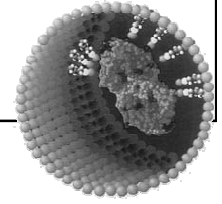
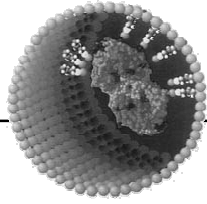


# BIOTECHNOLOGY

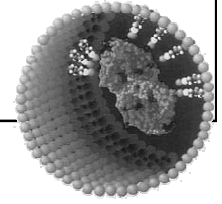


**Biotechnology is the integrated application of natural and engineering sciences for the technological use of living organisms, cells, parts thereof and molecular analogues for the production of goods and services**

[European Federation of Biotechnology, Bruxelles 2003]



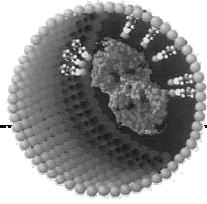
# BIOTECHNOLOGY



## Definitions of White Biotechnology

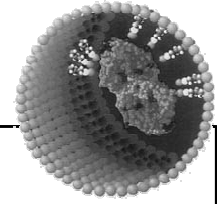
White biotechnology is based on the use of renewable resources and clean production and less polluting and less energy intensive processes in biological system, such as whole cells or enzymes, used as reagent or catalyst.

[European Commission, Bruxelles 2003]



# BIOTECHNOLOGY

## BIOCATALYTIC PROCESS



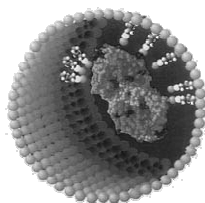
- The process refers to a reaction or a set of simultaneous reactions in which a pre-formed precursor molecule is converted

*(rather than a fermentation process with de novo production from a carbon and energy source such as glucose via primary metabolism)*

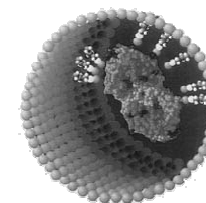
- The process must involve the use of enzymes and/or whole cells, or combination thereof, either free or immobilized

- The process should lead to the production of a fine chemical or commodity product that is usually recovered after the reaction.

# METODOLOGIE SINTETICHE



## CHIMICA DEGLI ENZIMI



Synthetic methods Enzyme chemistry

**L'USO DEGLI ENZIMI NELLA CHIMICA ORGANICA CONTINUA A CATTURARE INTERESSE PER L'ALTA SELETTIVITA' CATALITICA IN CONDIZIONI BLANDE. IL RANGE E LO SCOPO DELLE BIOTRASFORMAZIONI STA AUMENTANDO CON LA POSSIBILITA' DI OTTENERE NUOVI CATALIZZATORI MEDIANTE SOVRAESPRESSIONE DI PROTEINE NEI CEPPI RICOMBINANTI.**

The use of enzymes in synthetic chemistry continues to capture the imagination of organic chemists interested in using highly selective catalysts under mild conditions. The range and scope of biotransformations is being extended by the increasing availability of the enzymes and in particular the ability of gene technology to provide the catalysts in larger quantities through overexpression of the proteins in recombinant strains.

**In recent years, biocatalysis has become an increasingly attractive support to conventional chemical methods. Enzymes quite often display high chemo-, regio- and enantioselectivity, which makes these catalysts attractive for the pharmaceutical and agrochemical area, where the interest for enantiomerically pure and specifically functionalized compounds is continuously growing.**

**Enzyme catalyzed reactions are normally carried out under mild temperature and pH conditions, thus minimizing the problems related to product isomerization, racemization or epimerization. In addition, the enzymes can be very efficient catalyst, capable to increase reaction rates up to  $10^{12}$ .**

**Finally, biocatalytic processes are often less hazardous, polluting and energy consuming than conventional chemistry-based methodologies, especially those making use of heavy-metal catalyst. All these reasons have made biocatalysis a popular topic for research and a tremendous number of enzymes have been described in the literature with a claimed potential for practical exploitation.**

**Clearly, most of the described conversions are bound to remain mere laboratory curiosities, but an important part of them already has or will have an industrial application. To further widen the versatility of the biological approach, the so-called “non-aqueous enzymology”, represent an important area of research and biotechnological development nowadays.**



**This methodology is especially suitable for the modification of precursors of pharmaceutical compounds and fine chemicals, which, in most cases, are insoluble or poorly soluble in water.**

**Enzymes are often more stable and can catalyze reactions that are impossible or difficult in water.**

**Enzyme selectivity can also differ from that in water and change, or even reverse, moving from one solvent to another. This phenomenon, called “medium” engineering, can be exploited as a valid alternative to protein engineering.**

**Table 1**

Indication of the industrial focus reflected by selected<sup>a</sup> patents published in the area of biocatalysis and biotransformation.

Compound class	Subdivision/identified technologies	Number of patents
Alcohols	Ketone reduction	35 <sup>b</sup>
	(Dynamic) kinetic resolution	13
	Aldol condensation	5
	Oxygenases	3
	Other	1
Amino acids/peptides	Hydantoin/carbamoyl hydrolysis	17
	Amide hydrolysis	7
	Transamination	3
	$\beta$ -Amino acids	5
	Peptide synthesis by kinetic coupling	8
	Other	1
Acids	Resolution of esters	14
	Nitrile/cyanohydrin hydrolysis	5
	Oxidation by oxygenases	4
	Acids from lyase reactions	3
	Resolution of amides	2
Sugars	Other	1
	Glycosyltransferases/oligosaccharides/glycoconjugates	10
	Glycoprotein remodeling	9
	Activated monosaccharides	3
	Monosaccharides	1
	Others	4
	Antibiotics	9
Complex natural products	Steroids	5
	Other modification of complex scaffolds	2
Others	Epoxides/peroxides/diols	9
	Amines	9
	Aldehydes	6
	Cyanohydrins	6
	Others	5

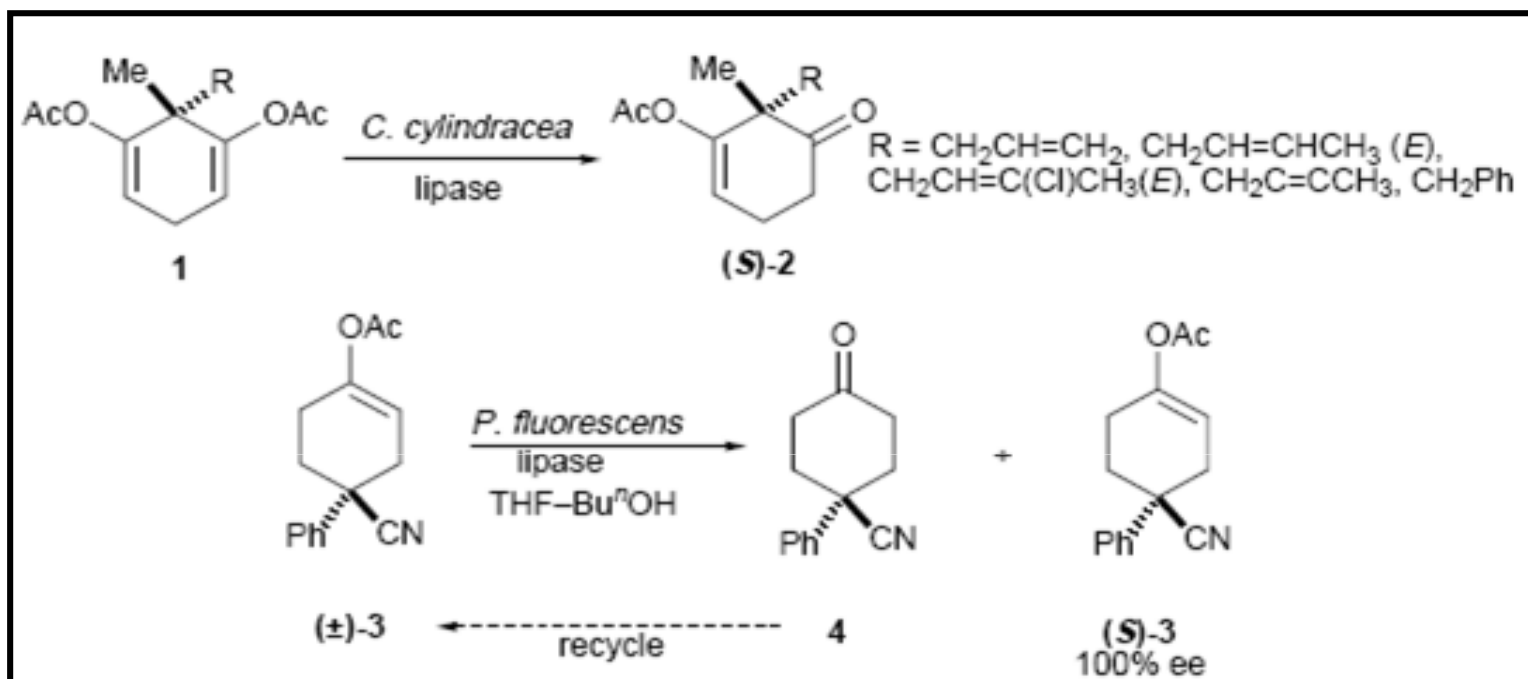
<sup>a</sup>Patents with priority date after 2000 were retrieved from Chemical Abstracts by SciFinder using enzym\*, biocatal\* and biotransform\* search keys and were cross-checked for all companies known to be active in the field. Fermentation processes were excluded. <sup>b</sup>Including nine patents that primarily deal with aspects of cofactor regeneration.

# HYDROLYTIC ENZYMES

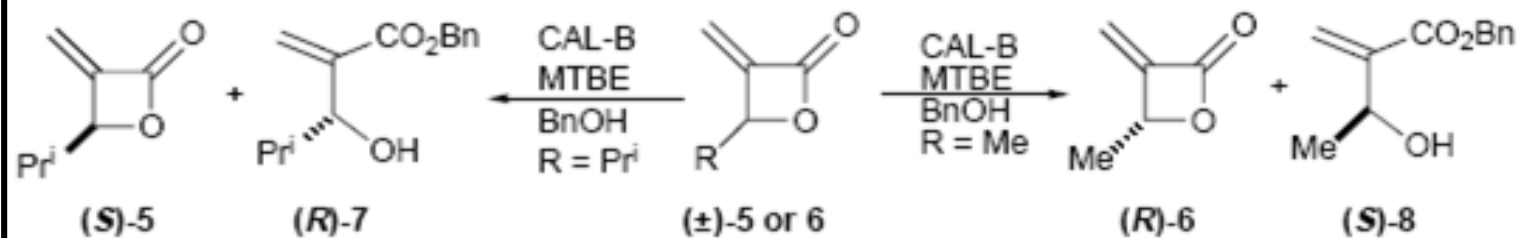
**APPARENTEMENTE NON CI SONO LIMITI ALL'USO DELLE LIPASI PER LA RISOLUZIONE DI ALCOLI E ACIDI CARBOSSILICI. SI RICHIEDE LA DISPONIBILITA' DI MOLTI ENZIMI E LA PAZIENZA DI TESTARLI IN DIVERSE CONDIZIONI DI IDROLISI O FORMAZIONE DI ESTERI IN SOLVENTI ACQUOSI, ORGANICI O MISCELE**

**There seems to be almost no limit to the use of lipases for resolution of alcohols and carboxylic acids. All that is required is access to enough enzymes and the patience to test them under a variety of conditions in the hydrolysis and ester forming modes in aqueous, organic or mixed solvents.**

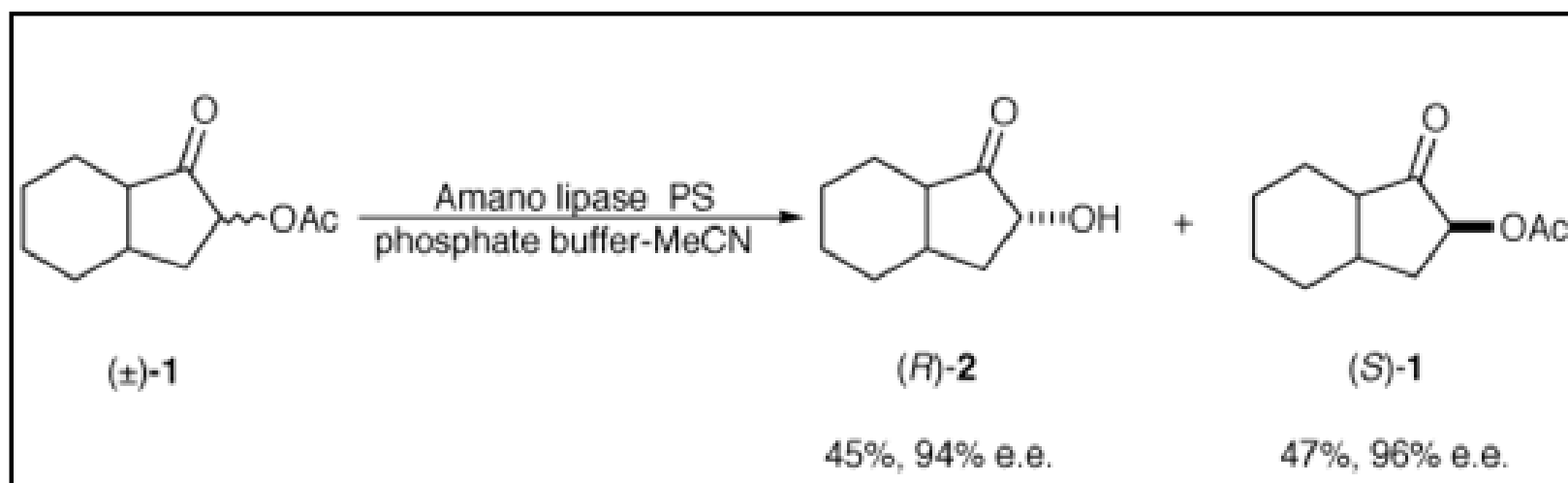
# LIPASES

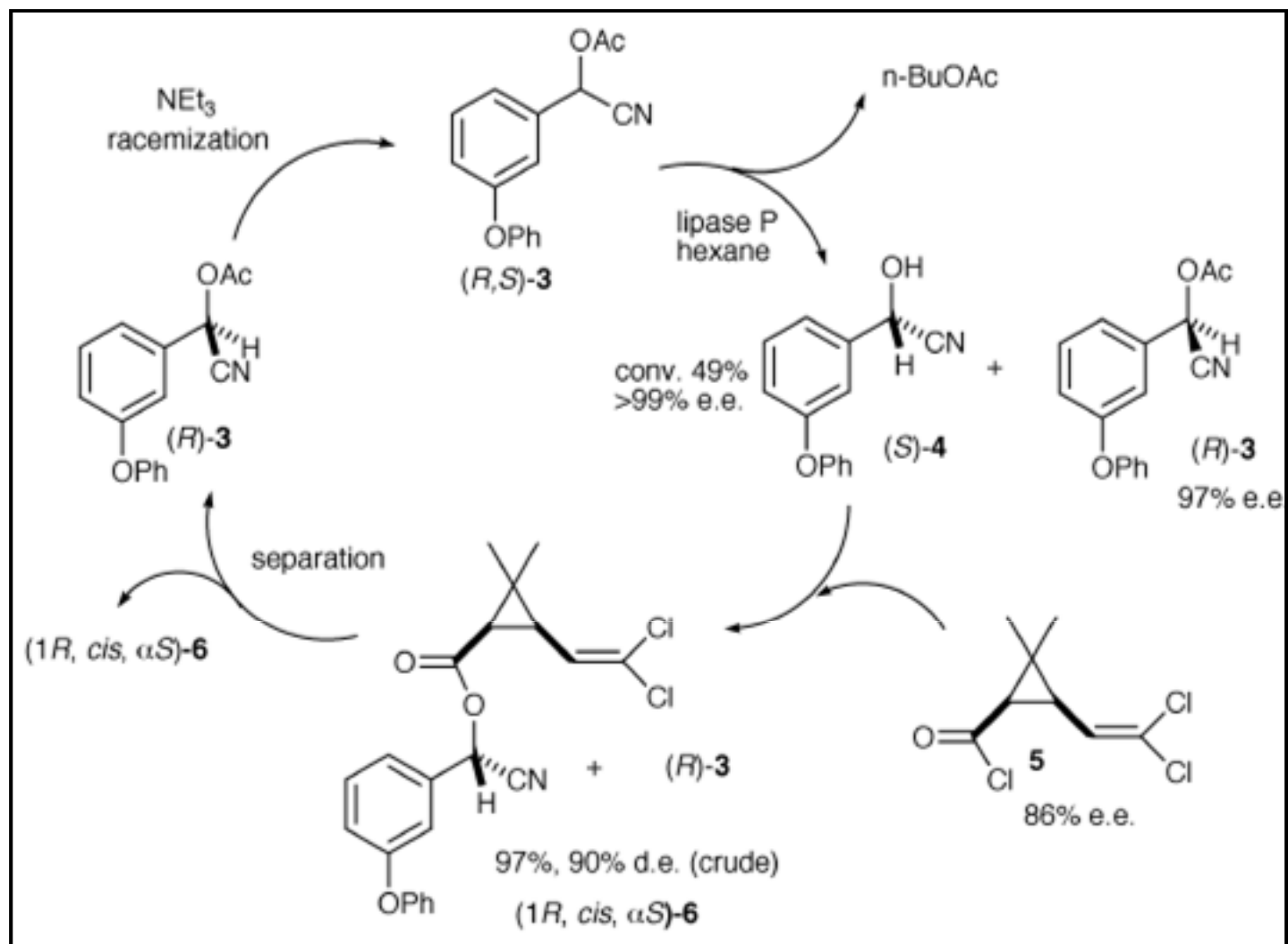


Scheme 1



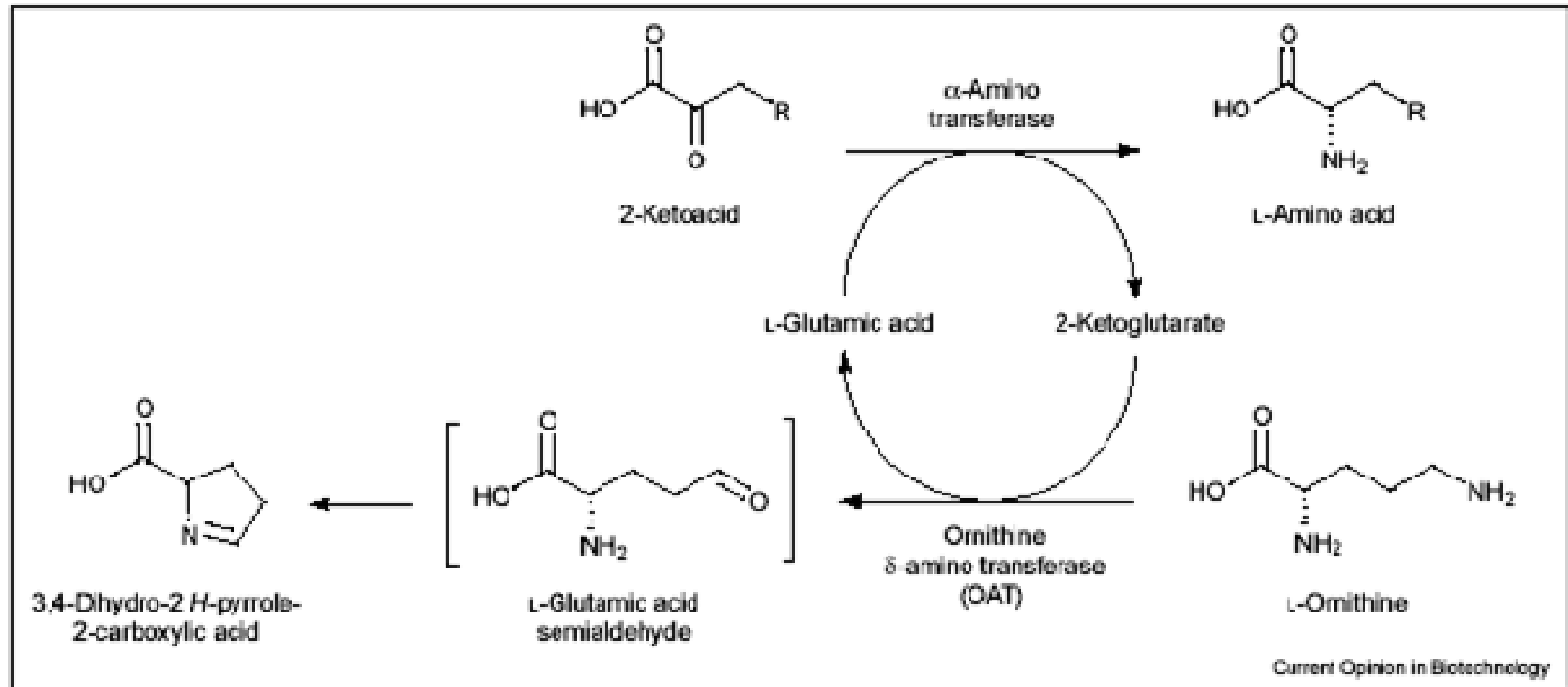
# LIPASES





Continuous chemoenzymatic process for the preparation of cypermethrine 6

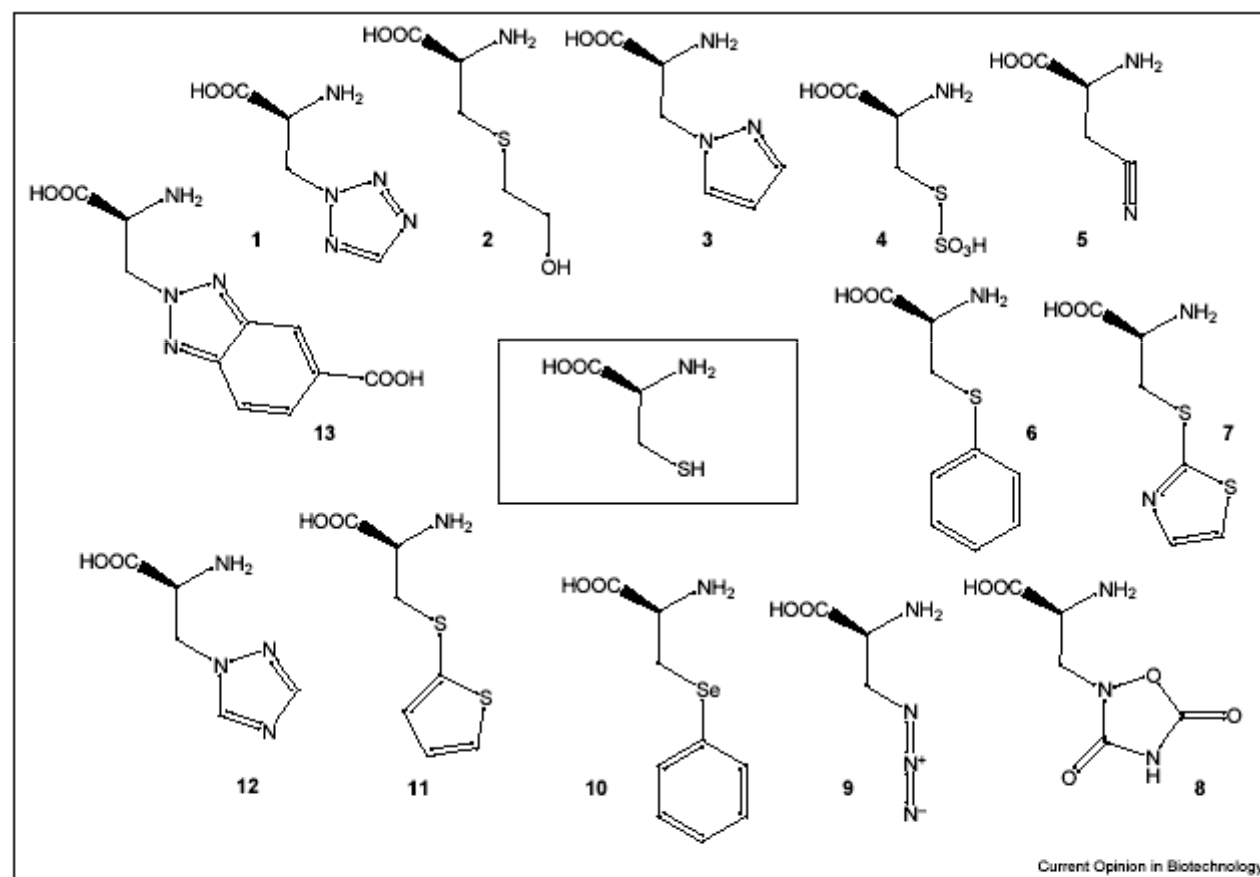
# PREPARATION OF $\alpha$ - e $\beta$ -amino acids



Great Lakes process for the combined utilization of  $\alpha$ -amino transferases with L-ornithine- $\delta$ -amino transferase to produce  $\alpha$ -amino acids [2].

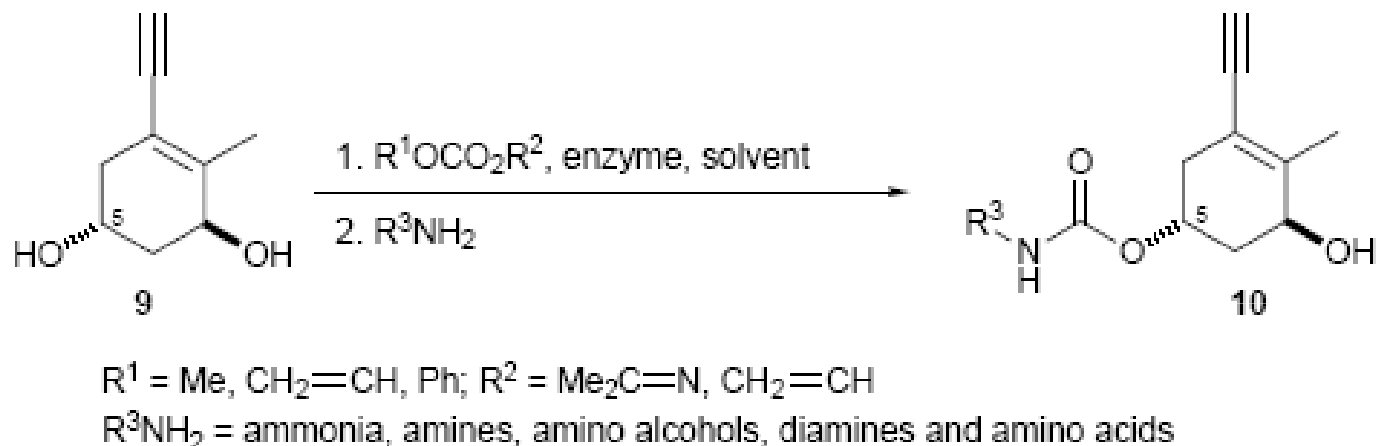


A production method for the manufacture of cysteine by fermentation — to replace cysteine produced by extraction from human hair — has been developed by Wacker Chemie (<http://www.wacker.com/>). Building on this technology in an engineered *Escherichia coli* strain, the enzyme

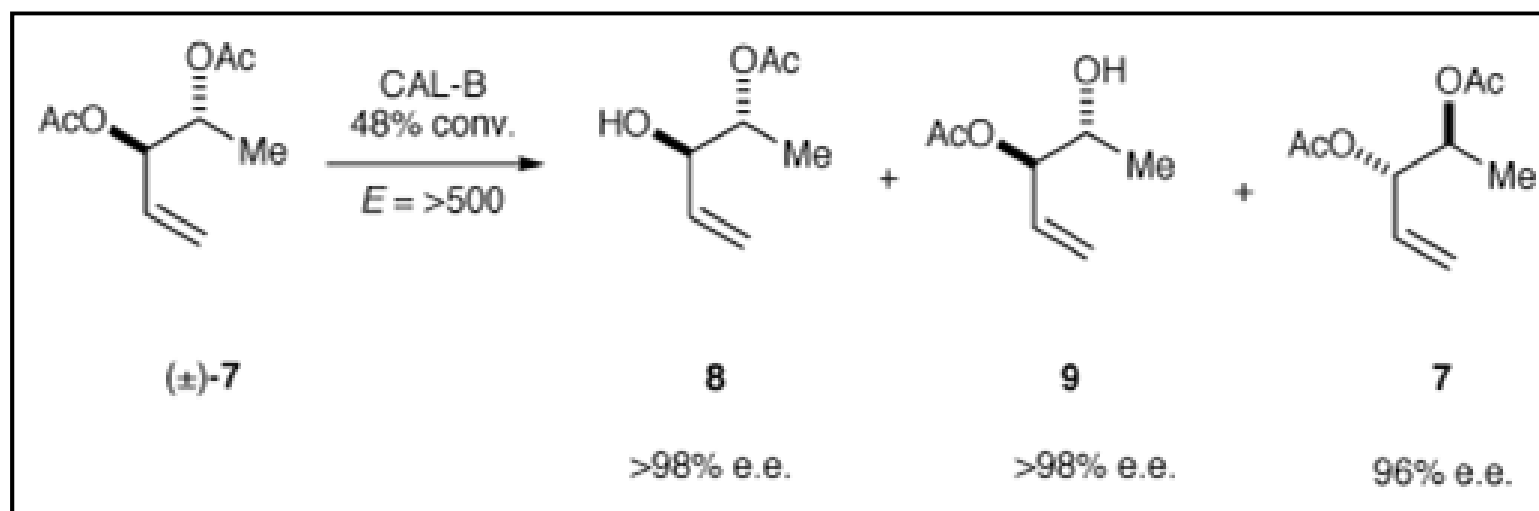


The Wacker Chemie process for L-cysteine (shown in box) production, which provides access to a wide range of unnatural (S)-α-amino acids [5]. Compounds that have been made include the (S)-enantiomers of tetrazole-2-yl alanine 1, hydroxyethylcysteine 2, pyrazole-1-yl-alanine 3, sulfo-cysteine 4, cyanoalanine 5, phenylcysteine 6, thiazole-2-yl-cysteine 7, quisqualic acid 8, azidoalanine 9, phenylselenocysteine 10, thien-2-yl-cysteine 11, triazole-1-yl-alanine 12 and 5-carboxybenzo-triazole-2-yl-alanine 13.

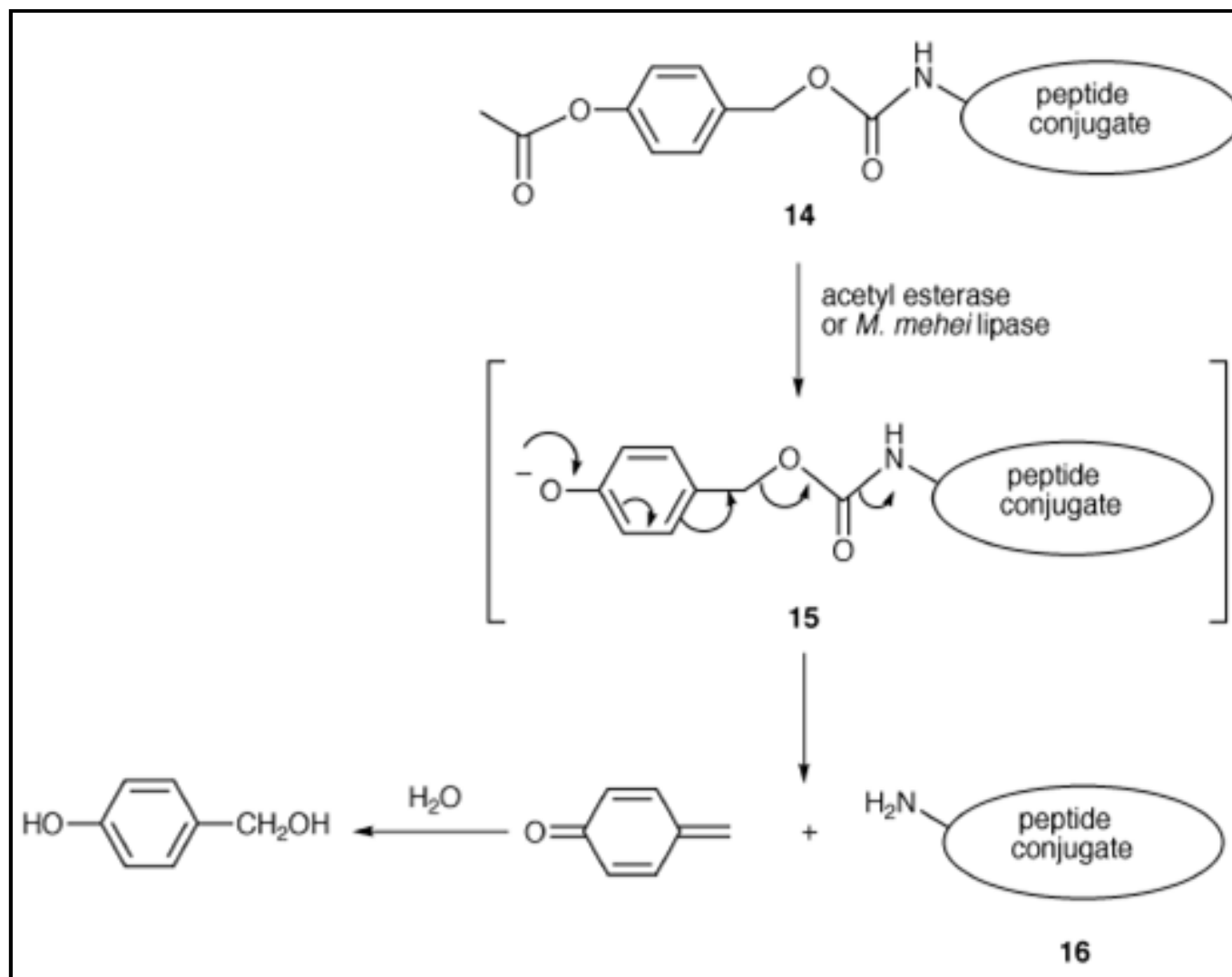
# ENZYME-CATALYZED PROTECTIONS OF HYDROXY GROUPS IN MILD CONDITIONS



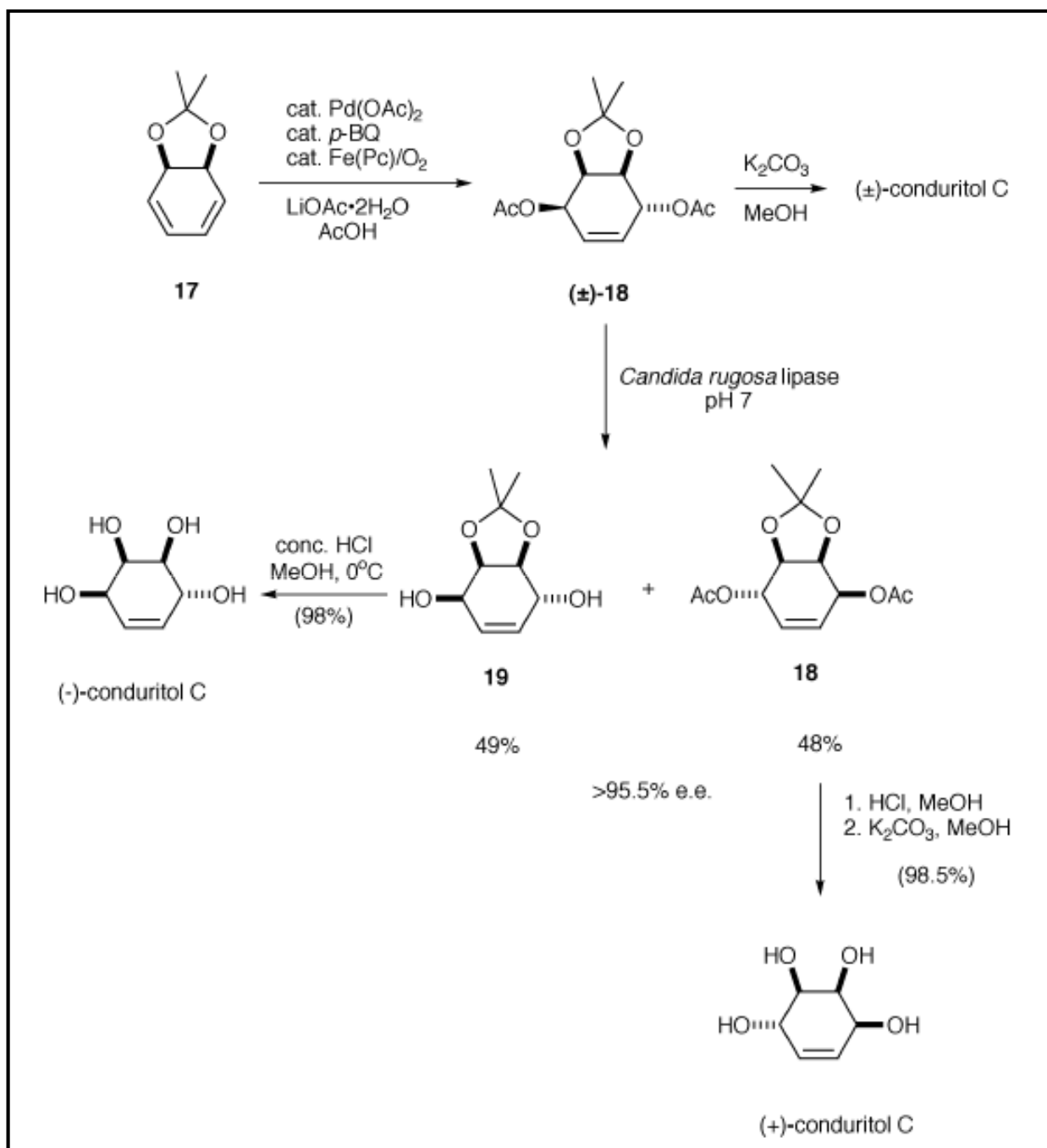
An efficient synthesis of 1a,25-(OH)<sub>2</sub>-vitamin D<sub>3</sub> A-ring carbamate derivatives 10 was developed using a two-step chemoenzymatic approach involving enzymatic synthesis of C-5 carbonates followed by reaction with amino derivatives. *Candida antartica* lipase in toluene was found to be the best enzyme for differentiating the C-3 and C-5 positions of compound 9.

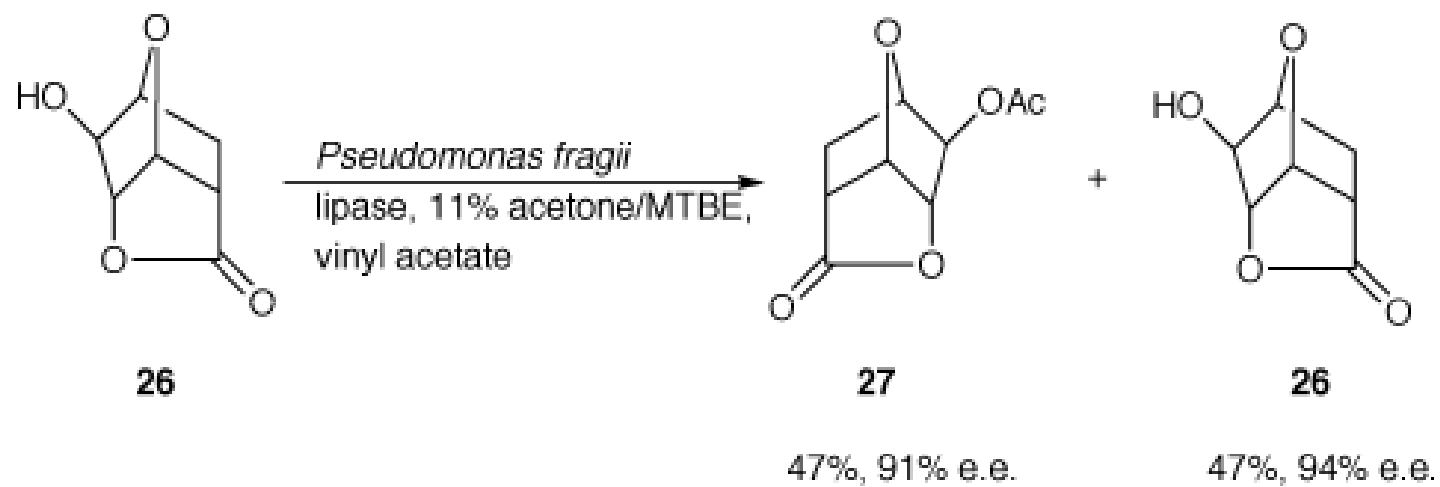


**Best results were obtained using *Candida antarctica* lipase fraction B (CAL-B) hydrolysis to afford enantiopure regioisomeric acetates 8 and 9 and highly enriched unreacted diacetate 7.**

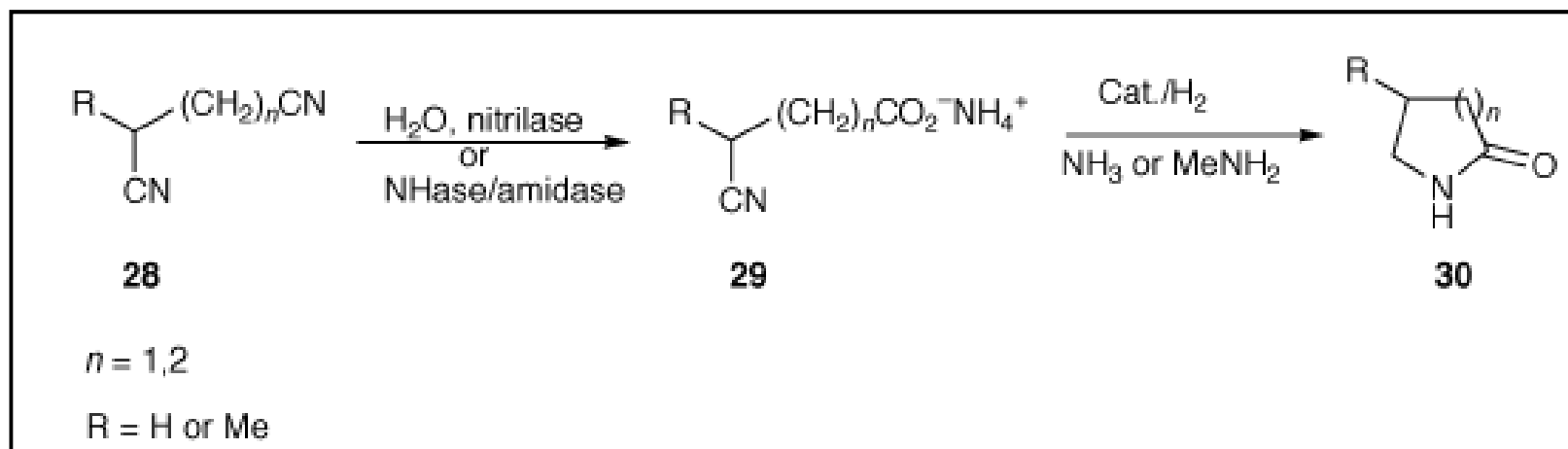


**Cleavage can be carried out under neutral conditions with acetyl esterase from oranges or *Mucor miehei* lipase.**

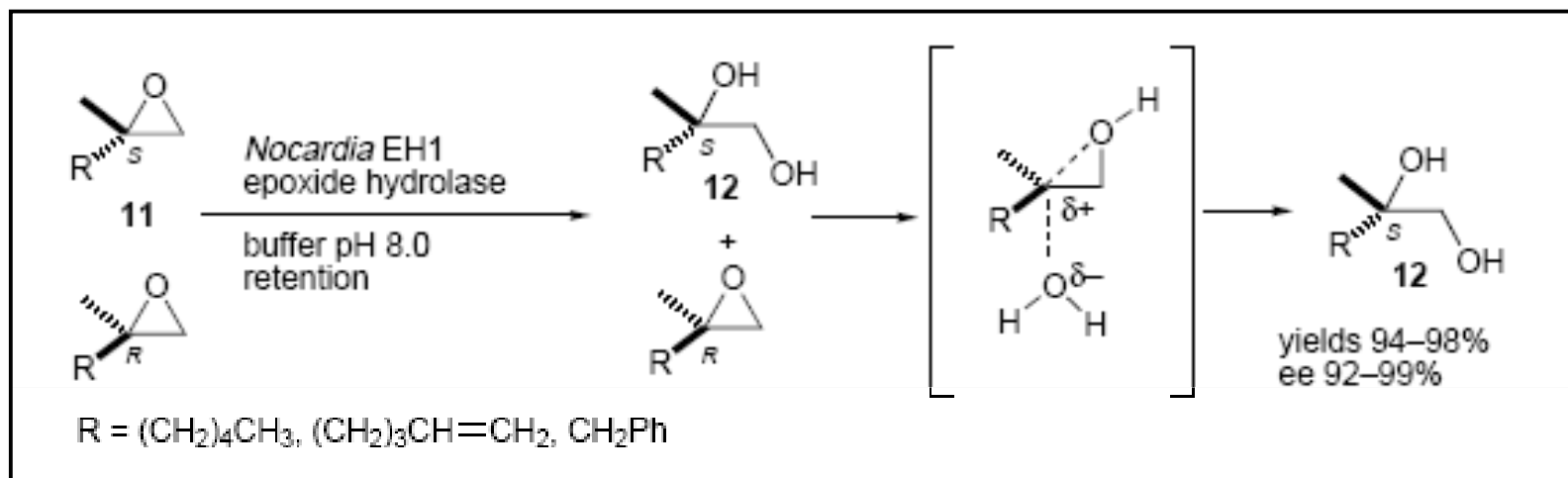




# NITRILE AND EPOXIDE HYDROLYSIS



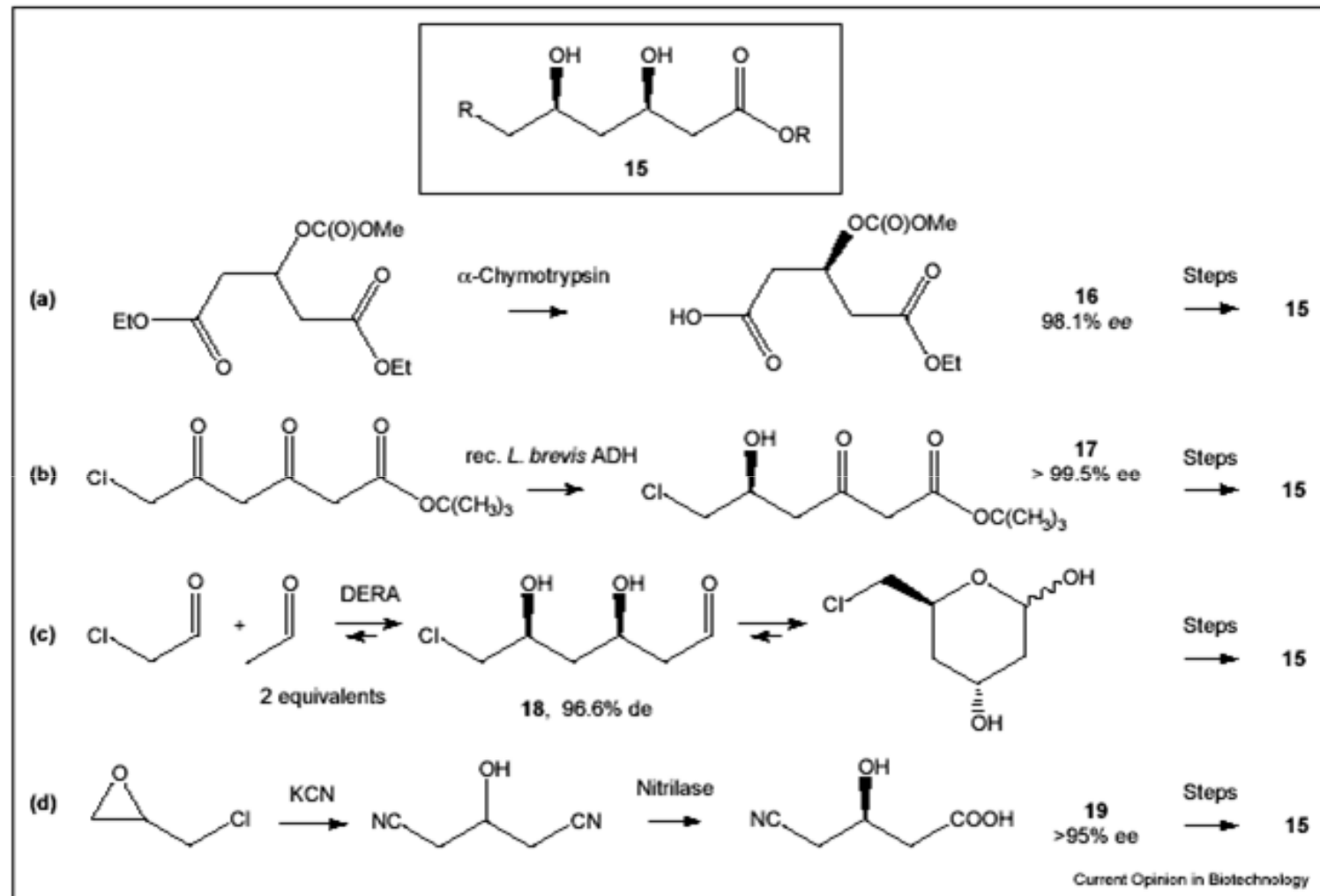
Only one of the two possible lactam products was produced from  $\alpha$ -alkylsubstituted  $\alpha,\omega$ -dinitriles resulting from  $\omega$ -cyano group hydrolysis



**Racemic epoxides 11 were resolved with the enzyme to give (*S*)-diols 12 and unreacted (*R*)-epoxides.**



# Dihydroxy esters as precursors for the synthesis of statins

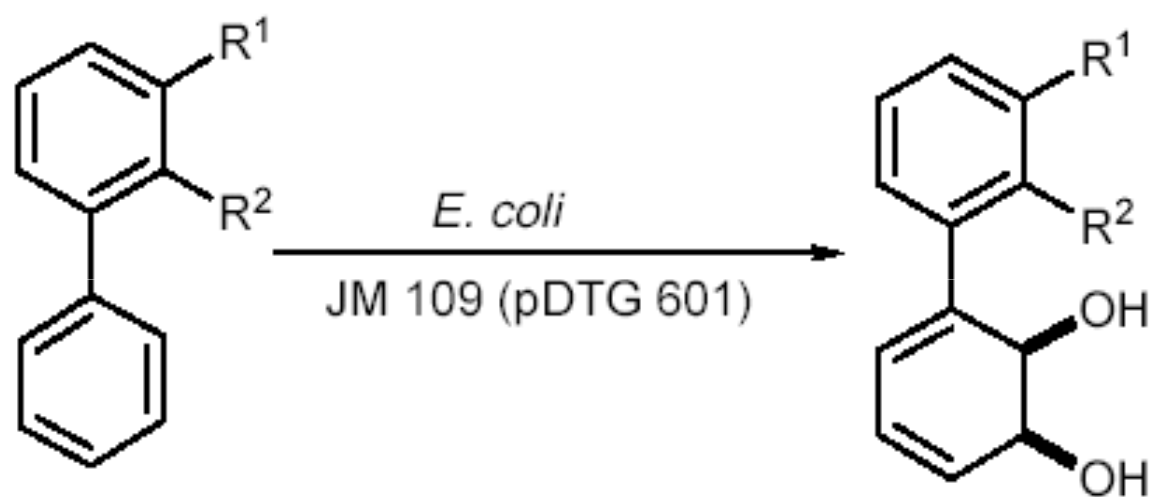


Various biocatalytic routes to (*R,R*)-3,5-dihydroxy-hexanoate ester derivatives (shown in box; **15**), that serve as precursors for the synthesis of statins. **(a)** Prochiral 3-substituted glutarates undergo desymmetrization by  $\alpha$ -chymotrypsin to (*R*)-3-methoxyacetylglutaryl monoethyl ester **16** [12]. **(b)** The reduction of 3,5-dioxo-hexanoic acid *tert*-butyl ester to (*S*)-6-chloro-5-hydroxy-3-oxo-hexanoic acid *tert*-butyl ester **17** [13]. The reaction is catalysed by *L. brevis* ADH. **(c)** The condensation of chloroacetaldehyde with two molecules of acetaldehyde to form (*3R,5S*)-6-chloro-3,5-dihydroxy-hexanal (**18**) is catalyzed by DERA [16]. The hexanal spontaneously cyclizes with retention of the stereochemistry to the hemi-acetal, which drives the equilibrium in the desired direction. **(d)** Nitrilase-catalyzed desymmetrization of 3-hydroxy-glutarodinitrile, which is easily accessible via epichlorohydrin, yields (*R*)-4-cyano-3-hydroxy-butyric acid **19** [19]. The enantiomeric excess (ee) values are given as percentages.

**Effenburger *et al.* have used polyurethane immobilized resting cells of *Rhodococcus erythropolis* MP50 for the resolution of naproxen amide to give (*S*)-naproxen in 99%ee. The free cells were inactive in organic solvents. Optimized conditions involved use of immobilized cells in butyl acetate containing 3 vol% DMSO and residual water.**

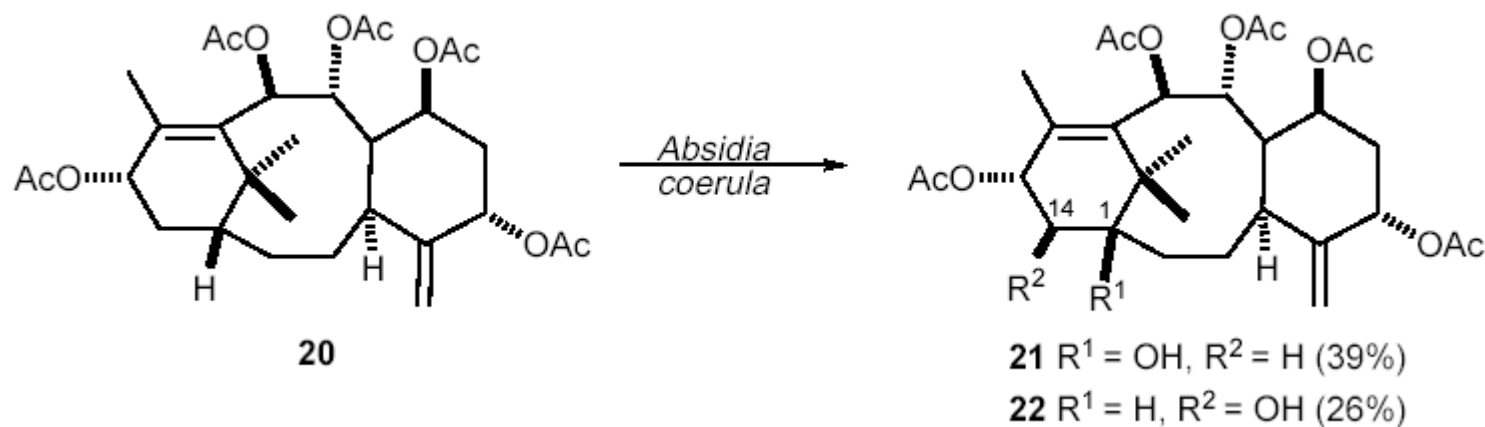
# OXIDATIONS

Biooxidations continue to attract much interest, transformations often being carried out with whole-cell systems due to the instability of the isolated enzymes or the need for cofactor recycling. *Escherichia coli* JM109 (pDTG601) is a recombinant organism which overexpresses the enzyme toluene dioxygenase.



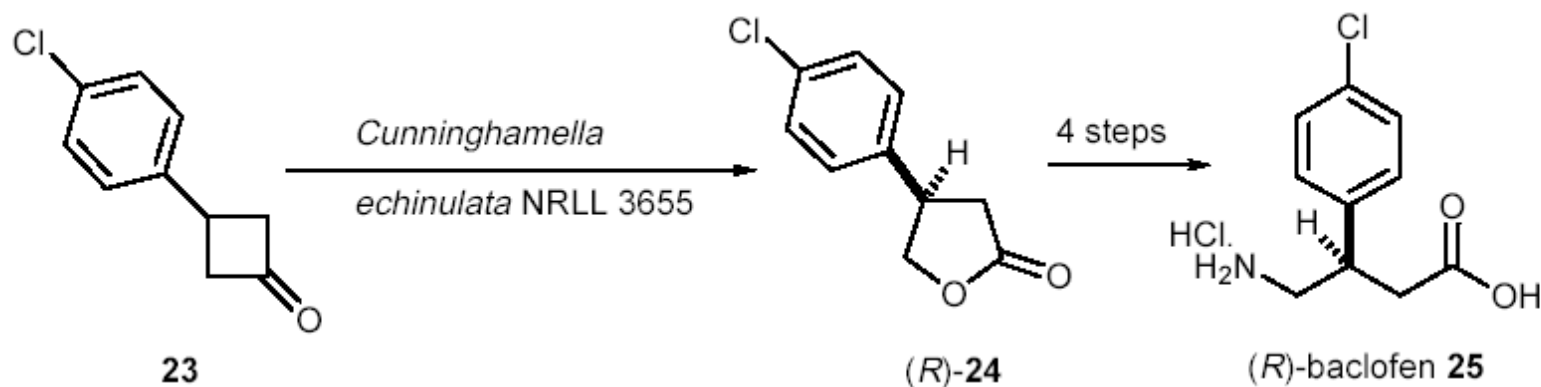
**13,14**  $R^1 = \text{H}$ ,  $R^2 = \text{MeO}$  (2.5 g l<sup>-1</sup>)

**15,16**  $R^1 = \text{MeO}$ ,  $R^2 = \text{MeO}$  (0.8 g l<sup>-1</sup>)



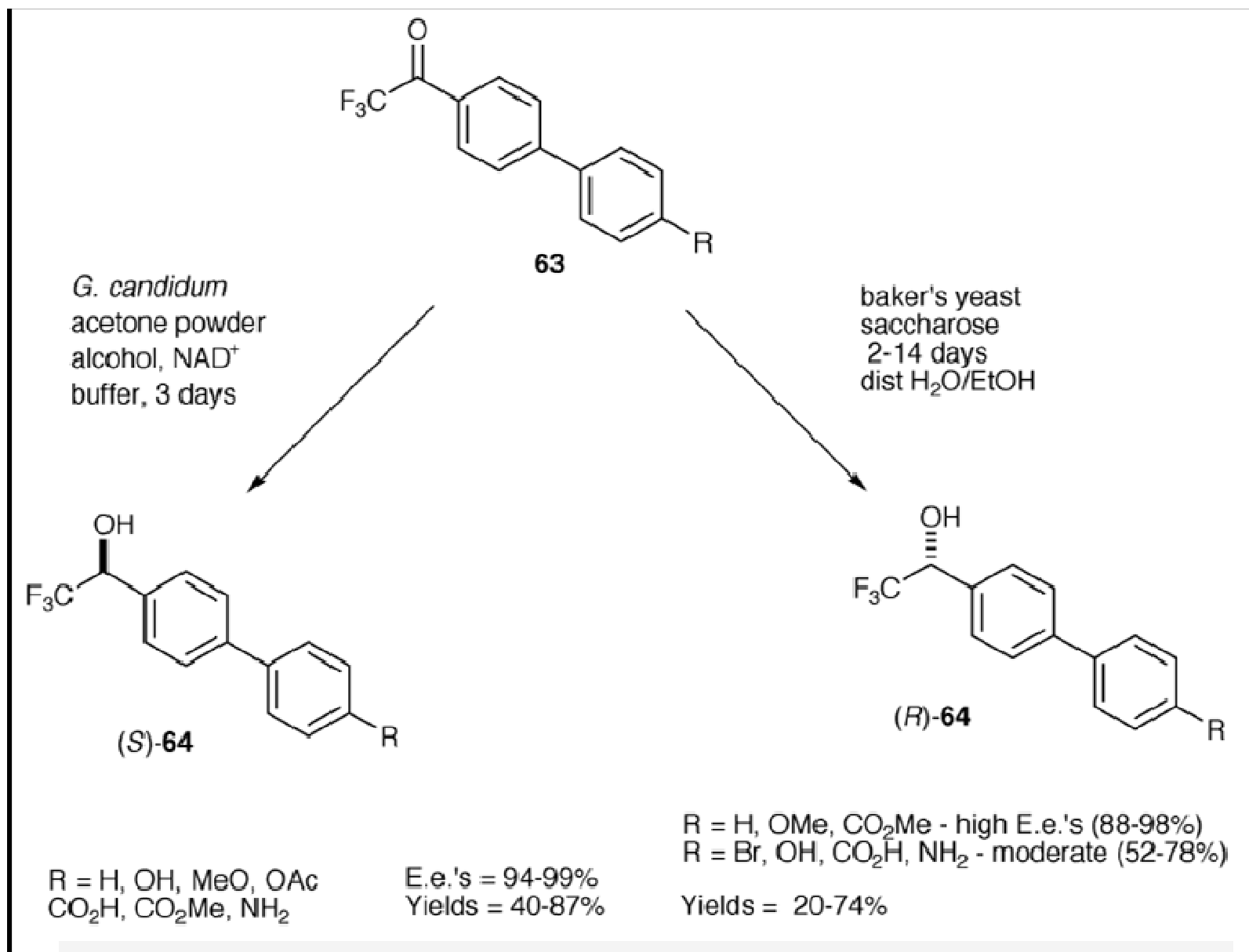
***Absidia coerula* have been used to hydroxylate the taxane skeleton in compound 20 giving novel C-1 and C-14 hydroxylated derivatives 21 and 22**

In the synthesis of the GABA B agonist (*R*)-(-)-baclofen 2534 asymmetric Baeyer–Villiger oxidation of the prochiral cyclobutanone 23 with *Cunninghamella echinulata* NRLL 3655 gave the enantiomerically pure (*R*)-chlorobenzyl lactone 24 in 30% yield



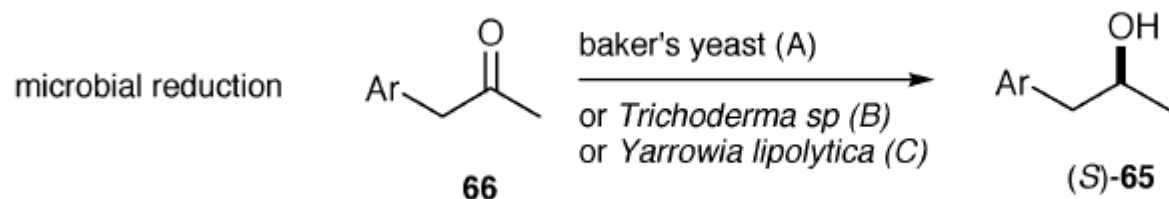
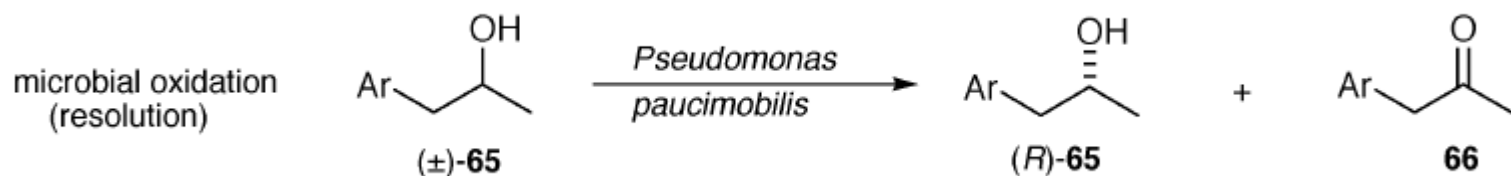
# REDUCTIONS

**A novel cofactor recycling system for NADH and FMNH has been devised by Bhaduri *et al.* using H<sub>2</sub> as the terminal reductant. This was coupled to lactate dehydrogenase and the conversion of pyruvate to lactate demonstrated with NADH being regenerated.**

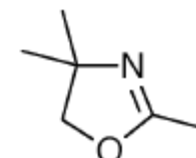
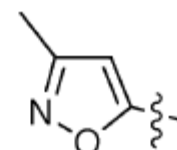
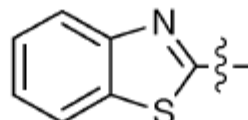
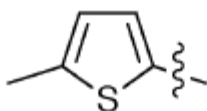
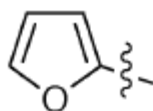


***Geotrichum candidum* acetone powder in phosphate buffer—alcohol with supplemental NAD<sup>+</sup> afforded variable yields and high e.e.'s for formation of the (S)-configured alcohols 64.**





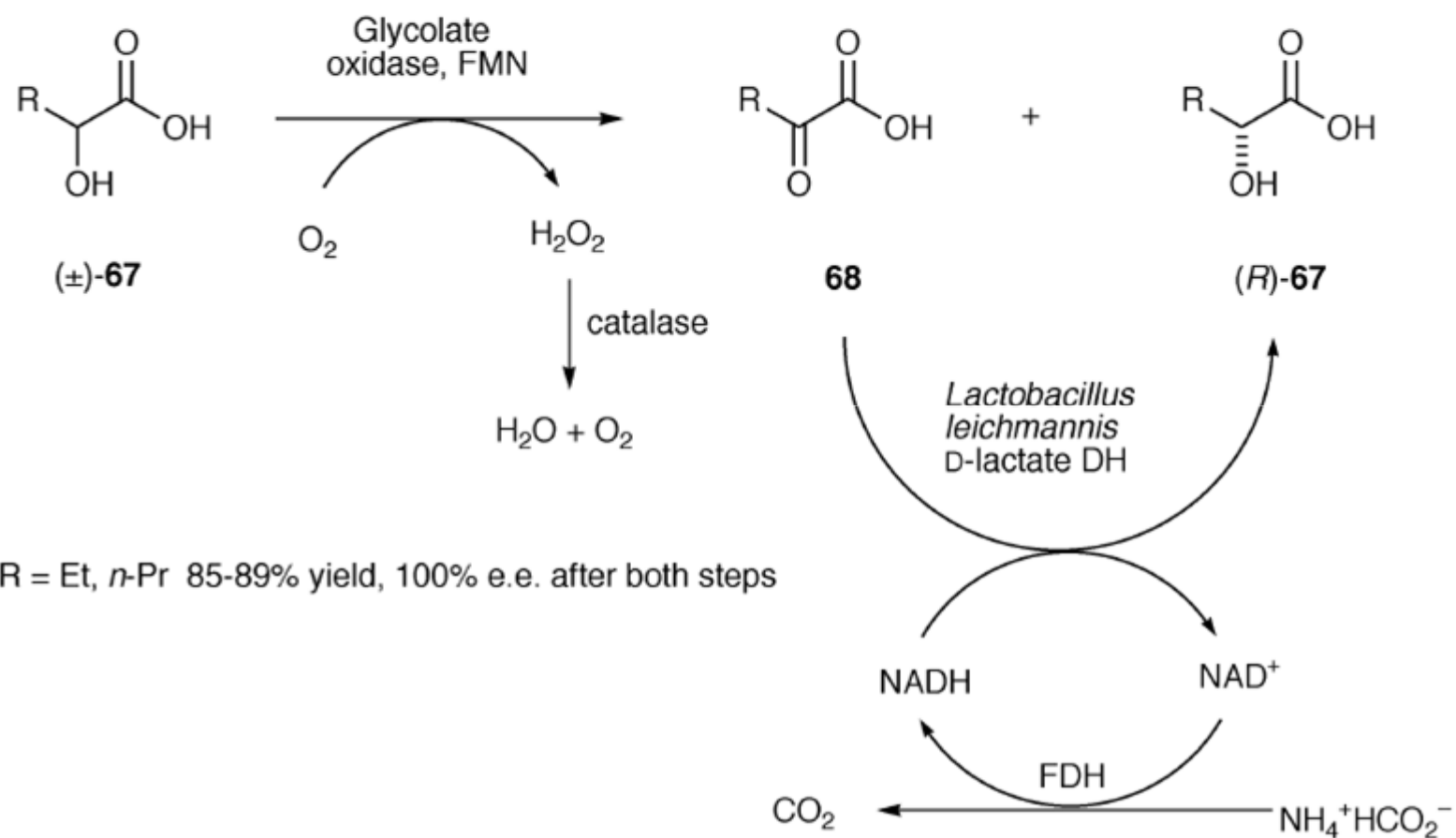
Ar =



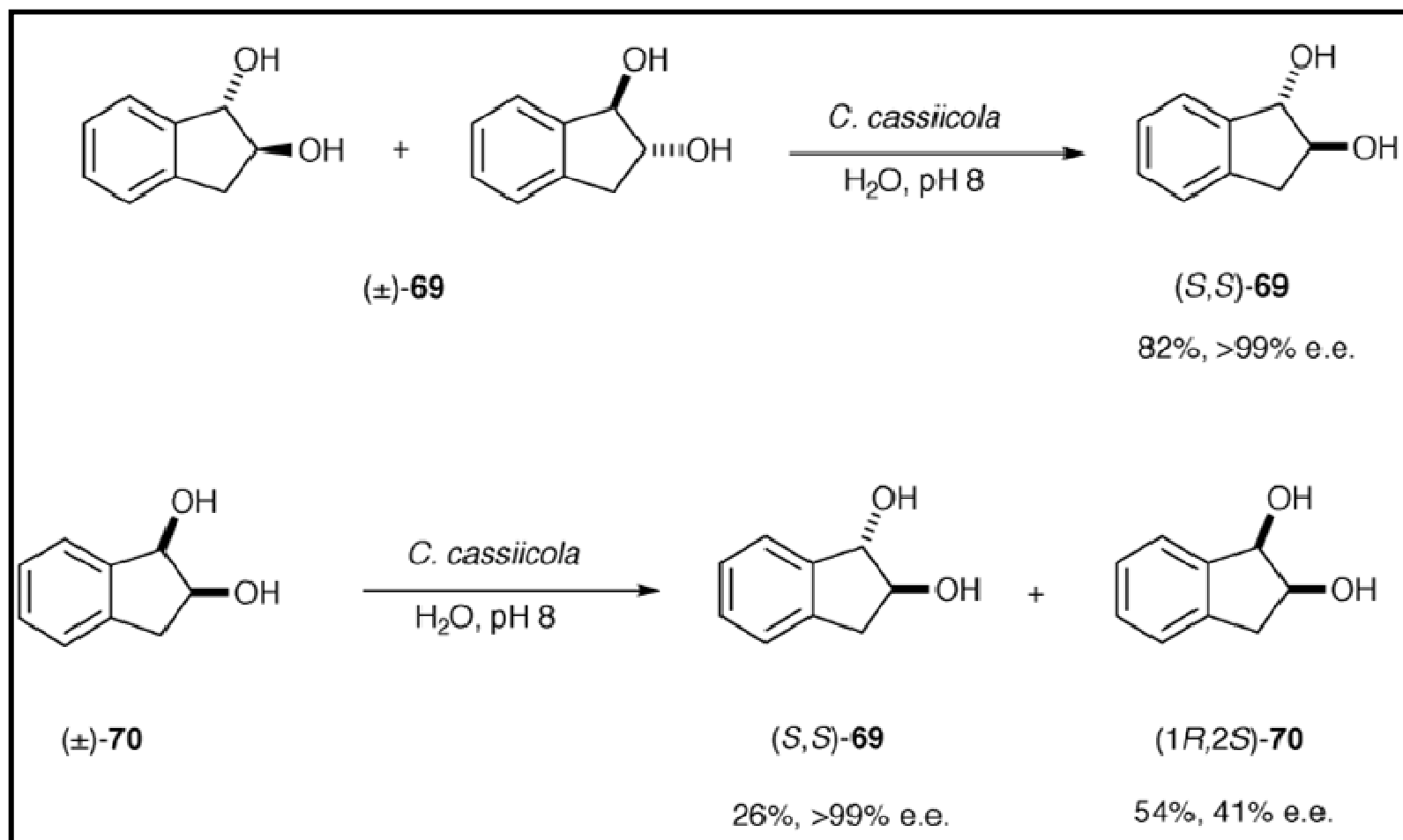
oxidation	40%, 100% e.e.	40%, 100% e.e.	43%, 100% e.e.	40%, 90% e.e.	47%, 95% e.e.
reduction	81%, 100% e.e.(A)	89%, 100% e.e. (B)	80%, 100% e.e. (A)	77%, 100% e.e. (A)	

**The use of redox enzymes for the deracemization of alcohols is an attractive approach. One can employ isolated enzymes with matched selectivity and the requisite cofactors in a two step procedure or a whole cell system which contains the necessary enzymes and cofactors to drive the deracemization to completion.**

**Adam has reported the combination of glycolate oxidase from spinach and D-lactate dehydrogenase from *Lactobacillus leichmannii* for the deracemization of 2-hydroxyacids 67 *via* the  $\alpha$ -ketoacids 68. The first enzyme requires flavin mononucleotide and oxygen to function. The oxygen is reduced to hydrogen peroxide which can be decomposed with catalase *in situ*. The NADH required by the lactate dehydrogenase was recycled using the well established formate dehydrogenase system.**

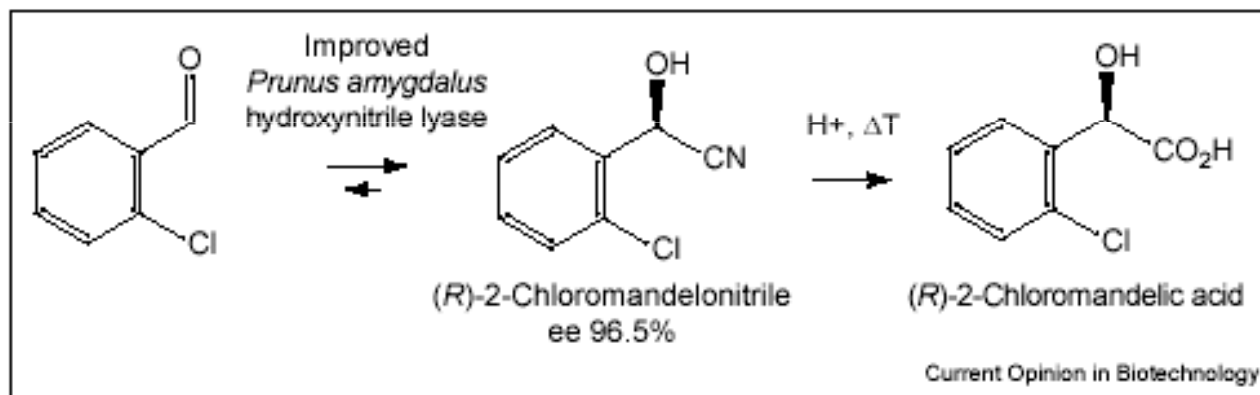


Carnell *et al.* have recently shown that *trans* or *cis* indane-1,2-diols **69** and **70** can be deracemized using whole cells of *Corynesporia cassiicola*.

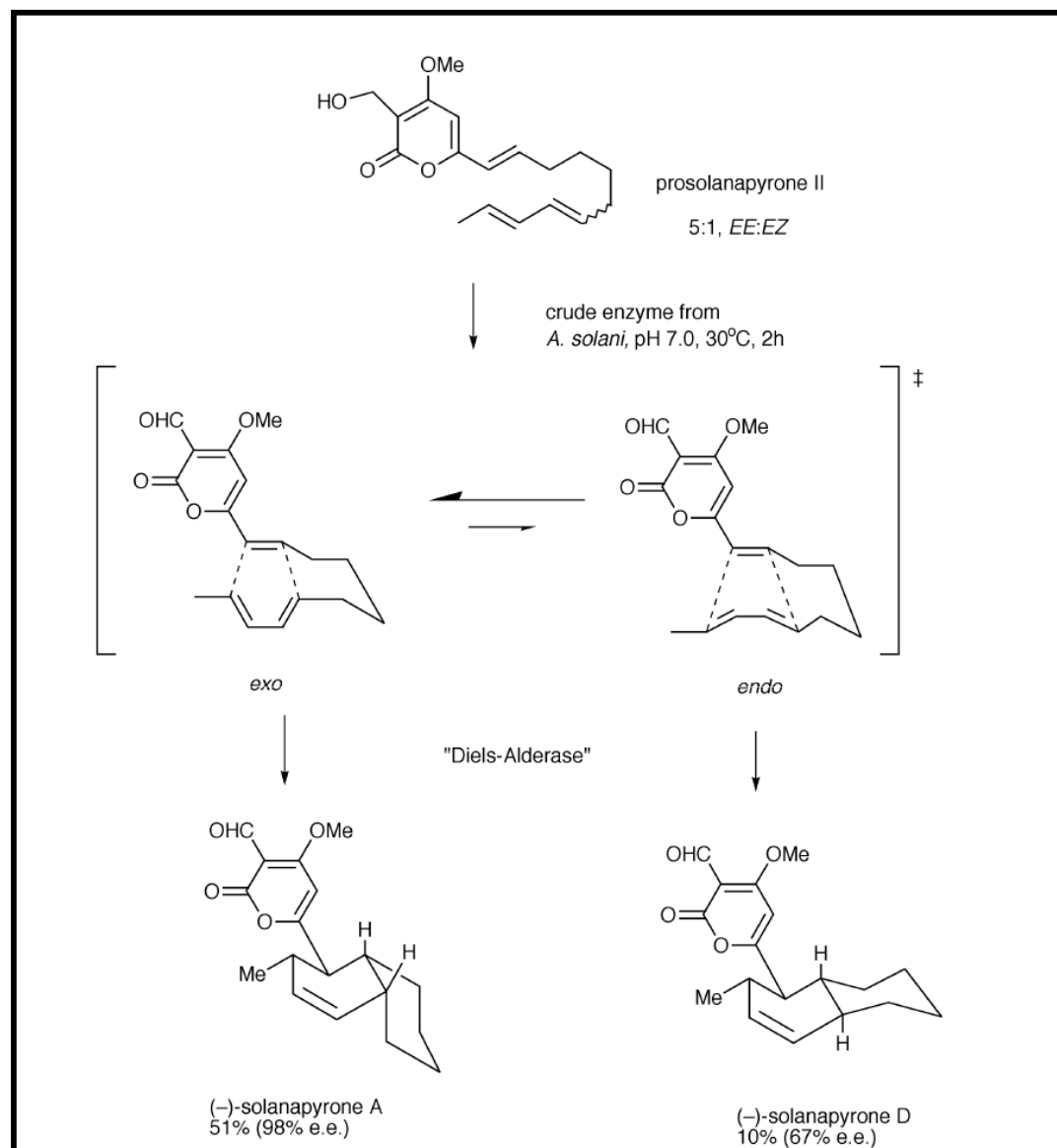


# **CARBON–CARBON BOND FORMATION**

## SYNTHESIS OF BOTH (R)- AND (S)-CYANOHYDRINS, IMPORTANT STARTING COMPOUNDS FOR THE PRODUCTION OF PYRETHROID INSECTICIDES



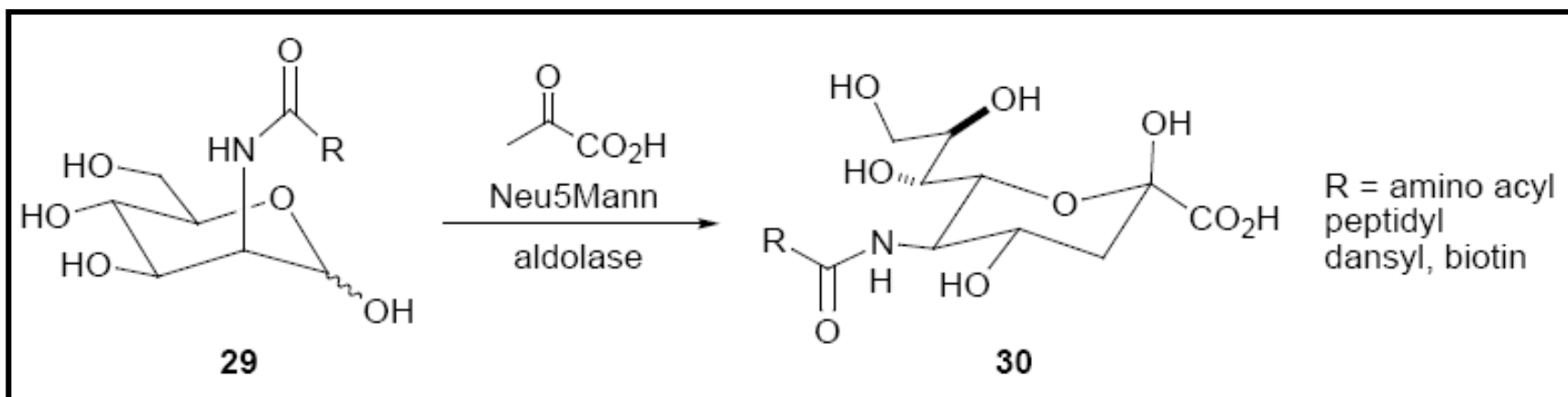
Hydroxynitrile lyase is used for the production of (R)-2-chloromandelonitrile. The enzyme has been engineered for several improvements: the inclusion of a secretion signal, active-site modification and recombinant expression of only one of the almond tree (*Prunus amygdalus*) isoenzymes [45\*\*]. The recombinant enzyme is also stable at acidic pH.



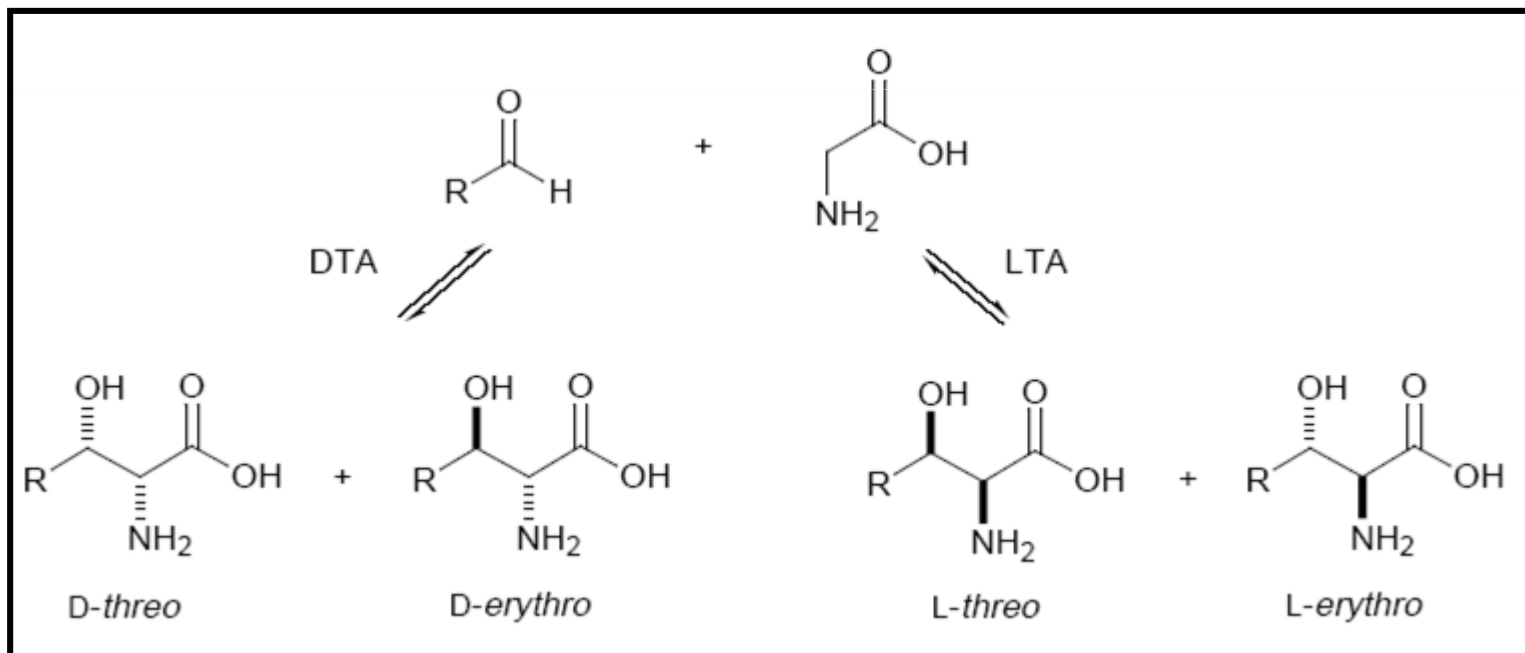
**Oikawa *et al.* have used a crude preparation of an enzyme from the fungus *Alternaria solani* to catalyse the two step conversion of prosolanapyrone II into (-)-solanapyrones A and D**



The synthesis of *N*-acetyl-D-neuraminic acid (Neu5Ac) **30** (R\CH **3**) using the corresponding aldolase to catalyse the reaction between *N*-acetyl-D-mannosamine (MannNAc) **29** (R\CH **3**) and pyruvic acid has been scaled up by the Glaxo Wellcome group.



**Recombinant D- and L-threonine aldolases for the enzymatic synthesis of a range of  $\beta$ -hydroxy- $\alpha$ -amino acids. L-Threonine aldolase (LTA) from *E. coli* and D-threonine aldolase (DTA) from *Xanthomonas oryzae* were cloned and overexpressed in *E. coli*.**



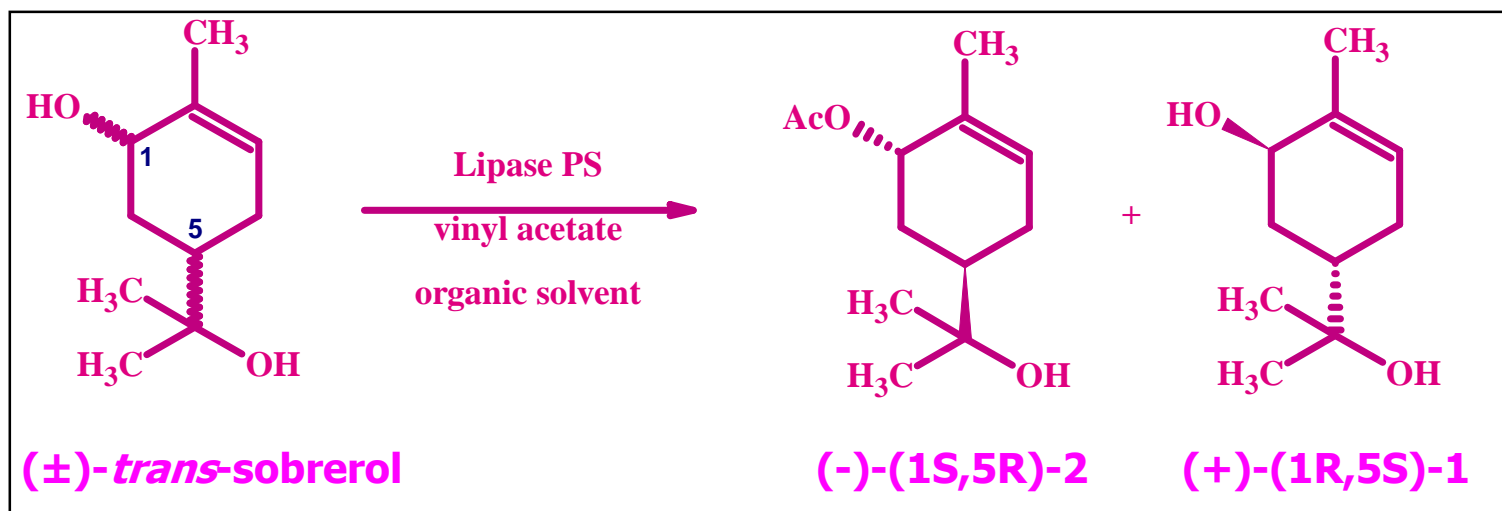
# Experimental

- Lipase-catalyzed resolution of 2-benzylpropionic acid
- Resolution of 2-[6-methoxy-2-naphthyl]propionic acid (Naproxen)
- Resolution of ( $\pm$ )-*trans*-5-(1-hydroxy-1-methylethyl)-2-methyl-2-cyclohexen-1-ol] ( $\pm$ )-*trans*-Sobrerolo by Lipase PS-Catalyzed transesterification
- Chemo-enzymatic synthesis of levodropropizine

**Resolution of ( $\pm$ )-*trans*-5-(1-hydroxy-1-methylethyl)-2-methyl-2-cyclohexen-1-ol] ( $\pm$ )-*trans*-Sobrerol by Lipase PS-Catalyzed transesterification**

**( $\pm$ )-*trans*-Sobrerol is a mucolytic drug and, in spite of differences in the pharmacologic activity between the (+)- and (-)-form, it is produced and marketed as a racemate. The two enantiomers have been isolated by asymmetric synthesis or by resolution of the racemate through crystallization of diastereomeric salts. However, both methods afford low yield.**

# Resolution of ( $\pm$ )-*trans*-Sobrerol by enantioselective acylation catalyzed by free Lipase PS in organic media

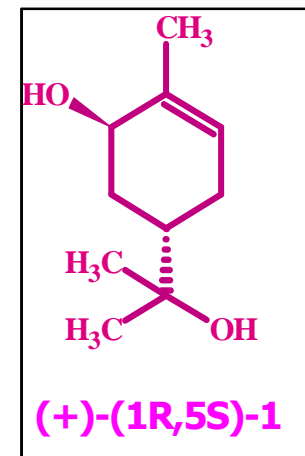


**For the enantioselective acylation of ( $\pm$ )-*trans*-Sobrerol with vinyl acetate several enzymes and organic solvents were tested and Lipase PS was the most active.**

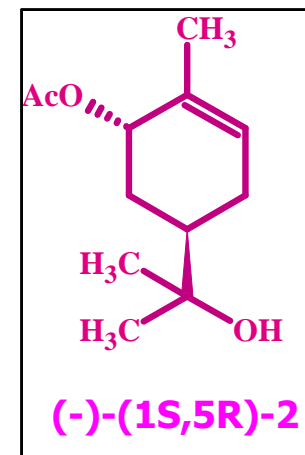
## **Resolution of ( $\pm$ )-trans-Sobrerol with free Lipase PS**

**To a solution of ( $\pm$ )-trans-Sobrerol (1 g, 5.87 mmol) in *t*-amyl alcohol (50 ml) containing vinyl acetate (10.8 mmol), Lipase PS (1.5 g) and molecular sieves (3 Å, 3 g) were added. The suspension was shaken at 45°C, and the reaction progress followed by TLC. After 5 h (approximately 50 % conversion), the enzyme and the molecular sieves were filtered off and the solution evaporated under reduced pressure. The residue was then flash-chromatographed on a silica-gel column (eluent: n-hexane/ethyl acetate 3:1) to separate unreacted (+)-1 from (-)-2**

- **(+)-1, mp 150°C;  
[ $\alpha$ ]<sub>D</sub><sup>20</sup>+149.3 (c 5,  
EtOH)**

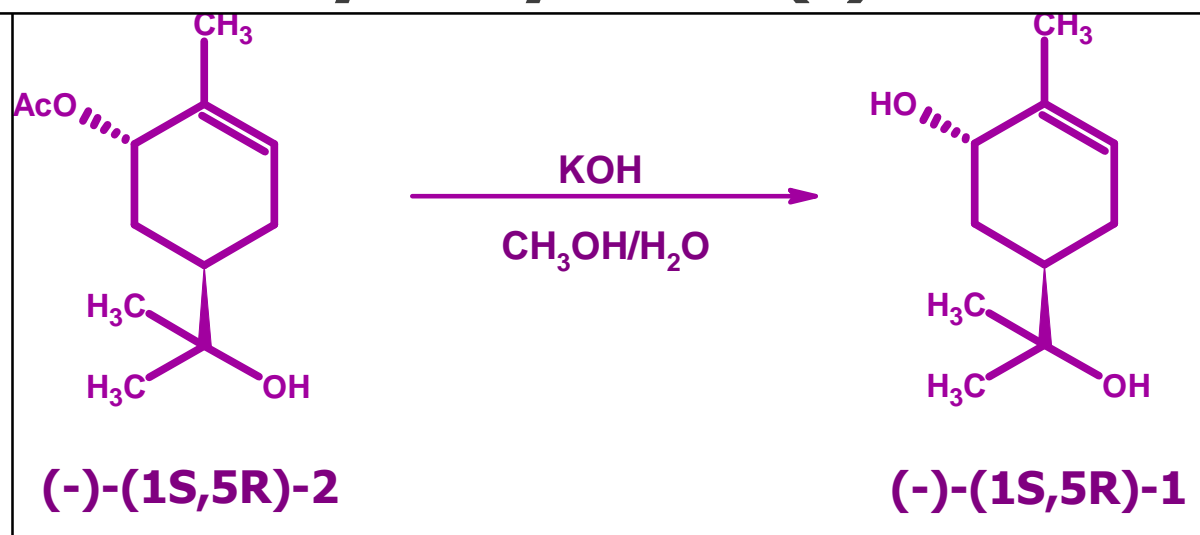


- **(-)-2; mp 70°C;  
[ $\alpha$ ]<sub>D</sub><sup>20</sup>-120.7 (c 4,5,  
EtOH)**



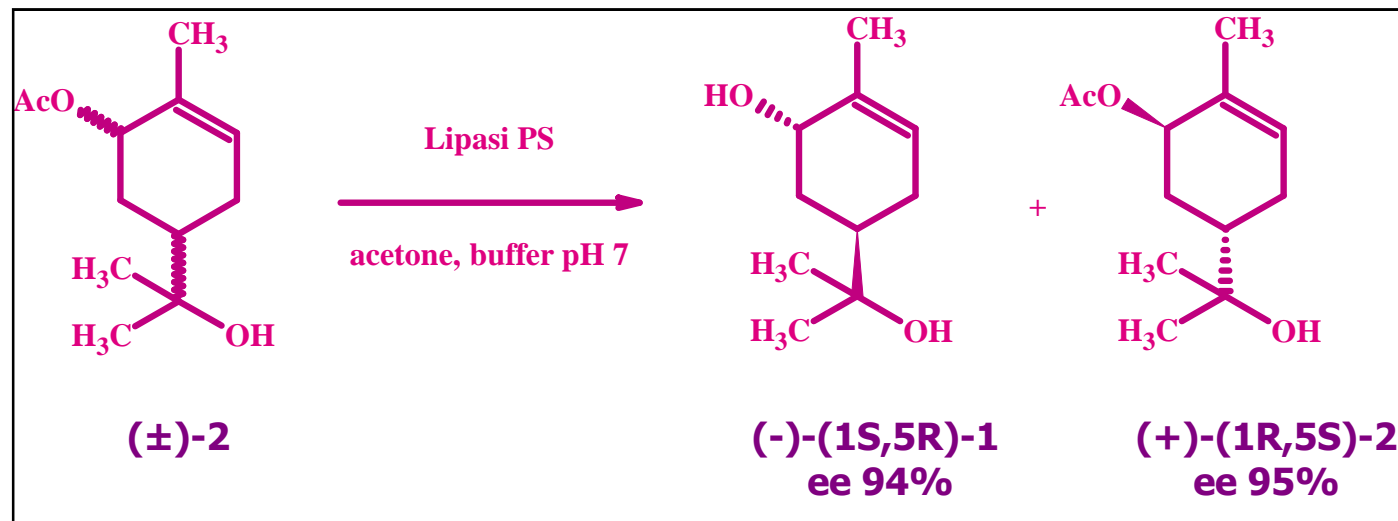


## Hydrolysis of (-)-2



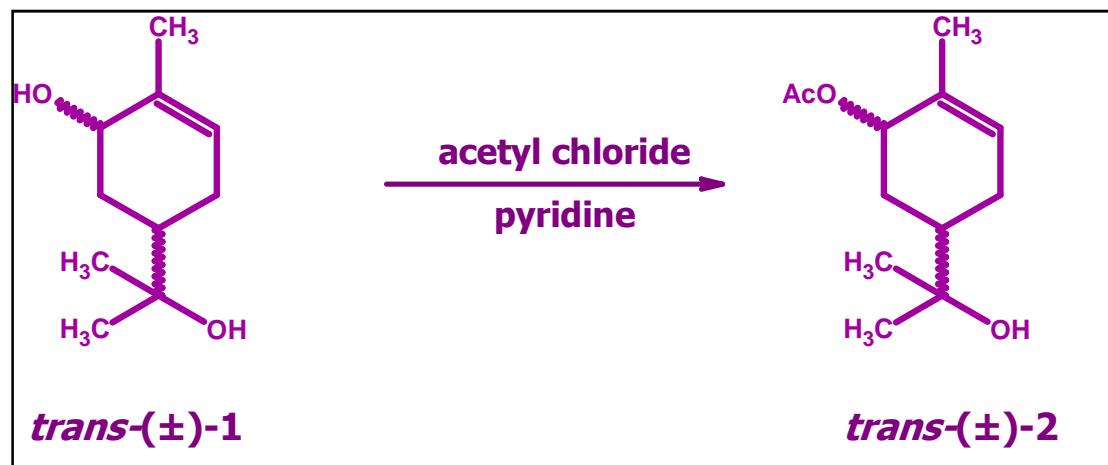
**(-)-2 (400 mg) was treated, at room temperature, with 15 ml of methanol/water (10:5) containing 2 % KOH, up to complete hydrolysis. The solution was evaporated, and the residue dissolved in 8 ml of water and extracted with CH<sub>2</sub>Cl<sub>2</sub>. The organic extract was washed with water, desiccated and evaporated to give (-)-1, mp 149°C;  $[\alpha]_{\text{D}}^{20}$ -147.0 (c 5.6, EtOH)**

Resolution of ( $\pm$ )-1 was also attempted by enzymatic hydrolysis of the acetate of ( $\pm$ )-*trans*-sobrerol in aqueous buffer.



( $\pm$ )-2 (900 mg, 4.24 mmol) dissolved in 4.5 ml of acetone was added to 40 ml of 0.1 M potassium phosphate buffer, pH 7, and treated with Lipase PS (3 g), under stirring, at 25°C. After 4 days (approximately 50% conversion), the reaction mixture was extracted with ethyl acetate and the organic extract was flash-chromatographed (eluent: n-hexane/ethyl acetate 3:1) to separate *trans*-sobrerol (-)-1 from its acetate (+)-2.

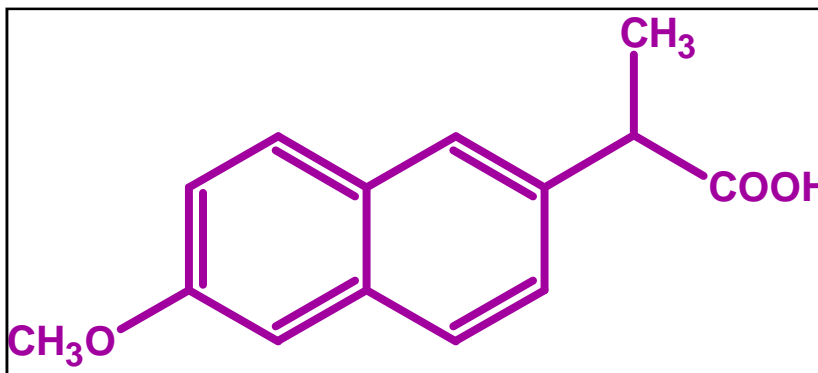
# Synthesis of acylated ( $\pm$ )-*trans*-sobrerol ( $\pm$ )-2



( $\pm$ )-2 was prepared by reacting ( $\pm$ )-1 (6 mmol), dissolved in 10 ml of dry pyridine, with 6 mmol of acetyl chloride for 3 h at room temperature. The products were purified by flash chromatography on silica gel (eluent: n-hexane/ethyl acetate 3:1).

## Enantioselective hydrolysis of racemic naproxen methyl ester by *Candida* Lipase

(S)-Naproxen or (S)-(+)-2-[6-methoxy-2-naphthyl] propionic acid belongs to non-steroidal anti-inflammatory drugs. The (S)-form of naproxen is 28-fold more active than the corresponding (R)-form. The preparation of (S)-naproxen has been accomplished by resolution of racemic naproxen through diastereoisomeric crystallization, asymmetric synthesis or chromatographic separation.



## **Synthesis of (R,S)-naproxen methyl ester**

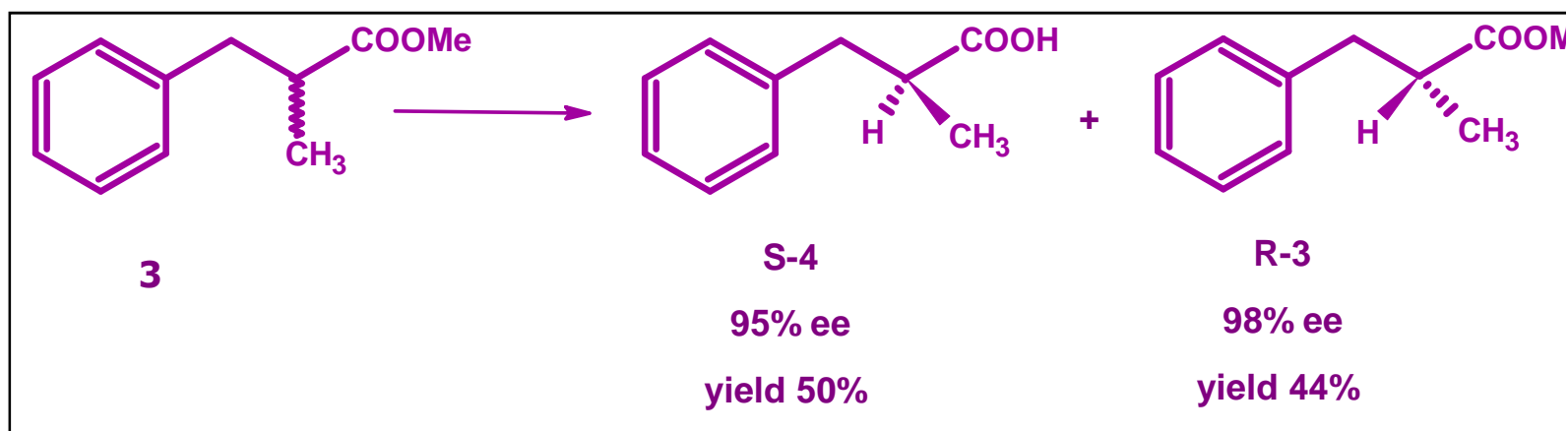
The (R,S)-naproxen was solubilized in methanol in a round-bottom flask. Sulphuric acid was used as a catalyst for esterification and added into the solution. The mixture was refluxed for 5 h. The residual methanol was removed by vacuum evaporation and the residue was washed three times with aqueous 1 M  $\text{NaHCO}_3$  solution and water to eliminate unreacted naproxen, sulphuric acid and methanol. After drying, (R,S)-naproxen methyl ester powder was obtained.

## **Enantioselective hydrolysis of racemic naproxen methyl ester by *Candida cylindracea* Lipase**

The racemic ester substrate (200 mg) was suspended in 2 ml of 0.2 M phosphate buffer (pH 7), and enzyme powder (50 mg) was added. The suspension was stirred at room temperature. The extent of conversion was analyzed by thin-layer chromatography using hexane-EtOAc (3:1 or 5:1) as eluent. The compounds were detected by UV light. When the conversion was near 50%, the reaction was terminated by adjusting the pH of the solution to 2 with 1 N HCl. The product (acid) and remaining substrate (ester) were extracted with ether. The ether layer was collected and dried. Then 2 mL of 0.25 M NaHCO<sub>3</sub> and 2 mL of hexane were added and the mixture was stirred violently to separate ester and acid. The ester was collected in the hexane layer; the acid was isolated after the aqueous phase was acidified and extracted into ether.

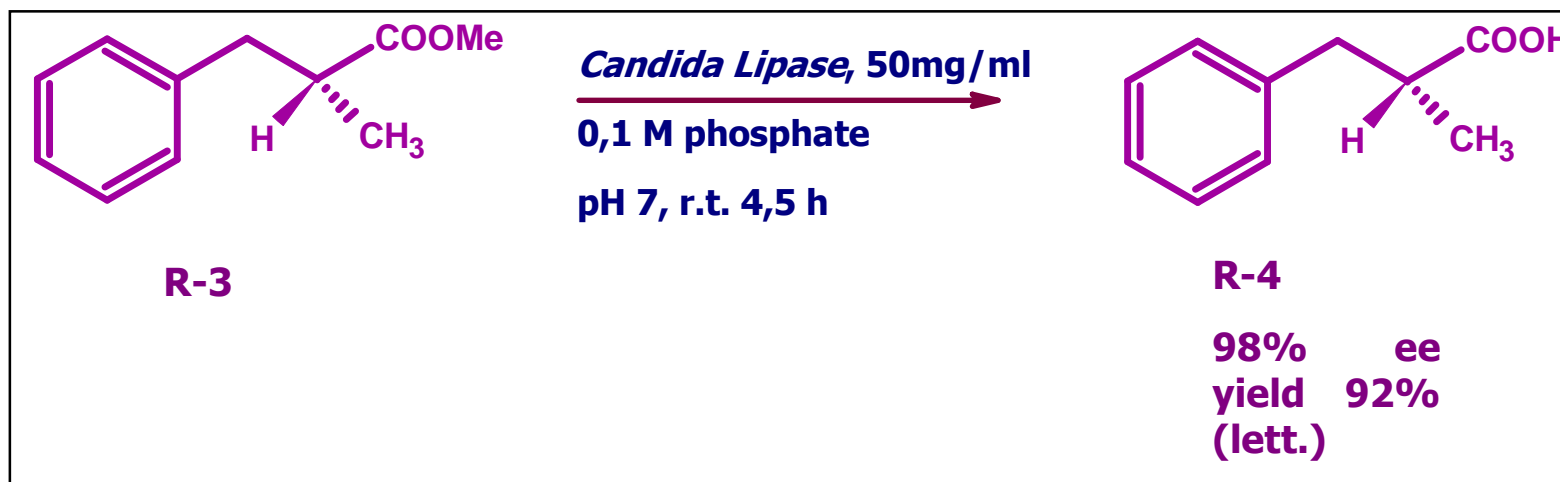
**(S)-(+)-Naproxen: mp 158°C;  $[\alpha]_D^{20} +66.0$  (c 1, CHCl<sub>3</sub>)**

## Lipase-catalyzed resolution of 2-benzylpropanol and 2-benzylpropionic acid



Until now the best method for the preparation of S-4 has been the repeated recrystallization of (+)-methylbenzylamine and/or quinine salts of 4.

Since R-3 is prone to racemization, hydrolysis cannot be carried out under basic conditions. *Candida cylindracea* lipase easily hydrolyzes R-3 without racemization to give R-4 in good yield.





## Lipase-catalyzed resolution of 2-benzylpropionic acid

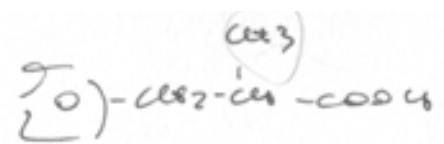
0,3 g of **3** was suspended in 30 ml buffer (pH 7; 0,1 M), lipase P (0,3 g) was added, and the mixture was stirred. After 12 h (the extent of conversion was analyzed by thin-layer chromatography using hexane/EtOAc 9:1 or CHCl<sub>3</sub>/EtOH 9:1) ester was extracted with hexane from basic solution (pH 8-9); and acid was extracted with EtOAc from acidic solution (pH 2).

(S)-**4**  $[\alpha]_D^{25} = +25,6$  (c 1; CHCl<sub>3</sub>)

(R)-**4**  $[\alpha]_D^{25} = -26,2$  (c 1; CHCl<sub>3</sub>)

(S)-**3**  $[\alpha]_D^{25} = +35,9$  (c 1; CHCl<sub>3</sub>)

(R)-**3**  $[\alpha]_D^{25} = -33,7$  (c 1; CHCl<sub>3</sub>)



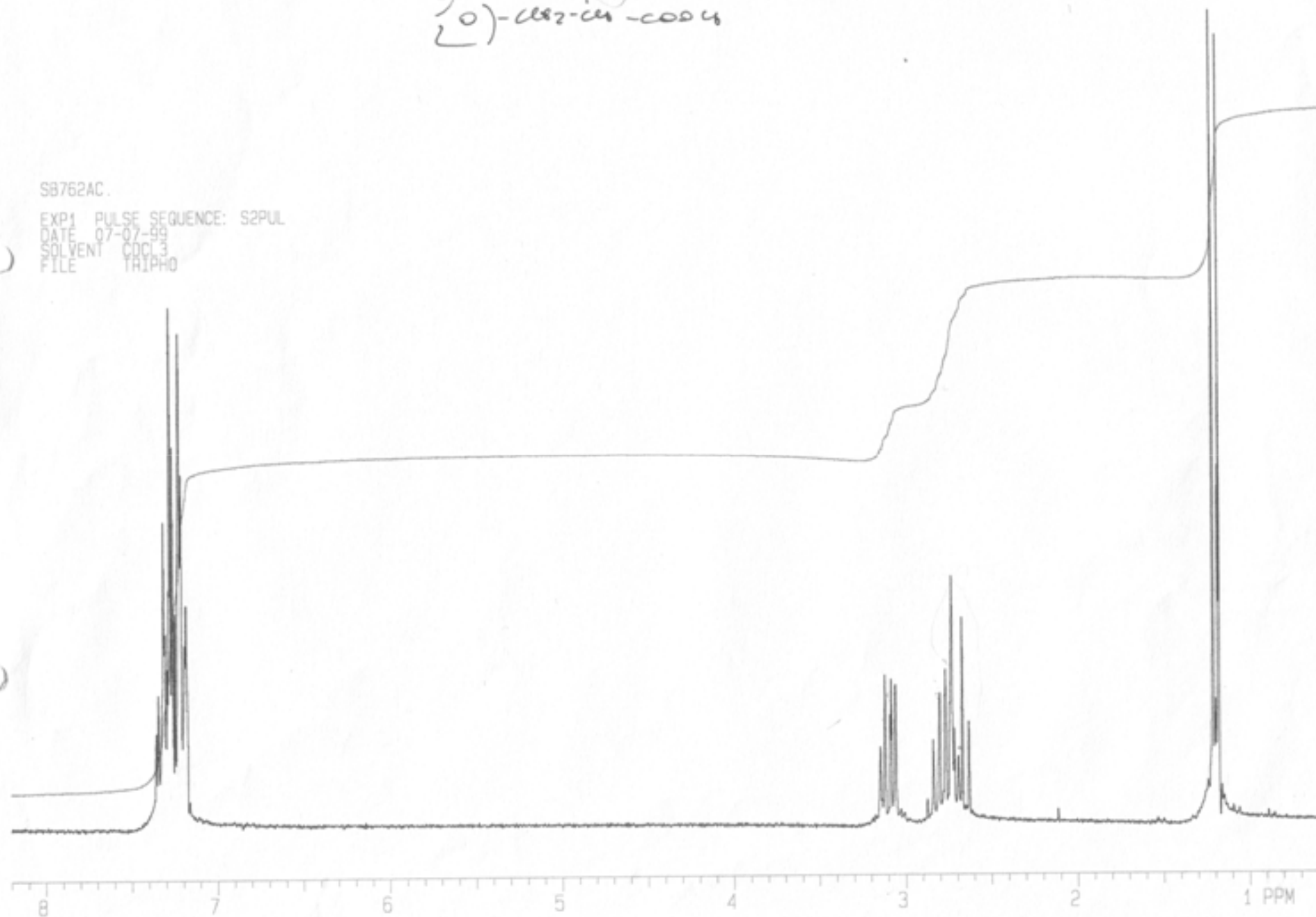
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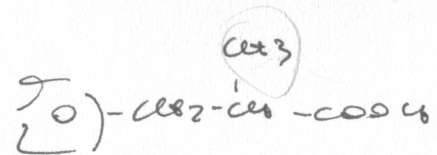
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DATE 07-07-99

SOLVENT CDCl<sub>3</sub>

FILE TRIPH0





RAC + CHININA

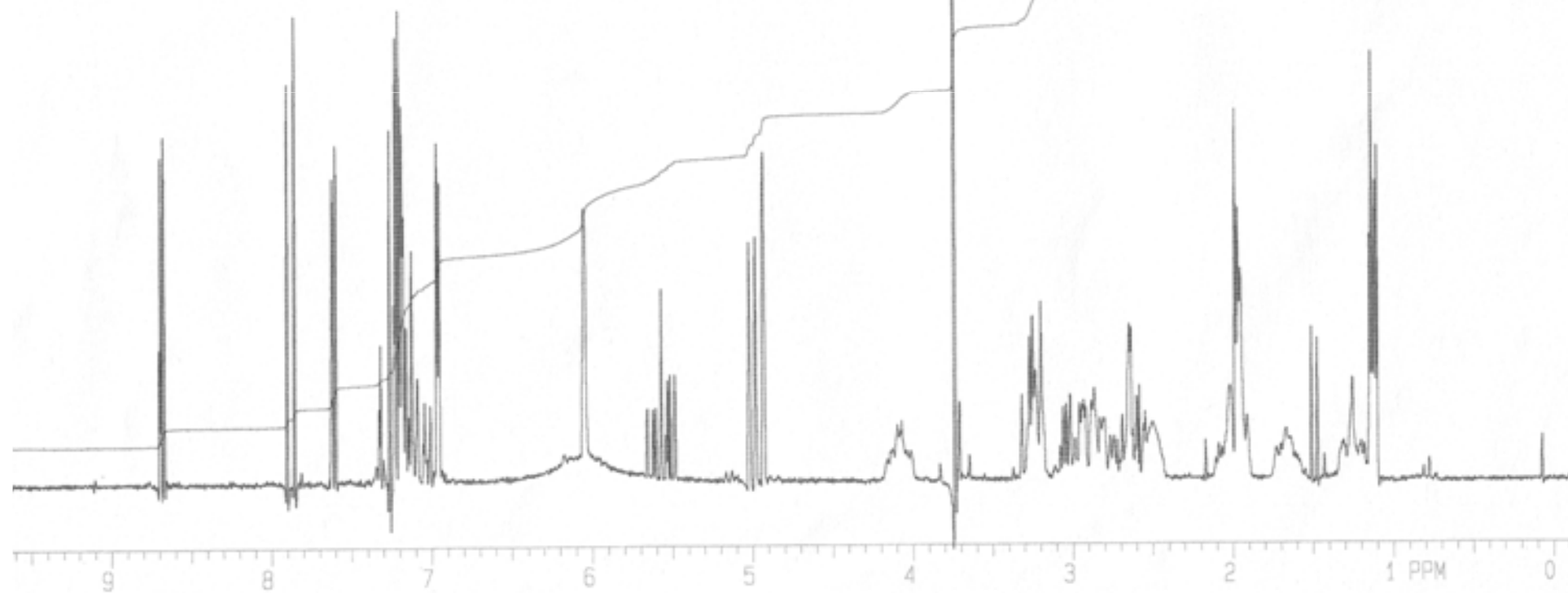
PIGINI

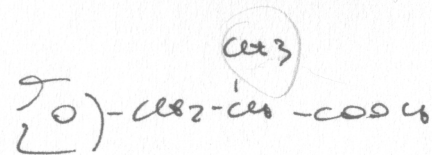
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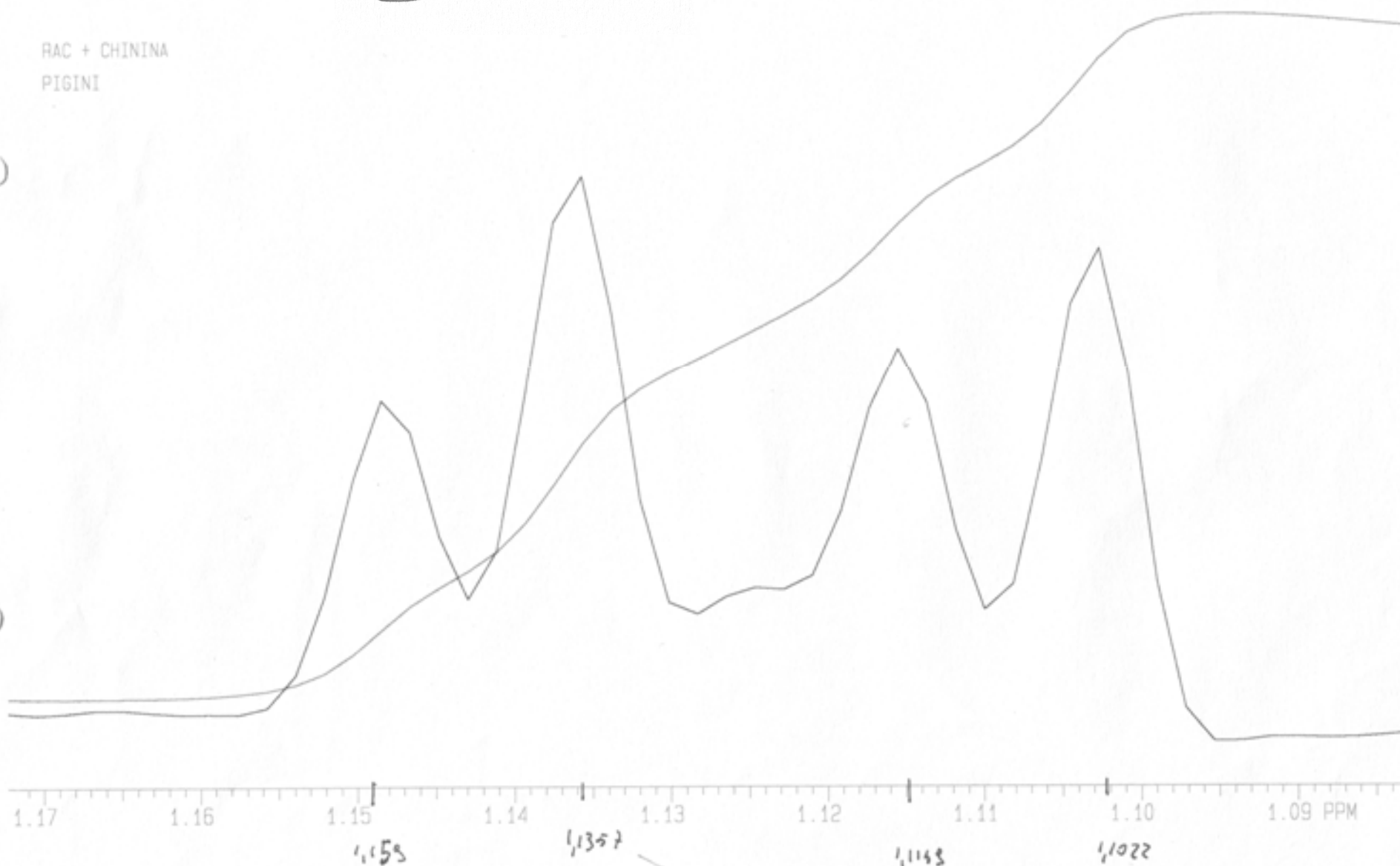
SOLVENT CDCL3

FILE H



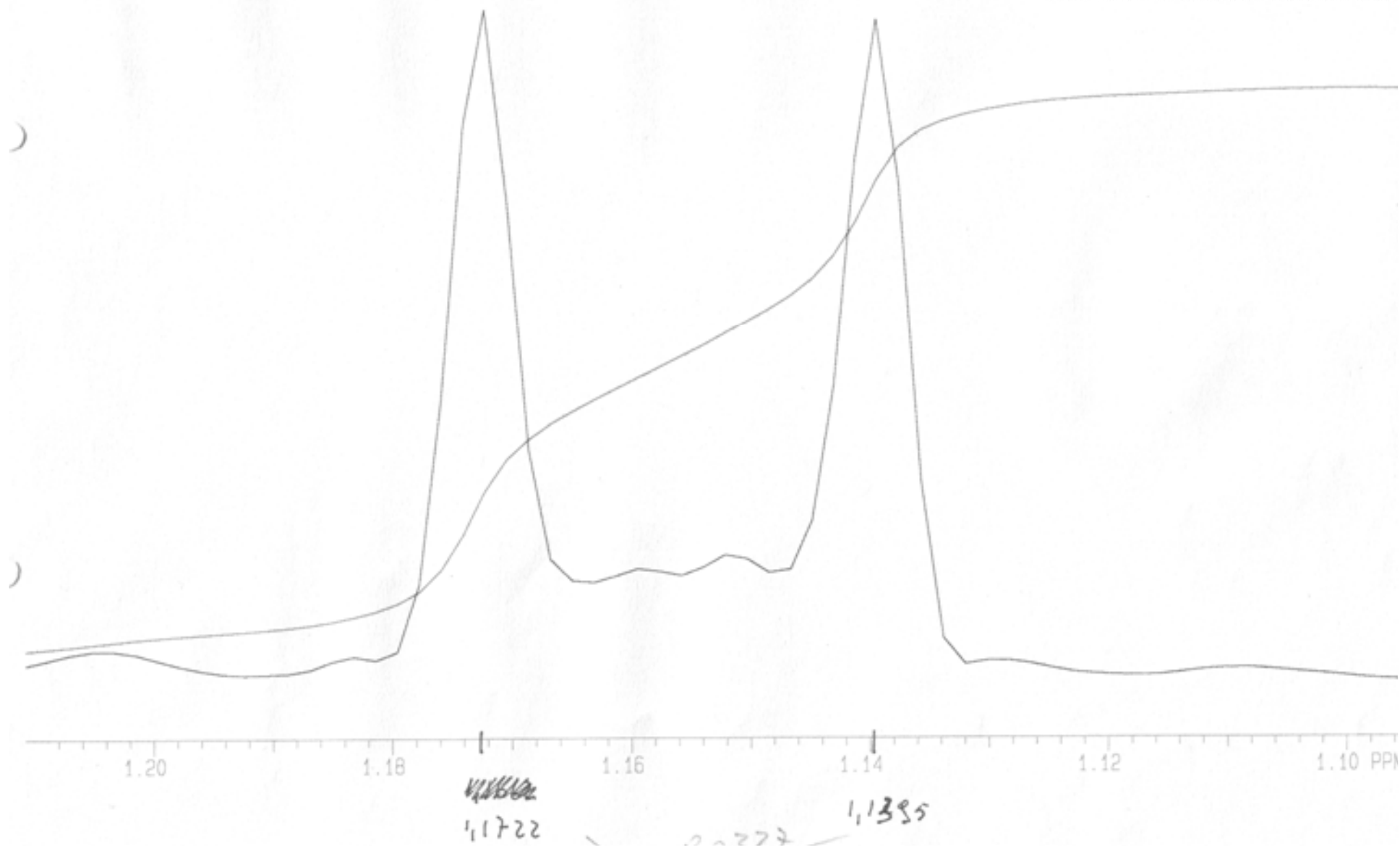
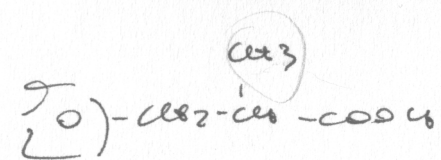


RAC + CHININA  
PIGINI

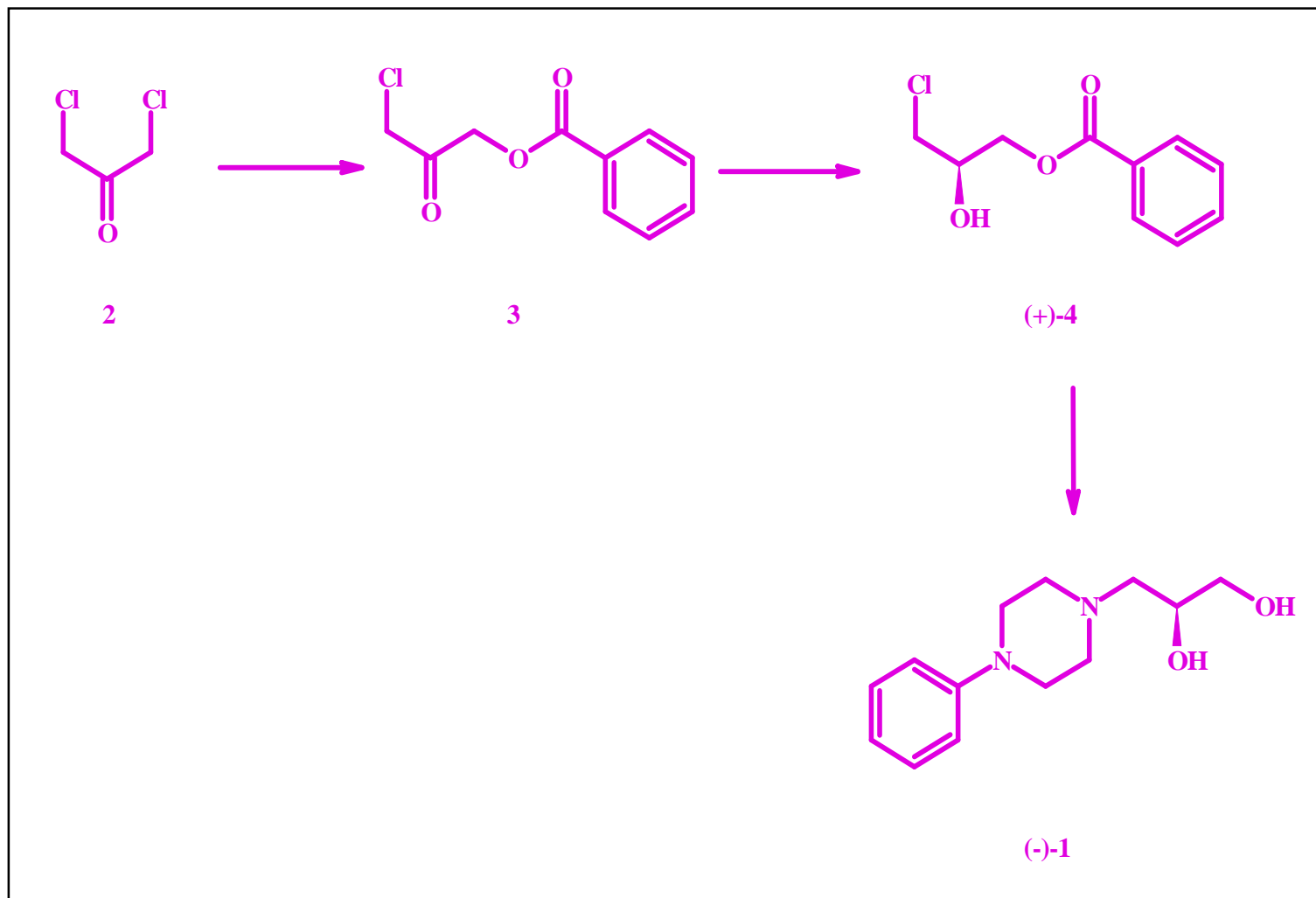


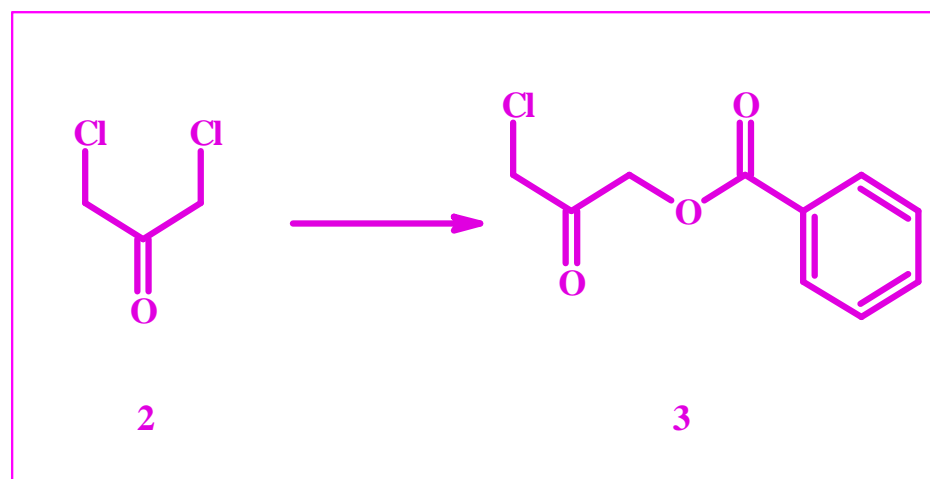
EN + CH<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>

(A)

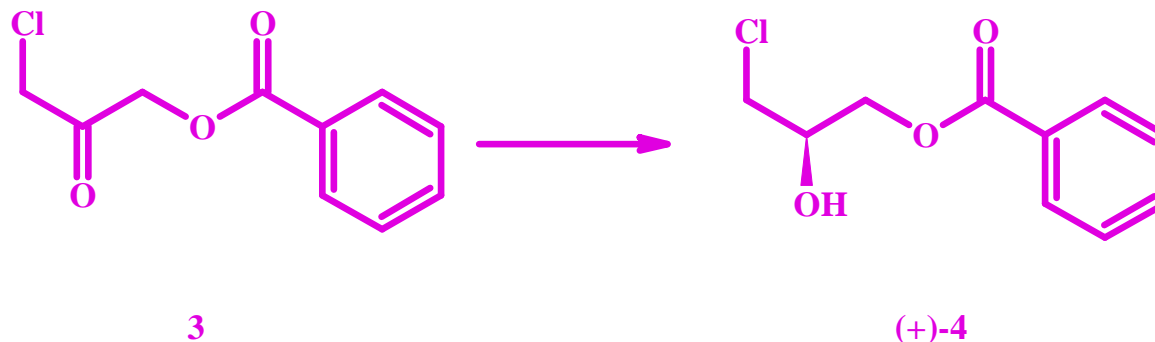


# Chemo-enzymatic synthesis of levodropropizine





- **1-Benzoyloxy-3-chloropropan-2-one (3)**
- **Dichloroacetone 2 (10 g, 79 mmol) was added in one portion to a solution of NaHCO<sub>3</sub> (4 g, 48 mmol) and benzoic acid (4.8 g, 39 mmol) in dry DMF (150 ml) at 0 °C. The reaction was stirred at this temperature for 3 h, then at room temperature (r.t.) for a further 12 h. The reaction mixture was diluted with H<sub>2</sub>O and extracted with light petroleum/ethyl acetate (95:5); the collected organic phases were washed with water and brine and dried over MgSO<sub>4</sub>. The solvent was removed under reduced pressure and the residue purified by crystallization with boiling n-hexane (250 ml) to give 3 as pale yellow solid, m.p. 89-92 °C.**
- **<sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 4.28 (2H, s, CH<sub>2</sub>Cl), 5.17 (2H, s, CH<sub>2</sub>O), 7.46-7.57 (2H, m, *meta* Ph), 7.59-7.70 (1H, m, *para* Ph), 8.08-8.18 (2H, m, *ortho* Ph).**

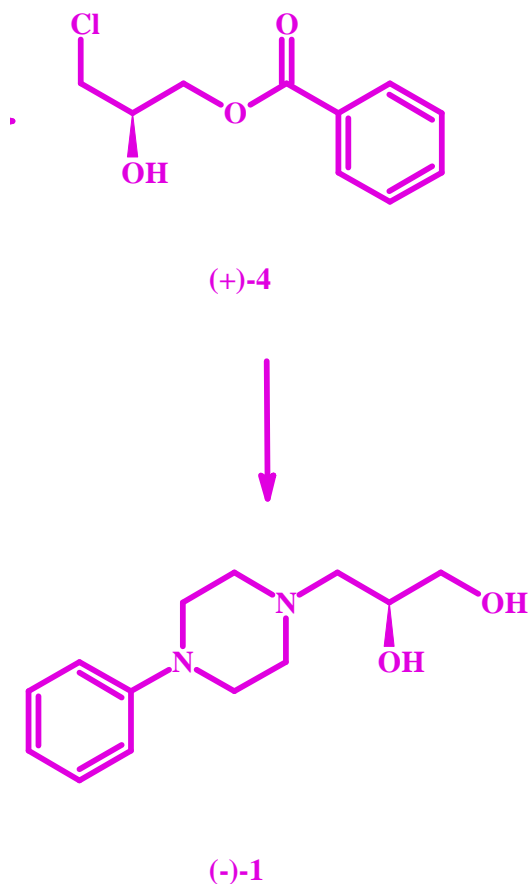


• **1-Benzoyloxy-3-chloropropan-2-ol (4)**

• A suspension of baker's yeast (43 g) in water (850 ml) was preincubated at 30 °C for 30 min; thereafter, a solution of the chloroketone **3** (2 g, 94 mmol) in DMSO (5 ml) was added and the resulting mixture was magnetically stirred at 30 °C for 3 h, until a GLC analysis showed up to 95% conversion. The mixture was centrifuged, the yeast re-suspended in water (50 ml) and re-centrifuged. The collected aqueous phases were extracted with ethyl acetate and the organic phases washed with water, brine and dried (MgSO<sub>4</sub>). The solvent was removed under reduced pressure and the residue purified by column chromatography (light petroleum-ethyl acetate 7:3) to afford compound **4**, which was recovered as a pale yellow oil,  $[\alpha]_D = +2.0$  (c 1, CHCl<sub>3</sub>), 73% ee.

• (+)-**4**: <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 3.13 (1H, b, OH), 3.70 (1H, dd, J 11.3, 5.0, CH<sub>2</sub>Cl), 3.75 (1H, dd, J 11.3, 5.7, CH<sub>2</sub>Cl), 4.24 (1H, quintet, J 5.3, CHOH), 4.39-4.58 (2H, m, CH<sub>2</sub>O), 7.38-7.51 (2H, m, *meta* Ph), 7.52-7.65 (1H, m, *para* Ph), 8.01-8.11 (2H, m, *ortho* Ph).





- Levodropropizine (1)
- Phenyl-piperazine (0.71 ml, 4.65 mmol) was added to a solution of (+)-4 (500 mg, 2.33 mmol), 73% ee in isopropyl alcohol (10 ml). The resulting solution was refluxed for 24 h, when TLC analysis showed disappearance of 4 and formation of a new product, which was occasionally isolated and identified as 1-benzoyloxy-3-(4-phenyl-piperazin-1-yl)-2-propanol (6). NaOH (93 mg, 2.33 mmol) was added and the solution was refluxed for a further 1.5 h. The resulting mixture was cooled at room temperature, the white precipitate (sodium benzoate) filtered off and the solvent removed under reduced pressure. The crude residue was purified by column chromatography (EtOAc:EtOH:NH<sub>4</sub>OH, 8:2:0.3) to give (-)-1 as pale yellow solid,  $[\alpha]_D = -17.7$  (c 3.0, CH<sub>2</sub>Cl<sub>2</sub>), ee 73%.
- Crystallization from dichloromethane/hexane afforded
- (-)-1, ee 95%.
- (-)-1: <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  2.47 (1H, dd, J 12.5, 4.1, NCH<sub>2</sub>CHOH), 2.55-2.95 (5H, m, CH<sub>2</sub>CH<sub>2</sub>NPh+NCH<sub>2</sub>CHOH), 3.24 (4H, t, J 5.0, CH<sub>2</sub>NPh), 3.40 (2H, b, 2 OH), 3.57 (1H, dd, J 11.4, 4.8, CH<sub>2</sub>OH), 3.77 (1H, dd, J 11.4, 3.7, CH<sub>2</sub>OH), 3.90 (1H, sextuplet, J 4.4, CHOH), 6.90 (1H, t, 7.2, arom. *para*), 6.95 (2H, d, J 8.8, arom. *ortho*), 7.30 (2H, dd, J 7.2, 8.8, arom. *meta*).

## **Sintesi del ( $\pm$ )-Naprossene metil estere**

**Solo l'(*S*)-Naprossene è reperibile in commercio, quindi:**

**Naprossene sodico (3 g) viene sciolto in glicole etilenico (12 ml). Si fa rifluire per 2 h a 150°C. La miscela di reazione viene raffreddata a t.a. e 12 ml di HCl 1N vengono aggiunti. L' (*R,S*)-naprossene precipita come polvere bianca che viene filtrata. Il solido viene lavato con H<sub>2</sub>O e seccato. L'acido viene solubilizzato in metanolo. Viene aggiunto acido solforico (poche gocce) e la miscela viene fatta rifluire per 5 h. Si fa raffreddare e si distilla il metanolo. Il residuo viene ripreso con acetato d'etile e lavato con NaHCO<sub>3</sub> sat./ H<sub>2</sub>O. Si secca su Na<sub>2</sub>SO<sub>4</sub> e si porta a secco.**