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Synthesis of Enantiopure Building Blocks for Biologically Active Compounds by Enzyme Catalysis

Optimization of reaction conditions for increased enantioselectivity and activity

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Agenda lecture 1

- Chirality and enantiopurity of molecules
- Need for enantiopure biologically active compounds
- Biocatalysis in industry
- Theory of biocatalysis
- Enzyme catalyzed kinetic resolutions of secondary alcohols and halohydrins
- Improvement of enantioselectivity
- Enantioselective enzyme inhibition
- Asymmetrization of prochiral diesters

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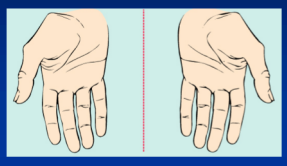
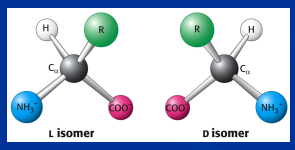
Chiral Nature and chiral molecules

Cheir: Greek for hand

Hands are mirror images of each other but not alike



A molecule with a C-atom (other atoms also) with 4 different groups is chiral.
It excists as two different forms: enantiomers.

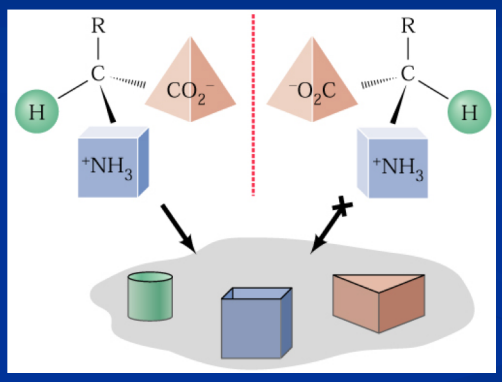


Enantiopure: Only one of the enantiomers, %ee 99

The American Food and Drug Administration (FDA) considers the wrong enantiomer as an impurity and demands for pure enantiomers as marketed drugs, not racemates

Enantiomers interact differently with other chiral molecules

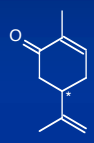
(F. inst. enzymes, receptors)



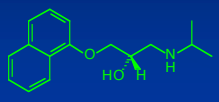
Proteines are made up of 20 amino acids, 19 are chiral

Significance of chirality for biological activity

Eudismic ratio (ER): Ratio of the one enantiomer more active than the other

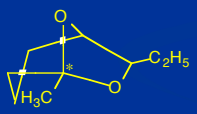


(*S*)-carvone, caraway
(*R*)-carvone, spearmint

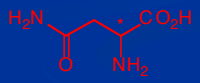


(*S*)-Propranolol, ER = 130

(*S*)-Ketamin
(*S*)-anesthetic
(*R*)-halucinogen



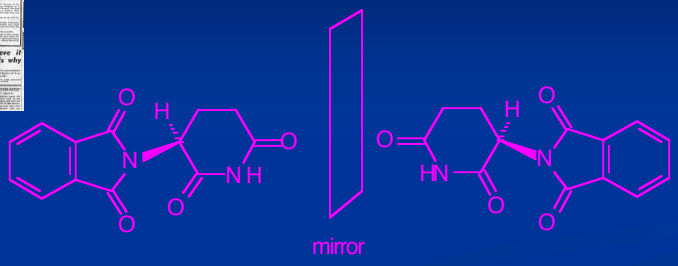
exo-brevicomin



(*S*)-Asparagine, bitter
(*R*)-Asparagine, sweet

(1*R*, 5*S*, 7*R*)-exo-Brevicomin is produced by females of the pine beetle *Dendroctonus brevicomis* as male attractant

Thalidomide



(*S*)-enantiomer of thalidomide

(*R*)-enantiomer of thalidomide

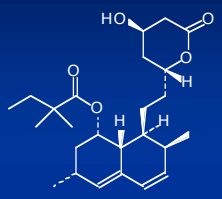
Racemic Thalidomide- 50 % of each- was used as a sedative by pregnant women in the 1960´s. The (*S*)-enantiomer caused the deformed babies.

Top 10 medicines in Norway 2006

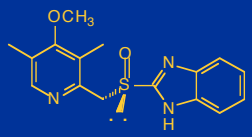
Rank 2006 (2005)	Medicine (Single enantiomer)	Molecule	Sales Mill. NOK	Main indication
1 (2)	Enbrel	Etanercept (TNF Fusion protein)	329	Rheumatoid arthritis
2 (1)	Lipitor	Atorvastatin	278	High cholesterol
3 (4)	Nexium	Esomeprazole	253	Gastric ulcer
4(3)	Seretide	Salmeterol & Fluticason	252	Asthma
5 (5)	Remicade	Infliximab (Immunoglobulin G)	196	Rheumatoid arthritis Psoriasis
6 (6)	Zyprexa	Olanzapin	153	Schizophrenia
7 (7)	Symbicort	Formoterol & Budesonid	141	Asthma
8 (15)	Humira	Adalimumab (Immunoglobulin G1)	125	Rheumatoid arthritis
9 (8)	Cozaar Comp	Lozartan & diurethica	121	High blood pressure
10 (9)	Metroprolol	Metroprolol (sold as racemate)	121	High blood pressure

Chiral medicines marketed as pure enantiomers

Lipitor® active molecule atorvastatin



Zocor® active molecule simvastatin



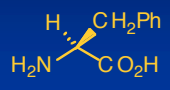
Nexium® active molecule esomeprazole

Single enantiomer compounds are preferred by FDA because they exhibit lower toxicity and higher efficacy. For pharmaceutical industry this may facilitate patent life extensions. However, higher manufacturing costs may occur.

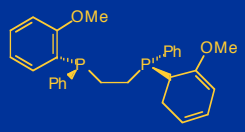
SynZymes Synmax

How to provide enantiopure molecules?
(Achiral synthesis gives both enantiomers)

1. Chiral natural products:
-carbohydrates,terpenoids,
hydroxy acids, alkaloids..



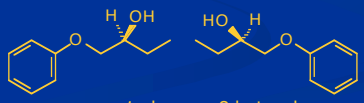
(*S*)-Phenylalanine



(*R*,*R*)-DIPAMP

2. Asymmetric synthesis: -chirality from substrate, chiral auxilliary, reagent or catalyst

3. Resolution of racemate:
-via diastereomeric derivatives or kinetic



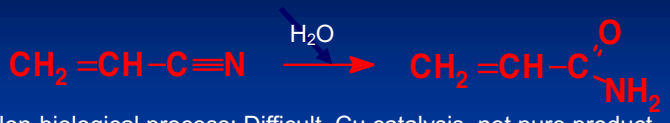
rac-1-phenoxy-2-butanol

Why Biocatalysis ?

- Enzymes are chiral molecules!
  - Selectivity -chemo
    -regio
    -stereo

- Both asymmetric synthesis and resolution
  - Taylor made new enzyme catalysts
    - Green Chemistry

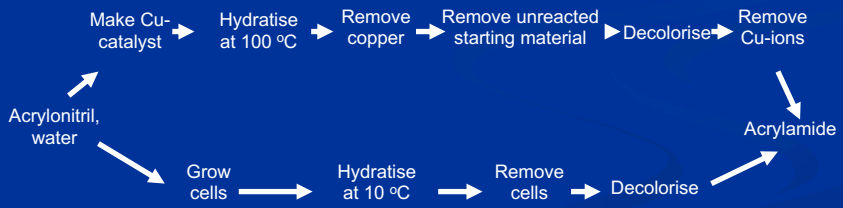
Industrial acrylamide process



Non-biological process: Difficult, Cu catalysis, not pure product

Whole cells from *Brevibacterium*, *Pseudomonas*, *Rhodococcus*
Pure product, > 99% yield., Nitto Japan > 30 000 tonnes/year

Traditional process



Green Chemistry

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08.10.2019

J. Chem. Education, 76, 1999, 1658-1660

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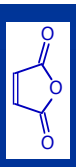
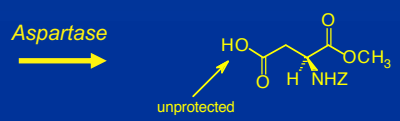
Biocatalytic process for Aspartame

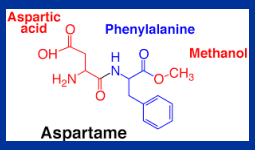
150-200 sweeter than sucrose, discovered by Searle 1965, approved by FDA 1981,
US patent (NutraSweet) expired 1992. Prod. by Holland Sweetener Company

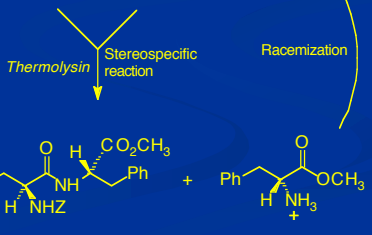
4 Stereoisomers, only one is sweet, the others are bitter



Maleic anhydride







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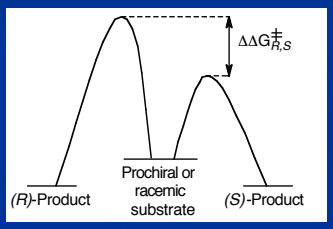
Resolution or asymmetric synthesis?

Asymmetric synthesis
Substrate: prochiral or meso-compound
May give 100% yield and 100% ee,
but is it the right enantiomer?
Enantiomeric excess independent
of degree of conversion



$\Delta\Delta G^{\#} = -RT\ln k_R/k_S$, F. inst. = 7.3 kJ/mol,
$k_R/k_S = 19 = 95/5$, ee = 90 %

Kinetic resolution
Substrate: racemic mixture
Maximum 50% of each enantiomer
Enantiomeric excess depends on degree of conversion

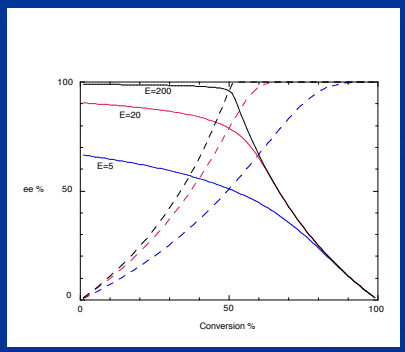
$\Delta\Delta G^{\#} = -RT\ln E$, F. inst. = 7.3 kJ/mol, $E = 19$

Kinetic resolution
Enantiomeric Ratio, *E*

E: Ratio of the spesificity constants (k_{cat}/K_M) of the enzyme for the two enantiomers

An *E*-value of 50: One enantiomer reacts 50 times faster than the other

ee= enantiomeric excess



$$\Delta\Delta G^{\#} = -RT\ln E$$

Calculation of *E*:

$$E = \frac{\ln\left[ee_P(1-ee_S)\right]/(ee_P+ee_S)}{\ln\left[ee_P(1+ee_S)\right]/(ee_P+ee_S)}$$

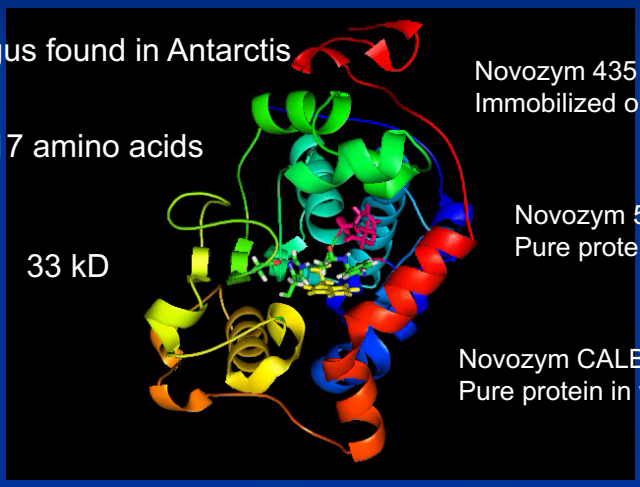
At the start of the reaction $ee_s = 0$, $ee_p = 90$ % (*i.e.* 95 : 5, 19 : 1, $E = 19$)

Lipase B from *Candida antarctica*

Fungus found in Antarctis

317 amino acids

33 kD



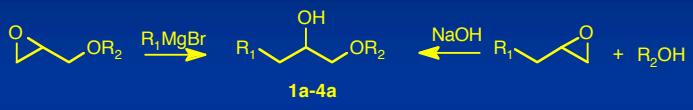
Novozym 435
Immobilized on resin

Novozym 525 F
Pure protein

Novozym CALB L
Pure protein in water

Secondary alcohols and halohydrins

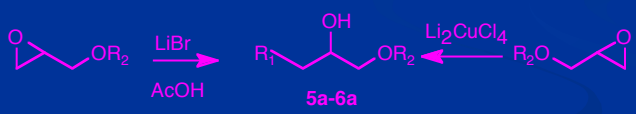
Synthesis of racemic substrates for kinetic resolutions



1a-4a

1a $R_1 = CH_3$, R_2 = Ph
2a $R_1 = CH_2CH_3$, R_2 = Ph
3a $R_1 = CH_2CH_2CH_3$, R_2 = Ph
4a $R_1 = CH_3$, $R_2 = CH_2Ph$

Yields: 24-50 %
Purity: 96-100 %

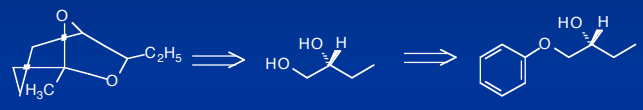


5a-6a

5a R_1 = Br, R_2 = Ph
6a R_1 = Cl, R_2 = Ph

Yields: 85 %
Purity: 99 %

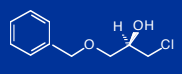
Building blocks for pheromones and pharmaceuticals



exo-brevicomin

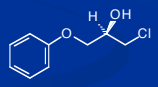
(*R*)-1,2-butanediol

(*R*)-1-phenoxy-2-butanol, (*R*)-**1a**



(*R*)-1-benzyloxy-3-chloro-2-propanol

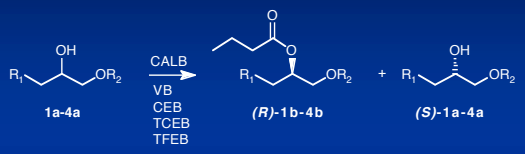
Cidofovir



(*R*)-3-chloro-1-phenoxy-2-propanol, (*R*)-**6a**

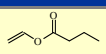
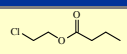
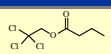
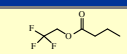
Transesterification reactions of 1a-4a

Effect of acyl donor on *E* and K_{eq}



1a-4a **(*R*)-1b-4b** **(*S*)-1a-4a**

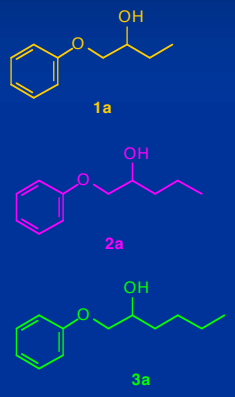
1a $R_1 = CH_3$, $R_2 = Ph$
2a $R_1 = CH_2CH_3$, $R_2 = Ph$
3a $R_1 = CH_2CH_2CH_3$, $R_2 = Ph$
4a $R_1 = CH_3$, $R_2 = CH_2Ph$

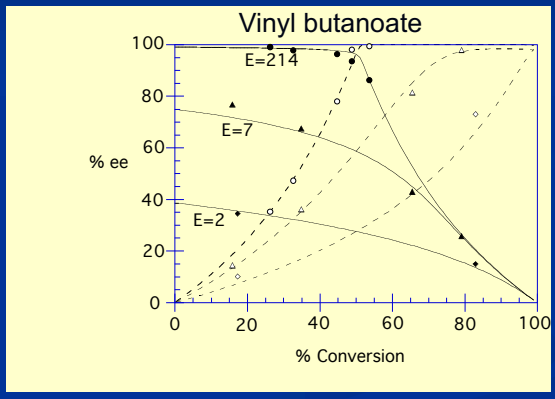
Substrate								
	E	K_{eq}	*E*	K_{eq}	*E*	K_{eq}	*E*	K_{eq}
1a	214	>10000	245	0.27	293	4.79	233	5.72
2a	7	>10000	21	0.63	31	6.35	40	>10000
3a	2	>10000	1.7	1.02	2.1	>1000	2.5	0.40
4a	13	>10000	84	0.41	106	6.59	128	3.33

Jacobsen, E. E. *et al. Chirality* 2000, *12*, 654-659.

Effect of substrate structure on *E*

Small group

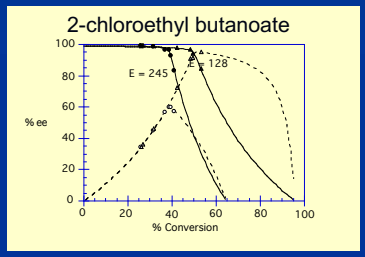


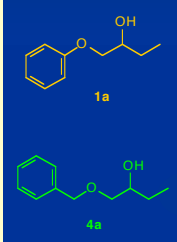


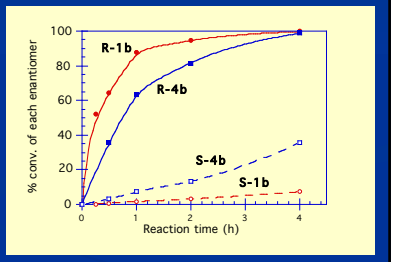
Number of carbons (size) of small group affect selectivity

Effect of substrate structure on *E*

Large group



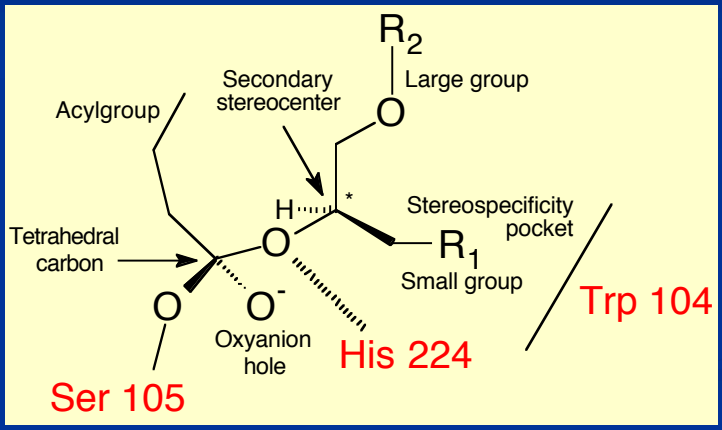




The slow enantiomer of **4a** reacts faster
The fast enantiomer reacts slower; *E* decreases

Detailed view of substrate binding

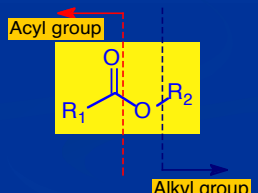
Faster reacting enantiomer of butanoate of derivative of 1,2-diol



Jacobsen, E. E. *et al*. *Chirality* 2000, *12*, 654-659.

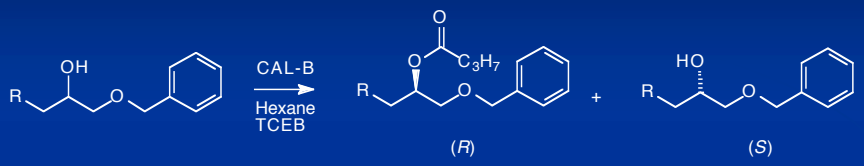
Ways to Improve Selectivity

- The catalyst
  New enzyme, engineered enzyme, new microorganism, immobilization
- The acyl donor
  The starting ester in organic media, acyl or alkyl group
- The substrate
  Protecting groups
- The medium
  Solvent, mixture of solvents, water activity of organic media
- The cofactor
  In microbial red-ox reactions



Effect of substrate structure on *E*

Halogens in small group



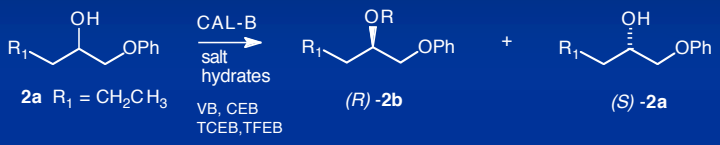
Substrate	R	E
1-benzyloxy-2-propanol	-H*	3
1-benzyloxy-3-bromo-2-propanol	-Br*	9
1-benzyloxy-3-fluoro-2-propanol	-F*	6
1-benzyloxy-3-chloro-2-propanol	-Cl*	15
1-benzyloxy-2-butanol, **4a**	$-CH_3$	106

Electronegative properties of small group affect selectivity

*Hoff, B. H. *et al. J. Mol. Catal., B: Enz.* 2000, *8*, 51

Improving enantioselectivity I

The water activity (a_w) in hexane



2a $R_1 = CH_2CH_3$

VB, CEB
TCEB,TFEB

(*R*) -**2b**

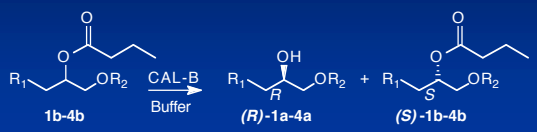
(*S*) -**2a**

Acyl donor	$a_w \approx 0$	$a_w = 0.18$	$a_w = 0.39$	$a_w = 0.65$
VB	7	12	15	16
CEB	21	20	17	Rx. stopped
TCEB	31	30	32	9
TFEB	40	23	19	Rx. stopped

Increased *E* by increased a_w in resolution of **2a** with VB in hexane

Improving enantioselectivity II

Hydrolysis of ester in buffer

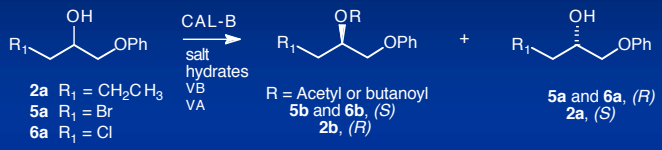


1b $R_1 = CH_3$, $R_2 = Ph$
2b $R_1 = CH_2CH_3$, $R_2 = Ph$
3b $R_1 = CH_2CH_2CH_3$, $R_2 = Ph$
4b $R_1 = CH_3$, $R_2 = CH_2Ph$

Substrate	Phosphate buffer conc.	*E*	Conv. /rx. time
1b	0.05 M, pH 7.00	158	51 % / 24 h
2b	0.1 M, pH 7.00	326	46 % / 96 h
3b	0.1 M, pH 7.00	No reaction	-
4b	0.1 M, pH 7.00	600	49 % / 8 h

Increased *E* in hydrolysis of corresponding esters of **2** and **4**

Effect of water activity in different solvents on *E*



2a $R_1 = CH_2CH_3$
5a $R_1 = Br$
6a $R_1 = Cl$

R = Acetyl or butanoyl
5b and **6b**, *(S)*
2b, *(R)*

5a and **6a**, *(R)*
2a, *(S)*

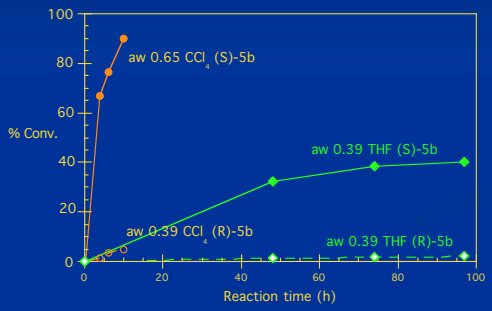
Solvent	Log P	a_w = 0.18	a_w = 0.39	a_w = 0.65
1,4-Dioxane	-1.1	25	47	conv. <1%
Acetonitrile	-0.3	40	69	conv. <1%
THF	0.5	30	73	conv. <1%
Et_2O	0.9	23	24	conv. <1%
Benzene	2.0	22	31	48
Toluene	2.5	35	42	52
CCl_4	3.0	52	63	69
Hexane	3.5	16	25	55

Improved selectivity by increased water activity

Jacobsen, E. E. and Anthonsen, T. *Can. J. Chem.* **2002**, *80*, 577-581

Effect of a_w in different solvents on reaction rate

Esterification of **5a** in CCl_4 and THF



No conversion of **5a** in THF when a_w = 0.65

5a in THF a_w 0.39: 40 % conv. in 100 h *E* = 73

5a in CCl_4 a_w 0.65: 90 % conv. in 5 h *E* = 69

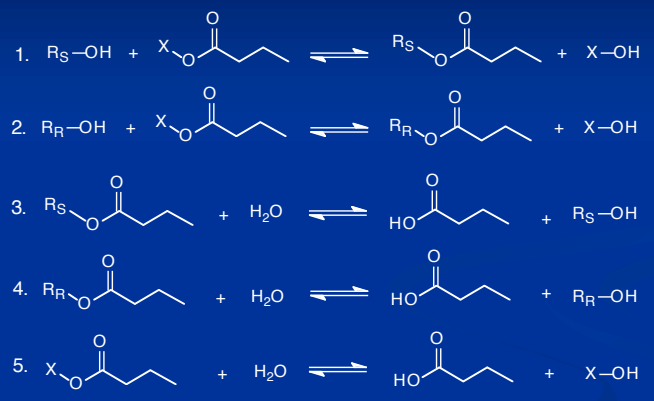
Water activity *vs.* water content

Water content (ppm, μg water per g solvent) Karl Fisher method

Solvent	Log P	a_w=0.18	a_w=0.39	a_w=0.65
1,4-Dioxane	-1.1	360	433	5820
Acetonitrile	-0.3	1641	1862	12391
THF	0.5	882	904	13003 (1.3%)
Et_2O	0.9	471	543	7980
Benzene	2.0	134	179	627
Toluene	2.5	138	142	510
CCl_4	3.0	45	55	84
Hexane	3.5	27	28	74

Increase of water activity results in higher increase of water content in polar solvents than in apolar solvent

Equilibria of transesterifications



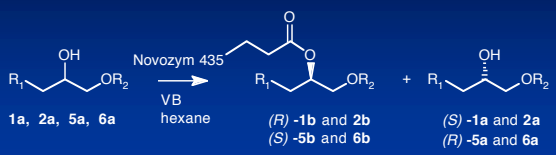
Ester products and acyl donors are hydrolyzed when water content is high

Synthesized enantiopure compounds

Compound	*ee*, %	Concentration/solvent	Optical rotation
(R)-**1a**	96	c 1.37, $CHCl_3$	$[\alpha]_D^{25} = -6.57$
(R)-**1a** (ref.)	99	c 1.40, $CHCl_3$	$[\alpha]_D^{25} = -6.44$
(S)-**1a**	99	c 1.40, $CHCl_3$	$[\alpha]_D^{25} = +5.84$
(S)-**1b**	99	c 1.50, $CHCl_3$	$[\alpha]_D^{25} = -6.57$
(R)-**2a**	99	c 1.14, $CHCl_3$	$[\alpha]_D^{30} = -12.25$
(R)-**2a** (ref.)	99	c 1.17, $CHCl_3$	$[\alpha]_D^{20} = -6.86$
(R)-**3a** (ref.)	99	c 0.90, $CHCl_3$	$[\alpha]_D^{25} = -5.55$
(R)-**4a**	94	c 2.95, EtOH	$[\alpha]_D^{25} = +4.74$
(S)-**4a**	100	c 2.20, $CHCl_3$	$[\alpha]_D^{25} = +4.03$
(S)-**4a**	100	c 2.20, EtOH	$[\alpha]_D^{25} = -4.03$
(S)-**4a**(ref.)	100	c 4.50, EtOH	$[\alpha]_D^{25} = -4.35$

Enantiopreference of CAL-B: *R*-enantiomer

E not constant!

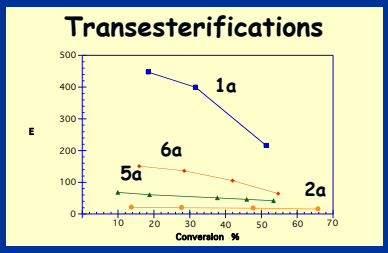


1a, 2a, 5a, 6a

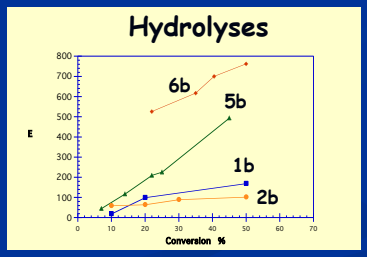
(R) **-1b** and **2b**
(S) **-5b** and **6b**

(S) **-1a** and **2a**
(R) **-5a** and **6a**

1a $R_1 = CH_3$, $R_2 = Ph$
2a $R_1 = CH_2CH_3$, $R_2 = Ph$
5a $R_1 = Br$, $R_2 = Ph$
6a $R_1 = Cl$, $R_2 = Ph$



Decrease of *E*



Increase of *E*

Jacobsen, E. E. *et al., Tetrahedron Lett.* 2003, *44*, 8453-8455

Possible reasons for change of *E*-value

- **Calculation of *E* assumes that experimental conditions do not change during reaction**
  hydrolyses and transesterifications:
  Decrease in substrate concentration, increase in product concentration

- **Enantioselective inhibition by enantiopure esters?**
  Increasing amount of *(R)*-ester in transesterification: decrease of *E*
  Decreasing amount of *(R)*-ester in hydrolysis: increase of *E*

- **Enantioselective inhibition by *(R)*-alcohol?**
  Decreasing amount of *(R)*-alcohol in transesterification: decrease of *E*
  Increasing amount of *(R)*-alcohol in hydrolysis: increase of *E*

- **Influence of immobilization?**
  Compare with pure enzyme preparation

Addition of enantiopure esters

Esterification reaction of **1a:**

- High starting selectivity (*E*=450), not much ester formed
- Low end selectivity (*E*=50), much *R*-ester formed

Start of reacton:
Addition of*(R)*-**1b** (faster) to the transesterification reaction of **2a**
No effect on *E*-value

Addtion of *(R)*-**6b** (slower) to the transesterification reaction of **1a**
No effect on *E*-value

Conclusion : No inhibition by the esters

R-alcohols interact with enzyme

SaturationTransferDifference-NMR studies:

***Enzyme preparation*: Novozym 525 F from Novozymes AS, freeze dried**

***Additives*: (*R*) and (*S*)-2-methyl-1,4-butanediol, mw 104.05 g/mol, Merck**

NMR Sample I: (*R*)-2-methyl-1,4-butanediol in 0.5 mL D_20 and pure enzyme
Result: Shows interaction with enzyme

NMR Sample II: (*S*)-2-methyl-1,4-butanediol D_20 with pure enzyme
Result: Shows no interaction with enzyme

Further experiments are under investigation

University of Bucharesti
08.10.2019

H.W. Anthonsen, E.E. Jacobsen and T. Anthonsen, to be published 34

How can the *R*-alcohols increase selectivity in CALB?

- Possible allosteric site (another site than active site) on the surface of CALB

- **Binding of the *R*-alcohols make the active site more suited for the faster reacting enantiomer giving enhanced selectivity**

However, the added enantiomers from our experiments are also esterified by time, indicating that the allosteric binding is reversible.

Challenge: to make an irreversible ACTIVATOR

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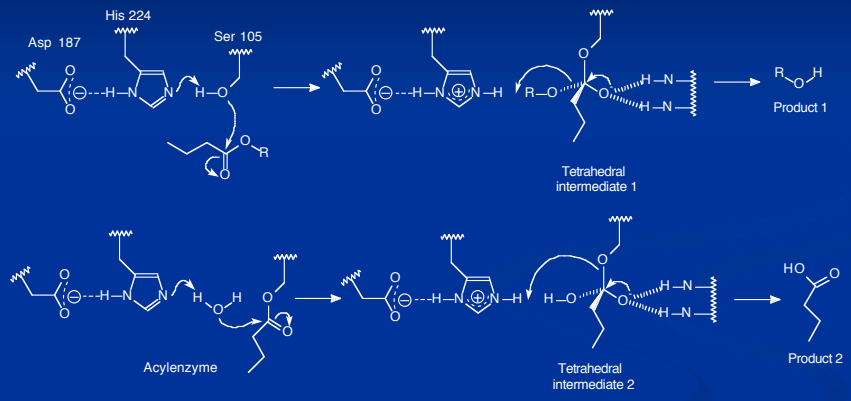
CAL-B catalysis mechanism

Hydrolysis of racemic ester



Change of amounts of different substances in the reaction mixture will affect the results

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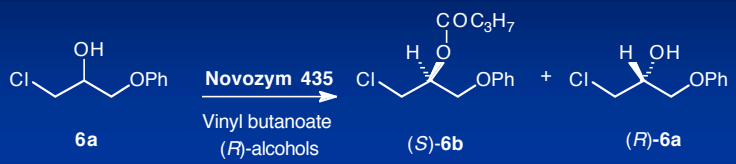
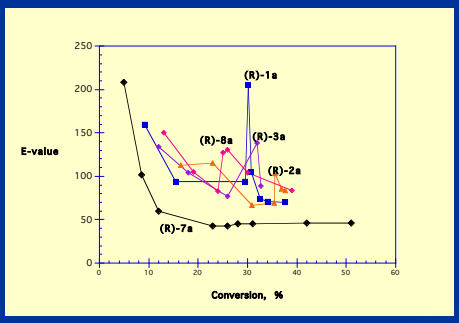
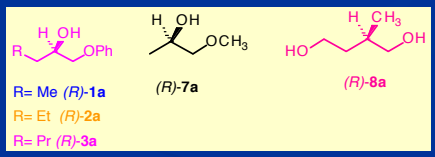
08.10.2019
36

Addition of enantiopure alcohols





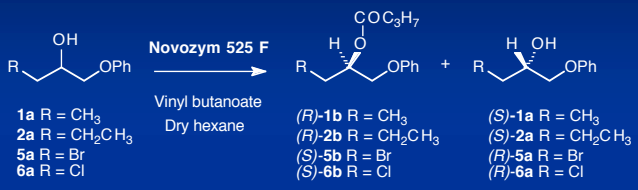


E increased by addition of the enantiopure alcohols (*R*)-**1a**-(*R*)-**6a** and (*R*)-**8a** but not when (*R*)-**7a** was added

Jacobsen, E. E. *et al., Tetrahedron Lett.* 2003, *44*, 8453-8455

Novozym CAL-B 525 F: E-value changes

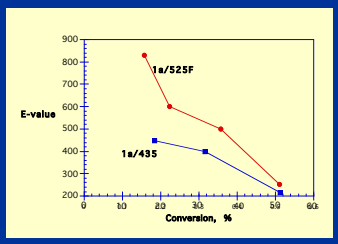
Pure protein-freeze dried-no immobilisation

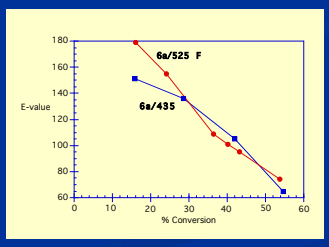


1a R = CH_3
2a R = CH_2CH_3
5a R = Br
6a R = Cl

(*R*)-**1b** R = CH_3
(*R*)-**2b** R = CH_2CH_3
(*S*)-**5b** R = Br
(*S*)-**6b** R = Cl

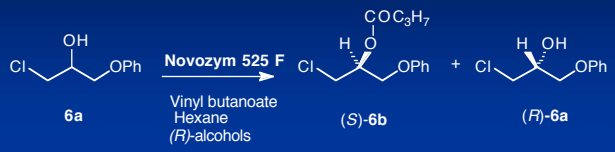
(*S*)-**1a** R = CH_3
(*S*)-**2a** R = CH_2CH_3
(*R*)-**5a** R = Br
(*R*)-**6a** R = Cl



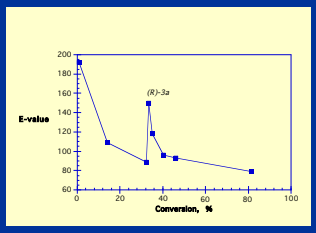


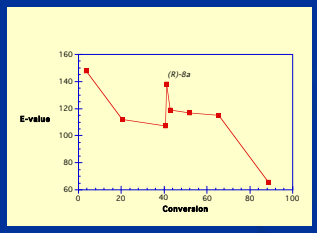
Enantioselectivity decreased with increasing conversion also in esterifications of **1a**, **2a**, **5a** and **6a** catalyzed by CAL-B Novozym 525 F

Addition of enantiopure alcohols in CAL-B 525 F reactions



6a (S)-6b (R)-6a

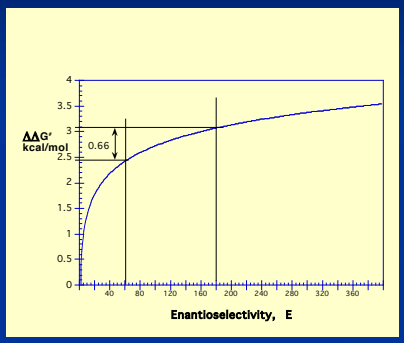




Enantioselectivity increased by addition of (*R*)-alcohols also in esterifications of **6a** catalyzed by CAL-B Novozym 525 F

Jacobsen, E.E, Andresen, L.S. and Anthonsen, T. Tetrahedron: *Asymmetry* 16 (2005) 847–850

Decrease in *E*, difference in $\Delta G^{\#}$



A decrease of *E* from 180-60 is due to a decrease of $\Delta G^{\#}$ of 0.66 kcal/mole

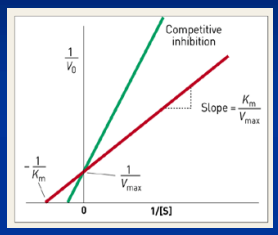
Change in enzyme conformation due to allosteric effect?

Ways to reveal an allosteric effect

Enzyme kinetics:

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

$$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$



But: Substrate is the "inhibitor"!

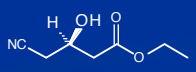
- NMR-studies of the CAL-B binding pattern
- Molecular modelling
- X-ray crystallography

• Guo, Z.-W. and C.J. Sih, J. Am. Chem. Soc., 1989: 6836-6841.
• Itoh, T., Ohira, E. Takagi, Y., Nishiyama, S., and Nakamura, K., Bull. Chem. Soc. Jpn., 1991. **64**: 624-627.
• Ammon, R. and H. Fischgold, Biochem.Z, 1931. **234**: 54.

Building blocks for drugs by asymmetric synthesis

Atorvastatin:Tissue selective inhibitor of HMG-CoA Reductase

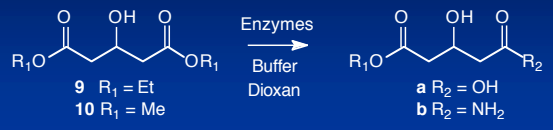
Atorvastatin



ethyl *(3R)*-4-cyano-3-hydroxybutanoate

α-Chymotrypsin gave the *R*-configuration of the mono ester
But: ee only 50 %

Enzyme catalyzed asymmetrisation of diesters of 3-hydroxyglutaric acid



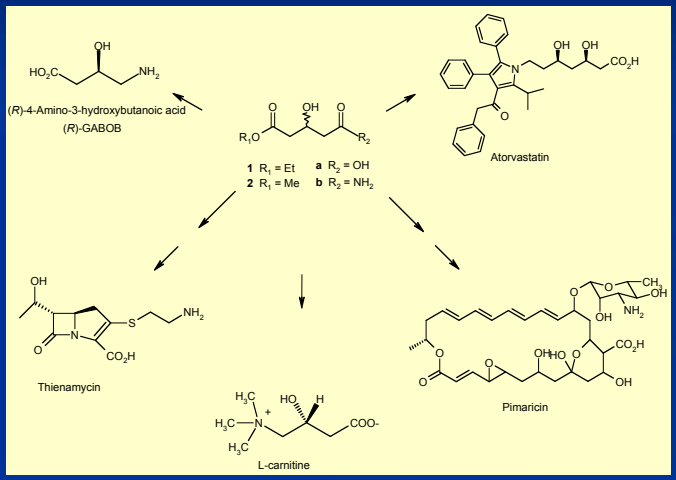
9 R_1 = Et
10 R_1 = Me

a R_2 = OH
b R_2 = NH_2

Prod.	Enzyme	Activity	% *ee*	% yield	$[\alpha]_D^{20}$	Conf.
9a	CAL-B	7 PLU/mg	91	80	+ 1.8 (c11.5, acetone)	*(S)*
9a	CAL-A		91	77	+ 1.8 (c11.5, acetone)	*(S)*
9a	CLEC-CAL-B	17 U/mg	86	80		*(S)*
9a	HLL		72	89		*(S)*
9a	RML	60 U/g	74	89		*(S)*
9a	PLE	15 U/mg	35	76	+ 0.2 (c 11.5, acetone	*(S)*
9a	α-Chymotrypsin	70 U/mg	50	65		*(R)*
9a	*A. lwoffii*	(cell cult.)	56			*(S)*
10a	CAL-B	7 PLU/mg	90	70	+ 0.8 (c11.5, acetone)	*(S)*
10a	PLE		22	75		*(S)*
10a	α-Chymotrypsin		45	59		*(R)*
10a	MCL	cell prep.	75	70		*(S)*
9b	CAL-B	7 PLU/mg	98	95	– 6.9 (c10.0, dioxan) – 6.5 (c1.3, $CHCl_3$)	*(S)*
10b	CAL-B	7 PLU/mg	98	95	– 2.0 (c3.5, dioxan)	*(S)*

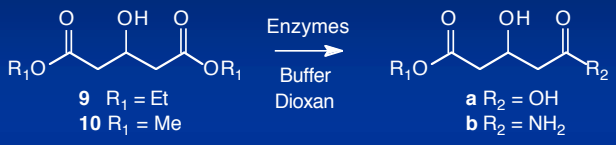
Jacobsen, E. E. *et al*, *J. Mol. Catal. B: Enz.* **2003,** *21*, 55-58

Industrial use of enantiopure monoesters of of 3-hydroxyglutaric acid



(*S*)-enantiomers also valuable synthons

Hydrolysis *vs.* ammonolysis



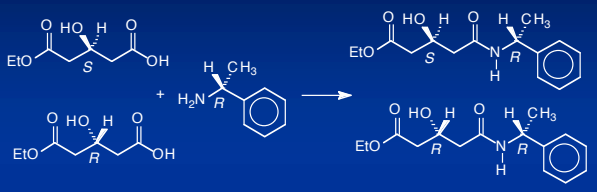
9 R_1 = Et
10 R_1 = Me

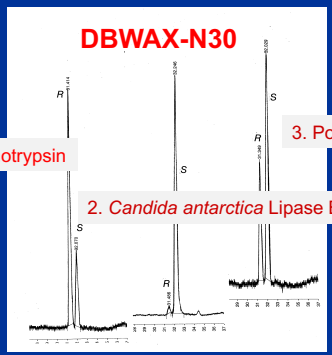
a R_2 = OH
b R_2 = NH_2

Product	Enzyme	Medium	% ee	% yield	Config.
1a	CALB	Buffer	91	80	*(S)*
2a	CALB	Buffer	90	70	*(S)*
2a	PLE	Buffer	22	75	*(S)*
1b	CALB	Dioxane/NH_3	98	95	*(S)*
2b	CALB	Dioxane/NH_3	98	95	*(S)*

Ammonolysis gives better selectivity than hydrolysis
BUT: Dioxane not safe solvent

GLC of diastereomeric derivatives

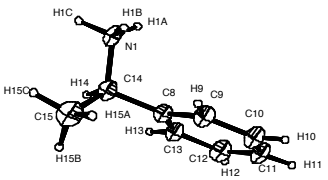


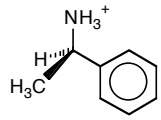


α-Chymotrypsin was reported to give 100 % ee of the (*R*)-enantiomer

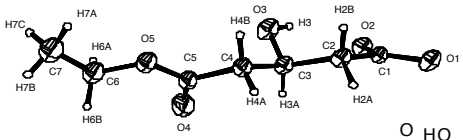
α-Chymotrypsin in our hands gave 50 % ee of the (*R*)-enantiomer

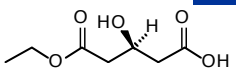
Configuration from X-ray of diastereomeric salt





(*R*)-phenylethylamine



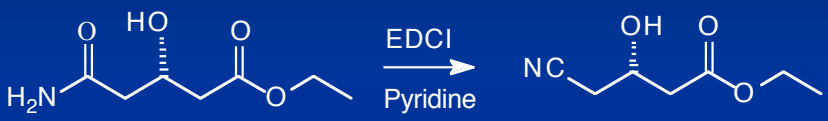


(*3S*)-3-hydroxy pentanedioic acid ethyl ester

Large scale: We have synthesized 200 g of this enantiopure acid

Moen, A. R. *et al.*, Tetrahedron:*Asymmetry.* 2004, *15*, 1551-1554

Synthesis of ethyl (3*S*)-4-cyano-3-hydroxybutanoate



(*S*)-**9b** (*S*)-**11**

Yield: 90 %
ee: 98 % GLC Chirasil DEX

Inversion of configuration:

- Mitsunobu esterification (possibly not effective due to elimination)
- Via mesylate ester

***N*-Ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride**

Conclusions I

(*R*)- and (*S*)-1-Phenoxy-2-butanol, (*R*)- and (*S*)-**1a**, and (*R*)- and (*S*)-1-benzyloxy-2-butanol, (*R*)- and (*S*)-**4a**, have been produced in gram scale by CAL-B catalysed esterifications in 99 % ee
(*R*)-1-Phenoxy-2-pentanol, (*R*)-**2a**, were produced in gram scale by CAL-B catalysed hydrolysis in 99 % ee

The enantioselectivity, *E*-value, depends on:

- the chain length and the electronegativity of the small substituent and also of the size of the large substituents in secondary alcohols
- the different acyl donors in transesterification reactions of 1-phenoxy-2-pentanol (**2a**) and 1-benzyloxy-2-butanol (**4a**)
- the water content and not water activity in polar solvents in esterifications of 3-bromo- and 3-chloro-1-phenoxy-2-propanol (**5a** and **6a**)