

Chemo-enzymatic synthesis of enantiopure stiripentol and

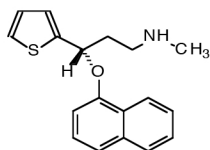
β -antagonists atenolol, metoprolol, practolol and pindolol and β -agonist clenbuterol precursor

Disclaimer: This was realised with the EEA Financial Mechanism 2014-2021 financial support. Its content (text, photos, videos) does not reflect the official opinion of the Programme Operator, the National Contact Point and the Financial Mechanism Office. Responsibility for the information and views expressed therein lies entirely with the author(s).

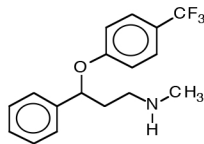
Enantiopure drugs in Trondheim

both enantiomers synthesised by lipase catalysed kinetic resolution (99 % ee)

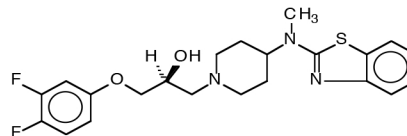
Non-tricyclic antidepressants
Inhibits serotonin re-uptake



(S)-(+)-Duloxetine
ER=2
Cymbalta by Lilly



Fluoxetine
Prozac, Fontex
Racemate by Lilly



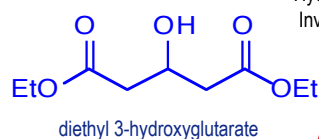
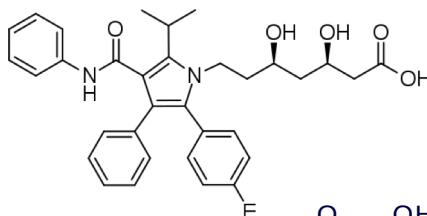
(2S)-Lubeluzole
Prosynap by Johnsen & Johnsen

Liu et al. *J. Chem. Soc. Perkin Trans. 1*, 2000, 1767 and *Chirality*, 13, 2001, 135

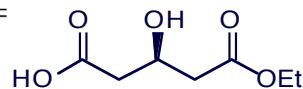
Chiral side chain of Atorvastatin by asymmetrisation of prochiral diethyl 3-hydroxyglutarate



Lipase B from *Candida antarctica*

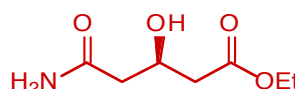


Asymmetric
Hydrolysis
Inversion



(*R*)-5-ethoxy-3-hydroxy-5-oxopentanoic acid

Asymmetric
Ammonolysis
Inversion



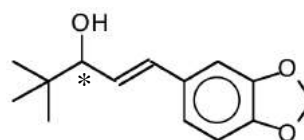
91% ee

ethyl (*R*)-5-amino-3-hydroxy-5-oxopentanoate

Jacobsen et al. *J. Mol. Catal. B, Enzymatic*, 21, 2003, 55-58, Riise Moen et al. *Tetrahedron: Asymmetry*, 2004, 15, 1551-1554

NTNU

rac-Stiripentol – allylic aromatic alcohol 1 stereocenter

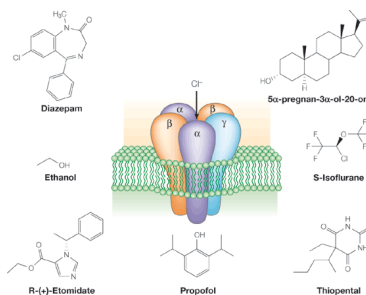


Diacomif™ by Bloodex

- unlike other antiepileptic drugs
- potent for children's epilepsy; infants with Dravet syndrome
- under investigation since 1970's, used in France and Canada since 1995, FDA appr. 2008
- used together with valproate and clobazam (onfi),
- also useful in autism treatment

➤ acts directly on the GABA_A receptor as a positive allosteric modulator at the $\alpha 3$ subunit: increases γ -aminobutyric acid in brain

➤ also indirect mechanism; inhibits liver microsomal enzymes (CYT P450): increases plasma conc. of other drugs



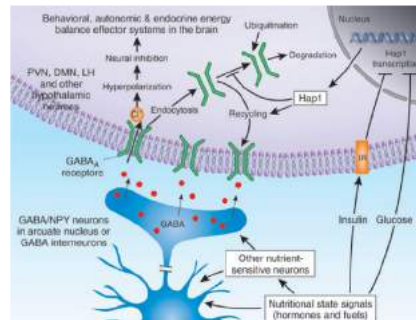
Nature Reviews | Neuroscience

Fischer, *J. Neuropharmacology* 2009, 56, 190-197, Chiron, C. *Expert Opin. Investig. Drugs* 2005, 14, 905-911

NTNU

Epilepsy

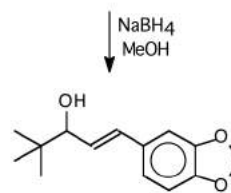
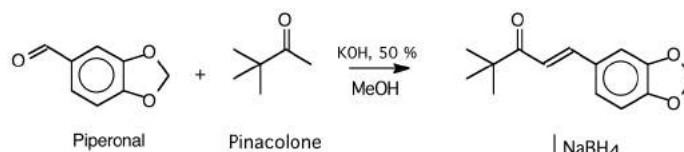
- central nervous system pathology characterised by seizures, affects 1-2 %
- treated by several drugs, f. inst acetazolamide, clonacepam, carbamacepine, pregabalin, clobazam, valproate, stiripentol
- several act on gamma aminobutyric acid receptor (GABA)
- epilepsy patiens may have mutations in GABA_A



NTNU

Stiripentol synthesis

(±)-1-(benzo[d][1,3]dioxol-5-yl)-4,4-dimethylpent-1-en-3-ol ((±)-1)



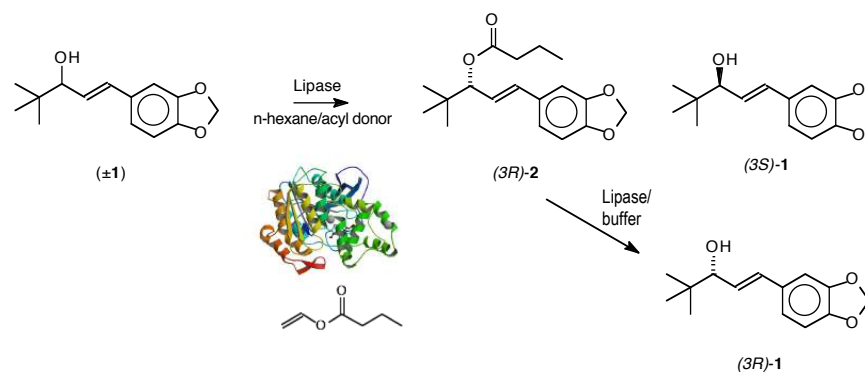
(+)-enantiomer 2.5 times more potent than (-)-enantiomer
 (-)-enantiomer accumulates in tissue
 However, racemisation observed in rats

Yield: 8.0 g, 79 %

Vallet F. M. J. US 3910959, 1975, Trojnar, M. K. et al., *Pharmacological Reports* 2005, 154-160, Arends, R.H. et al. *Epilepsy Res.* 1994, 18,91-96, Davies, N.M., 2004

NTNU

(R)-Stiripentol from esterification and hydrolysis

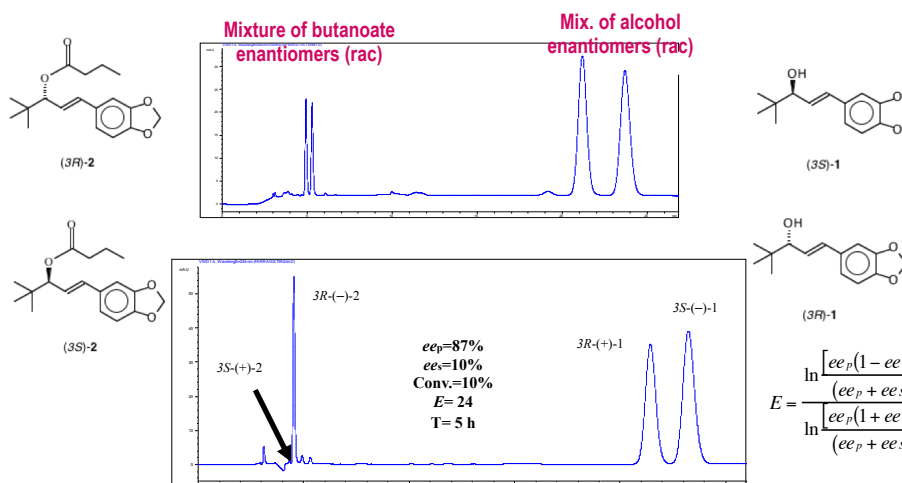


Candida antarctica lipase A (Novozym 735) and vinyl butanoate, E-value 24
 E-value: Ratio of the specificity constants (k_{cat}/K_M) of the enzyme for the two enantiomers
 An E-value of 24: One enantiomer reacts 24 times faster than the other

Jacobsen, E.E. et al *Lipase Catalysed Kinetic Resolution of Stiripentol*. *Int. J. Chem.* **2012**, 4 (1) 7-13

NTNU

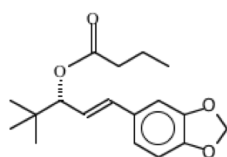
Chiral HPLC Chirasil OD-H, n-Hex/MTBE/2-PrOH



enantiomers identified from lipase preference and from optical rotation value comparisons

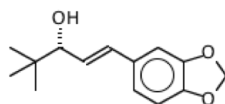
NTNU

ee profile of kinetic resolution of *rac*-1, 106 h *E*-value 24



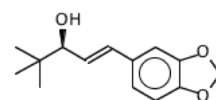
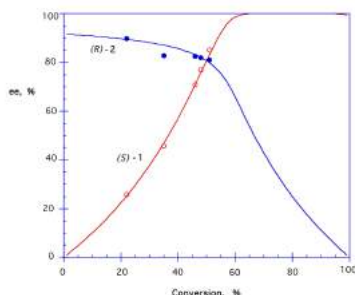
(3*R*)-2

Ester product
(3*R*)-2, *ee*_p, 87 % *ee*



(3*R*)-1

(3*R*)-1, 94 % *ee*



(3*S*)-1

Remaining alcohol
(3*S*)-1, *ee*_s, 86 % *ee*

50 % waste?

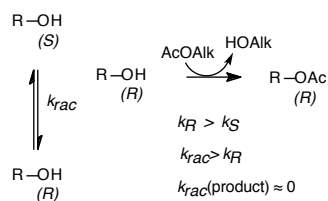
**Low *E*-value:
Low yield of high *ee*-enantiomer product
Always high *ee* of unconverted
enantiomer!**

NTNU

Deracemization

100 % yield and 100 % *ee* from racemate

1. Dynamic Kinetic Resolution



(*R*)- is the faster reacting
enantiomer
(*S*)- is racemized

The enzyme always encounters
a racemic mixture

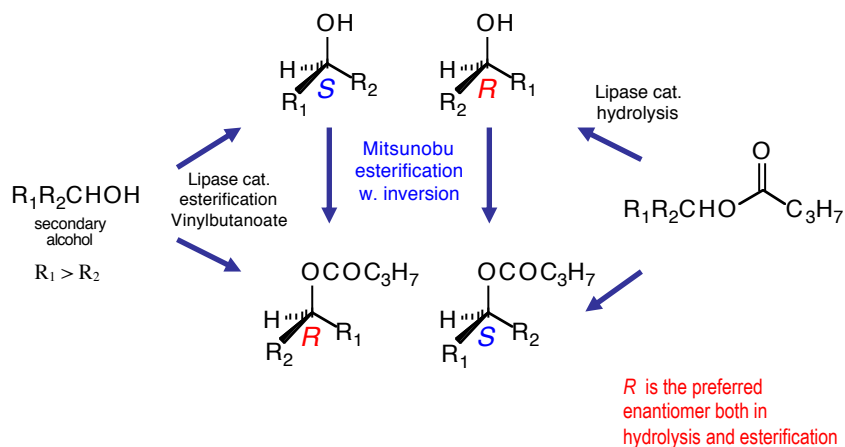
2. Stereoinversion by Mitsunobu esterification

(*R*)- Substrate is converted to (*R*)-product

(*S*)- Substrate is converted to the same (*R*)-product in another reaction without
separation ("one pot")

NTNU

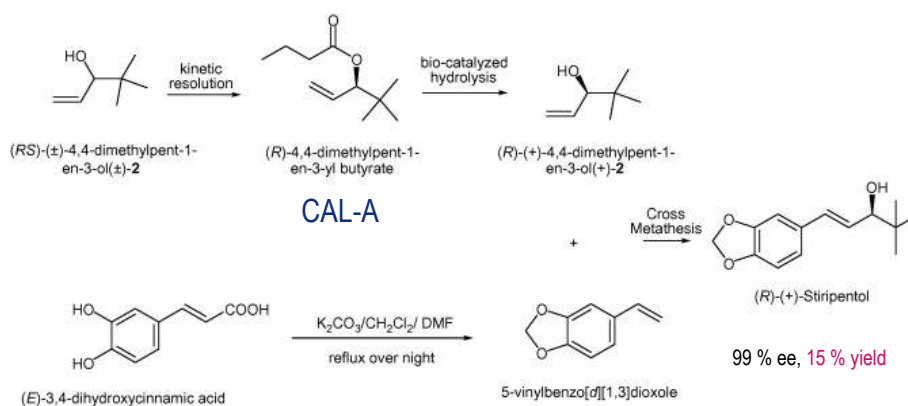
Stereoinversion



Liu & Anthonsen, *Chirality*, 14, 2002, 25

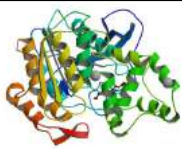
NTNU

(*R*)-Stiripentol by combination of lipase catalysed resolution and alkene metathesis



El-Behairy, M et al. *Tetrahedron: Asymmetry* 24, 2013 285–289

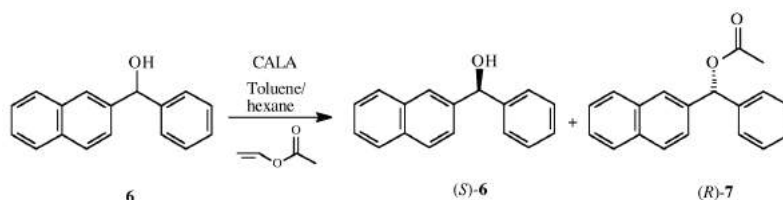
NTNU



Lipase A from *Candida antarctica*, CALA

R-selective catalyst in resolutions of secondary and tertiary alcohols with bulky groups around the stereocenter

Resolution of phenyl-(2-naphthyl) methanol by CALA, *E*-value 67:



Tjosås, F., Anthonsen, T., Jacobsen, E. E. *ARKIVOC* 2008, vi, 81-90.

Crystal structure of CALA (441 amino acids) has been determined:

Ericsson, D. J. et al. *J. Mol. Biol.* 2008, 376, 109-119.

NTNU

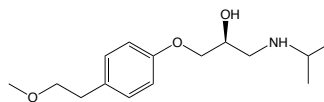
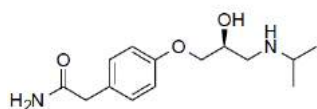
Conclusions

- (*R*)-Stiripentol has been synthesised in 94% *ee* in 60 % yield by kinetic resolution of *rac*-Stiripentol catalysed by lipase A from *Candida antarctica*.
- (*R*)-Stiripentol was also synthesised by a combination of lipase catalysed resolution and alkene metathesis in 99 % *ee* and 15 % yield.
- Wanted: 99 % *ee* AND 60 % yield!
- Lipase catalysis is a green, mild and enantioselective method to obtain pure enantiomers both from prochiral substrates and from secondary alcohols etc

NTNU

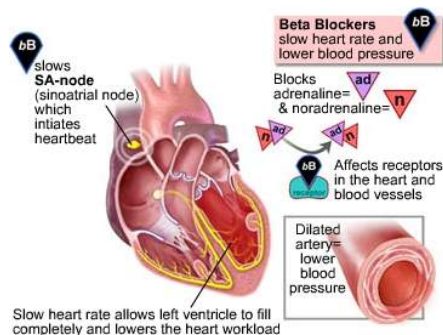
Enantiopure cardioselective β -blockers atenolol and metoprolol

Only the *S*-enantiomers have the desired effect



Atenolol is manufactured as Atpure® with enantiopure active pharmaceutical ingredient (API).

Mylan® has the racemic API



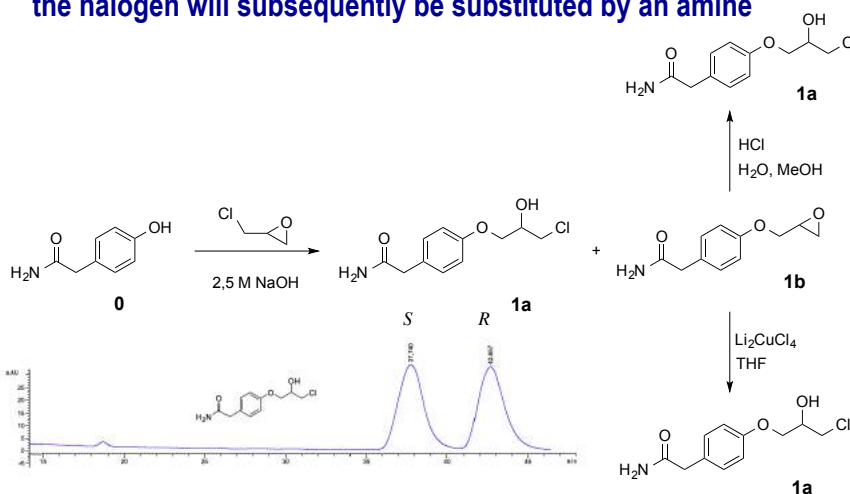
Metoprolol was the 14th most sold drug in Norway in 2017, and the most sold drug against hypertension in USA.

Marketed as Toprol-XL® by Astra Zeneca and as Lopressor® by Novartis, both with racemic API.

<http://www.cardiachealth.org/heart-disease-treatment/heart-disease-medications/blood-pressure-medications/beta-blockers>

NTNU

Organic synthesis of racemic building block for atenolol- the halogen will subsequently be substituted by an amine



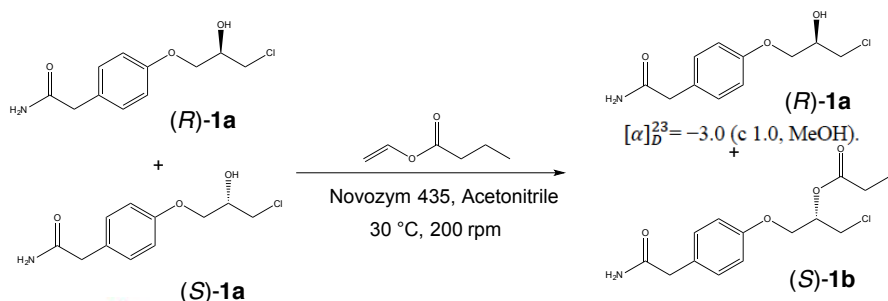
Enantiomers of **1a** were separated by HPLC on a Chiralcel OD-H column, eluent hexane:2-propanol (83:17, v:v) flow 1.000 mL min⁻¹, UV 254 nm.

Yield (80%):

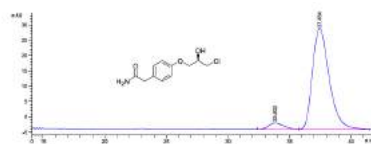
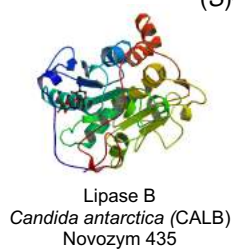
1a sticky and slimy compound

NTNU

Kinetic resolution of racemic building block by CALB



both ester and alcohol in 98-99 % ee



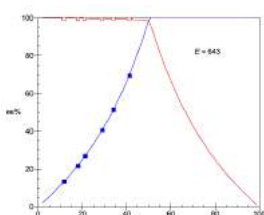
HPLC chromatogram of $(R)\text{-1a}$ after separation from $(S)\text{-1b}$ by flash chromatography.

Enzyme preference determines the right enantiomer:

the S-enantiomer will be converted

NTNU

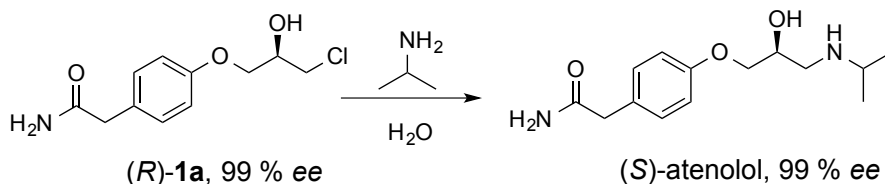
Enantiopure (S)-atenolol



$$E = \frac{k_{sp}^R}{k_{sp}^S} = \frac{(k_{cat} / K_M)^R}{(k_{cat} / K_M)^S}$$

$$E = \frac{\ln \left[\frac{ee_p(1 - ee_s)}{ee_p + ee_s} \right]}{\ln \left[\frac{ee_p(1 + ee_s)}{ee_p + ee_s} \right]}$$

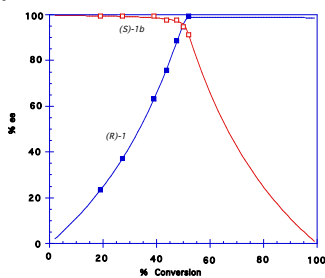
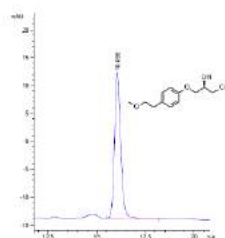
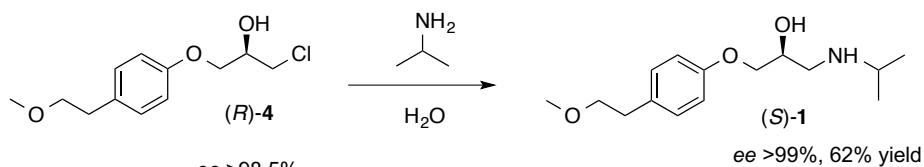
High E -value (>200):
 One enantiomer is converted 200 times
 faster to the ester than the other



Lund, I.T., Bøckmann, P.L., Jacobsen, E.E. *Tetrahedron* **2016**, 72, 7288-7294

NTNU

Enantiopure (S)-metoprolol, (S)-1



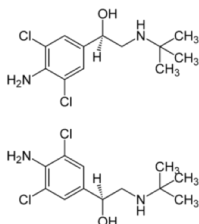
$E = > 200$

$[\alpha]_D^{20} = -39.78^\circ (c 0.88, \text{MeOH})$

Blindheim, F.H. Bøckmann, P.L. Jacobsen, E.E. Manuscript in preparation

NTNU

(R)-clenbuterol, a β_2 -agonist - opposite of β -blocker



Widely used as medication for horses [1]

Ventipulmin®

Reduces fat and increase muscle mass.

«Superclen» (clen+tyroxine)

Clenbuterol is a β_2 -agonist which has been widely used as bronchodilator in treatment of asthma and chronic obstructive pulmonary disease in Spain, Germany, Italy and Austria [2,3].

- **HOWEVER it is on the WADA list! Alberto Contador tested positive on clenbuterol**

The $R(-)$ -enantiomer is responsible for this activity and the $S(+)$ -enantiomer has no effect.

[1] Norton JL, Jackson K, Chen JW, et al. *J Vet Intern Med* **2013**, *27*, 1523.

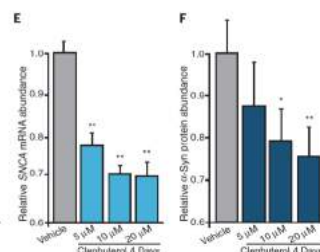
[2] Boner, AL, Vallone, G., Brighenti, C., Schiassi, M., Miglioranza, P. and Richelli, C. *Pediatr. Pulmonol.* **1988**, *4*, 197.

[3] Papiris, S. Galvotti, V. and Sturani, C. *Respiration* **1986**, *49*, 101.

NTNU

β 2-Adrenoreceptor is a regulator of the α -synuclein gene driving risk of Parkinson's disease

No.	Name	Class	Structure	FDA approved	Indication	Blood-brain penetrant
1.	Metaproterenol	β 2-Adrenoreceptor Agonist		Yes	Asthma	No
2.	Clenbuterol	β 2-Adrenoreceptor Agonist		No	Asthma	Yes
3.	Salbutamol	β 2-Adrenoreceptor Agonist		Yes	Asthma	Yes



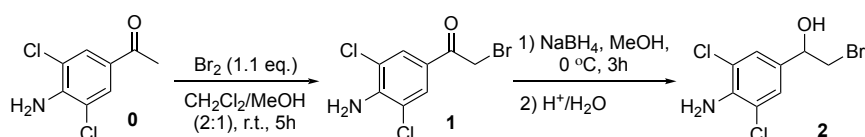
β 2AR agonist clenbuterol lowered the expression of SNCA mRNA (E) and α -Syn protein (F) in a dose-dependent manner in neuroblastoma cells

However, which of the enantiomers of clenbuterol is causing this effect??
We are under way of making them separately-just wait for it!

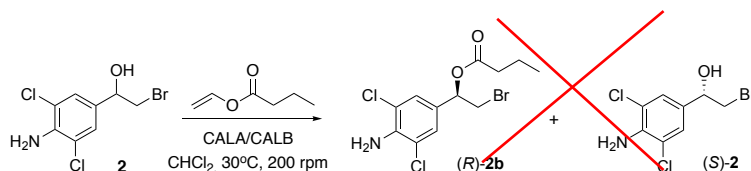
Mittal *et al.*, Science **2017**, 357, 891-898

NTNU

1-(4-Amino-3,5-dichlorophenyl)-2-bromoethan-1-ol (**2**)



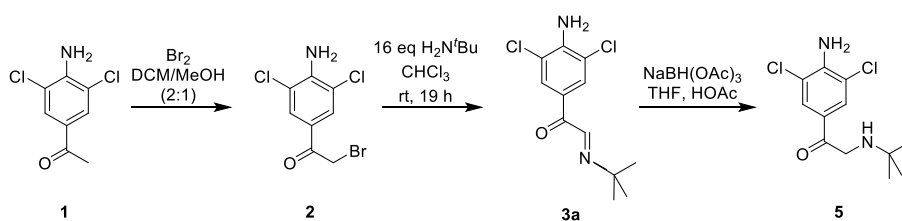
Transesterification of **2** with lipases



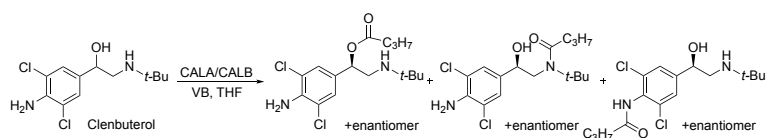
Several lipases used in kinetic resolutions-no conversion
Let's synthesise racemic clenbuterol and perform kinetic resolution

NTNU

However, problems with imine formation by addition of *t*-butylamine

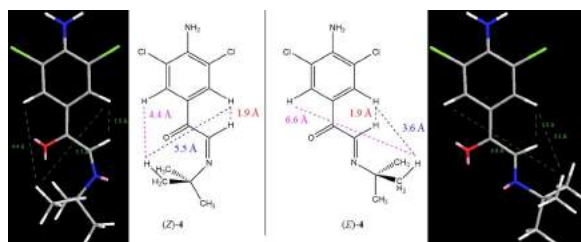


Transesterification of clenbuterol with lipases

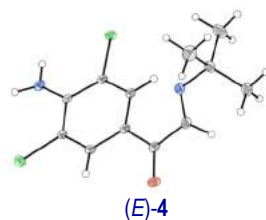


NTNU

(Z)- and (E)-4 modelled with their optimal geometries (minimal energy calculation - MM2), and distances measured in Chem3D (PerkinElmer Inc.)

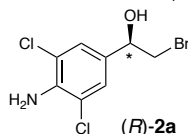
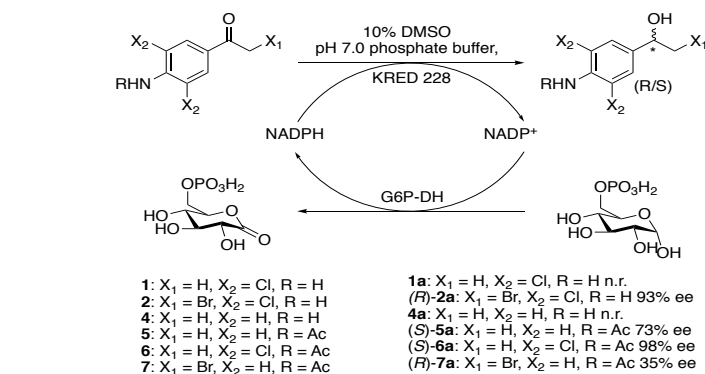


(E)-4 determined by X-ray analysis



NTNU

Building block for (*R*)-clenbuterol in 93% ee by KRED 228



Ketoreductase, KRED228

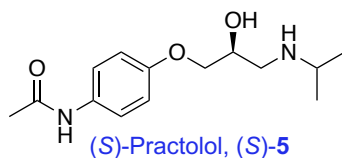
Syn Zymes
尚科生物医药

Blindheim, F.H., Hansen, M.B., Evjen, S., Zhu, W. and Jacobsen, E.E. Chemo-Enzymatic Synthesis of Enantiopure Synthons as Precursors for (*R*)-Clenbuterol and other β -2 agonists. *Catalysts*, 2018, 8 (11), 516 doi:10.3390/catal8110516

NTNU

Enantiopure cardioselective β -blockers practolol and pindolol

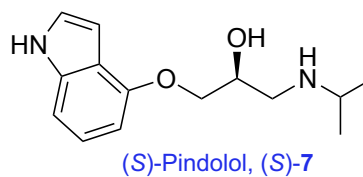
Only the *S*-enantiomers have the desired effect



Practolol

Selective β_1 -antagonist
First β_1 -selective β -blocker in treatment of cardiovascular diseases (1970s)

1. effective treatment of hearth failure
 2. arrhythmic hearth rate
 3. critical side-effects: oculomucocutaneous syndrome (dry eyes)
- Then withdrawn from the market.



Pindolol

β_1 -antagonist that reduces high blood pressure.

1. also used in treatment of angina pectoris
2. also been studied for use in treatment of depression as an add-on therapy to selective serotonin re-uptake inhibitors (SSRIs).

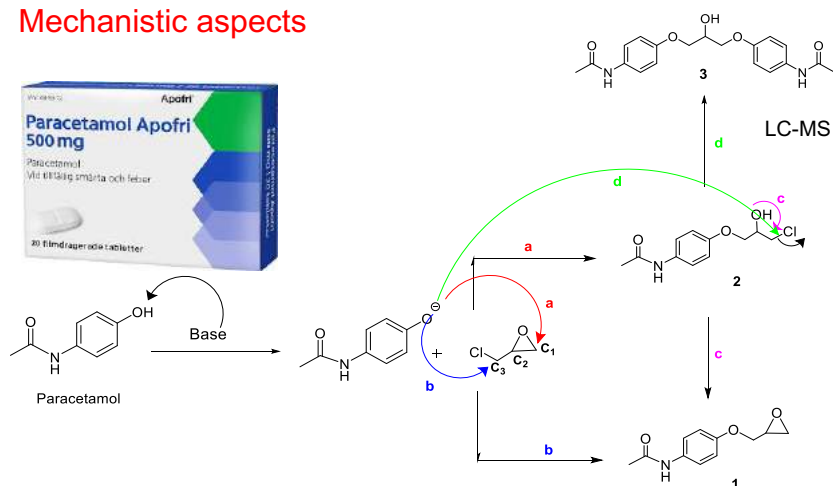
<http://www.cardiachealth.org/heart-disease-treatment/heart-disease-medications/blood-pressure-medications/beta-blockers>

NTNU

Step 1 Practolol:

N-(4-(3-chloro-2-hydroxypropoxy)phenyl)acetamide, **2**

Mechanistic aspects



Step 1: Different concentrations of NaOH

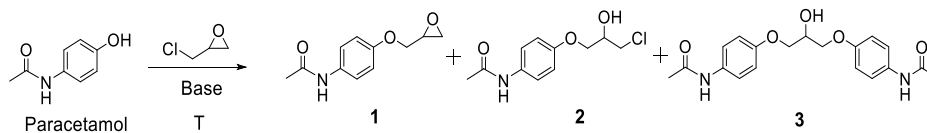
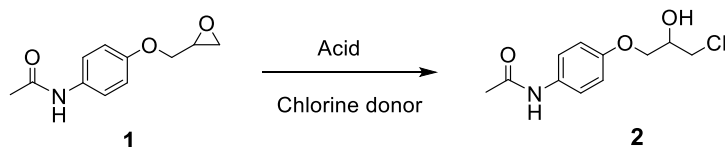


TABLE 1. Measured areas (amount) by GC, DB 1701 COLUMN, 100-270°C/10°C

Base	Equivalent	T	Time [h]	Amount 1 [%]	Amount 2 [%]	Amount 3 [%]
NaOH	0.1	r.t.	48	29	38	10
NaOH	0.5	r.t.	8	37	43	8
NaOH	1.0	r.t.	7	81	12	4
NaOH	2.0	r.t.	7	36	0	64
NaOH	10.0	r.t.	18	0	3	24

2 eq. Epichlorohydrin

Step 2: Addition of LiCl and Acetic Acid

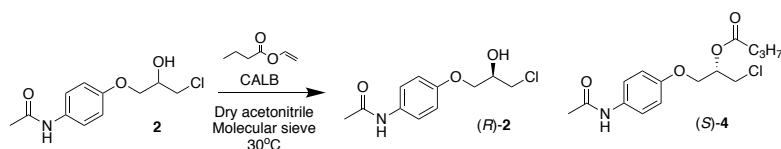


Equivalents LiCl	Equivalents Acetic acid	Time [h]	Amount 2 in GC [%]	Yield [%]
1.0	6.0	120	82	-
2.0	6.0	120	>99	43
4.0	6.0	48	82	-
4.0	10.0	26	98	62

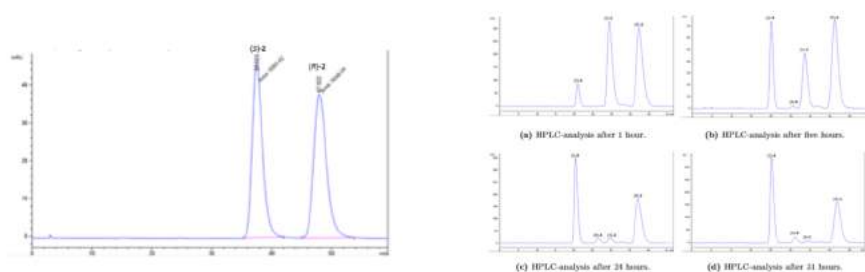
Similar results for pindolol building block, **6**

NTNU

Transesterification of **2** with CALB



Novozym 435: 93% ee
Syncozymes CAL-B: 96% ee



Chiralcel OD-H column, hexane-2-propanol (85:15), 1 mL/min

NTNU

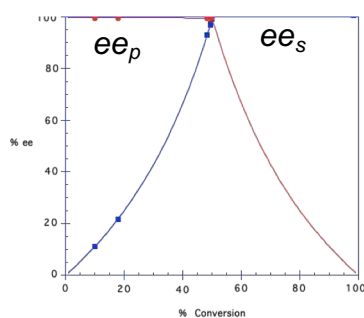
ee-values and E-values

Plotting ee-values into E & K Calculator : average E-value

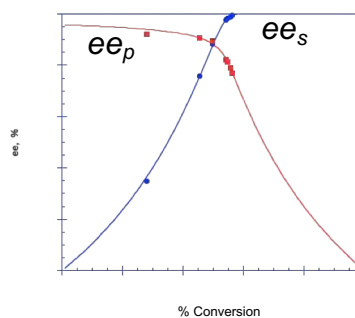
$$E = \frac{(k_{cat}/K_M)_R}{(k_{cat}/K_M)_S} \quad \Delta\Delta G^\ddagger = -RT \ln E$$

$$E = \frac{\ln \left[\frac{ee_p(1-ee_s)}{ee_p+ee_s} \right]}{\ln \left[\frac{ee_p(1+ee_s)}{ee_p+ee_s} \right]}$$

Practolol E=1000

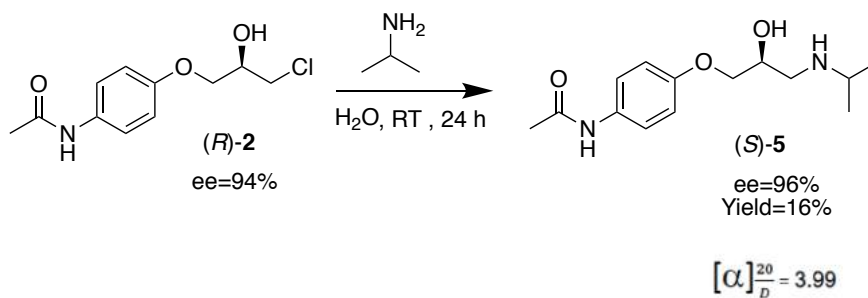


Pindolol E=46



NTNU

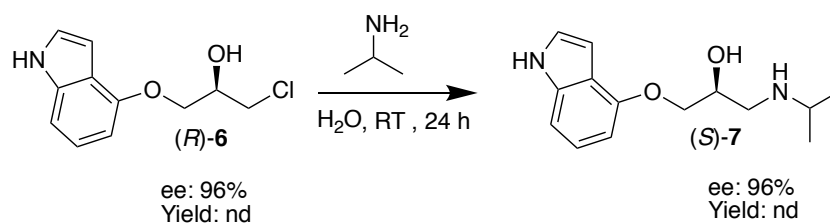
Synthesis of (S)-practolol, (S)-5



Hansen, Mari B., Austli, Guro B., Jacobsen, Elisabeth E. *Manuscript in preparation*

NTNU

Synthesis of (S)-pindolol, (S)-7

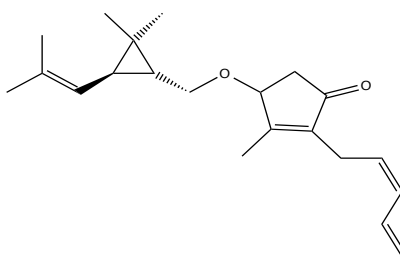


- Yield not determined due to residue of isopropylamine-will be improved
- Optical rotation not determined

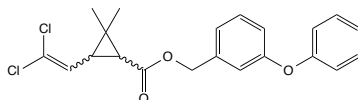
Dale, Oskar and Jacobsen, Elisabeth E. *Manuscript in preparation*

NTNU

Insect killers: Active ingredients pyrethrin and permethrin, inhibit insects' central nervous system



Pyrethrin-I from *Chrysanthemum cinerariaefolium*

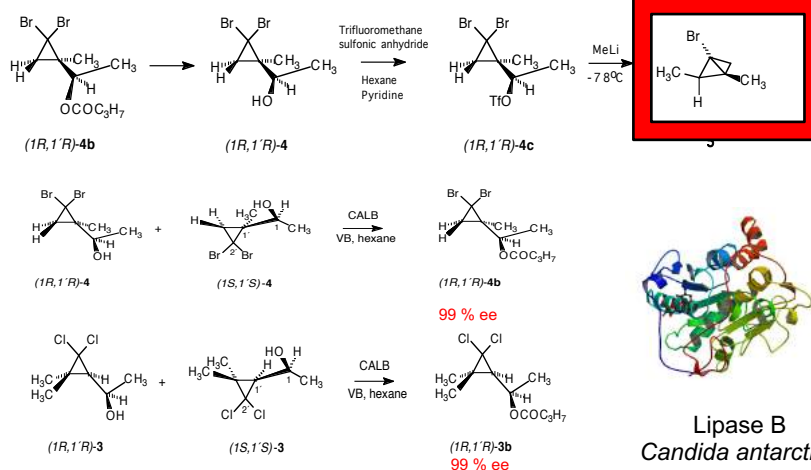


Permethrin has four stereoisomers. Industrial process

Martel, J. The development and manufacture of pyrethroid insecticides In: *Chirality in Industry*; Collins, A. N.; Sheldrake, G. N.; Crosby, J. Eds.; John Wiley & Sons: New York, 1992

NTNU

Enantiopure dihalobicyclobutanes – new building blocks



Jacobsen, E.E.; *et al.* Enantiopure dihalocyclopropyl alcohols and esters by lipase catalyzed kinetic resolution *J. Biotechnol.* **2013**, *168*, 284-288

NTNU

Thank you for your attention!



The Research Council of Norway (contract Grant Number 202903/11) and EEA grants (contract Grant Number 18-COP-0041) are thanked for funding

The Research Council of Norway

Iceland
Liechtenstein
Norway grants

viazym®

Syn Zymes
尚科生物医药

novozymes®

NTNU