substrate specificity was alterable by substituting amino acids with polar side chain (*R*, *S*, *D*) for 81st tyrosine (*Y*) of farnesyl diphosphate synthase from *Bacillus stearothermophilus*, showing that the mutants tested can easily accept the following substrate analogs, which are hardly accepted by the wild enzyme.

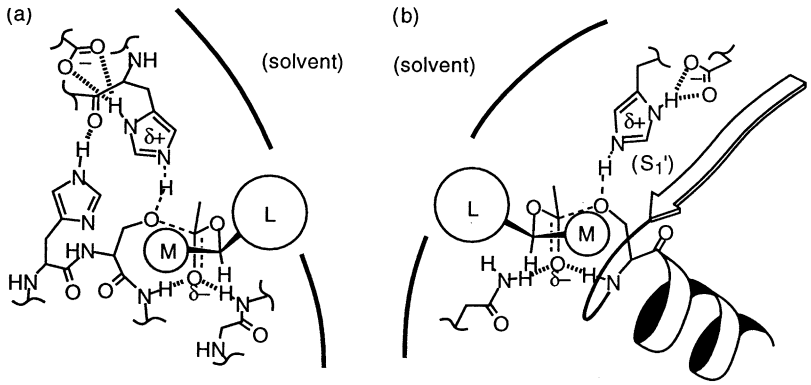


Origin of the enantioselectivity in the lipase- and subtilisin-catalyzed kinetic resolutions of secondary alcohols

Tadashi Ema*, Kunihiro Yamaguchi, Yuji Wakasa, Nobuaki Tanaka, Takashi Sakai, Masanori Utaka

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

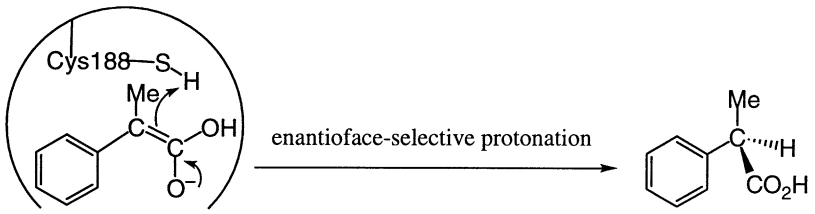
The transition-state models shown below were able to explain the *R*- and *S*-preferences of lipases and subtilisin, respectively, toward secondary alcohols as well as the thermodynamic parameters determined by the temperature-variable measurements.



Studies on the function of arylmalonate decarboxylase

Kaori Matoishi[a], Mamoru Miyazaki[a], Satoshi Hanzawa[b], Hitoshi Kakidani[b], Hiromichi Ohta[a]*
[a]Department of Chemistry, Keio University, 3-14-1 Hiyoshi, Yokohama 223-8522, Japan
[b]Tokyo Research Center, Tosoh Corp., 2743-1 Hayakawa, Ayase 252-1123, Japan. E-mail: hohta@chem.keio.ac.jp

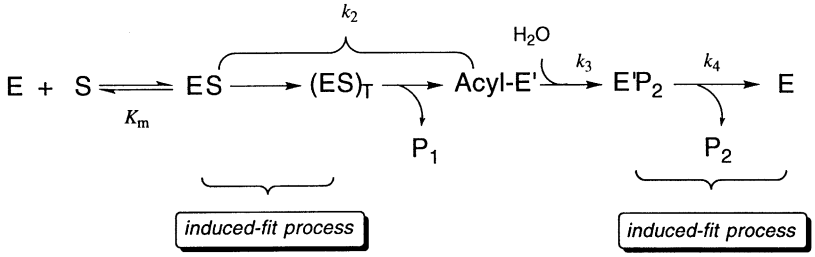
The Cys188 in arylmalonate decarboxylase (EC 4.1.1.76) was estimated to be working as the proton donor to the intermediate enolate resulting in the formation of enantiomerically pure α-substituted arylacetate from the corresponding malonate.



Reaction mechanism of alpha-chymotrypsin from the viewpoint of fluctuations of enzyme

Yasushi Kawai[a]*, Takashi Matsuo[a], Atsuyoshi Ohno[b]
[a]Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan
[b]Fukui University of Technology, 3-6-1 Gakuen, Fukui 910-8505, Japan. E-mail: kawai@scl.kyoto-u.ac.jp

Effects of medium viscosity on kinetics for hydrolysis catalyzed by α-chymotrypsin have been investigated in order to clarify the significance of the conformational change of the enzyme for its catalytic activity.

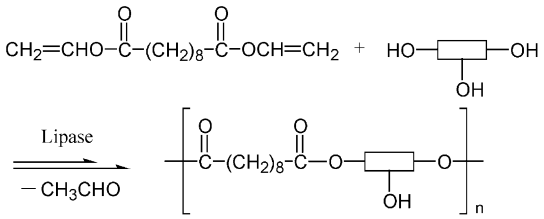


Regioselective polymerization using lipase as catalyst

Hiroshi Uyama*, Kojiro Inada, Satoshi Wada, Shiro Kobayashi

Department of Materials Chemistry, Graduate School of Engineering, Kyoto University, Kyoto 606-8501, Japan. E-mail: uyama@mat.polym.kyoto-u.ac.jp

Regioselective polymerization of divinyl sebacate and triols was achieved by using *Candida antarctica* lipase as catalyst to give soluble polyesters having a reactive hydroxy group in the main chain.



Construction of three consecutive chiral centers with a reductase

Yasushi Kawai*, Kouichi Hida

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

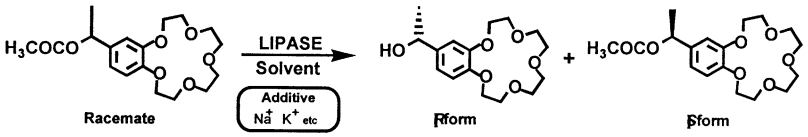
Asymmetric synthesis of hydroxy esters having three consecutive chiral centers with a reductase isolated from baker's yeast (YKER-I) was investigated.

Lipase-mediated resolution of racemic crown ether derivatives

Tatsuro Kijima*, Takanori Moriya, Taeko Izumi

Department of Materials Science and Technology, Faculty of Engineering, Yamagata University, 4-3-16 Jonan, Yonezawa 992-8510, Japan. E-mail: kijima@chem.yz.yamagata-u.ac.jp

Racemic crown ether compounds were resolved enzymatically using hydrolysis by *Candida antarctica* lipase.



Solvent	Additive	Product Alcohol	Remaining Ester
Water	None	45% (59% ee)	33% (>99% ee)
Water	NaCl	36% (79% ee)	38% (59% ee)
Water	KCl	39% (80% ee)	46% (70% ee)
Toluene	None	35% (90% ee)	40% (>99% ee)

Synthesis of optically active novel 1,1,2-trifluoro-1-alken-3-ols through lipase-catalyzed reaction

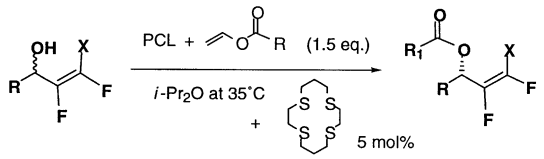
Yumiko Takagi[a]*, Takiko Nakatani[a], Hiroshi Kihara[a], Toshiyuki Itoh[b]

[a]Department of Natural Science, Hyogo University of Teacher Education, Yashiro, Hyogo 673-1494, Japan.

[b]Department of Chemistry, Faculty of Education, Okayama University, Okayama 700-8530, Japan. E-mail: ytakagi@sci.hyogo-u.ac.jp

The first synthesis of several types of optically active 1,1,2-trifluoro-1-alken-3-ols has been accomplished through the *Pseudomonas cepacia* lipase-catalyzed trans-esterification using vinyl chloroacetate as the acyl donor, which

provided the corresponding fluorinated-allyl alcohols that possess an aromatic functional group with sufficient enantioselectivity.



Oxidative polymerization of phenols by tyrosinase and its model complex

Shuhei Namekawa[a,b]*, Hideyuki Higashimura[a,b], Masaaki Kubota[a,b], Akinobu Shiga[a,b], Kiyoshi Fujisawa[c], Yoshihiko Moro-oka[d], Hiroshi Uyama[e], Shiro Kobayashie[f]

[a]Joint Research Center for Precision Polymerization, Japan Chemical Innovation Institute, Tsukuba 305-8565, Japan

[b]Sumitomo Chemical Co. Ltd., Japan

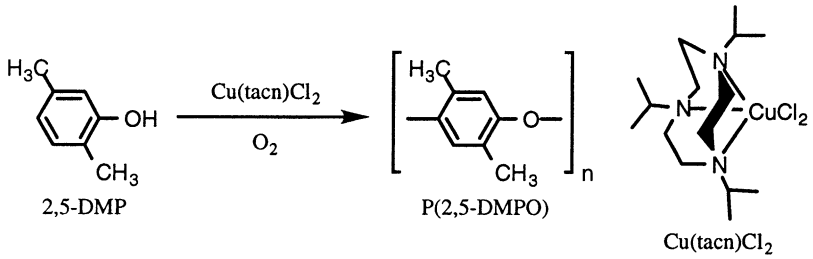
[c]University of Tsukuba, Tsukuba, Japan

[d]Fukui University of Technology, Fukui, Japan

[e]Kyoto University, Kyoto, Japan

[f]National Institute of Materials and Chemical Research, Japan. E-mail: namekawa@nimc.go.jp

Radically controlled oxidative polymerization of 2,5-dimethylphenol (2,5-DMP) catalyzed by a tyrosinase-model complex proceeded regioselectively to give a new crystalline polymer, poly(2,5-dimethyl-1,4-phenylene oxide) (P(2,5-DMPO)).



Homogeneous enzymatic polymerization catalyzed by a lipid-coated enzyme in organic solvent

Kouta Isoyama, Toshiaki Mori, Yoshio Okahata*

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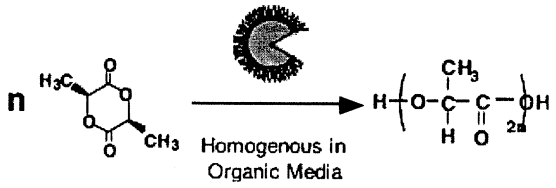
Enzymatic polymerization by lipid-coated enzymes of L-, D,L-, and D-lactide in benzene gave a low molecular-weight-dispersion product (M_w=1200 and M_w/M_n=1.05).



Microbial hydrolysis of C2-symmetrical cyclic carbonates

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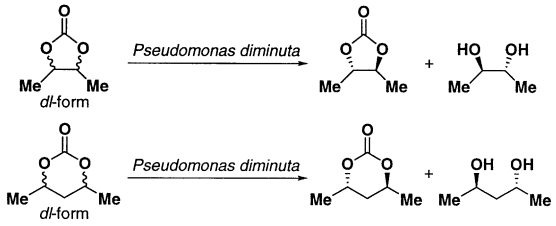
During the screening of the five-membered cyclic carbonate (D,L-4,5-dimethyl-1,3-dioxolan-2-one), *Pseudomonas diminuta* gave the best result to afford optically active compounds. This reaction is also applicable to the six-membered cyclic carbonate.



Application of D-threonine aldolase to the synthesis of some β-hydroxy-α-amino acids

Nobuya Itoh[a]*, Mine Odania, Ji Quan Liu[a], Tohru Dairi[a], Michihiko Kataoka[b], Sakayu Shimizu[b]

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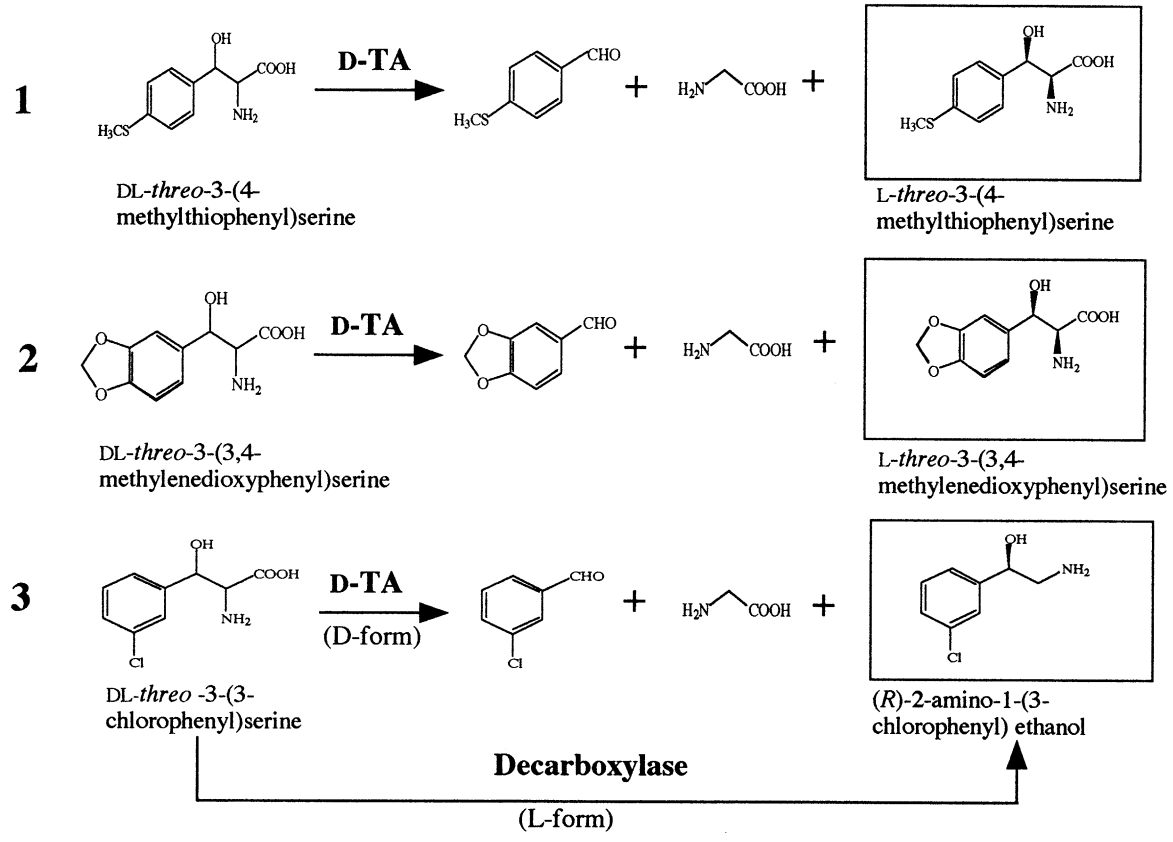
The recombinant D-threonine aldolase was applied to the optical resolution of the following compounds. It gave the optically pure L-*threo* form products and the aldehydes with a high yield.



Biocatalytic one-pot synthesis of octyl β-glycoside in supercritical fluid

Takehiro Nakamura, Kazunobu Toshima, Shuichi Matsumura*

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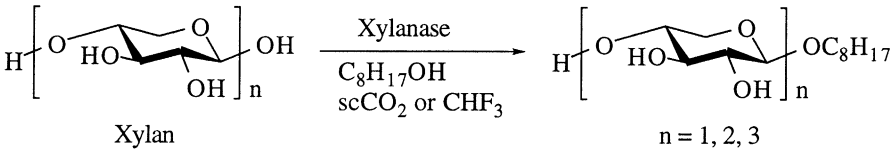
n-Octyl β-D-xylotrioside, xylobioside and xyloside, which are effective next generation surfactants, were prepared by the one-pot reaction of xylan and *n*-octanol using acetone powder (acetone-dried cells) of *Aureobasidium pullulans* KK415 (ATCC 201145) as the enzyme source of xylanase in supercritical carbon dioxide ($scCO_2$) and fluoroform (CHF_3).



Catalytic action of lipid-coated lipase in supercritical carbon dioxide

Atsushi Kobayashi, Mori Toshiaki, Okahata Yoshio*

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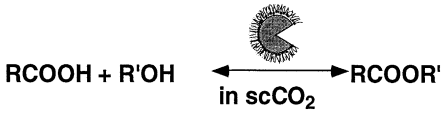
A lipid-coated lipase D from *Rhizopus delemar* catalyzed not only esterification but also hydrolysis in supercritical carbon dioxide about 10-fold faster than in organic media.



Effective transglycosylation catalyzed by a lipid-coated enzyme in supercritical fluoroform

Mariko Funasaki, Toshiaki Mori, Yoshio Okahata*

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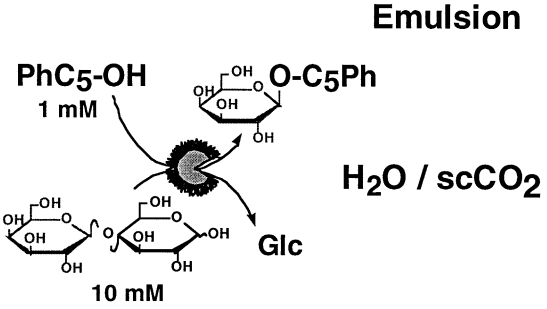
We report on the effective galactosylations catalyzed by a lipid-coated-galactosidase in water-containing supercritical fluid.



Synthesis of bioactive dehydrodiketopiperazines catalyzed by actinomycetous enzyme system

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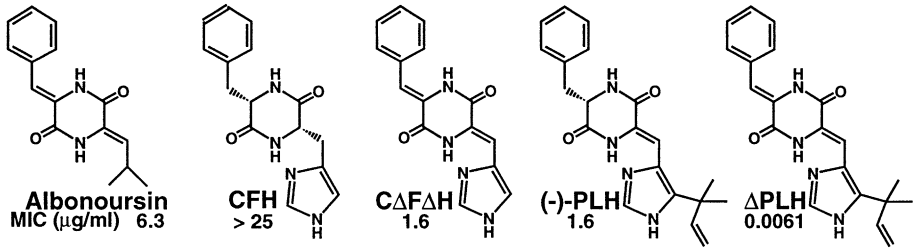
Novel and potent cytotoxic compounds dehydrophenylahistin (DPLH) and CDFDH were effectively synthesized from phenylahistin and CFH, respectively, by a novel enzyme system of an albonoursin-producing actinomycete *Streptomyces albulus* KO_{23}.



Asymmetric reduction by liver cytosol phenylbutanone derivatives

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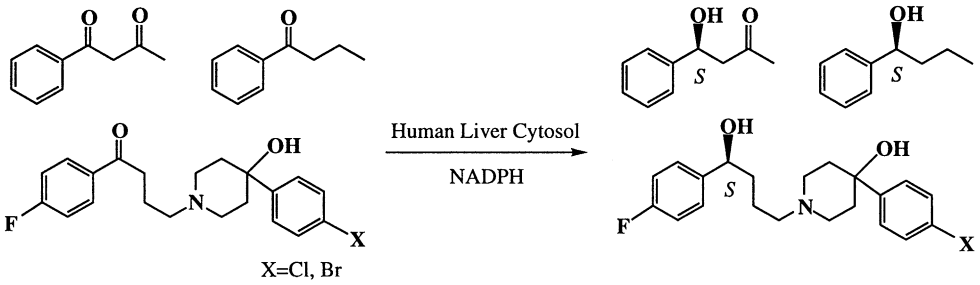
In human liver cytosol, the reduction of phenylbutanone derivatives (1-phenyl-1,3-butadione, 1-phenyl-1-butanone, holoperidol and bromoperidol) was given (*S*)-alcohols in good optical yield (90%, 78%, 99% and 99% ee, respectively).



Oxidative coupling of dibenzylbutanolides catalyzed by *C. sinensis* peroxidase

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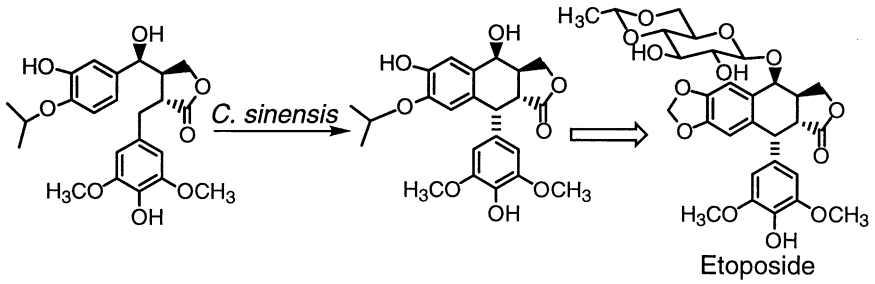
Oxidative coupling of dibenzylbutanolides was carried out using peroxidase enzymes within plant cell culture of *Camellia sinensis*.



Enzymatic synthesis of soluble polyphenol

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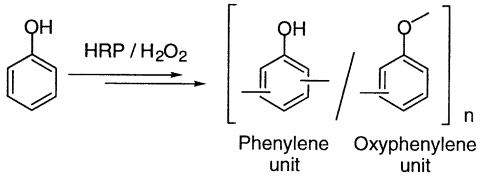
A soluble polyphenol was synthesized by using horseradish peroxidase (HRP) as catalyst in an aqueous organic solvent, whose structure (unit ratio of phenylene/oxyphenylene) could be controlled by changing the solvent composition.



Construction of oligosaccharide library by cells (3)

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[b]Japan Institute of Leather Research, Japan. E-mail: hanyoji@bio.titech.ac.jp

For constructing saccharide library, production of oligosaccharide was carried out by adding simple saccharide primers (*n*-dodecyl lactoside) to living cells such as COS-7.