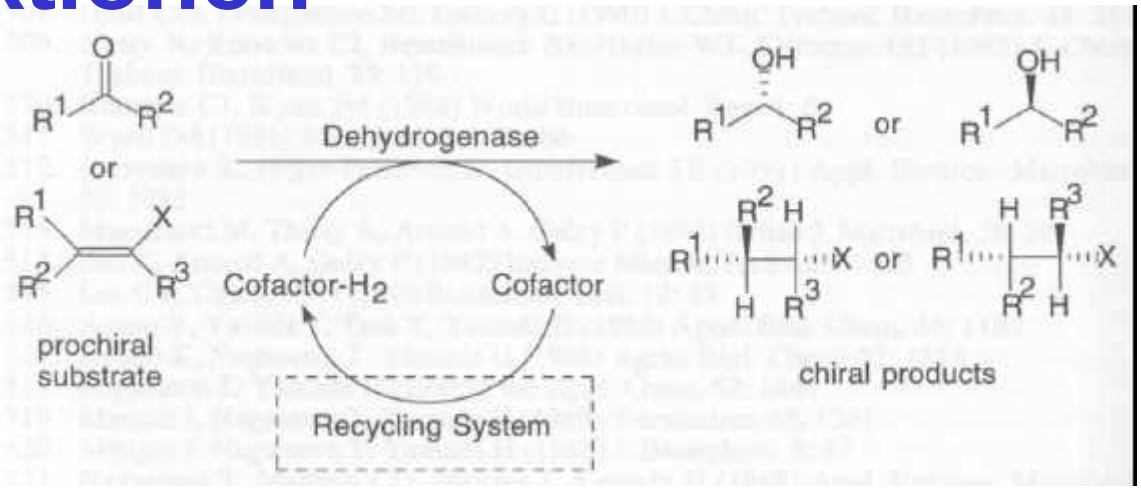
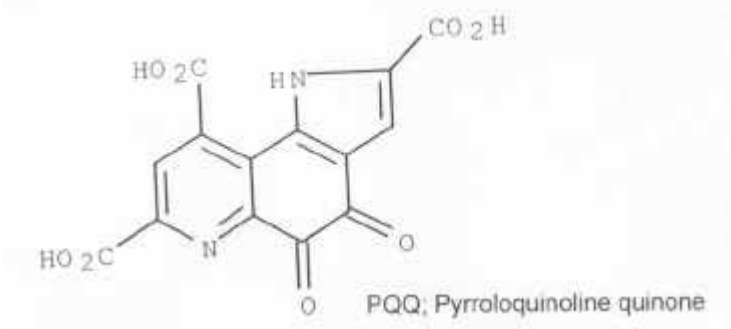
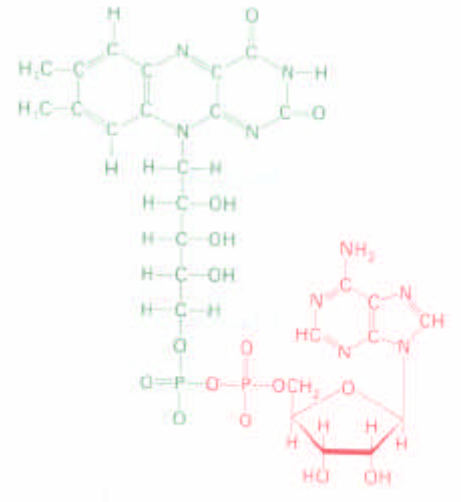
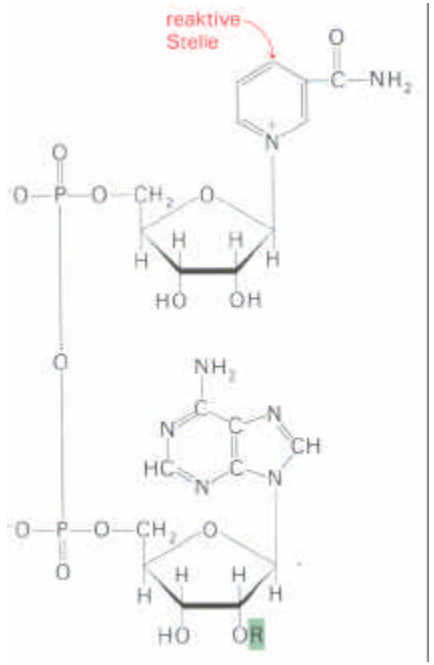
Reduktionen



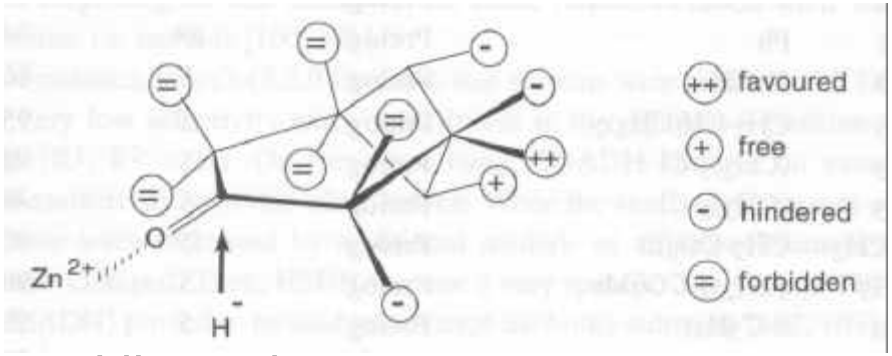
- Dehydrogenasen (650+ bekannt)
- Reduktion C=Hetero und C=C
- Cofaktoren
  - NADH (80%)
  - NADPH (10%)
  - FMN, FAD, PQQ



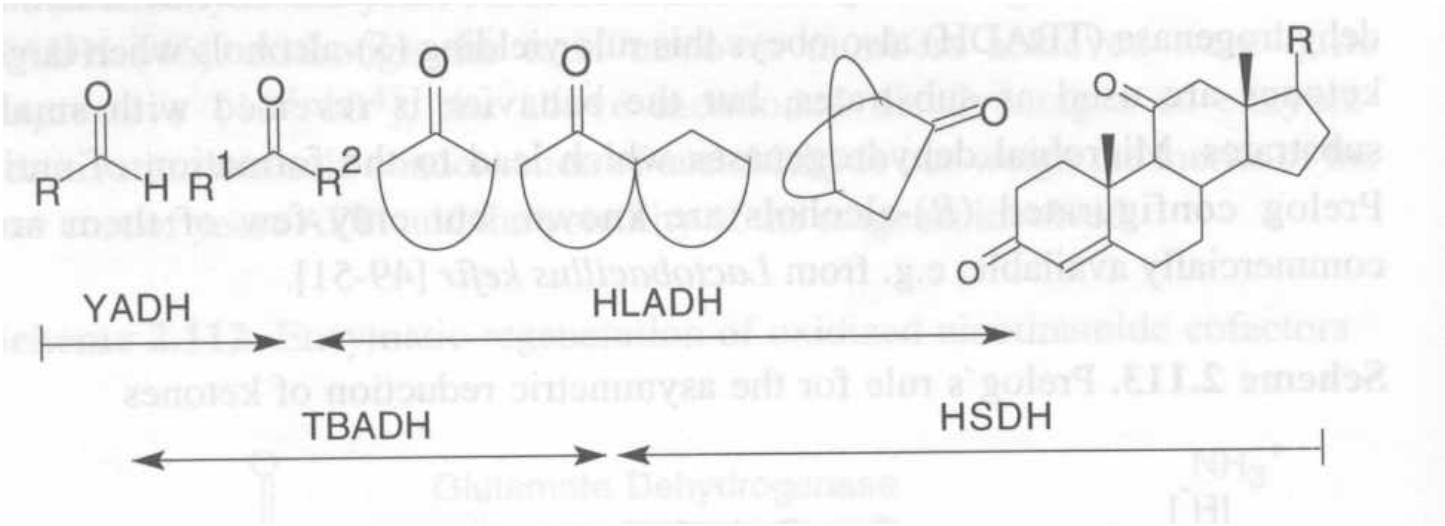




Dehydrogenasen



- HLADH (Pferdeleber ADH)
- YADH (Hefe ADH)
- TBADH (Thermoanaerobium brockii ADH)
- HSDH (Hydroxysteroid DH)
- CPCR (Candida parapsilosis Reduktase)



Dehydrogenasen

Enzyme	Coenzyme	1° Alc	2° Alc	ALD.	Acycl keto.	Simple cyclic keto.	Bulky Cycl. keto.	Arom. keto.	Diketo.	Unsat. keto.	2-Oxo ester.	3-Oxo ester.	4-Oxo ester.	5-Oxo ester.
HLADH	NADH	yes	yes	yes		yes	yes	yes	yes			yes	yes	
YLADH	NADH	yes	yes	yes	yes					yes				
TBADH	NADPH	yes	yes	yes	yes	yes	yes			yes	yes	yes	yes	yes
HSDH	NAD(P)H						yes					yes		
CPCR*	NADH	yes	yes	yes	yes	yes		yes	yes		yes	yes	yes	yes

*CPCR is one of the new reductases recently described it is not as yet commercially available but technical-grade enzyme can be apparently relatively easily isolated.

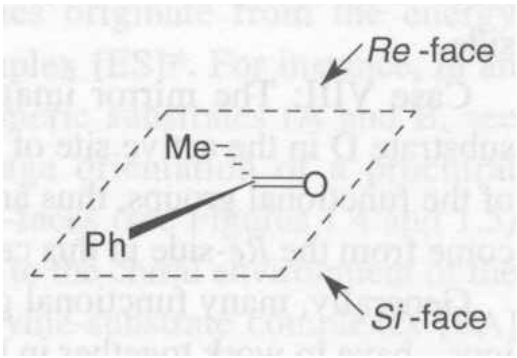
Dehydrogenasen

Enzyme	T Optim.	T Stabil.	pH Optim. Red.	pH Optim. Oxid.	pH Stabil.	Stereo specificity	Specific activity (Red)	Org. Solv accept.	Detrgent accept.	Inhibitors
HLADH	37	25	7	8-9	5-10	*re*-face	2.7	yes	yes	chelators, SH blockers, heavy metsl ions.
YLADH	30	10-25	7.15	8	6.6-9.5	*re*-face	>300	yes	no	
TBADH	50	65	7.5-8	7.8-9	7-9	*re*-face	8 (25°)	yes	no	SH blockers
HSDH	25	25	6.4-7	8-9	7.5-9.5	*re*-face	5-350	no	no	heavy metal ions
CPCR*	36-40	25	7-8.5	9-10.5	6-8	*re*-face	1800	yes	yes	chelators metal ions

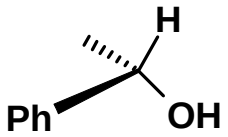
CPCR (*Candida parapsilosis*) is not commercially available, but the technical grade enzyme (40Umg^{-1}) is apparently quite easy to isolate in approximately 70% yield.

Dehydrogenasen

Prelog-Regel

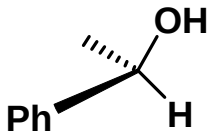


L-Reduktase



S-Alkohol

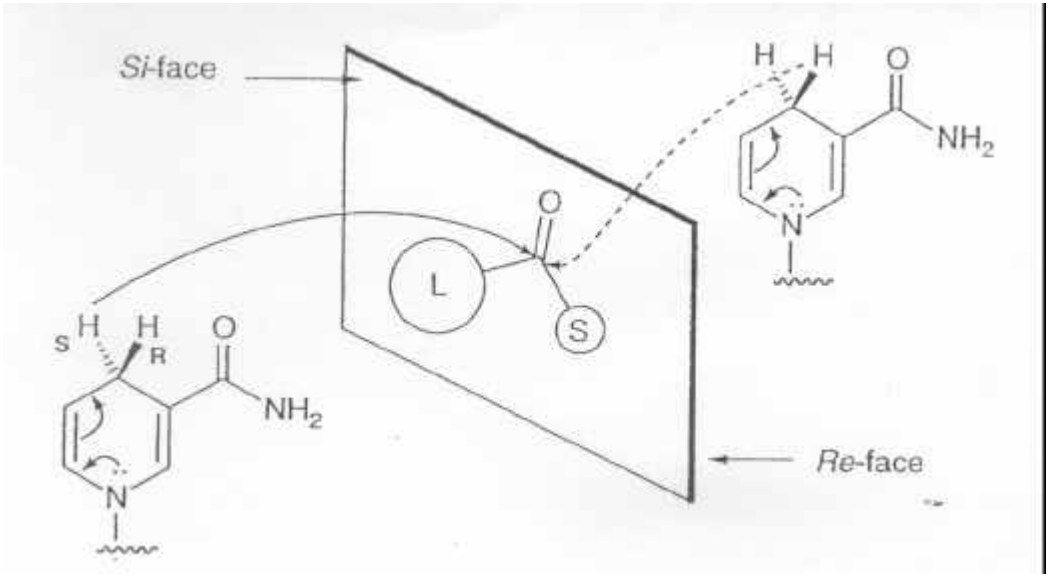
D-Reduktase



R-Alkohol

Dehydrogenasen

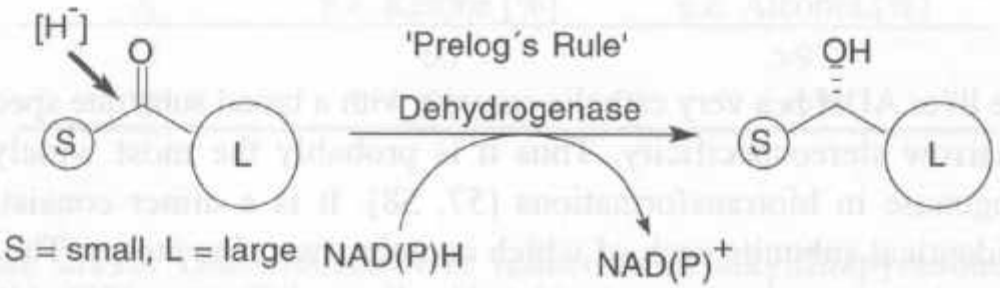
Prelog-Regel



- Hydrid-Angriff erfolgt von re-Seite
- Nomenklatur baut auf sterischen Effekten auf
- aufgestellt für Curvularia falcata Zellen
- Mehrzahl der Reduktasen folgt Regel

Dehydrogenasen

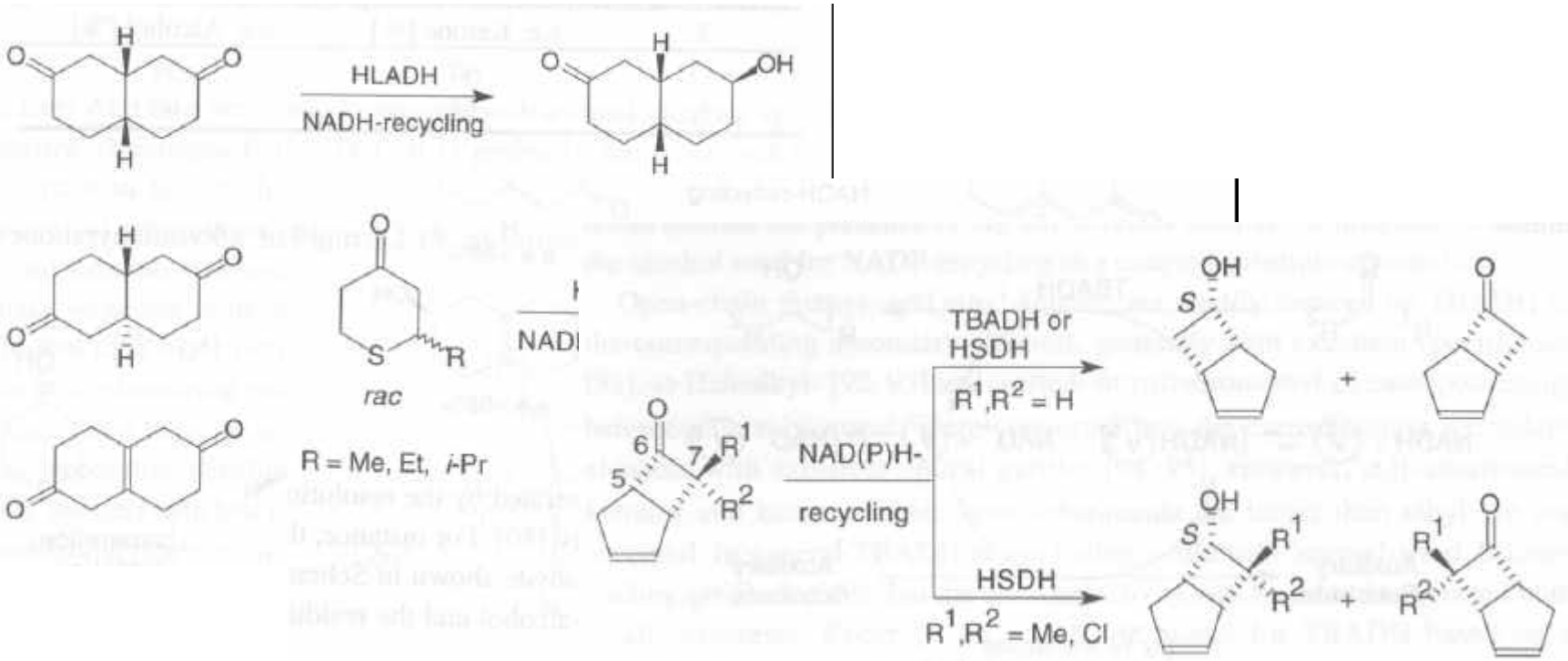
Prelog-Regel



Dehydrogenase	Specificity	Cofactor	Commercially Available
yeast-ADH	Prelog	NADH	+
horse liver-ADH	Prelog	NADH	+
Thermoanaerobium brockii-ADH	Prelog[a]	NADPH	+
Hydroxysteroid-DH	Prelog	NADH	+
Curvularia falcata-ADH	Prelog	NADPH	-
Lactobacillus kefir-ADH	Anti-Prelog	NADPH	+
Mucor javanicus-ADH	Anti-Prelog	NADPH	-
Pseudomonas sp.-ADH	Anti-Prelog	NADH	-

[a] The specificity is reversed when small ketones are used as substrates.

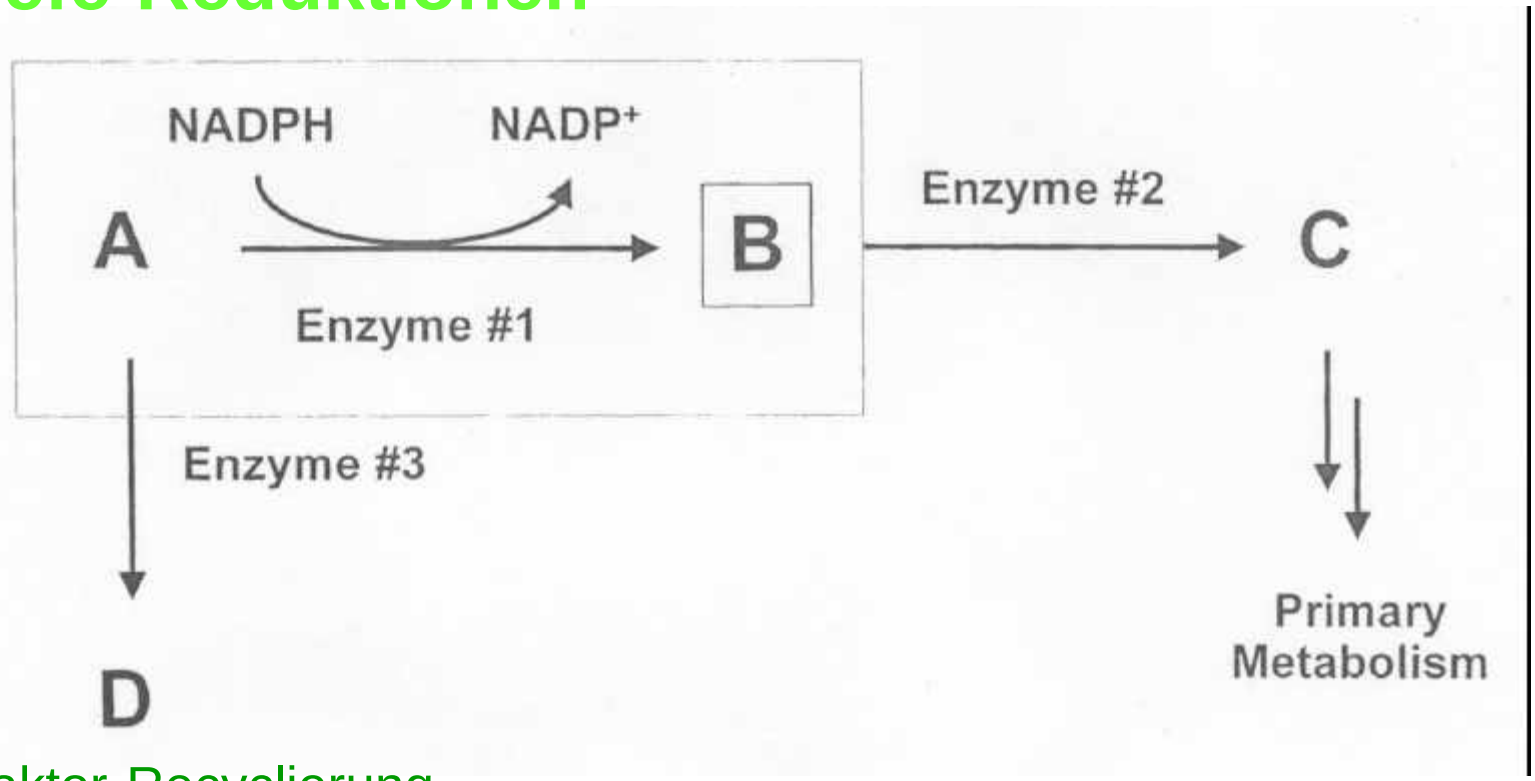
Dehydrogenasen



R^1	R^2	Enzyme	e.e. Alcohol [%]
H	H	HSDH	≤10
H	H	TBADH	>95
H	Cl	HSDH	>90
Cl	Cl	HSDH	>95
Me	Cl	HSDH	>98
Me	Me[a]	HSDH	>95

[a] No reaction was observed with HLADH or TBADH.

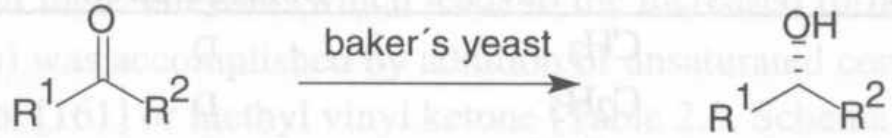
Hefe-Reduktionen



- Kofaktor-Recyclierung
- Enzymproduktion
- Enzyme in natürlichem Milieu
- günstige C-Quellen (Glucose, Saccharose) für enantioselektive Synthesen
- Toxizität nicht-natürlicher Substrate
- Transporteffekte
- Nebenreaktionen

Hefe-Reduktionen

Stereopräferenz

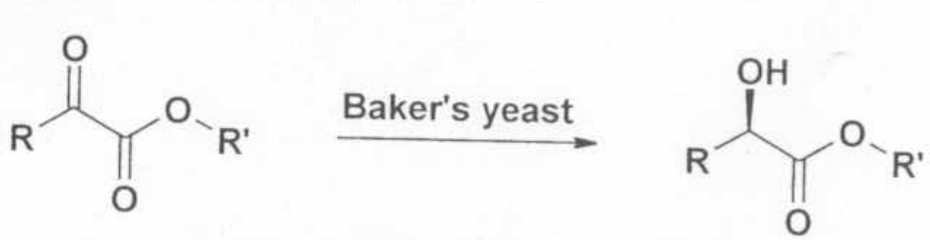


R^1	R^2	Configuration	e.e. [%]
Me	Et	*S*	67
Me	*n*-Bu	*S*	82
Me	Ph	*S*	89
Me	CF_3	*S*	>80
CF_3	CH_2-Br	*S*	>80
Me	$C(CH_3)_2$-NO_2	*S*	>96
Me	CH_2-OH	*S*	91
Me	$(CH_2)_2$-CH=$C(CH_3)_2$	*S*	94
Me	*cyclo*-C_6H_{11}	*S*	>95

- Prelog-Regel (S-Produkte)
- signifikante Größendifferenz
- Limitierung auf sterisch nicht zu anspruchsvolle Substituenten

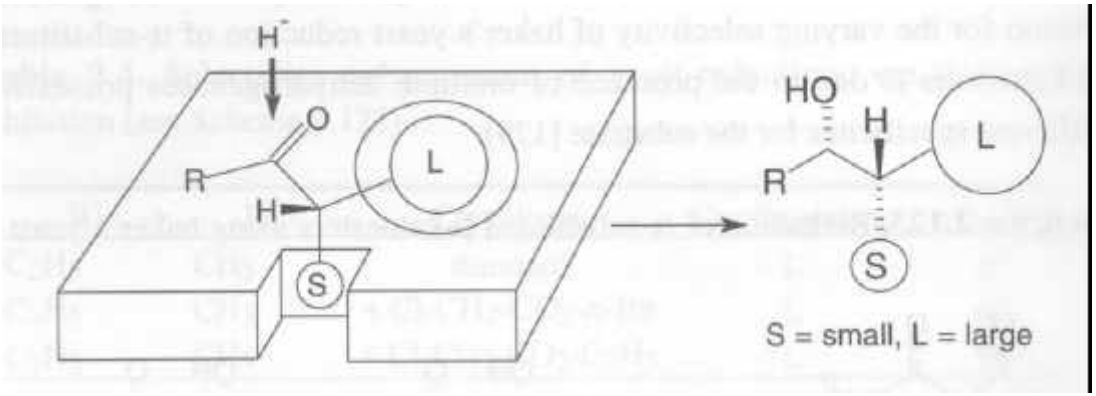
Hefe-Reduktionen

a-Ketoester



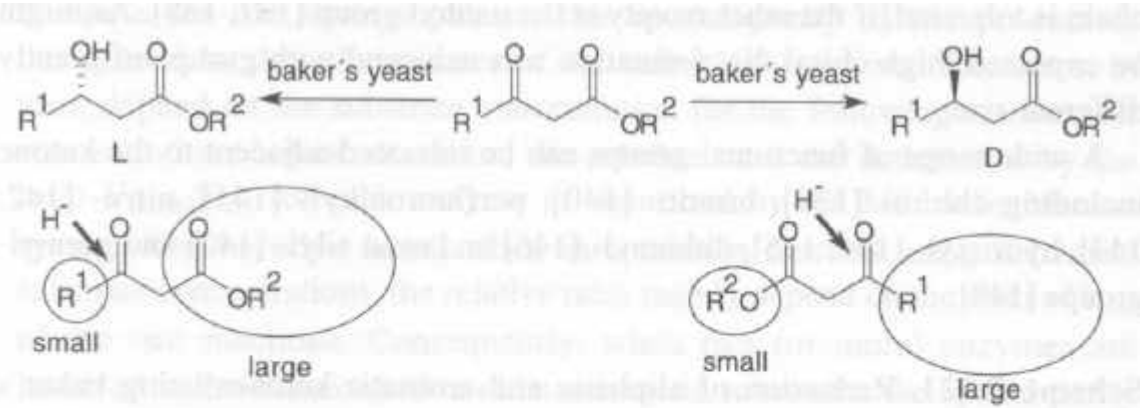
- Ester = „großer Substituent“
  ⇓ R-Produkte
- Lipophilie erhöht Ausbeuten
- kurzkettige Alkoholkomponenten bevorzugt

R	R'	yield	e.e.
Me	Me	36%	92%
Me	Et	38%	90%
Me	*n*-Pent	24%	48%
Me	*n*-Oct	17%	18%
Ph	Me	91%	98%
Ph	Et	68%	82%
Ph	*n*-Pent	47%	52%
Ph	*n*-Oct	46%	20%



Hefe-Reduktionen

b-Ketoester



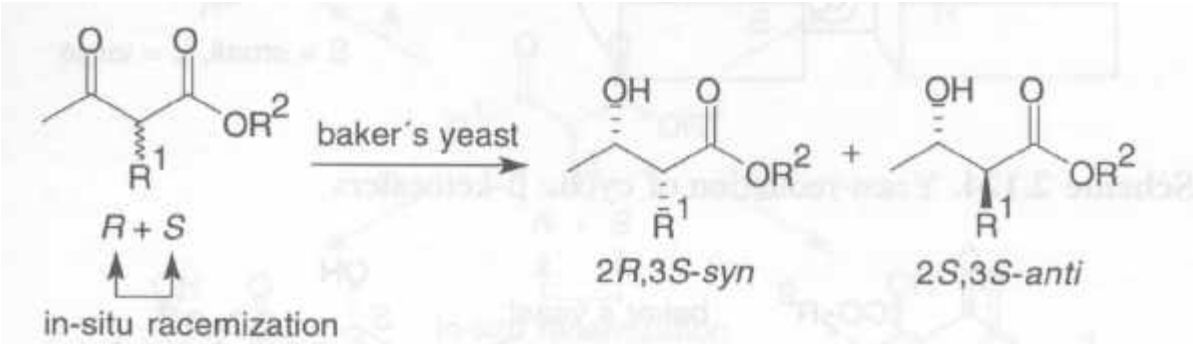
R^1	R^2	Configuration	e.e. [%]
Cl-CH_2-	CH_3	D	64
Cl-CH_2-	C_2H_5	D	54
Cl-CH_2-	*n*-C_3H_7	D	27
Cl-CH_2-	*n*-C_5H_{11}	L	77
Cl-CH_2	*n*-C_8H_{17}	L	97[a]
$(CH_3)_2C$=CH-$(CH_2)_2$-	CH_3	D	92
CCl_3	C_2H_5	D	85
CH_3	C_2H_5	L	>96
N_3-CH_2-	C_2H_5	L	80
Br-CH_2-	*n*-C_8H_{17}	L	100
C_2H_5-	*n*-C_8H_{17}	L	95

[a] Low yield.

- Größe von R1 & R2 bestimmend für Selektivität

Hefe-Reduktionen

b-Ketoester - dynamische Resolution

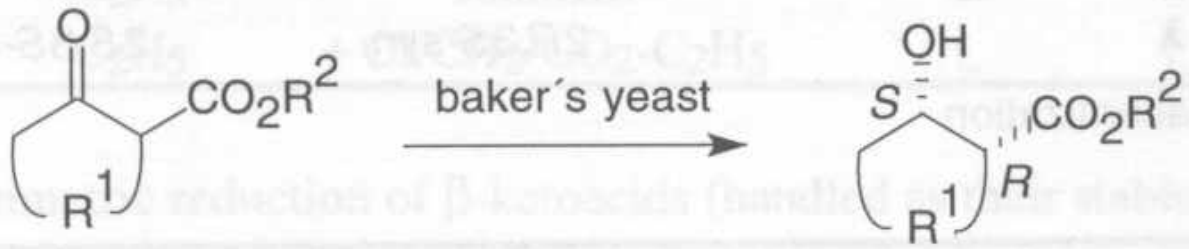


R^1	R^2	e.e. [%]		Ratio
		syn	*anti*	*syn/anti*
CH_3	C_2H_5	100	100	83:17
CH_3	Ph-CH_2-	100	80	67:33
CH_2=CH-CH_2-	C_2H_5	100	100	25:75
Ph-CH_2-	C_2H_5	100	100	33:67
Ph-S-	CH_3	>96	>96	17:83

- Acidität unter physiologischen Bedingungen ausreichend für Racemisierung

Hefe-Reduktionen

b-Ketoester - dynamische Resolution

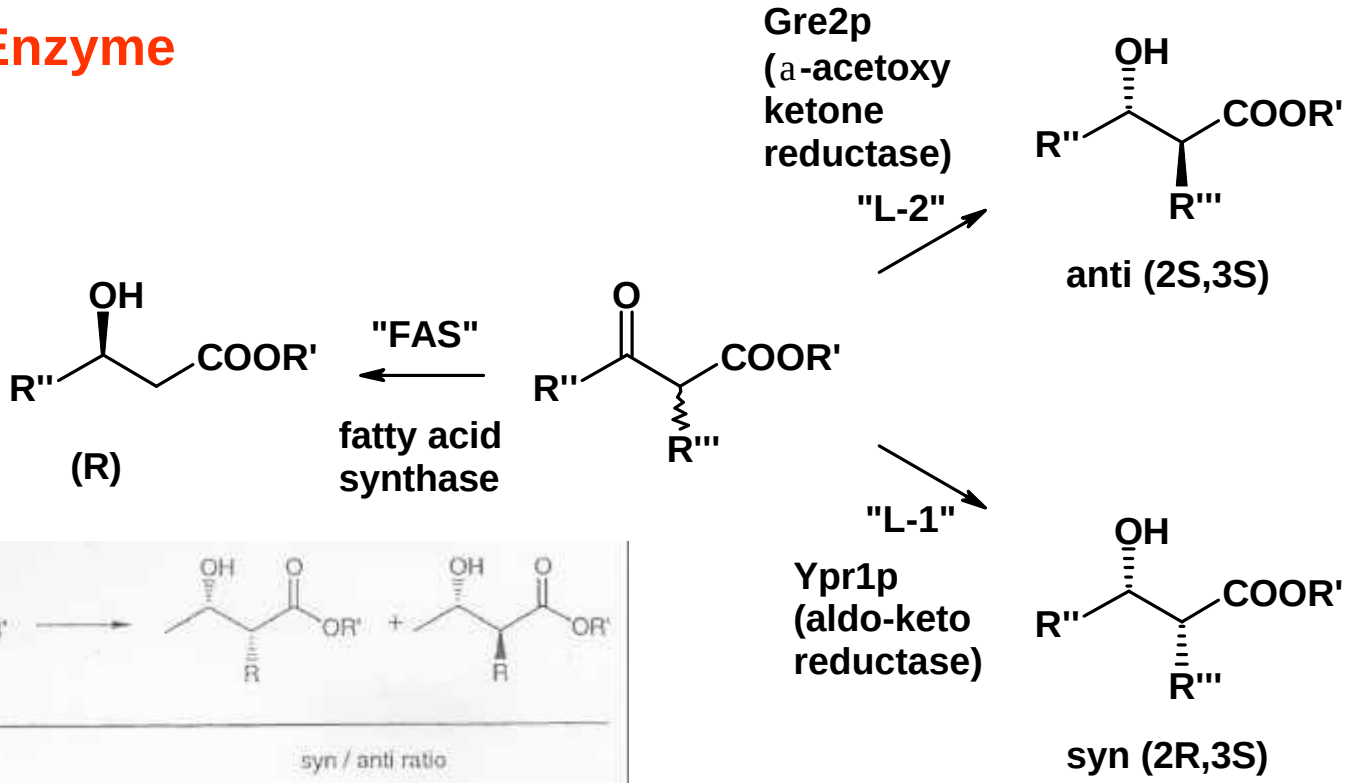


R^1	R^2	e.e. [%]
-$(CH_2)_2$-	C_2H_5	>98
-$(CH_2)_3$-	C_2H_5	86-99[a]
-S-CH_2-	CH_3	85
-CH_2-S-	CH_3	>95

[a] Depending on the yeast strain used.

Hefe-Reduktionen

D- & L-Enzyme

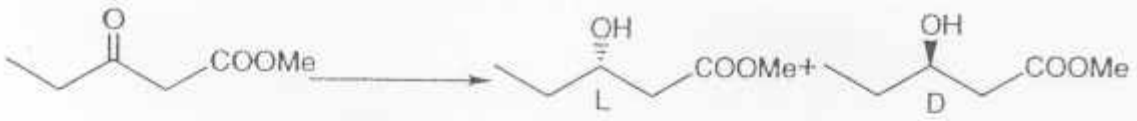


R, R'	syn / anti ratio		
	Baker's yeast	L-1	L-2
Me, Et	87 : 13	>99 : 1	82 : 18
Allyl, Et	35 : 65	>99 : 1	6 : 94

- 50+ Reduktasen in Hefe
  (ca. 500 Enzyme mit vermuteter Reduktaseaktivität)

Hefe-Reduktionen

Selektivitätsmodifikation - Inhibition



Additive	Config.	ee %
none	D (R)	19
glucose	D	59
allyl alcohol	D	60
glucose + allylalcohol	D	96

Table 2.4. Selectivity enhancement of yeast-reductions via D-enzyme inhibition (see Scheme 2.122)

R^1	R^2	Conditions	Configuration	e.e. [%]
C_2H_5	CH_3	standard	L	15
C_2H_5	CH_3	+ Cl-CH_2-CO_2-*n*-Bu	L	69
C_2H_5	CH_3	+ Cl-CH_2-CO_2-C_2H_5	L	91
C_2H_5	C_2H_5	standard	D	40-50
C_2H_5	C_2H_5	+ allyl bromide	L	>98
Cl-CH_2-	C_2H_5	standard	D	43
Cl-CH_2-	C_2H_5	+ Cl-CH_2-CO_2-C_2H_5	L	80

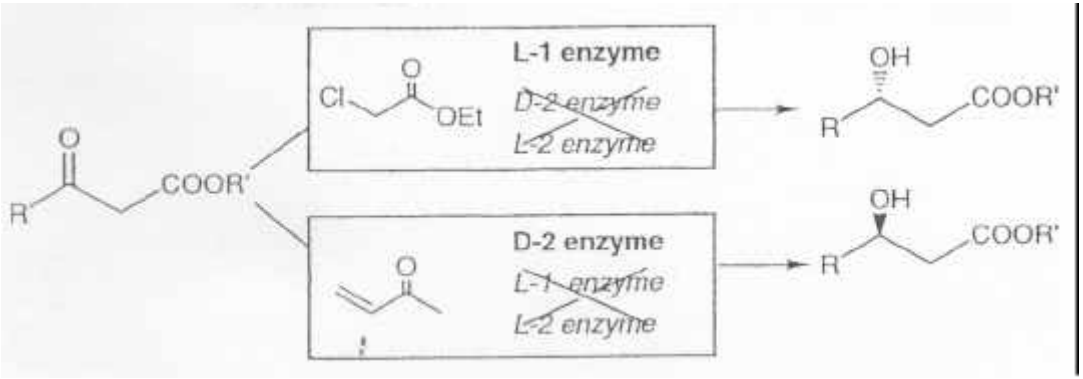
Hefe-Reduktionen

Selektivitätsmodifikation - Inhibition

Table 2.3. Selectivity enhancement of yeast-reductions via L-enzyme inhibition (see Scheme 2.122)

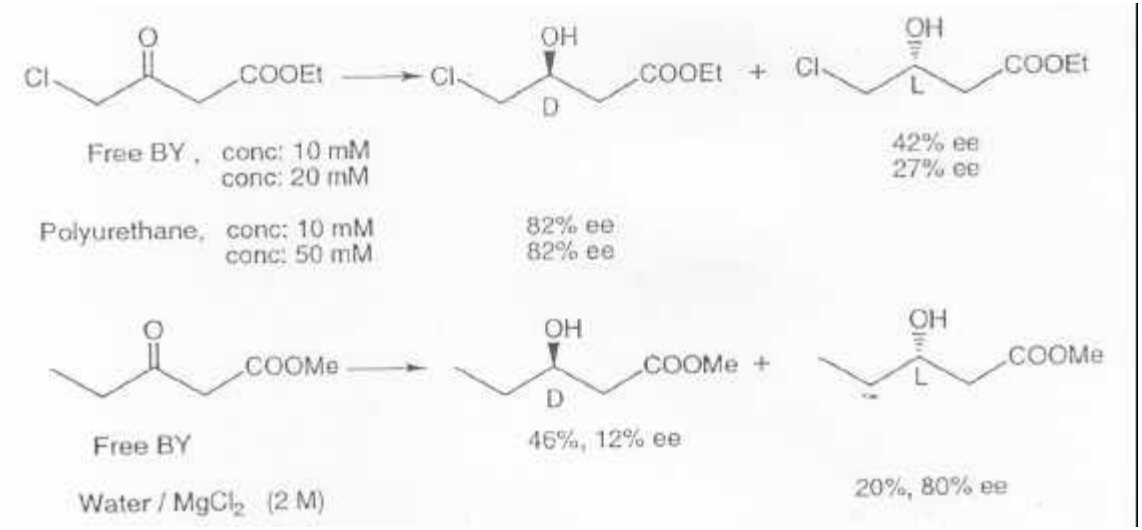
R^1	R^2	Conditions	Configuration	e.e. [%]
$Cl\text{-}CH_2\text{-}$	C_2H_5	standard	D	43
$Cl\text{-}CH_2\text{-}$	C_2H_5	+ allyl alcohol	D	85
C_2H_5	CH_3	standard	D	37
C_2H_5	CH_3	+ $CH_3\text{-}CO\text{-}CH{=}CH_2$	D	89
CH_3	C_2H_5	standard	L	>98
CH_3	C_2H_5	PU-immobilized[a]	L	60
C_2H_5	CH_3	standard	D	5
C_2H_5	CH_3	PU-immobilized[a]	D	86

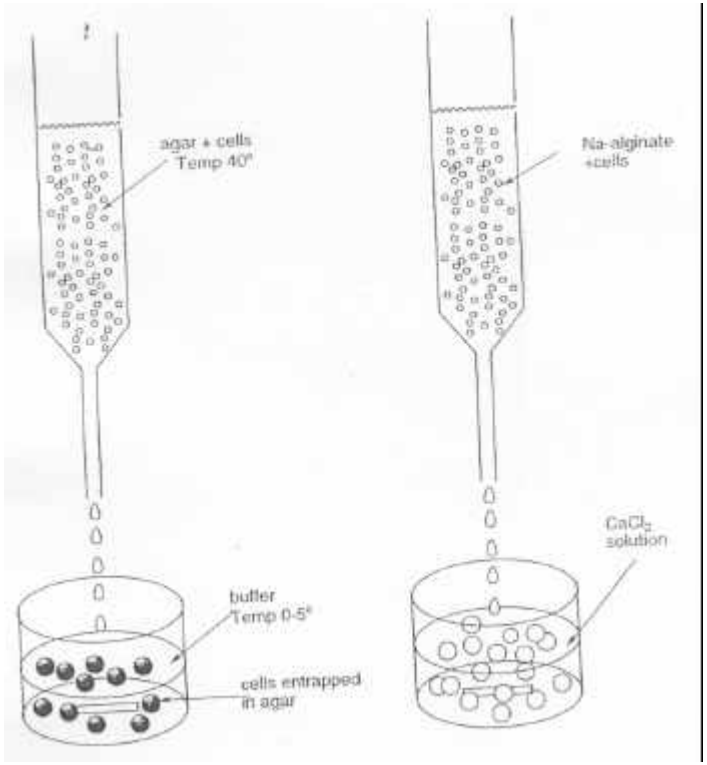
[a] Polyurethane-immobilized.



Hefe-Reduktionen

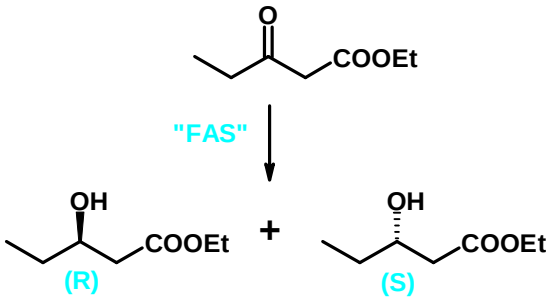
Selektivitätsmodifikation - Immobilisierung

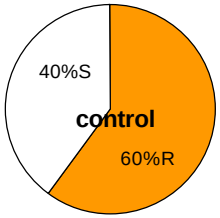


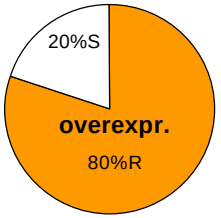


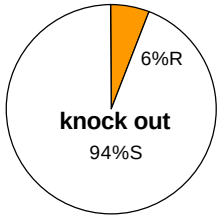
Hefe-Reduktionen

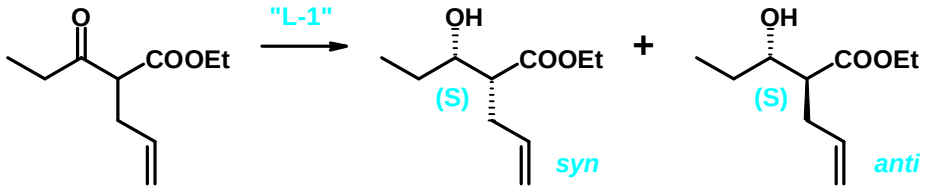
Selektivitätsmodifikation - genetische Veränderung

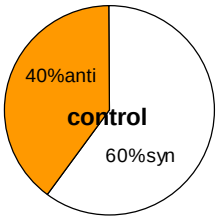


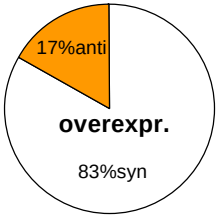


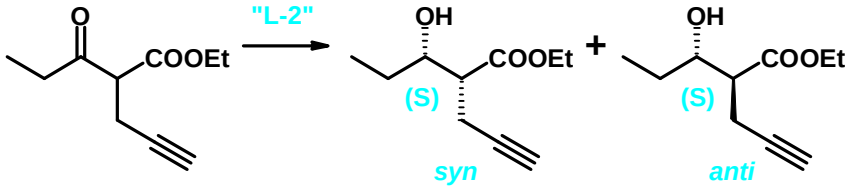


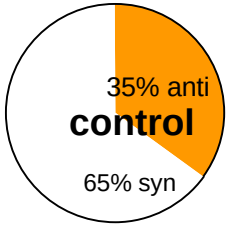


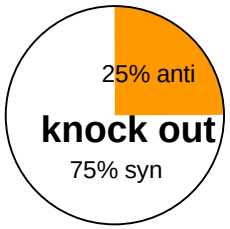


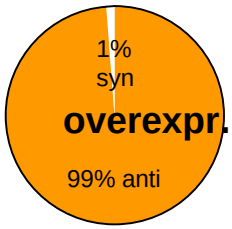






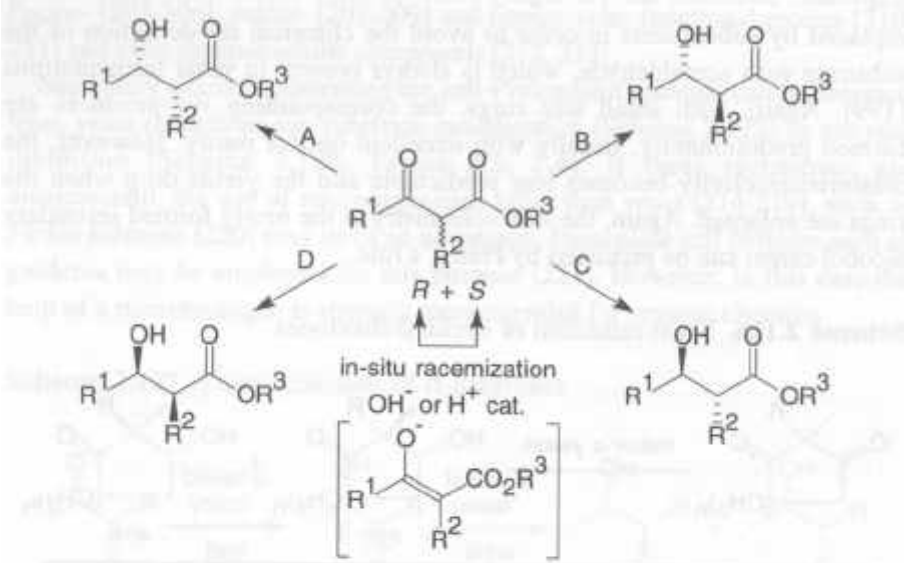






Hefe-Reduktionen

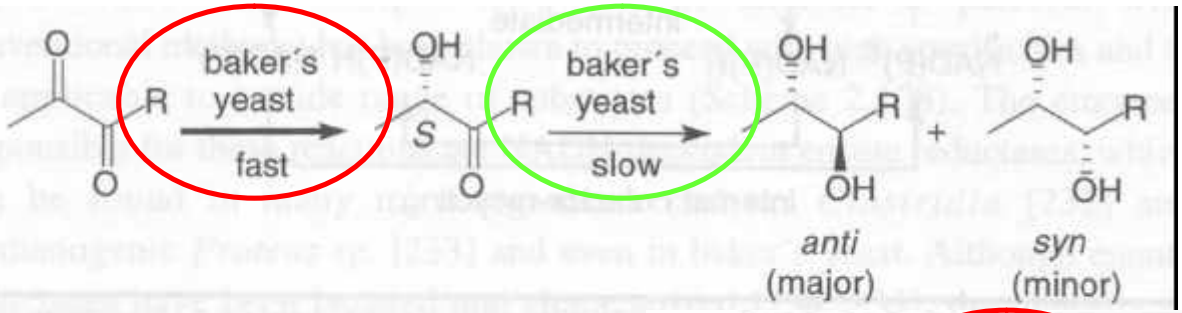
Selektivitätsmodifikation - natürliche Vielfalt



Path-way	R^1	R^2	R^3	Biocatalyst	yield [%]	d.e. [%]	e.e. [%]	Ref.
A	Me	allyl	Et	baker´s yeast	94	92	>99	[187]
A	Me	Me	*n*-Octyl	baker´s yeast	82	90	>98	[188]
B	Me	Me	Et	*Geotrichum candidum*	80	>98	>98	[189]
B	Et	Me	Et	*Geotrichum candidum*	80	96	91	[190]
C	4-$MeOC_6H_4$-	Cl	Et	*Sporotrichum exile*	52	96	98	[191]
D	4-$MeOC_6H_4$-	Cl	Me	*Mucor ambiguus*	58	>98	>99	[192]

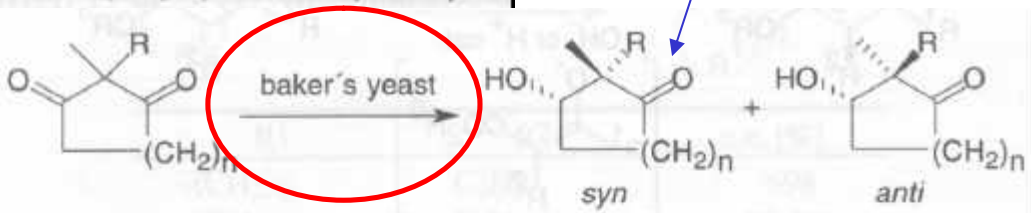
Hefe-Reduktionen

Diketone



R	e.e. *anti*-diol
Ph-	94
1,3-dithian-2-yl-	97
Ph-S-CH_2-	>97

zumeist **keine** weitere Reduktion

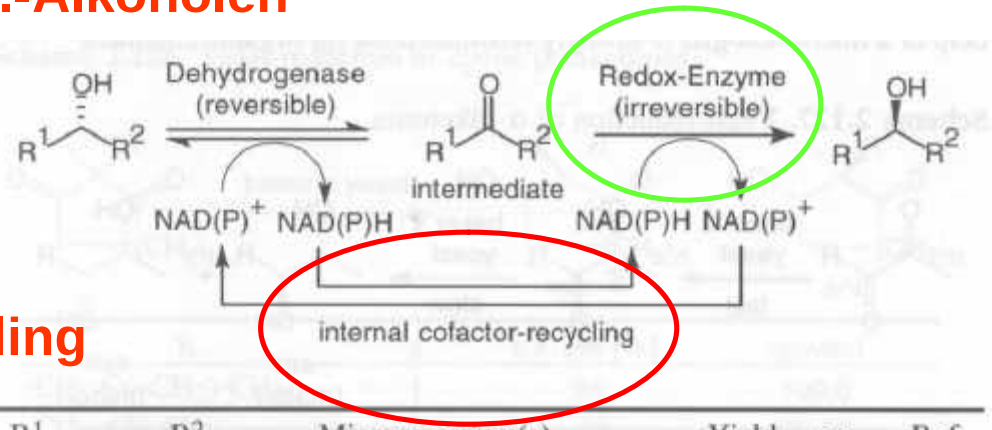


R	n	e.e. *syn* [%]	*syn/anti*
CH_3-C(=CH_2)-CH_2-	1	98	100:0
CH_2=CH-CH_2-	1	>98	90:10
HC≡C-CH_2-	1	>90	100:0
N≡C-$(CH_2)_2$-	1	>98	96:4
CH_3-C(=CH_2)-CH_2-	2	>98	24:76
CH_2=CH-CH_2-	2	>98	45:55
HC≡C-CH_2-	2	>98	27:73
N≡C-$(CH_2)_2$-	2	>98	30:70
CH_2=CH-CH_2-	3	>98	100:0
CH_2=CH-CH_2-	4	>98	82:18
CH_2=CH-CH_2-	5	>98	no reaction

Redox-Kopplungen

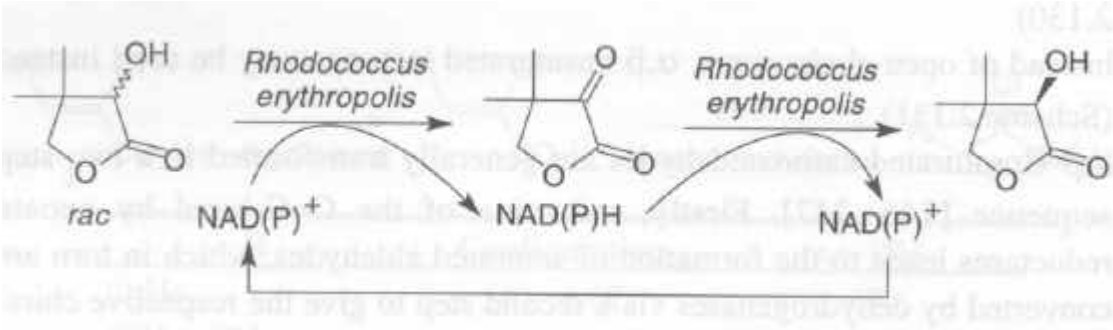
Deracemisierung von sec.-Alkoholen

- 2. Redox-Enzym muß irreversibel transformieren
- Netto-Elektronentransfer = 0
  ß **internes Kofaktor-Recycling**



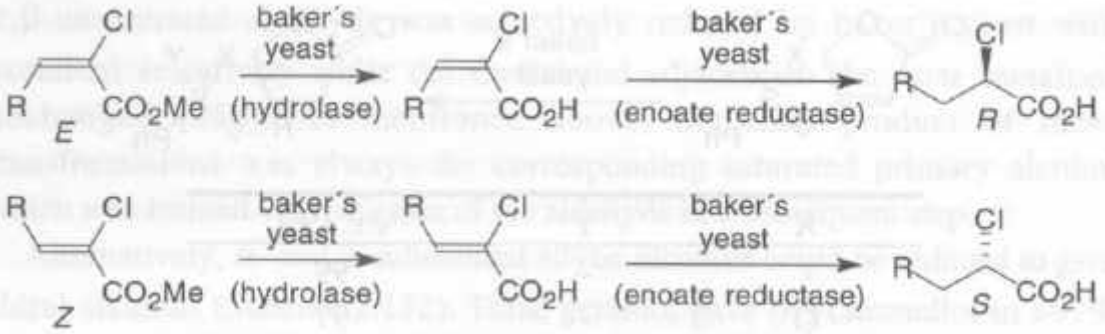
R^1	R^2	Microorganism(s)	Yield [%]	e.e. [%]	Ref.
Me	CH_2CO_2Et	*Geotrichum candidum*	67	96	[224]
Me	p-Cl-C_6H_4	*Geotrichum candidum*	97	96	[225]
Ph	CH_2OH	*Candida parapsilosis*	~100	~100[a]	[226]
Me	$(CH_2)_2CH{=}CMe_2$	*Bacillus stearothermophilus* + *Yarrowia lipolytica*	91	~100	[227]
Ph	CO_2H	*Pseudomonas polycolor* + *Micrococcus freudenreichii*	70	>99	[228]

[a] Opposite configuration as shown.



C=C Reduktionen

ungesättigte Ester

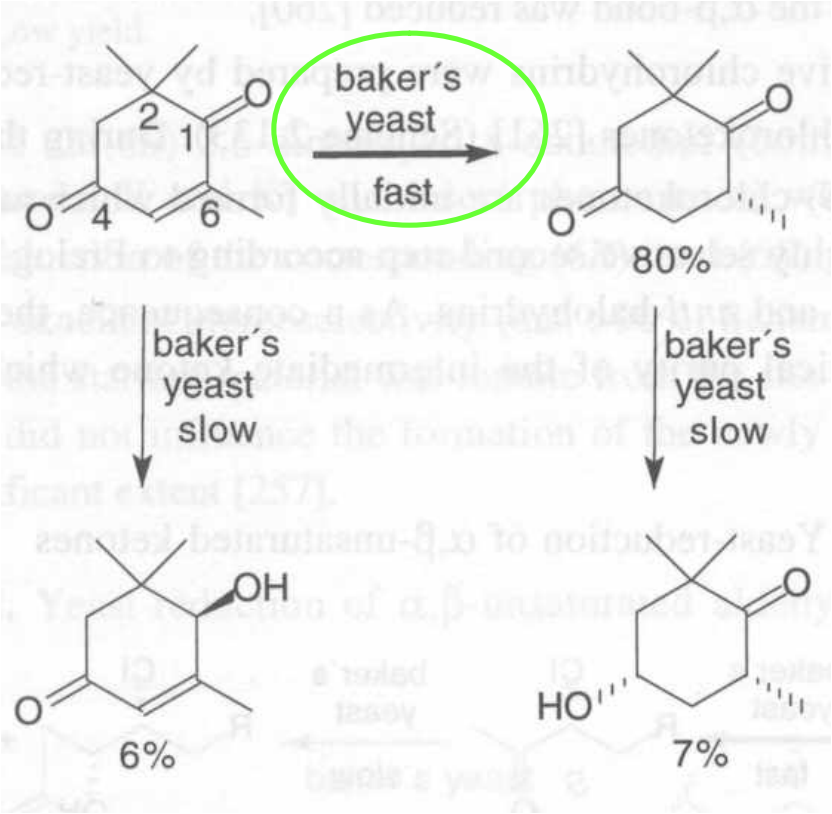


R	Configuration	e.e. [%]
C_2H_5-	(E) → (R)	47
$(CH_3)_2CH$-	(E) → (R)	68
$CHCl_2$-	(E) → (R)	92
C_2H_5-	(Z) → (S)	>98
$(CH_3)_2CH$-	(Z) → (S)	>98
$CHCl_2$-	(Z) → (S)	98
CCl_3-	(Z) → (S)	>98

- zunächst Hydrolyseaktivität, dann Reduktaseaktivität
- Chiralität abhängig von E/Z-Positionierung
  ß **Erkennung von Prochiralität**
- aktivierte DB Voraussetzung
  (Michael-Akzeptor, nucleophiler Angriff)

C=C Reduktionen

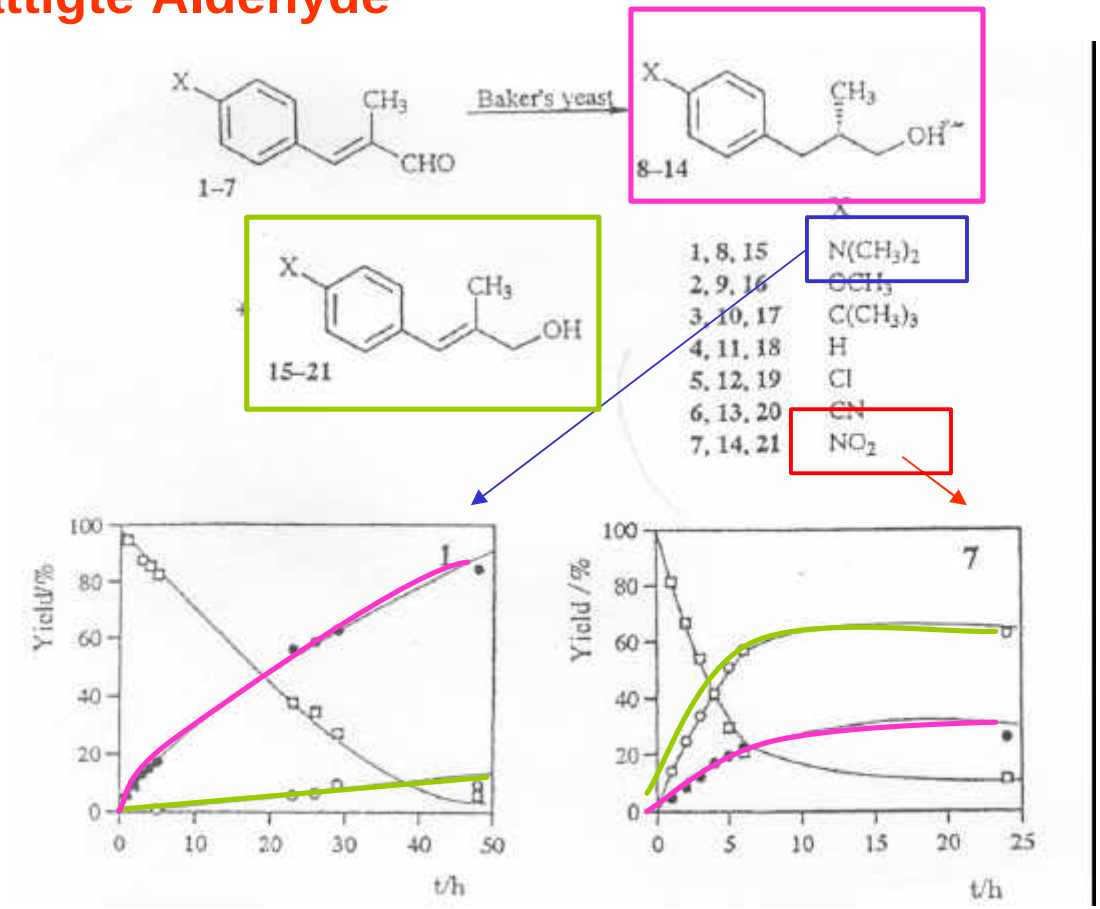
ungesättigte Ketone



- Angriff an DB üblicherweise rasch
- weitere Reduktaseaktivität (C=Het) möglich

C=C Reduktionen

ungesättigte Aldehyde



- normalerweise zuerst DB-Reduktion (1,4-Angriff)
- bei stark elektronenziehenden Substituenten am Aromaten
  ⇓ starke Aktivierung von CO
  ⇓ zuerst Carbonylreduktion