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JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

Selected Abstracts from the 2nd Japanese Symposium on the Chemistry of Biocatalysis

Introduction

'The 2nd Japanese Symposium on the Chemistry of Biocatalysis' was successfully held in Toyama Ken'min Kaikan, Toyama, Japan, on January 21–22, 1999, organized by Professor Yasuhisa Asano of Toyama Prefectural University. There were five invited lectures, nine oral presentations, and 100 posters. About 230 participants from Universities, government research institutes, and companies, extensively discussed the screening of biocatalysts, novel biocatalysts, mechanisms of their reactions and their uses in organic synthesis, etc. The organizers expect more international researchers would join the Symposium. Shown below are the selected short abstracts (75 titles) of the presentation. Thanks are due to those who gladly sent the abstracts to me.

The selected abstracts of the 1st Symposium are reported in *J. Molec. Catal. B: Enzymatic*, **5**, 491–513 (1998).

Yasuhisa Asano, Editor

Plenary Lectures

Enzymatic resolution of racemic pantoyl lactone toward mass production

Tadanori Morikawa[a,*], Keiji Sakamoto[a], Koichi Wada[a], Shinji Kita[a], Kazuya Tuzaki[a], Michihiko Kataoka[b], Sakayu Shimizu[b], Hideaki Yamada[c]

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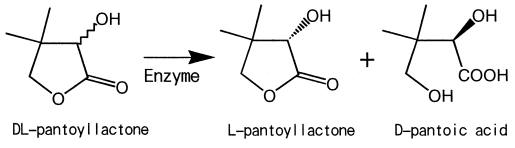
We have established a new process involving a microbial enzyme as a catalyst for asymmetric hydrolysis of DL-pantoyl lactone, which process is exceedingly evaluated from the following viewpoint: preparation of high-purity product, energy and man power saving, and environmental acceptability.



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Diltiazem synthesis, optical resolution and asymmetric synthesis

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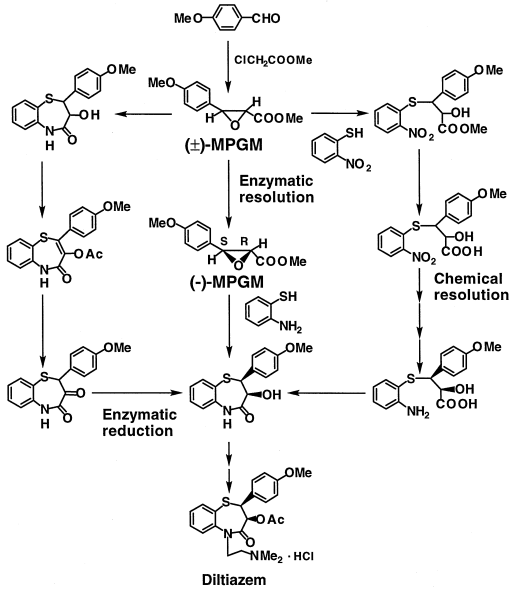
We focus on the asymmetric hydrolysis and the asymmetric reduction of the key intermediate in the synthesis of diltiazem which is used as a typical calcium channel blocker, and report the point of industrialization by the enzymatic reaction using a water-insoluble substrate.

The Synthetic Process for Diltiazem



How to use enzymatic reactions in enantioselective synthesis of bioregulators

Kenji Mori

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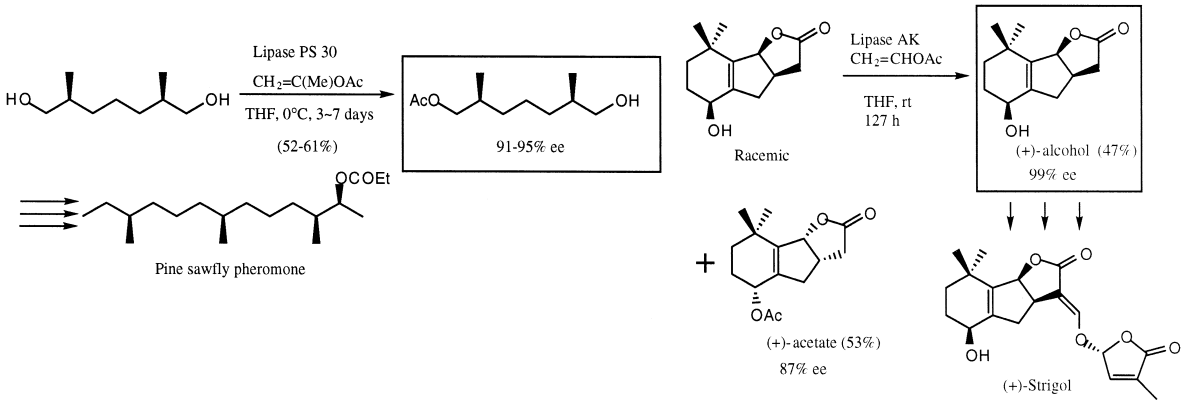
Lipases were employed to prepare various chiral and non-racemic building blocks, which were converted to important bioregulators such as pine sawfly pheromone and strigil, the germination inducer for the seeds of parasitic weeds.



Development of microbial enzymes and their uses in industrial synthesis

Hideaki Yamada

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Our laboratories in Kyoto University, Kansai University and Toyama Prefectural University have in the past 45 years carried out basic studies on the synthesis of various biologically and chemically

Table 1

Product	Enzyme (source)	Yield g/l (mol%)
Amino acids		
D-*p*-Hydroxyphenylglycine	Dihydropyrimidinase (*Bacillus* sp.)	5 (74)
D-Phenylglycine	Dihydropyrimidinase (*Bacillus* sp.)	6 (91)
L-Tyrosine	β-Tyrosinase (*Erwinia herbicola*)	61
L-Dopa	β-Tyrosinase (*Erwinia herbicola*)	53
L-Tryptophan	Tryptophanase (*Proteus rettgeri*)	100 (95)
L-Cysteine	Cysteine desulfhydrase (*Enterobacter cloacae*)	50 (86)
L-Cysteine	Cysteine synthase (*Bacillus sphaericus*)	70 (82)
D-Cysteine	β-Chloro-D-alanine chloride-lyase (*Pseudomonas putida*)	22 (88)
L-Cystathionine	Cystathionine γ-synthase (*Bacillus sphaericus*)	42 (92)
L-Serine	Serine hydroxymethyltransferase (*Hyphomicrobium* sp.)	52
Ethyl-(*R*)-4-chloro-3-hyroxybutanoate	Aldehyde reductase (*Sporobolomyces salmonicolor*)	88 (95)
Amides and acids		
Acrylamide	Nitrile hydratase (*Pseudomonas chlororaphis*)	400 (100)
Acrylamide	Nitrile hydratase (*Rhodococcus rhodochrous*)	650 (100)
Methacrylamide	Nitrile hydratase (*Pseudomonas chlororaphis*)	200
Crotonamide	Nitrile hydratase (*Pseudomonas chlororaphis*)	200
Nicotinamide	Nitrile hydratase (*Rhodococcus rhodochrous*)	1465 (100)
Acrylic acid	Nitrilase (*Rhodococcus rhodochrous*)	380 (100)
Nicotinic acid	Nitrilase (*Rhodococcus rhodochrous*)	172 (100)
6-Hydroxynicotinic acid	Hydroxylase (*Comamonas acidovorans*)	120 (96)
6-Hydroxypicolinic acid	Hyroxylase (*Alcaligenes faecaliss*)	116 (97)
D-Malic acid	Maleate hydratase (*Artrobacter* sp.)	87 (72)
Pyrogallol	Gallic acid decarboxylase (*Citrobacter* sp.)	23 (100)
Theobromine	Oxygenase (*Pseudomonas putida*)	20 (92)
D-Pantoyl lactone	carbonyl reductase (*Candida parapsilosis*)	100 (83)
D-Pantoic acid	Aldonolactonase (*Fusarium oxysporum*)	700 (95)
Coenzymes		
5′-IMP	Nucleoside phosphotransferase (*Pseudomonas trifolli*)	5.6 (80)
Coenzyme A	Multi-step enzyme system (*Brevibacterium ammoniagenes*)	115 (95)
Adenosylmethionine	AdoMet synthetase (*Saccharomyces sake*)	12 (45)
Adenosylhomocysteine	AdoHcy hydrolase (*Alcaligenes faecalis*)	74 (97)
FAD	FAD pyrophosphorylase (*Arthrobacter globiformis*)	18 (28)
Pyridoxal 5′-phosphate	PMP oxidase (*Pseudomonas flurescens*)	0.15 (98)
NADH	Formate dehydrogenase (*Arthrobacter* sp.)	30 (90)
NADPH	Glucose dehydrogenase (*Gluconobacter suboxydans*)	73 (100)
Polyunsaturated fatty acids		
Dihomo-γ-linolenic acid	Multi-step conversion (*Mortierella alpina*)	4.1
Arachidonic acid	Multi-step conversion (*Mortierella alpina*)	4.5
Eicosapentaenoic acid	Multi-step conversion (*Mortierella alpina*)	1.8

useful compounds, using new and novel microbial enzymes isolated from the screened microorganisms, as shown in Table 1.

Development of multifunctional asymmetric catalysis

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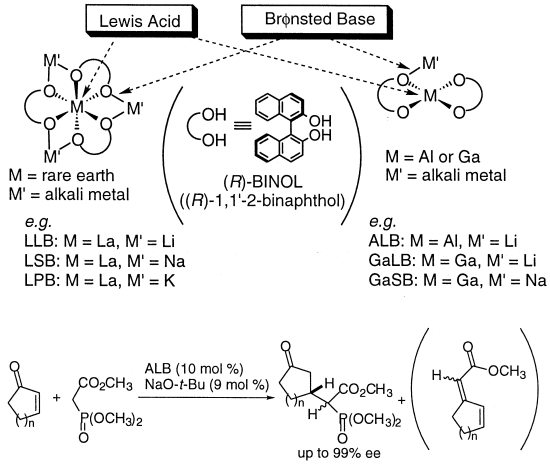
Several heterobimetallic catalyst can provide optically active compounds, which are difficult to synthesize even in a racemic form. For example, the reaction of cyclic enones with Horner – Wadsworth – Emmons reagents promoted by the combination of the



AlLibis(binaphthoxide) complex (ALB) and NaO-*t*-Bu gives Michael product exclusively[2]. In contrast, the usual basic reagent such as BuLi or NaO-*t*-Bu gives only 1,2-adducts (Horner – Wadsworth – Emmons product). This unique feature of the multifunctional catalyst is believed to be the result of a synergistic cooperation of metals in the asymmetric bimetallic complexes.

References

1. For a review: M. Shibasaki, H. Sasai, T. Arai, Angew. Chem. Int. Ed. Engl. 36 (1997) 1236–1256.
2. T. Arai, H. Sasai, K. Yamaguchi, M. Shibasaki, J. Am. Chem. Soc. 120 (1998) 441–442.

Oral Presentations

Simulation of industrial use of enzymes immobilized on EUPERGIT by laboratory-scale experiments

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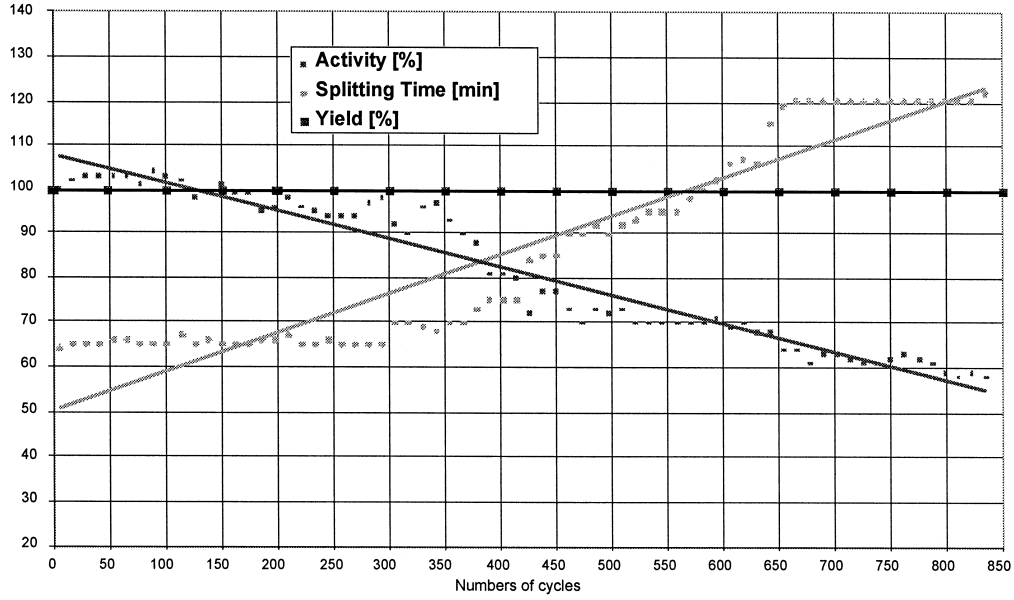
We focus on equipment and methods for judging the performance of immobilized penicillin amidase on laboratory scale, with an automatic titration system for determination of the operational stability of the biocatalyst.



Novel allosterically *trans*-activated ribozymes (maxizymes) with high potential as gene-inactivating agents

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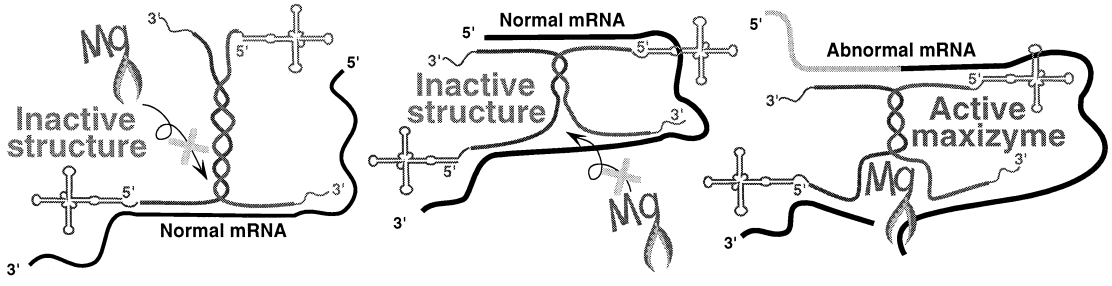
Our novel maxizymes derived from a hammerhead ribozyme, which is a small RNA with site-specific RNA cleavage activity, are capable of cleaving abnormal target mRNA specifically without damaging the normal mRNA in cultured cells, providing the first example of successful allosteric control of the activity of an artificially created allosteric enzyme.



Stereochemical control in microbial reduction

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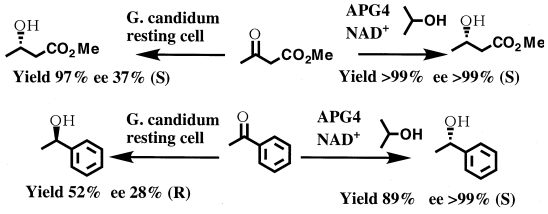
Stereoselectivity for the microbial reduction is improved dramatically by using an acetone powder of *G. candidum* (APG4), 2-propanol and coenzyme instead of the resting cell; we show a novel mechanism for stereochemical control, involving an activation of a specific enzyme.



Biotransformation of exogenous substrates by plant cultured cells — Enzyme system for the reduction of enones

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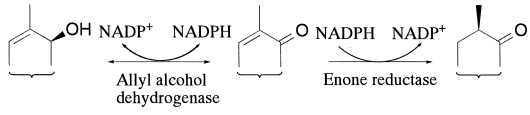
In the course of the study on the biotransformation of exogenous substrates by plant cultured cells, it was found that two enzyme systems participating in the reduction of enones exist in the cell cultures: one, alcohol dehydrogenase responsible for the oxidoreduction between allyl alcohol and enone, of which amino acid sequence predicted by coding nucleotide sequence had high homology with medium chain dehydrogenase/reductase and the other, enone reductases responsible for the reduction of the C–C double bond of enones, one of which catalyzes *syn*-addition of hydrogen atoms.



Synthesis of chiral difluorocyclopropanes through lipase-catalyzed reaction

Koichi Mitsukura, Miyuki Furutani, Satoshi Korekiyo, Toshiyuki Itoh*
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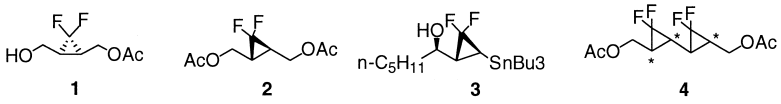
Efficient synthesis of chiral difluorocyclopropane building block has been accomplished; optically active difluorocyclopropane derivatives **1 ~ 4** were synthesized through lipase-catalyzed reaction with > 99% enantiomeric excess.



Transition-state analogue inhibitor of asparagine synthetase and its application to structural study

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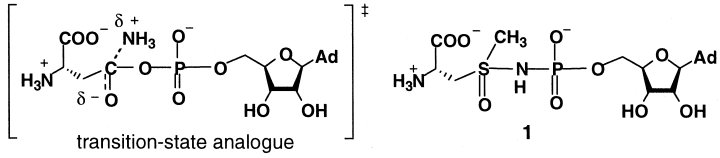
The transition-state analogue **1** of the reaction catalyzed by asparagine synthetase A (AMP-forming, EC 6.3.1.1) from *E. coli* was an extremely potent slow-binding inhibitor of this enzyme (K_i = 67 nM), and the structure of the enzyme-inhibitor complex was determined by X-ray diffraction analysis.



Mechanism of the enantioselectivity of lipases

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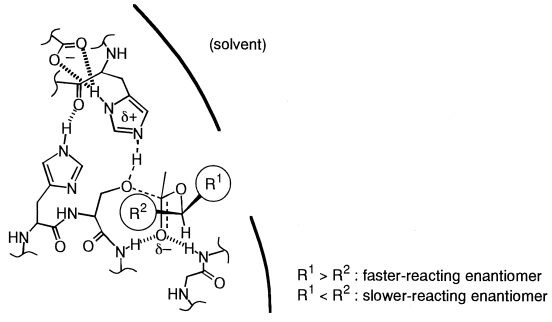
Based on the molecular orbital calculations on model reactions and on X-ray crystal structures of lipases, we proposed the transition-state model which can explain the enantioselectivity of lipases toward secondary alcohols such as the well-known empirical rule (R-preference).



Production of chiral alcohols with baker's yeast-mediated reduction

Tadashi Kometani

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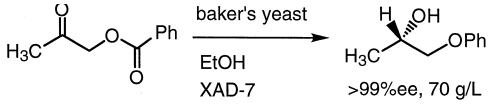
Baker's yeast-mediated reduction of four selected prochiral ketones using well-chosen procedures afforded chiral alcohols with high productivity and high optical purity.



Halogenating enzymes from marine organisms and their functions

Nobuya Itoh

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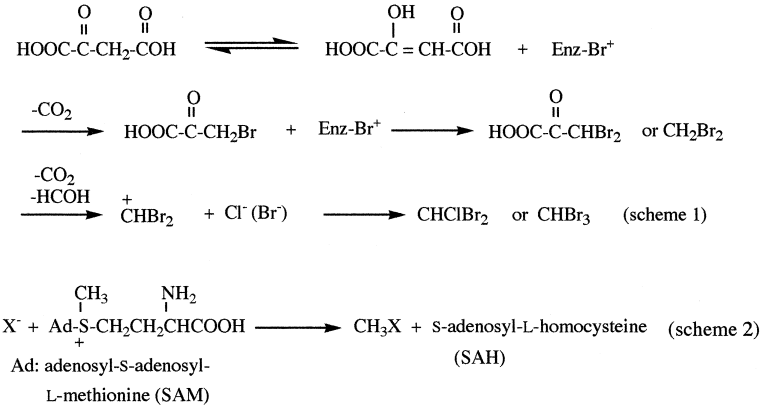
Marine algae including *Corallina pilulifera*, etc., produce di- and trihalomethanes by the bromoperoxidase reaction (Scheme 1). On the other hand, monohalomethanes are synthesized by SAM: halide ion methyltransferases in some marine microalgae (Scheme 2). Volatile halogenated compounds in marine environment originate from these enzymatic reactions.



Posters

Deracemization of racemic alcohols by a biocatalyst

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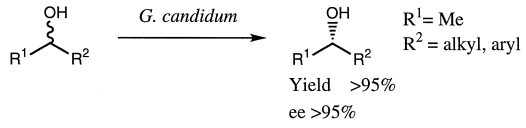
Optically active secondary alcohols of excellent enantiomeric excess were obtained in high yields by deracemization of the corresponding racemic alcohols with *Geotrichum candidum* IFO 5767.



Optical resolution of racemic primary alcohols with a double coupling system

Shinobu Oda[a,*], Takeshi Sugai[b], Hiromichi Ohta[b]

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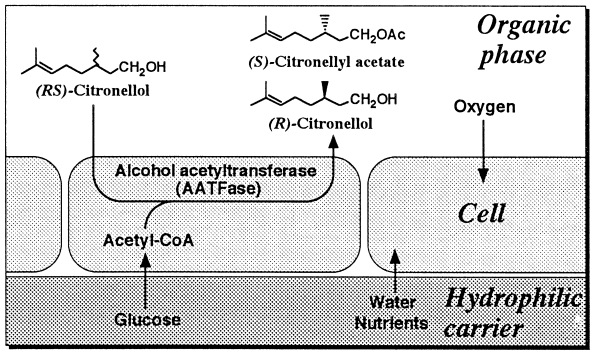
Racemic primary alcohols, such as (*RS*)-citrinellal were resolved in a double coupling system consisting of the acetyl-CoA formation *via* the metabolism of glucose and the following acetylation of the alcohols with the acetyl-CoA by the aid of alcohol acetyltransferase.



Enzymatic conversion of bioactive compounds by glucosylation and esterification: (Part II) Multi-enzymatic glucosylation using *Eucalyptus* UDP-glucosyltransferase coupled UDPglucose-fermentation by bakers' yeast

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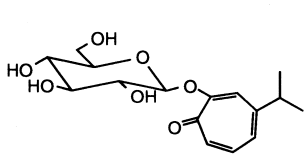
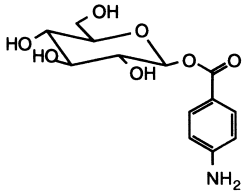
Some aromatic compounds were converted effectively to the corresponding monoglucosides (**1**, **2**) using UDP-glucosyltransferase from *Eucalyptus perriniana* in a coupled reaction with UDPglucose-fermentation by bakers, yeast, and polyphenol was also glucosylated (**3**) in the coupled enzymatic system.



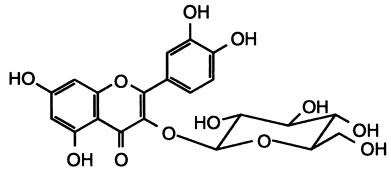
1 Hinokitiol glucoside

(β-Thujaplicin β-D-glucopyranoside)



2 PABA glucoside

(*p*-Aminobenzoyl β-D-glucopyranoside)



3 Isoquercitrin

(Quercetin 3-*O*-β-D-glucopyranoside)

Artificial substrates for undecaprenyl diphosphate synthase (2)

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[b]Faculty of Science, Yamagata University

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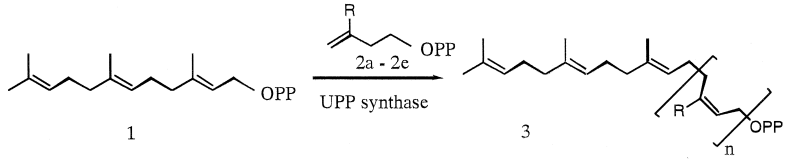
Substrate specificity of undecaprenyl diphosphate synthase from *Micrococcus luteus* B-P 26 was examined with the alkyl-group homologs of isopentenyl diphosphate to find two homologs [desmethyl-IPP(2b) and ethyl-IPP(2c)] to be acceptable as substrates.



2a IPP R = Me
2b desMe-IPP R = H
2c ethyl-IPP R = Et
2d propyl-IPP R = *n*-Pr
2e butyl-IPP R = *n*-Bu

n = 1~ 8

OPP = $OP_2O_6^{3-}$

Application of glycosylation reaction using β-glucosidase to the synthesis of naturally occurring β-glucosides

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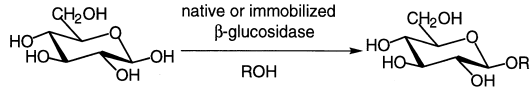
Enzymatic glucosidation of the various kinds of primary alcohols with glucose using β-glucosidase from almonds gave stereoselectively β-D-glucosides in moderate yield, which were converted to the naturally occurring β-glucosides such as cyanoglucosides.



Dramatic changes in the substrate specificity of *Bacillus stearothermophilus* farnesyl diphosphate synthase by replacement of tyrosine-81 with glycine or phenylalanine

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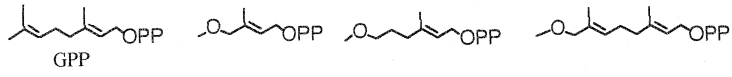
The replacement of tyrosine with glycine at 81 position caused the mutated farnesyl diphosphate synthase (FPS) to easily accept the substrate analogs involving oxygen atoms in their side chains, which were hardly accepted by the wild type enzyme, showing that the single mutation changes the substrate specificity of the bacterial FPS dramatically.



Identification of significant residues in the enzymatic catalysis of undecaprenyl diphosphate synthase of *Micrococcus luteus* B-P 26

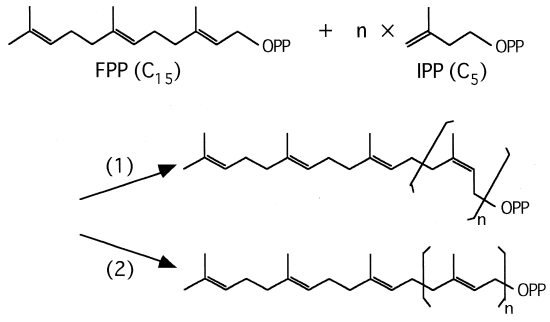
Hideki Yoshisaki[a], Naoto Shimizu[b], Yuan-Wei Zhang[b], Tokuzo Nishino[a], Tanetoshi Koyama[b,*]

[a]Department of Biochemistry and Engineering, Faculty of Engineering, Tohoku University

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

Random and site-directed mutagenesis studies revealed that Asn-77 and Trp-78 in Region of *Micrococcus luteus* B-P 26 undecaprenyl diphosphate synthase, which catalyzes the formation of

C_{55}-phenyl diphosphate with *E,Z* mixed stereochemistry (1) and shows no primary structural similarity with (*E*)-phenyl diphosphate synthases (2), were important for the enzymatic catalytic function.

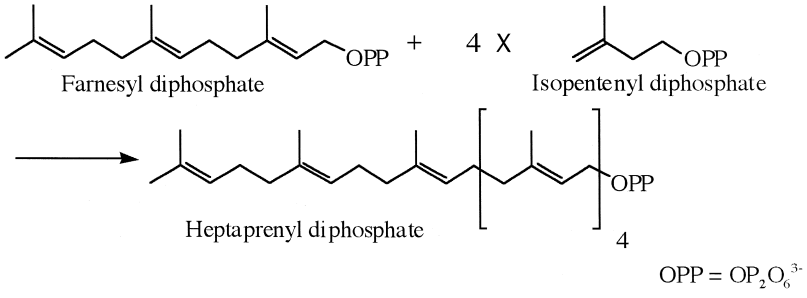


Identification of important amino acid residues in component I of *Bacillus subtilis* heptaprenyl diphosphate synthase

Hiroshi Sugawara, Yuan-Wei Zhang, Tanetoshi Koyama*

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

Ser-100 and Leu-102 in Region B and Leu-220 in C-terminal region of component I of *Bacillus subtilis* heptaprenyl diphosphate synthase, which is composed of two dissociable heterosubunits (component I and component II), were identified by random and site-directed mutagenesis to be important for the enzymatic catalysis as well as for determination of the chain length of the ultimate product.

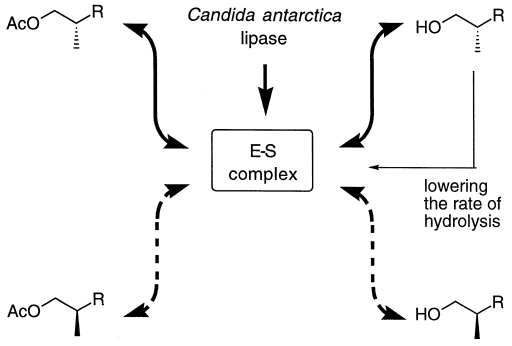


Enantioselectivity depending on the conversion in *Candida antarctica* lipase-catalyzed hydrolysis

Tomohiro Akeboshi, Hiromichi Ohta, Takeshi Sugai*

Department of Chemistry, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223-8522, Japan. E-mail: sugai@chem.keio.ac.jp

The *E* value of kinetic resolution on the occasion of *Candida antarctica* lipase-mediated hydrolysis of a primary alcohol was revealed to depend upon the conversion of the reaction. The product, an alcohol which was produced accompanied with the progress of the hydrolysis had an inhibitory effect on the hydrolysis of highly reacting enantiomer of acetate, which resulted in the lowering of *E* value.

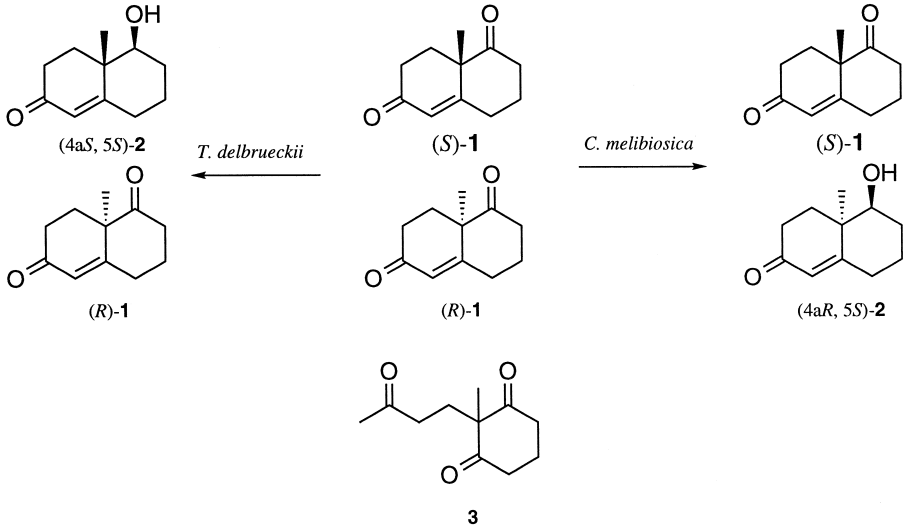


Yeast-mediated synthesis of enantiomerically enriched Wieland – Miescher ketone and related compounds

Ken-ichi Fushuku, Nobutaka Funa, Tomohiro Akeboshi, Hiromichi Ohta, Takeshi Sugai*

Department of Chemistry, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223-8522, Japan. E-mail: sugai@chem.keio.ac.jp

Through a screening of microorganism which can enantioselectively reduce racemic mixture of Wieland – Miescher ketone, two yeast strain was found: *Torulaspora delbrueckii* IFO 10921 and *Candida melibiosica* IAM 4488 showed opposite preference in regard to the enantiomers. *T. delbrueckii* was applied the prochiral triketone, 2-methyl-2-(3-oxobutyl)-1,3-cyclohexanedione, a precursor of Wieland – Miescher ketone.

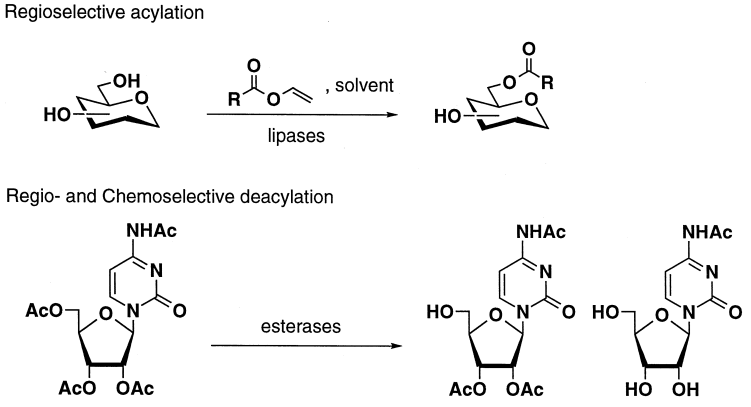


Enzyme-catalyzed regioselective de-acylation and acylation of carbohydrates

Atsuhito Kuboki*, Takashi Ishihara, Eiko Kobayashi, Hanako Okazaki, Hiromichi Ohta, Takeshi Sugai*

Department of Chemistry, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223-8522, Japan. E-mail: sugai@chem.keio.ac.jp

Enzyme-catalyzed regioselective de-acylation and acylation of carbohydrates was studied by using *Pseudomonas* and *Candida* lipase and some esterases. Wide range of substrates, such as monosaccharide aryl glycosides, monosaccharide glycals, and peracetylated forms of sugar nucleosides. In many cases, enzyme-catalysed reaction preferentially proceeded on the primary alcohol (or acetate) in the substrates.

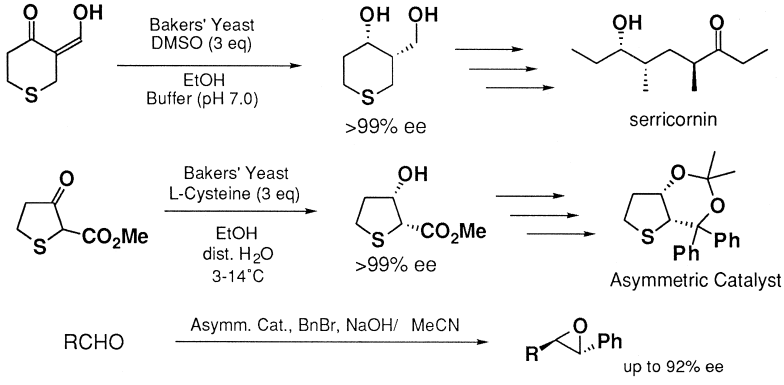


Bakers' yeast reduction in the presence of a sulfur compound and its application

Ryuuichirou Hayakawa, Makoto Shimizu, Tamotsu Fujisawa

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

The bakers' yeast reduction of β-keto aldehyde derivatives or 3-oxo-2-methoxycarbonyltetrahydrothiophene in the presence of a sulfur compound as an additive gave the enantiomerically pure reduction products, which in turn were applied to the synthesis of serricornin and asymmetric epoxidation of aldehydes as an asymmetric catalyst.

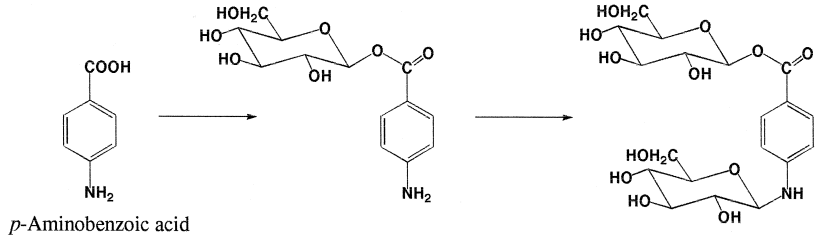


Biotransformation of foreign substrate by plant suspension cells

Hiroki Hamada, Tsutomu Furuya

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

We studied the biotransformation of foreign substrate by plant suspension cells and found that the plant cells have the selective conversion ability for foreign substrate.



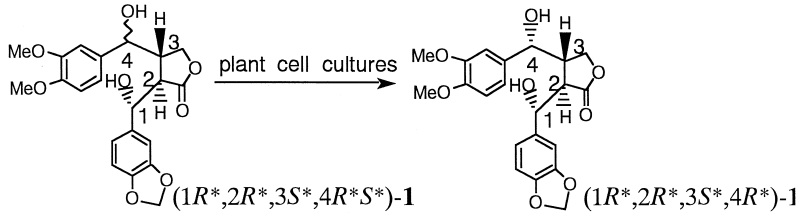
Biotransformation of *p*-aminobenzoic acid by cultured cells of *E. perriniana*

Dediastereomerization of lignans utilizing plant cell cultures

Masumi Takemoto*, Yuki Matsuoka, Kazuo Achiwa

School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Shizuoka 422-8526, Japan. E-mail: takemoto@ys2.u-shizuoka-ken.ac.jp

(1*R**,2*R**,3*S**,4*R***S**)-**1** was converted to (1*R**,2*R**,3*S**,4*R**)-**1** with plant cell cultures.

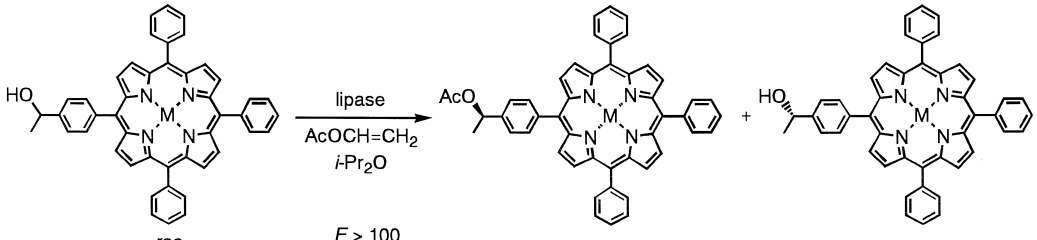


Lipase-catalyzed kinetic resolution of a chiral porphyrin designed by a transition-state model

Tadashi Ema*, Masahito Jittani, 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

A very large secondary alcohol having a tetraphenylporphyrin was successfully resolved using several lipases, supporting our transition-state model recently proposed to rationalize the enantioselectivity of lipases toward secondary alcohols.

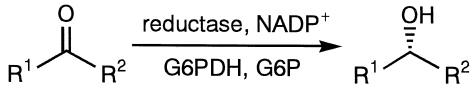


E > 100
Pseudomonas cepacia lipase, *Candida antarctica* lipase,
*Rhizomucor miehe*i lipase, *Pseudomonas aeruginosa* lipase

Catalytic asymmetric reduction of carbonyl compounds using a reductase purified from bakers' yeast

Tadashi Ema*, Hiroyuki Moriya, Toru Kofukuda, 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

Highly enantioselective asymmetric reductions of several carbonyl compounds were carried out using a reductase purified from bakers' yeast.

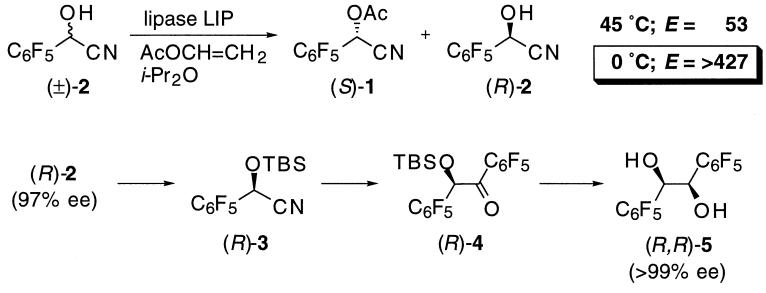


R^1	R^2	% ee
$ClCH_2$	n-C_4H_9	>99
n-C_5H_{11}	CH_2OAc	98
CH_3	CH_2CO_2Me	>99
CH_3	CO_2Et	>99
$ClCH_2$	CH_2COCH_3	>99
CH_3	$CH_2COC_2H_5$	>99

Lipase-catalyzed resolution of fluorine-containing cyanohydrin derivatives and its synthetic application

Takashi Sakai*, Yasushi Miki, Tomonori Harada, Tadashi Ema, Masanori Utaka
Department of Applied Chemistry, Faculty of Engineering, Okayama University, Tsushima-naka, Okayama 700-8530, Japan. E-mail: tsakai@cc.okayama-u.ac.jp

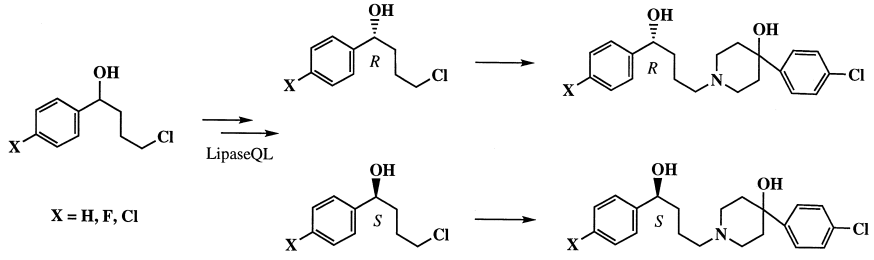
The kinetic resolution of racemic cyanohydrin (±)-**2** containing pentafluorophenyl group gave optically active cyanohydrin (*R*)-**2** and its antipodal ester (*S*)-**1**, the former of which was transformed into *threo*-diol (*R*,*R*)-**5**, a new chiral ligand for asymmetric synthesis, without loss of the optical purity.



Synthesis and pharmacological activity of optically active dihydrohaloperidol-related compounds

Masatomo Miura, Maki Kubota, Yuka Nakabayashi, Katsuyo Abe, Mitsuhiro Takeshita*
Tohoku College of Pharmacy, 4-4-1 Komatsushima, Aoba-ku, Sendai 981-0905, Japan.
E-mail: mtake@tohoku-pharm.ac.jp

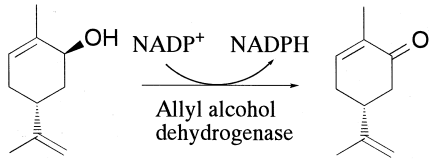
Optically active dihydrohaloperidol related compounds, which are reduced butyrophenone neuroleptics, were asymmetrically synthesized using lipaseQL and the difference in cataleptic activities in mice between these isomers was investigated.



Allyl alcohol dehydrogenase from the cultured cells of *Nicotiana tabacum*

Yoshitaka Tamura, Kei Shimoda, Toshifumi Hirata*
Department of Chemistry, Faculty of Science, Hiroshima University, Kagamiyama 1-3-1, Higashi-Hiroshima 739-8526, Japan. E-mail: thirata@sci.hiroshima-u.ac.jp

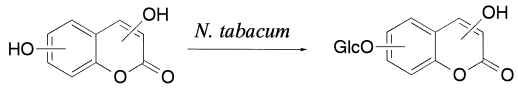
In the course of the study on the biotransformation of exogenous allyl alcohols such as carveol by the cultured cells of *Nicotiana tabacum*, it was found that the cell cultures contain alcohol dehydrogenase specific for the allyl alcohols, of which amino acid sequence predicted by coding nucleotide sequence had high homology with medium chain dehydrogenase/reductase.



Glucosylation of phenol compounds by the cultured cells of *Nicotiana tabacum*

Kei Shimoda[a], Takeshi Fujino[a], Shinji Ohta[b], Toshifumi Hirata[a,*]
[a]Department of Chemistry, Faculty of Science, Hiroshima University, Kagamiyama 1-3-1, Higashi-Hiroshima 739-8526, Japan
[b]Instrument Center for Chemical Analysis, Hiroshima University.
E-mail: thirata@sci.hiroshima-u.ac.jp

The cultured cells of *Nicotiana tabacum* were found to be capable of glucosylating exogenous hydroxycoumarins to yield their corresponding mono-β-glucosides, showing the regioselectivity to prefer the glucosylation of phenolic hydroxyl groups.

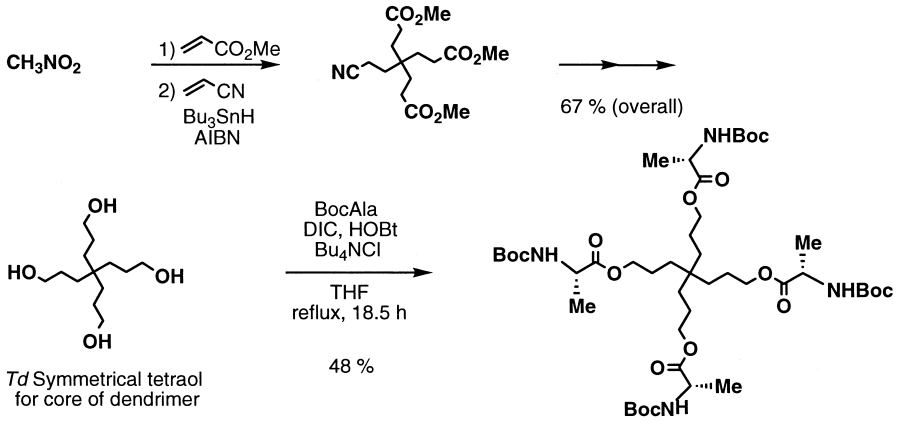


Synthesis of functionalized dendrimers: immobilized model of aldolase

Masayuki Shimagaki*, Susumu Koizumi, Kazuaki Shirota, Tadashi Nakata

The Institute of Physical and Chemical Research (RIKEN), 2-1 Hirosawa, Wako, Saitama 351-0198, Japan. E-mail: masayuki@riken.go.jp

Synthesis of dendrimer core was examined to explore new immobilized material for enzyme as model of functionalized dendrimer, and coupling reaction of the core and Boc alanine was studied for exploitation of immobilized model of aldolase, an enzyme.

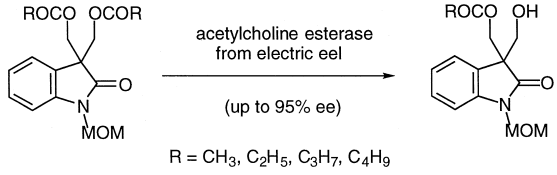


Asymmetric induction of oxindoles by enzymatic hydrolysis

Masaki Hayashi, Masakazu Tanaka, Hiroshi Suemune*

Faculty of Pharmaceutical Sciences, Kyushu University, Fukuoka 812-8582, Japan.
E-mail: suemune@lyra.phar.kyushu-u.ac.jp

Asymmetric hydrolysis of *meso*-diesters with 2-oxindole moiety afforded the corresponding monoesters in an enantioselective manner by using acetylcholine esterase from electric eel.



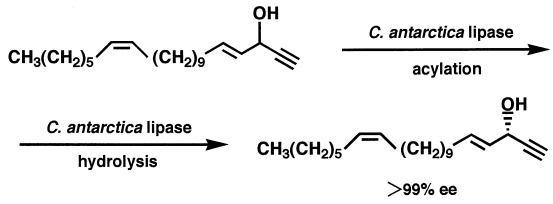
Asymmetric synthesis of biologically active natural products possessing chiral alkanol skeletons by lipase-catalyzed biotransformations

Fuminari Okimoto[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, 5-21, Nakagawa-cho, Takatsuki-shi, Osaka 569-0066, Japan. E-mail: naoshima@sp.ous.ac.jp

Biologically active compounds possessing chiral alkanol skeletons, such as (4*E*, 15*Z*)-docosa-4, 15-dien-1-yn-3-ol and related compounds, have been synthesized in highly enantiomerically pure forms by biocatalytic reactions with *Candida* and *Pseudomonas* sp. lipases.



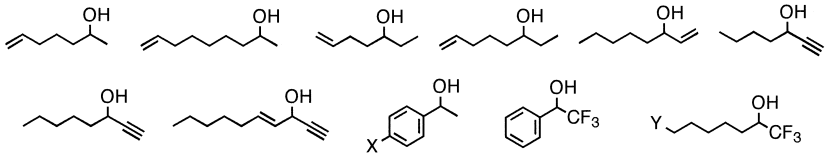
Enzymatic preparation of chiral secondary alcohols with *Candida antarctica* lipase as an asymmetric catalyst

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, 5-21, Nakagawa-cho, Takatsuki-shi, Osaka 569-0066, Japan. E-mail: naoshima@sp.ous.ac.jp

Preparation of chiral building blocks with an enantiomeric purity of $>98\%$ ee for the synthesis of natural products has been accomplished by the enantioselective biotransformation of racemic secondary alcohols in aqueous solutions or organic solvents with *Candida antarctica* (CHIRAZYME) and *Pseudomonas cepacia* (lipase PS) lipases.

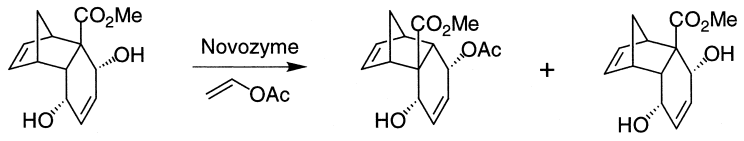


Preparation of chiral building blocks using lipase

Michiyasu Takahashi, Hiroyuki Konno, Kunio Ogasawara*

Pharmaceutical Institute, Tohoku University, Aobayama, Sendai 980-8578, Japan.
E-mail: konolmail.cc.tohoku.ac.jp

Synthetic equivalents of optically active cyclohexadienediols have been prepared from the racemic diol by lipase-catalyzed transesterification with vinyl acetate.

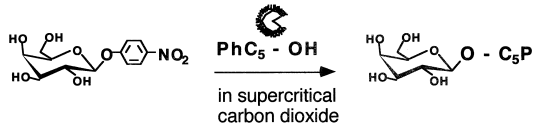


Transglycosylation catalyzed by a lipid-coated glycoside hydrolase in supercritical fluids

Toshiaki Mori, Yoshio Okahata*

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

The lipid-coated β-D-Galactosidase (from *Bacillus circulans*) was soluble in supercritical carbon dioxide, and showed a higher transgalactosylation (18 times) in supercritical carbon dioxide (40°C/150 atm) than in organic solvent for the reaction of 5-phenylpentanol with *p*-nitrophenyl-β-D-galacto-pyranoside.

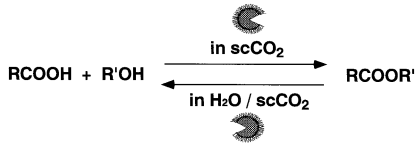


Catalytic activity of a lipid-coated lipase in supercritical carbon dioxide

Atsushi Kobayashi, Toshiaki Mori, Yoshio Okahata*

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

A lipid-coated lipase was soluble in supercritical carbon dioxide ($scCO_2$) and catalysed not only esterification in $scCO_2$ but also hydrolysis in water-containing $scCO_2$ about 10-fold faster than in organic media.

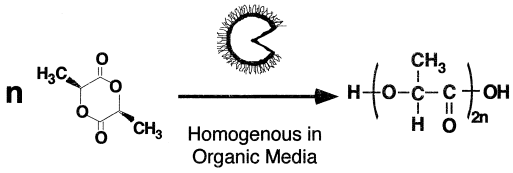


Preparation of poly(lactic acid) catalyzed by a lipid-coated enzyme in organic solvent

Kouta Isoyama, Toshiaki Mori, Yoshio Okahata*

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

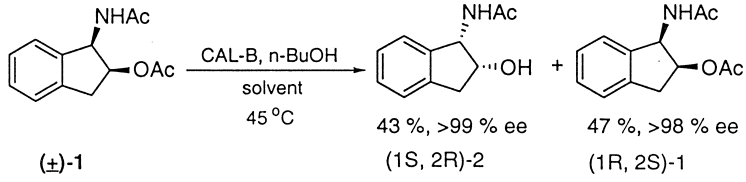
Lipid-coated enzymatic polymerization of L-, D,L-, and D-lactide in benzene gives a low molecular-weight-dispersion product ($M_w = 1200$ and $M_w/M_n = 1.05$).



Enzyme catalyzed resolution of cyclic 1,2-aminoalcohols

A.T. Anilkumar, Kouhei Goto, Harumi Kaga*
Hokkaido National Industrial Research Institute, 2-17-2-1 Tsukisamu-Higashi, Toyohira-ku, Sapporo 062-8517, Japan. E-mail: kaga@hniri.go.jp

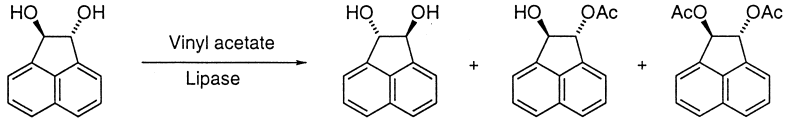
An efficient resolution of *cis*-1-amino-2-indanol, the key component of HIV protease inhibitor Indinavir, is reported using CAL-B catalyzed deacetylation of its *N*,*O*-diacetyl derivative in organic medium.



Lipase catalyzed transesterification of cyclic diols

Kunio Hirosawa, Tomiki Takahashi, Kouhei Goto, Harumi Kaga*
Hokkaido National Industrial Research Institute, 2-17-2-1 Tsukisamu-Higashi, Toyohira-ku, Sapporo 062-8517, Japan. E-mail: kaga@hniri.go.jp

During lipase catalyzed transesterification of *trans*-1,2-dihydroxyacenaphthene, resolution was accomplished by lipase PS or CAL-B affording both enantimers with high enantiomeric purity.



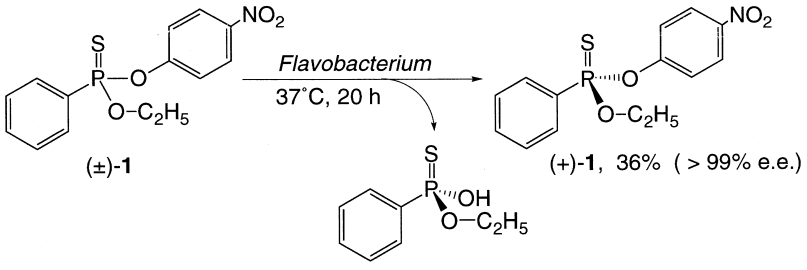
	%ee (%Y)	%ee (%Y)	%ee (%Y)
PS	- (trace)	95 (49)	92 (51)
CAL-B	89 (53)	99 (47)	- (0)

Optical resolution of *P*-chiral organophosphorous esters by *Flavobacterium* catalyzed hydrolysis

Hiroshi Kita, Tadahiro Uemura, Shokichi Ohuchi*

Department of Biochemical Engineering and Science, Kyushu Institute of Technology, Iizuka, Fukuoka 820-8502, Japan. E-mail: ohuchi@bse.kyutech.ac.jp

The *P*-chiral organophosphorous esters as an EPN were optically resolved in preparative scale by *Flavobacterium* sp. catalyzed hydrolysis.

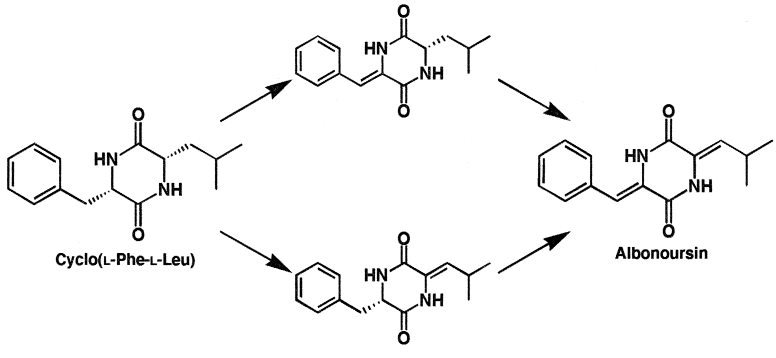


Enzymatic synthesis of bioactive dehydro cyclic dipeptides

Hiroshi Kanzaki*, Kazumi Akazawa, Satohiro Yanagissawa, Daisuke Imura, Teruhiko Nitoda, Kazuyoshi Kawazu

Laboratory of Bioresources Chemistry, Faculty of Agriculture, Okayama University, Tsushima-naka, Okayama 700-8530, Japan. E-mail: hkanzaki@cc.okayama-u.ac.jp

A novel enzyme system in the cell-free extract of an albonoursin-producing actinomycete catalyzing the conversion of cyclo(L-Phe–L-Leu) to albonoursin via one of the dehydro intermediates was found to be applicable for producing bioactive dehydro cyclic dipeptides other than albonoursin.



Enzymatic synthesis of D-amino acids with mutant D-amino acid aminotransferases

Nobuyoshi Esaki[a], Kazuo Shiomi[b], Aldo Gutierrez[a], Yoshihiro Fuchikami[a], Tohru Yoshimura[a]
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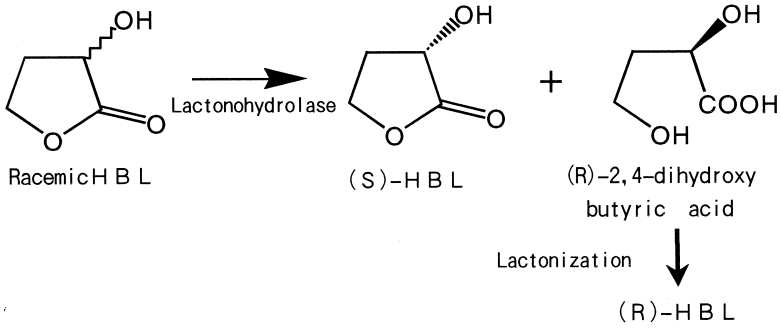
A simple method has been established for the microbial synthesis of D-amino acids from the corresponding α-keto acids and ammonium formate as substrates with the resting cells of the recombinant *E. coli* clone expressing simultaneously heterologous genes of formate dehydrogenase, L-alanine dehydrogenase, alanine racemase, and an appropriate mutant D-amino acid aminotransferase to obtain optically pure D-enantiomers of glutamate, leucine and other amino acids with high yields, respectively.

Enzymatic production of (*S*) or (*R*)-α-hydroxy-γ-butyrolactone

Koichi Wada, Shinji Kita, Kazuya Tuzaki, Keiji Sakamoto, Tadanori Morikawa*
Research Institute, Fuji Chemical Industries, 530 Chokeiji, Takaoka, Toyama 933-8511, Japan.
E-mail: morikawa@fuji-chemi.co.jp

We have established a convenient biocatalytic resolution method of α-hydroxy-γ-butyrolactone (HBL) that produces both enantiomers with high optical purity and good yield by a new fungal lactonohydrolase.



Production of an aspartame precursor using thermolysin mutants

Satoshi Hanzawa[a,*], Seigou Ooe[a], Toshio Miyake[a], Shin-ichiro Nakamura[a], Akira Tokuda[a], Shun-ichi Kidokoro[b], Kimiko Endo[b], Akiyoshi Wada[b]
[a]Tokyo Research Center, Tosoh
[b]Sagami Chemical Research Center. E-mail: hanzawa@tosoh.co.jp

Thermolysin mutants, of which Leu 144, Asp 150 and/or Asn 227 were replaced by Phe, Trp and/or His, respectively, showed higher stability and activity, and we successfully reduced enzyme consumption during condensation of *Z*-Asp and PheOMe into an aspartame precursor *Z*-Asp–PheOMe, using these mutants.

Table 1
Residual enzyme during condensation of *Z*-Asp and PheOMe into an aspartame precursor *Z*-Asp–PheOMe

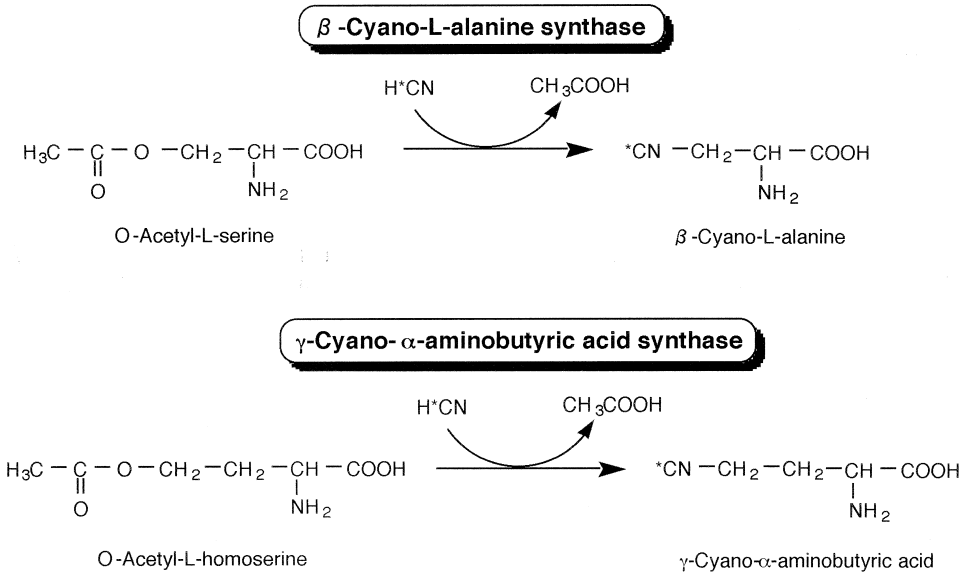
Conversion rate (%)	Residual Enzyme (%)		
	Wild type	D150W-N227H	L144F-D150W-N227H
47	82	92	97
63	61	74	82
$[E]_0$ (%)	2.1	0.25	0.25

$[E]_0$; Initial concentration of crude enzyme powders whose thermolysin contents were about 20%.

Cyan adding enzyme from *Bacillus stearothermophilus* CN332 and it's application

Shoji Kaneko[a], Masahiro Ikemoto[a], Hironori Omura[a,*], Kiichi Ishiwata[b], Michio Senda[b]
[a]Ikeda Food Research, 95-7 Minooki-cho, Fukuyama-shi, Hiroshima 721-8558, Japan
[b]Tokyo Metropolitan Institute of Gerontology, 1-1 Naka-cho, Itabashi, Tokyo 173-0022, Japan. E-mail: LEE03537@nifty.ne.jp

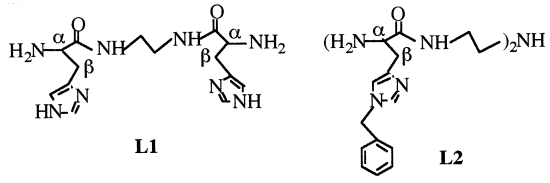
Two types of cyan adding enzyme, β-cyano-L-alanine synthase and γ-cyano-α-amino butyric acid synthase, were purified from *Bacillus stearothrmophilus* CN332, and applied to synthesis of amino acids labelled with a positron emitting radionuclide.



Zinc complexes of artificial histidine-containing dipeptides and their catalytic function on *p*-nitro phenylphosphates

M. Khabir Uddin, Kou Nakata, Kazuhiko Ichikawa*
Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 060-0810, Japan. E-mail: Ichikawa@earth.ees.hokudai.ac.jp

Zinc complex of *N,N′*-dihistidylethylenediamine L1 is inactive for hydrolysis of bis(*p*-nitrophenyl) phosphate (BNPP) and *p*-nitrophenylphosphate (NPP), while zinc complex of im-bzl-*N,N″*-dihistidyldiethylenetriamine L2 efficiently hydrolyzes the BNPP and NPP and their pseudo-first-order rate constants are 0.000011/s and 0.000021/s, respectively.



Asymmetric reduction of pyridyl ketones by *Geotrichum candidum*

Kaoru Nakamura[a,*], Ibuki Misawa[a], Tomoko Matsud[a], Atuyoshi Ohno[a], Tomoko Yokoi[a], Yuji Mikata[b], Junichi Uenishi[c]

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[b]Department of Chemistry, Faculty of Science, Nara Women's University, Nara 630-8506, Japan

[c]Department of Chemistry, Faculty of Science, Okayama University of Science, Ridai-cho 1-1, Okayama 700-0005, Japan.

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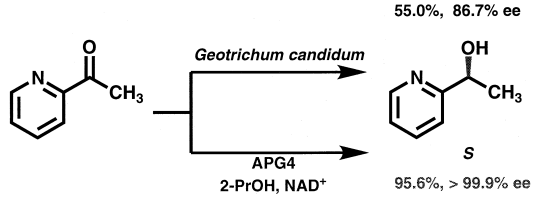
We found that a crude enzyme, acetone powder, from *G. candidum* IFO4597 (APG4) catalyzes the reduction of ketones giving the corresponding chiral alcohols with high enentioselectivitity.



Novel mechanism for the stereochemical control in microbial reduction

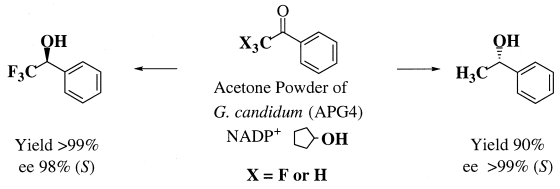
Kaoru Nakamura[a,*], Tomoko Matsuda[a], Nobuyoshi Nakajima[b]

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

[b]Department of Nutritional Science, Faculty of Health and Welfare, Okayama Prefectural University, Soja, Okayama 719-1112, Japan. E-mail: nakamura@boc.kuicr.kyoto-u.ac.jp

Reduction of trifluoromethyl ketones by an acetone powder of *Geotrichum* affords (*S*)-trifluoromethyl carbinols in excellent ee, whereas the reduction of methyl ketones gives the corresponding

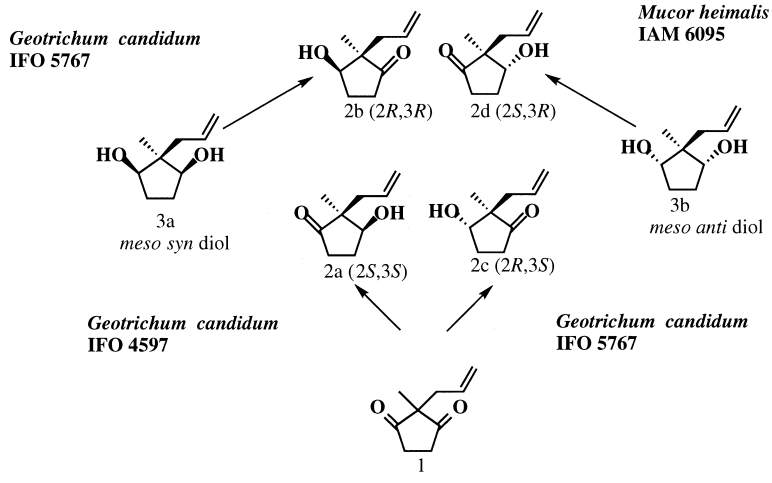
alcohols of the opposite configuration in excellent ee; the mechanism of this opposite selectivity will be reported.



Asymmetric synthesis of quaternary carbon centers by biocatalyst

Kaoru Nakamura, Minoru Takeuchi, Atsuyoshi Ohno
Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan.
E-mail: nakamura@boc.kuicr.kyoto-u.ac.jp

Reduction of 2,2-disubstituted 1,3-diketones by *Geotrichum candidum* affords the corresponding 3*S*-hydroxy ketones enantioselectively. Oxidation of prochiral *meso*-diols by *Geotrichum candidum* and *Mucor heimalis* gave the corresponding 3*R*-hydroxy ketones in excellent ee.



Asymmetric synthesis of chiral alcohols with the plant cell culture

Kaoru Nakamura[a,*], Rio Yamanaka[a], Atsuyoshi Ohno[a], Hiroki Hamada[b]
[a]Institute for Chemical Research, Kyoto University, Uji, Kyoto, 611-0011, Japan
[b]Okayama University of Science, 1-1 Ridaicho, Okayama, 700-0005, Japan. E-mail: nakamura@boc.kuicr.kyoto-u.ac.jp

The suspension cultured cells and an acetone powder of *Marchantia polymorpha* instead of the intact cells are used as biocatalyst to reduce fluorinated ketones, and the corresponding chiral alcohols are given in high yield or enantioselectively.

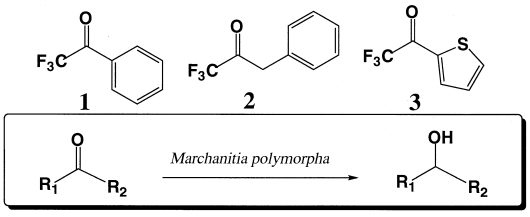
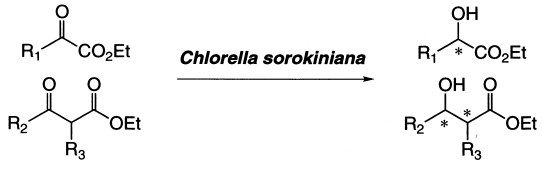


Figure Reduction of Various ketones with *M. polymorpha*

Stereoselective reduction of keto esters with micro green algae

Kohji Ishihara[a,*], Hitomi Yamaguchi[a], Masataka Ikeda[a], 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

Various α- and β-keto esters were reduced stereoselectively to the corresponding alcohols by the micro green algae, *Chlorella* sp., in particular, the reduction of ethyl 2-methylacetoacetate with *Chlorella sorokiniana* SAG 211-8k (thermophilic strain) gave the hydroxy ester in excellent diastereoselectively (*syn* > 99% d.e.).



Stereoselective reduction of α-keto esters with thermophilic actinomycetes: Purification and characterization of α-keto ester reductase from *Streptomyces thermocyaneoviolaceus* IFO 14271

Kohji Ishihara[a,*], Hitomi Yamaguchi[a], Nobuyoshi Nakajima[b], Kaoru Nakamura[c]
[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.
E-mail: kishi@wsml.kyokyo-u.ac.jp

α-Keto ester reductase was purified from a thermophilic actinomycete, *Streptomyces thermocyaneoviolaceus* IFO 14271, and the enzyme was found to reduce various α-keto esters to corresponding

alcohols, and had high stability toward a variety of additives such as organic solvents, surfactants and denaturizing agents.

Substrate specificity and K_m value

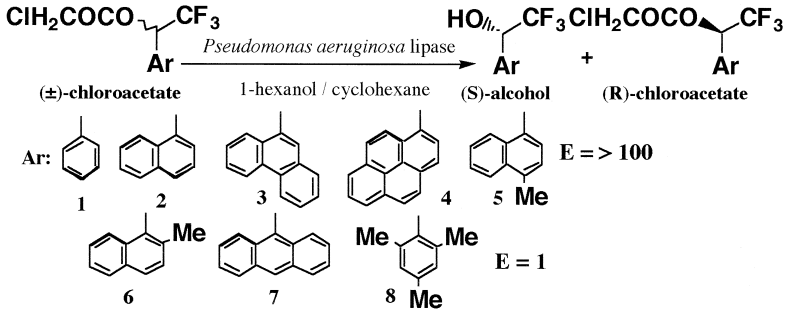
R$COCO_2Et$	Relative activity (%)	K_m (mM)	***R***$COCO_2Et$	Relative activity (%)	K_m (mM)
CH_3	100	0.079	*n*-C_4H_9	60	0.853
C_2H_5	96	0.121	*n*-C_5H_{11}	82	0.696
n-C_3H_7	50	1.55	$CH(CH_3)_2$	8.8	9.01

Lipase-catalyzed optical resolution of 2,2,2-trifluoro-1-(aryl)ethanols

Katsuya Kato*, Yue-Fa Gong, Satoko Tanaka, Masato Katayama, Hiroshi Kimoto

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

Optical resolution of 2,2,2-trifluoro-1-(aryl)ethanols by lipase from *Pseudomonas aeruginosa* (lipase LIP, Toyobo) was explored and the lipase selectivity was remarkably influenced by the structures of the aryl groups of the substrates.



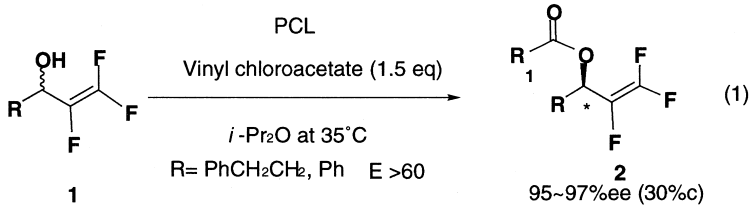
Synthesis of optically active novel 1,1,2-trifluoro-1-alken-3-ols through lipase-catalyzed reaction

Yumiko Takagi[a,*], Masaya Kashiwagi[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.

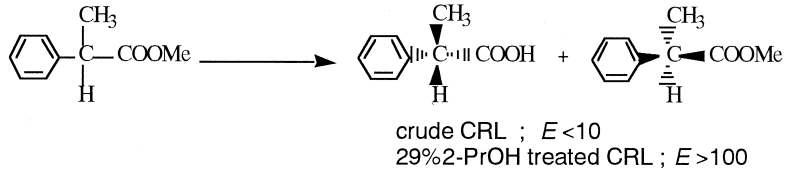


Organic solvent treatment of *Candida rugosa* lipase and its hydrolytic activity

Michimasa Goto, Hisato Ogawa, Hisae Urai, Tadashi Kometani

Department of Chemical and Biochemical Engineering, Toyama National College of Technology, Hongo 13, Toyama 939-8630, Japan. E-mail: kometani@toyama-nct.ac.jp

Treatment of *Candida rugosa* lipase with several concentrations of 2-PrOH solution caused a conformational change of the enzyme in 29% 2-PrOH and its enantioselectivity toward methyl 2-phenylpropanoate considerably increased.

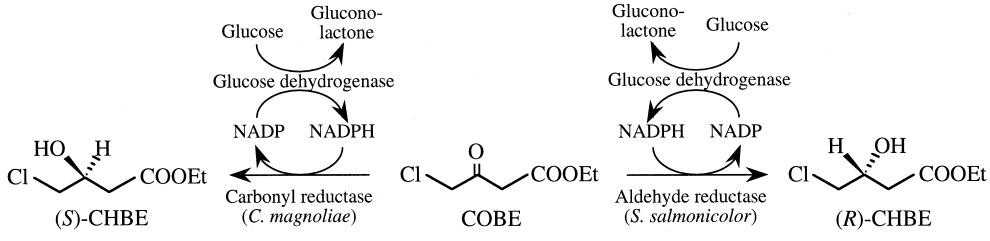


Microbial carbonyl reductases and their application to the chiral alcohol syntheses

Michihiko Kataoka*, Masaru Wada, Hiroshi Kawabata, 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

Novel enzymes reducing 4-chloro-3-oxobutanoate ethyl esters (COBE) to (*R*)- and (*S*)-4-chloro-3-hydroxybutanoate ethyl esters (CHBE) were found to be produced by *Sporobolomyces salmonicolor* and *Candida magnoliae*, respectively.



Asymmetric synthesis of 1-amino-2,2-difluorocyclopropanecarboxylic acid

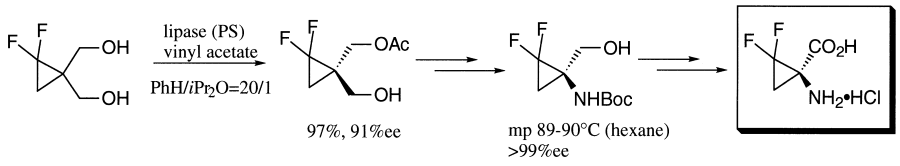
Masayuki Kirihara[a,*], Tomofumi Takuwa[a], Masashi Kawasaki[b], Hiroko Kakuda[c], Shun-ichi Hirokami[c]

[a]Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, Sugitani 2630, Toyama 930-0194, Japan

[b]Faculty of Engineering, Toyama Prefectural University, 5180 Kurokawa, Kosugi-Machi, Toyama 939-0398, Japan

[c]Laboratory of Chemistry, Toyama Medical and Pharmaceutical University, Sugitani 2630, Toyama 930-0194, Japan. E-mail: kirihara@ms.toyama-mpu.ac.jp

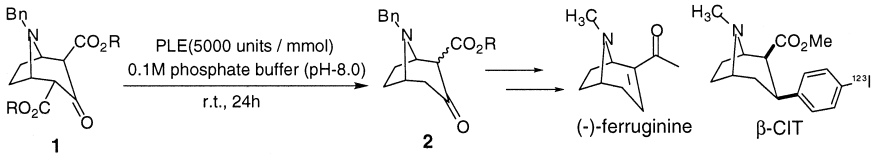
Asymmetric synthesis of (+)-(*R*)-1-amino-2,2-difluorocyclopropanecarboxylic acid was accomplished through lipase-catalysed asymmetric acetylation of a pro-chiral diol as the key step.



Asymmetric dealkoxycarbonylation by esterase and the application

Soichi Nakamura, Daisaku Nakamura, Takahiro Katoh, Takashi Nakai, Manabu Node*
Kyoto Pharmaceutical University, Misasagi, Yamashina, Kyoto 607-8414, Japan.
E-mail: node@mb.kyoto-phu.ac.jp

The asymmetric dealkoxycarbonylation of σ-symmetric tropinone diester **1** using esterase (PLE) afforded optically active monoester **2** (~95%ee), which was used in the formal asymmetric syntheses of (−)-ferruginine as tropane alkaloid and a (−)-cocaine derivative (β-CIT) as a radiodiagnostic drug.



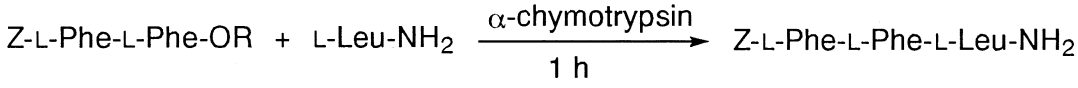
entry	substrate	R	product (yield %)	ee (%)	recovery of st. mat. (%)
1	**1a**	Me	**2a** (20)	43	54
2	**1b**	Et	**2b** (50)	93	21
3	**1c**	*i*-Pr	**2c** (15)	63	30
4	**1d**	Bn	**2d** (51)	74	31
5	**1e**	*n*-Pr	**2e** (30)	93	14
6	**1f**	*n*-Bu	**2f** (38)	95	24
7	**1g**	*n*-Pen	**2g** (29)	94	25

Carbamoylmethyl esters as excellent acyl donors for the protease-catalyzed peptide synthesis in organic media

Toshifumi Miyazawa*, Eiichi Ensatsu, Ryoji Yanagihara, Takashi Yamada
Department of Chemistry, Faculty of Science, Konan University, 8-9-1 Okamoto, Higashinada-ku, Kobe 658-8501, Japan. E-mail: miyazawa@base2.ipc.konan-u.ac.jp

In the kinetically controlled approach of peptide synthesis, the superiority of the carbamoylmethyl ester was demonstrated further in segment condensations and in couplings mediated by proteases other than α-chymotrypsin.

Ex. :



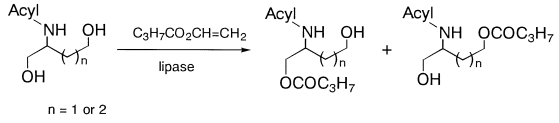
Peptide yield: R = CH_3, 12.2%; R = CH_2CF_3, 51.3%; R = CH_2CONH_2, 81.4%

Regioselectivity in the lipase-catalyzed acylation of unsymmetrical *N*-acylaminodiols

Toshifumi Miyazawa*, Koushi Hidaka, Ryoji Yanagihara, Takashi Yamada

Department of Chemistry, Faculty of Science, Konan University, 8-9-1 Okamoto, Higashinada-ku, Kobe 658-8501, Japan. E-mail: miyazawa@base2.ipc.konan-u.ac.jp

In the *Alcaligenes* sp. lipase-catalyzed acylation of 2-(*N*-acylamino)-1,4-butanediols or 1,5-pentanediols with vinyl butyrate in organic media, the regioselectivity was greatly influenced by the *N*-acyl group and the chain length of the diol.



Directed biosynthesis of fluorinated antibiotics

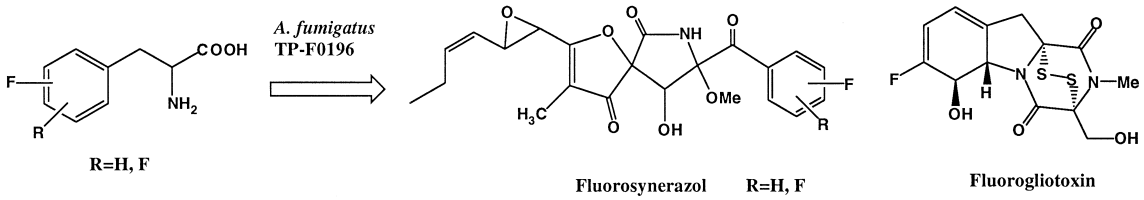
Yasuhiro Igarashi[a,*], Yukihiro Yabuta[a], Akira Sekine[a], Tamotsu Furumai[a], Toshikazu Oki[b]

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

[b]Applied Life Science Research, Tamagawa University, 6-1-1 Tamagawa-Gakuen, Machida, Tokyo 194-8610, Japan.

E-mail: yas@tpusv.pu-toyama.ac.jp

Fluorinated analogs of synerazol, pseurotin, and gliotoxin were prepared by feeding fluorinated phenylalanines as a biosynthetic precursor to a fungus, *Aspergillus fumigatus* TP-F0196 isolated from the deep sea water collected in Toyama Bay.

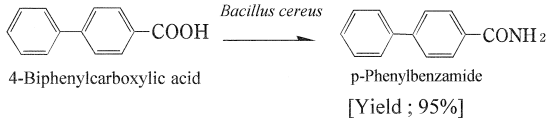


Amidation of aromatic carboxylic acids by *Bacillus cereus*

Reiji Maruyama, Kayoko Kishimoto, Masami Inoue*

Cell Engineering, Faculty of Engineering, Toyama University, Gofuku, Toyama 930-8555, Japan. E-mail: minoue@eng.toyama-u.ac.jp

The amidation of 4-biphenylcarboxylic acid was carried out by *Bacillus cereus* in yield 95% under the condition of 1 mM of substrate and 0.02 wet-g/ml of cell at 37°C for 24 h in aerobic atmosphere.

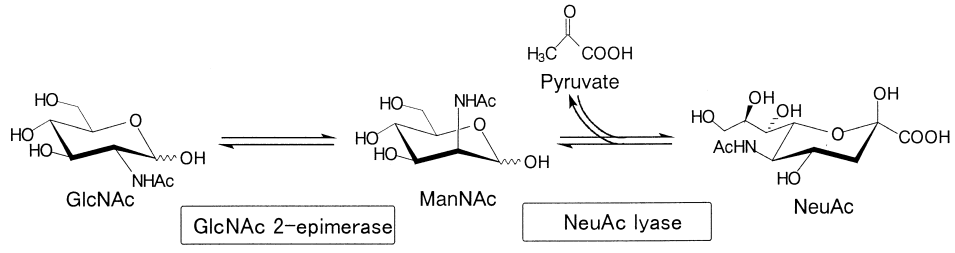


Why do we take up sialic acid for today's subject?

Yasuhiro Ohta*, Jun Ohnishi, Isafumi Maru, Yoji Tsukada

Kyoto Research Laboratories, Marukin Shoyu, 27 Monnomae, Todo, Uji, Kyoto 611-0013, Japan. E-mail: fvbf0882@mb.infoweb.or.jp

We have developed a method for the simple and large-scale production of *N*-acetylneuraminic acid from *N*-acetyl-D-glucosamine and pyruvate using two recombinant enzymes, *N*-acyl-D-glucosamine 2-epimerase and *N*-acetylneuraminate lyase.



Cloning and expression of the bromoperoxidase gene from *Pseudomonas putida*, and its application

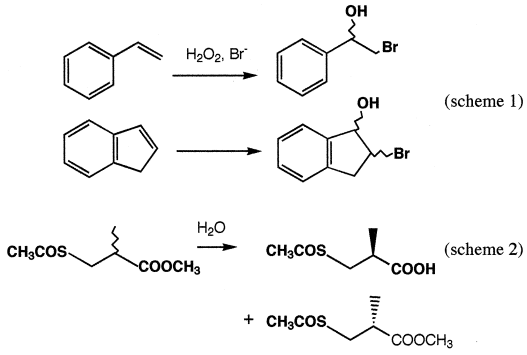
Nobuya Itoh[a,*], Takafumi Kawanami[a], Ji-Quan Liu[a], Tohru Dairi[a], Masao Miyakoshi[b], Chigusa Nitta[b], Yoshio Kimoto[b]

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

[b]Faculty of Engineering, Fukui University, Bunkyo 3-9-1, Fukui 910-8507, Japan.
E-mail: itoh@pu-toyama.ac.jp

The recombinant Co^{2+}-activating bromoperoxidase–esterase catalyzes the halogenation of various organic compounds such as styrene and indene to give racemic bromohydrin compounds (Scheme 1).

The enzyme also catalyzes specific esterase reaction, and hydrolyzes methyl D-acetylthioisobutyrate (Scheme 2).



Optical resolution of unnatural β-hydroxy-α-amino acids by microbial threonine aldolases

Ji-Quan Liu[a], Takaharu Yasuoka[a], Mine Odani[a], Tohru Dairi[a], Nobuya Itoh[a,*], Michihiko Kataoka[b], Sakayu Shimizu[b], Hideaki Yamada[a]

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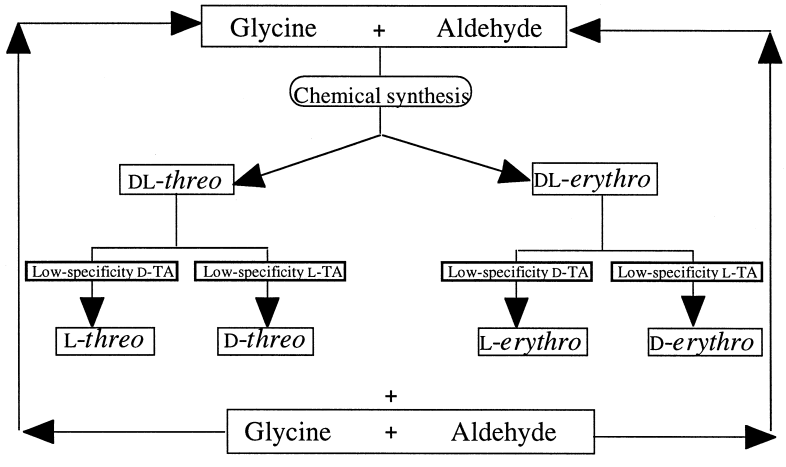
An enzymatic process was established for the production of industrially useful β-hydroxy-α-amino acids based on the use of threonine aldolases.



Synthesis of D-phenylalanine oligopeptides catalyzed by alkaline D-peptidase from *Bacillus cereus* DF4-B

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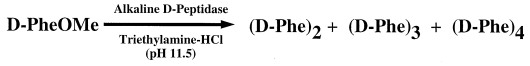
D-Phenylalanine oligopeptides, dimer, trimer and tetramer were synthesized from D-phenylalanine methylester under alkaline condition by use of alkaline D-peptidases (ADP and ADP2) from *Bacillus cereus* DF4-B.



Synthetic studies of optically active polyol derivative by enzymatic reactions

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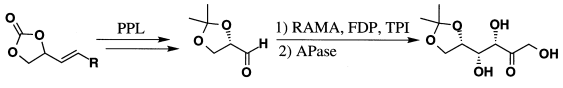
Optically active glycerol derivatives were effectively prepared by the reaction with PPL, and the products were transformed to the corresponding glyceraldehyde derivatives. Enzymatic aldol condensation between the glyceraldehyde derivatives and dihydroxyacetone phosphate gave acyclic polyol derivatives.



A new enzymatic method of selective phosphorylation of nucleosides

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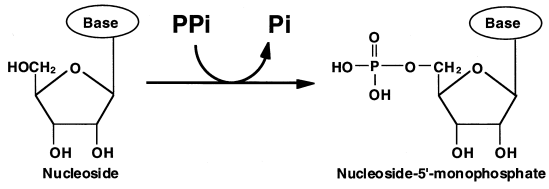
The nucleosides phosphorylation reaction, selective in the 5′-position to produce nucleosides-5′-monophosphate, was studied using the food additive pyrophosphate (PPi) as the phosphate donor.



Catalytic mechanism of opine dehydrogenase from *Arthrobacter* sp. 1C

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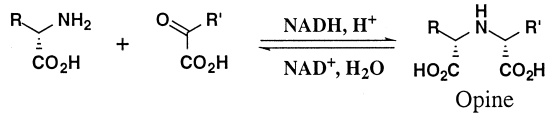
Mutational, crystallographic, and kinetic investigations showed that in the oxidative reaction the opine dehydrogenase from *Arthrobacter* sp. 1C binds the moiety of the opine substrate derived from hydrophobic or neutral amino acid side-chain in a hydrophobic pocket located in domain II, and the carboxyl group derived from keto acid by an arginine residue situated in the NAD-binding domain I.



Lipase-catalyzed effective kinetic resolution of primary alcohols with racemic mixture of vinyl esters as acyl donor

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By using a racemic mixture of vinyl esters lipase SL(Meito Sangyo)-catalyzed kinetic resolution of a primary alcohol was improved.