



Chiral Vision

Innovative biocatalysis

**Immobilised bulk enzymes for chiral
intermediate and chiral API production**

Chiral Vision

Company introduction:

- Dedicated to application of biocatalysis
- Founded in 2006
- Headquarters in Leiden, The Netherlands

Business model / Market:

- Off-shelf immobilized (bulk) enzymes
- Chiral Process Development service (enzymatic)
- Chiral compounds with high purity (>99% ee)



In 2006 it was 400 years ago that Rembrandt van Rijn was born in Leiden. This Dutch master could catch like no other light and darkness in paint



Currently, Leiden is host to a new generation of "Dutch masters":

Discovery & research	Product & process development	Clinical trials	Manufacturing	Distribution	Services, sales, other
Add2X Biosciences	Add2X	Astellas	Biocult	BIOKÉ	BaseClear
Astellas	Astellas	Bio-Imaging Technologies	Centocor	Danisco/Genencor	Biotop Medical
BIOKÉ	CAM Implants	Biometric Support	Crucell	Mentor Medical Systems	Cosine Research
Biometric Support	Chiral Vision	Centocor	Danisco/Genencor	Octoplus	Hogeschool Leiden
CAM Implants	Crucell	CHDR	FlexGen	Pharming Group	Holland Biotechnology
CIDRUX	Danisco/Genencor	LACDR	Katwijk Farma	Promega Benelux	Katwijk Farma
Crucell	Derphartox	TNO	Mentor Medical Systems	Sanquin blood bank	LAP&P
Danisco/Genencor	DNAge	Top Institute Pharma	Octoplus	ServiceXS	Microsafe
DeltaCell	FlexGen	Xendo	Promega Benelux	Verilabs Nederland	Promega Benelux
DNAge	Holland Biotechnology		Sanquin blood bank		Proxy Laboratories
Enzyscreen	Katwijk Farma		Xendo		Rainbow Oxidations
FlexGen	LACDR				Schenkelaars BC
Fytagoras	Mentor Medical Systems				Servier
Galapagos Genomics	Mucovax				STI management
Lactrys	Mycobics				Verilabs
LACDR	Octoplus				Xendo
Leiden University	Ondeo Nalco				
LUMC	Pharming Group				
LION	Prisna				
Lorentz Center	Promega Benelux				
Membrane Protein Lab.	Protein Labelling Inn.				
Mentor Medical Systems	Proteonic				
Naturalis	Sanquin blood bank				
Octoplus	Service XS				
Percuros	SU Biomedicine				
Pharming Group	TNO				


PhotoBioChem	To-BBB
PRISNA	Top Institute Pharma
Profibrix	Virosome Biologicals
Promega Benelux	Xendo
Prosensa	
Protein Labelling Inn.	
Sanquin blood bank	
ServiceXS	
Stem Cell Innovations	
TNO	
To-BBB	
Top Institute Pharma	
Virosome Biologicals	
Viruvation	
ZoBio	

60 companies make this Park the largest dedicated lifes sciences cluster in the Netherlands.



Enzymes as chiral catalyst


The dominant production method for a single enantiomer is resolution:

- 
- direct crystallization
 - crystallization of diastereomeric salts
 - chromatography
 - kinetic resolution (esterification / acylation or hydrolysis; enzymatic)

Kinetic resolution yields 50% max. Solution:

- racemisation of the undesired enantiomer
- in-situ racemisation by a dynamic kinetic resolution (DKR)

Enzymes costs



Enzyme	typical cost	cost / kg product	min. product scale
Protease	300 €/ kg	0.1 – 10 €	50 kg
Lipase	300 – 2000 €/ kg	0.1 – 10 €	50 kg - 1 ton
Special enzyme	10k – 50k €/ kg	50 – 1000 €	1 – 100 ton

Some facts:

Alcalase, the first detergent enzyme produced and improved since 1963


Savinase (the White Tornado) introduced in 1973

Lipolase, first GMO enzyme in detergent 1988

Enzymes as chiral catalyst

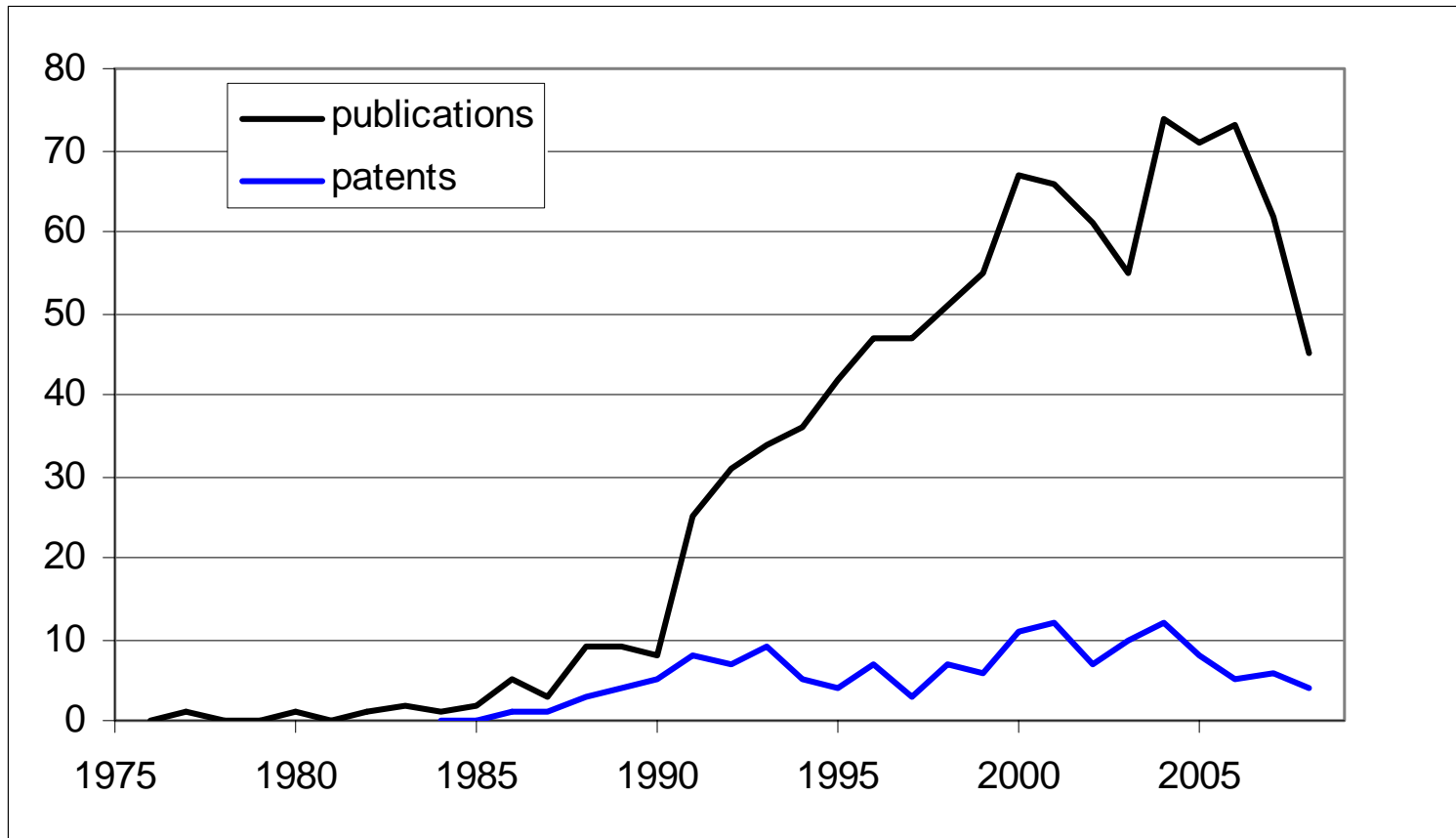
Selecting the right enzyme and conditions: not a paper exercise!

Smart throughput screening:

- 
- about 100 commercially available (bulk) hydrolytic enzymes
 - clever substrate derivatisation (yielding the same end product)
 - solvent system
 - temperature
 - pH
 - buffer salts, etc.

These yields a choice of at least 10,000 – 100,000 distinctively different process conditions with varying yield and enantioselectivity.

Enzymes in chiral resolution



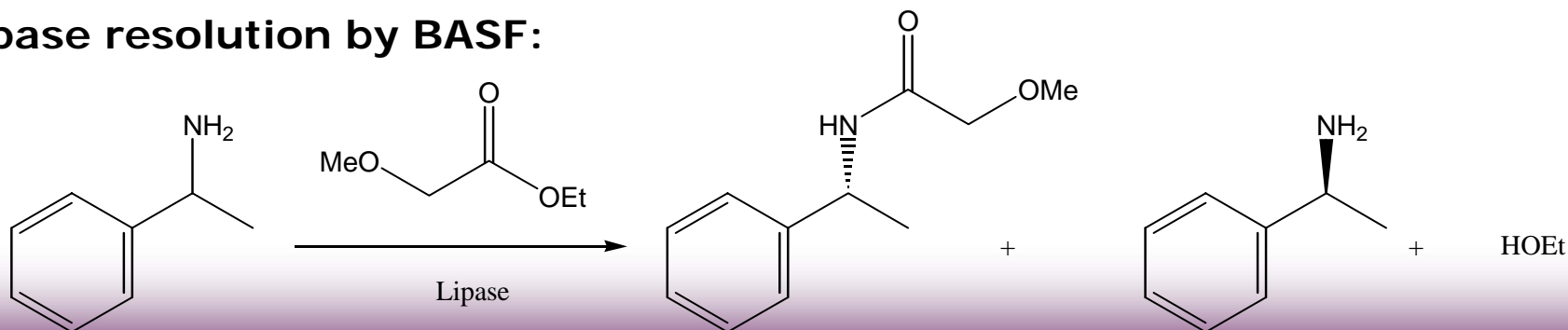
Articles and patents (annually) on “enzymatic resolution”

Enzymes used in chemical production

Manufacturer	compound	enzyme	scale (tons / y)
BASF	chiral amines	<i>C. antarctica</i> lipase B*	1000
BASF	(R)-mandelic acid	(<i>S</i>)- or (<i>R</i>)-nitrilases	multi ton
DSM	L- or D-amino acids	amidase	multi ton
DSM	chiral alcohol	lipase*	multi ton
DSM Chemie Linz	2-halopropionic acids	porcine lipase	0.1
DSM-Andeno	(R)-glycidyl ester	lipase	multi ton
Dowpharma	β -phenylalanines	lipase	0.1-0.5
Dowpharma	carbocyclic nucleosides intermediate	γ -lactamase	multi ton
Kaneka	D-amino acids	hydantoinase* / carbamoylase*	1-5000
Schering	protein farnesyl transferase inhibitor	lipase LIP-300	0.1
various	L-methionine	L-aminoacylase	1-5000
various	6-aminopenicillanic acid	penicillin G acylase*	20,000
	acryl amide	nitrile hydratase	

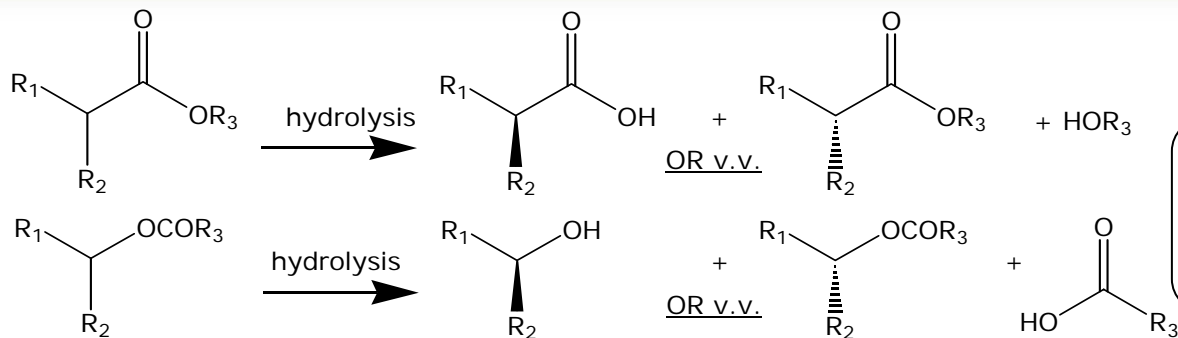
* immobilized

Lipase resolution by BASF:

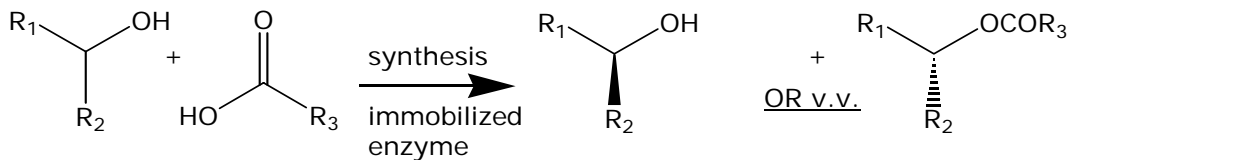


Enzymatic resolution with hydrolases

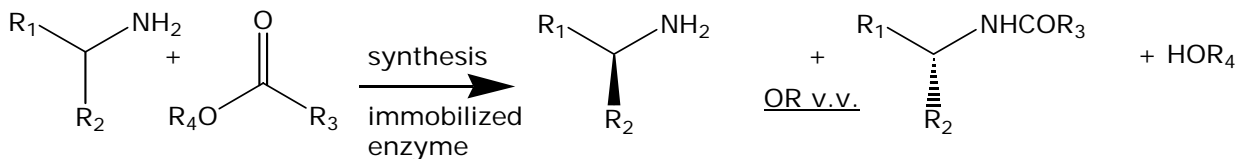
Lipases
+
Proteases
+
Esterases



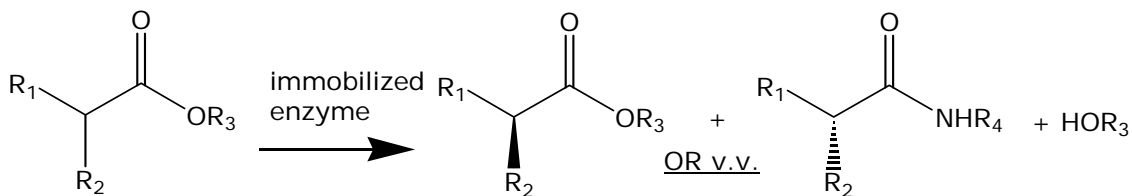
chiral:
carboxylic acids
amino acids
hydroxy acids



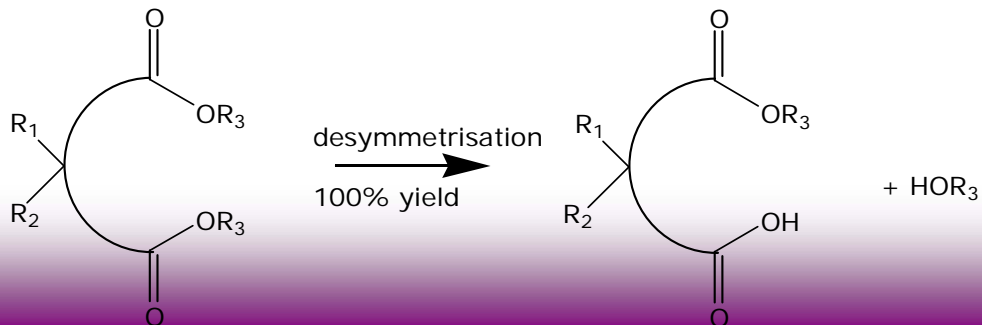
chiral alcohols



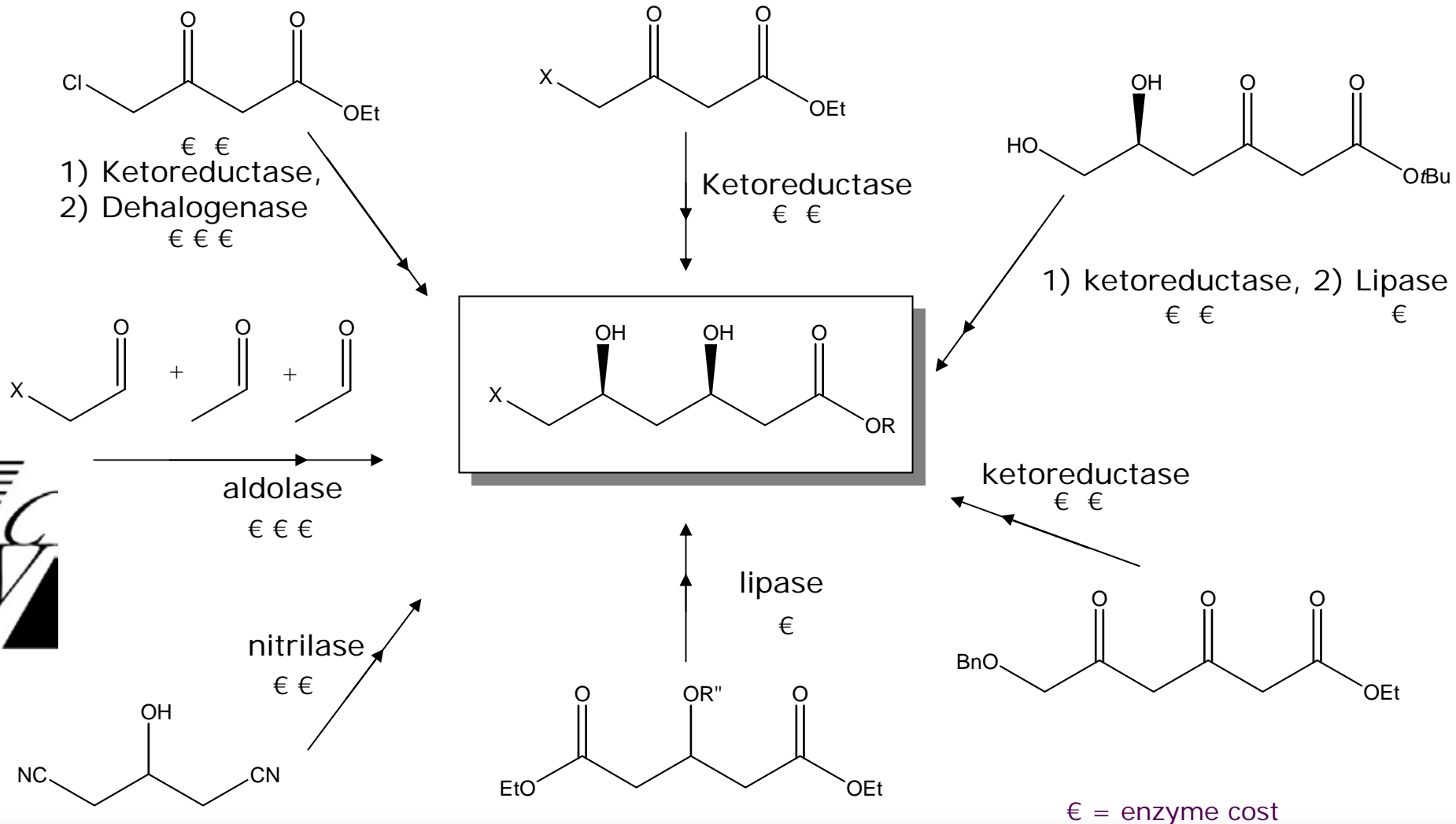
chiral amines



chiral:
acids / amides
amino acids
hydroxy acids
peptide coupling



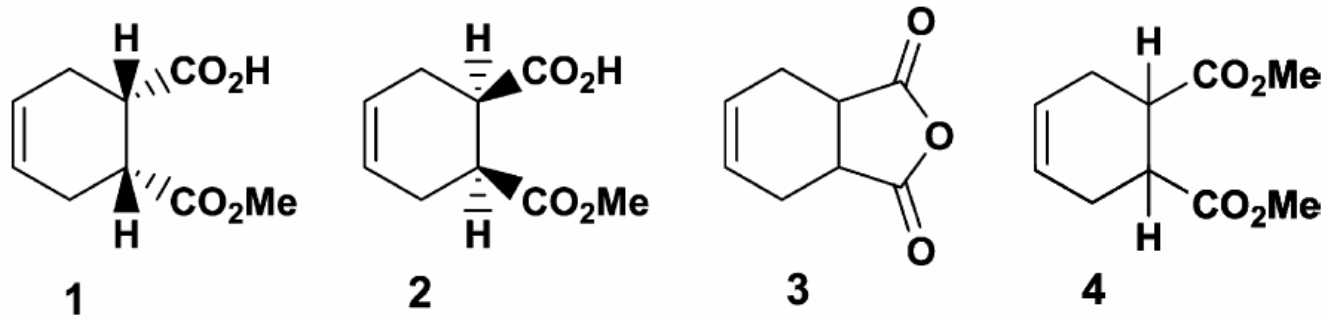
Enzymatic approach: Lipitor intermediate



(3R,5S)-dihydroxyhexanoates (by various companies)

Example I:

Lipase-catalyzed enzymatic desymmetrization of dimethyl esters

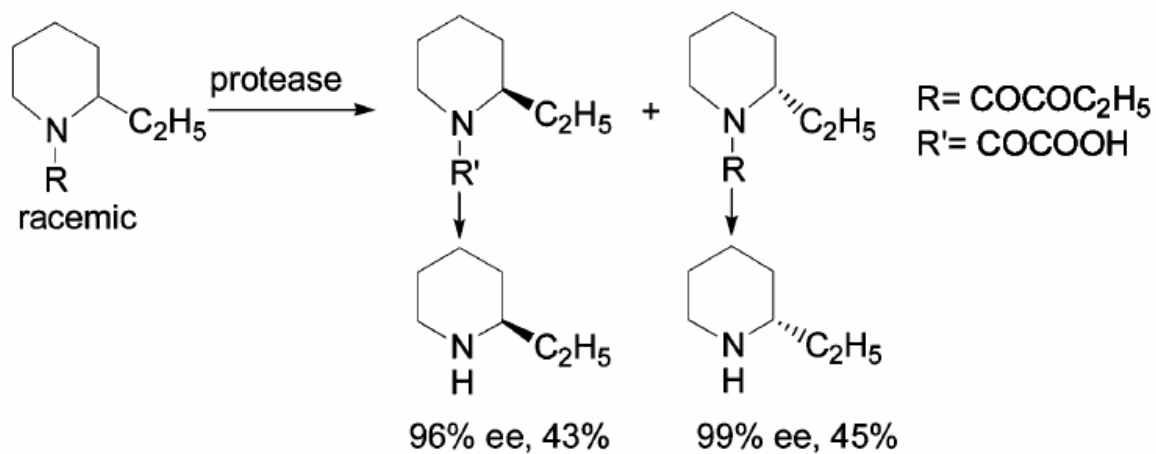


100% yield, 2 substrates and 11 lipases tested

Goswami et al, OPRD 2009 (BMS)

Example II:

Protease mediated preparation of optically pure secondary amines building blocks

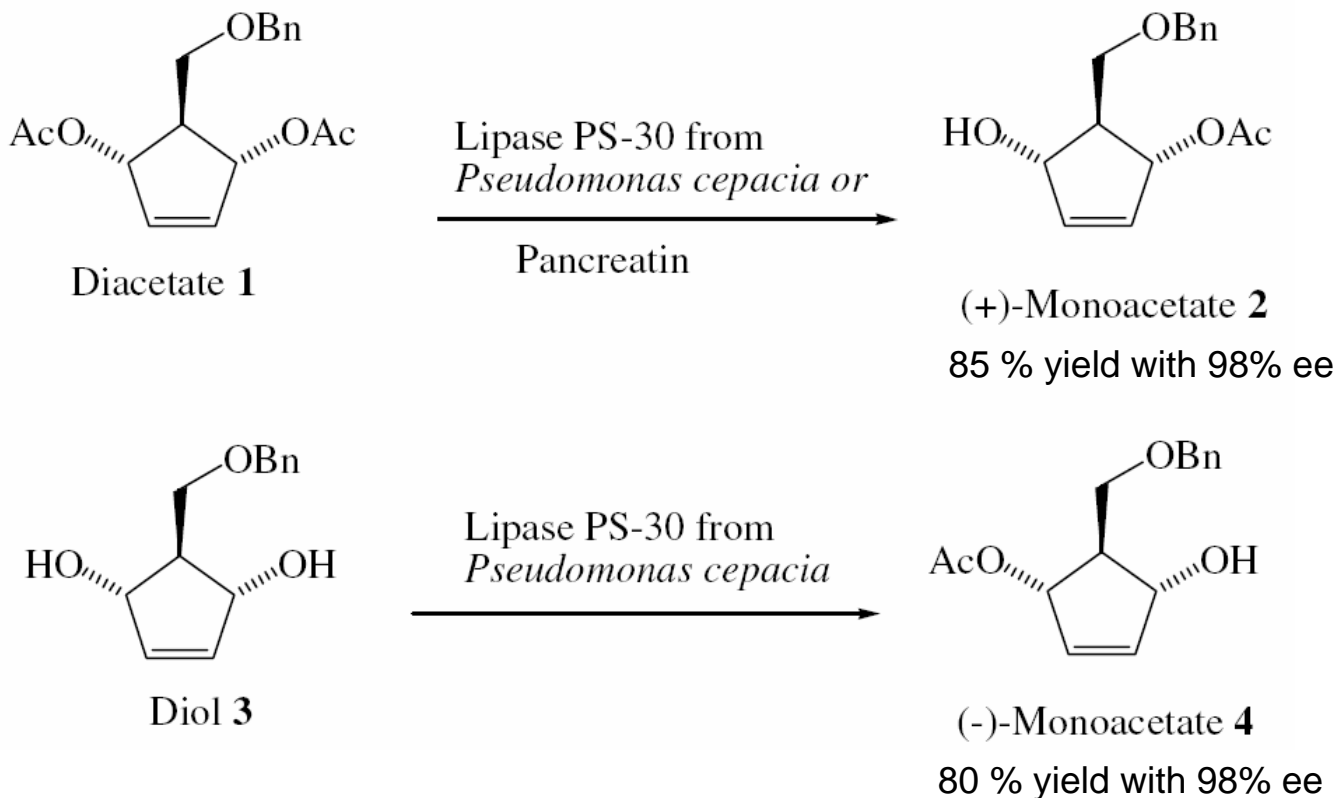


Various substrates and 12 proteases tested

Hu et al, Organic Letters 2005 (Pfizer)

Example III

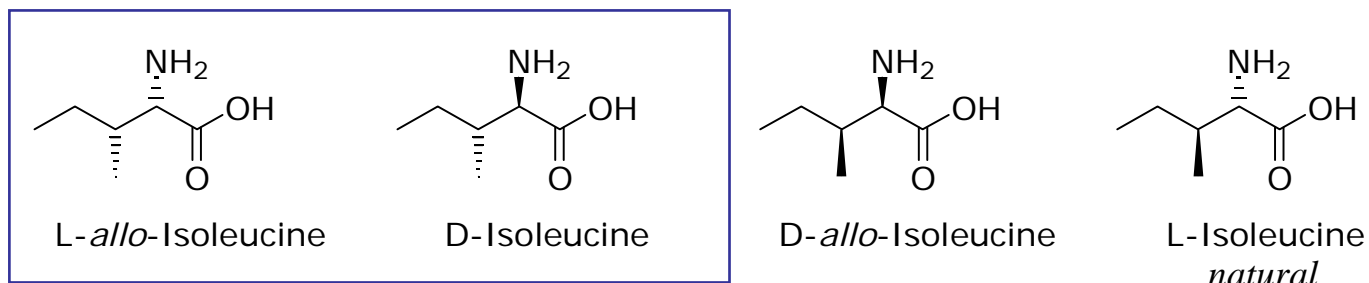
Lipase mediated desymmetrisation of a chiral synthon for the HBV inhibitor Monc



Patel et al, Tet. Asymm 2006 (Bristol-Myers Squibb)

Example IV

Production of non-natural amino acids with high chiral purity

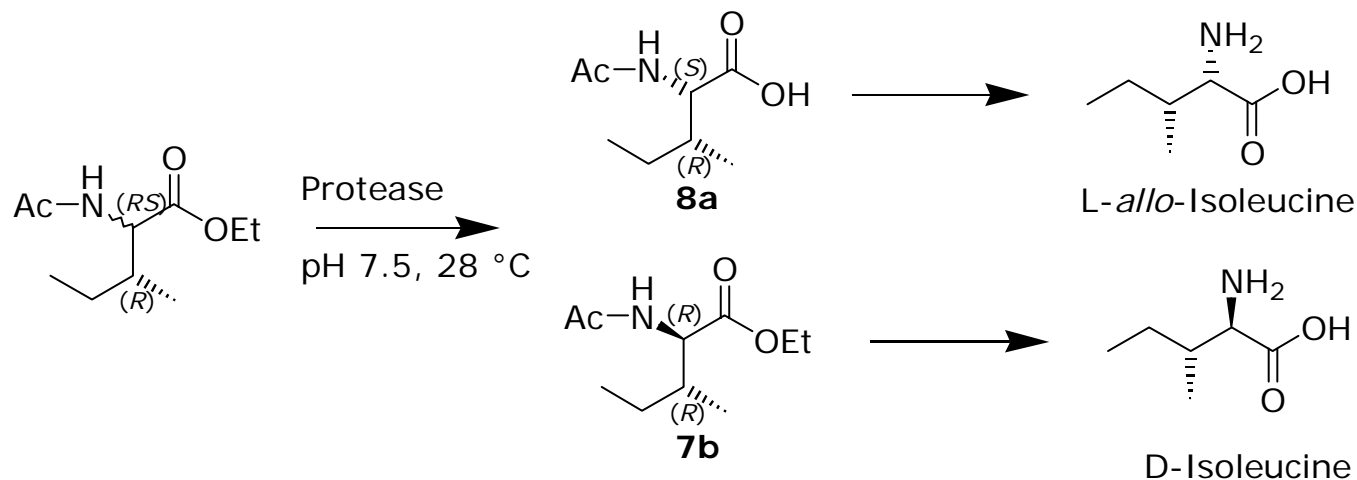


L-Isoleucine is one of the smallest natural occurring α -amino acids with 2 chiral centers.

While the D-*allo*-isoleucine is accessible via epimerization of natural L-isoleucine, no such easy access is available for the 2 other diastereomers.

To develop a multi kg scale procedure of L-*allo*-isoleucine and D-isoleucine a 2-step chemoenzymatic approach was developed with commercially available proteases.


Example IV



Entry	Enzyme	8a (% de)	7b (% de)	"E" ^a
1	IMMP6	97.4	96.9	325
2	IMMP30	97.6	66	160
3	IMMP40L	97.6	78	210
4	IMMP40X	96.5	33	79
5	IMMP51	n.d.	< 10	-
6	IMMP89	97.5	68	160
7	IMMALC	97.3	98.7	320
8	IMMSAV	97.6	76	190
9	IMMESP	97.1	78	160
10	IMMEVE	n.d.	12	-

Screening of immobilized proteases yields many candidates


General economic evaluation



Parameter	productivity*	enzyme cost**
1 st enzyme screening	10	200
substrate analogues	25	20
various conditions	50	10
solvent system	160	4
temperature	180	3.5
pH	190	3
buffer	200	2.5
recycling	200 - 600	0.5 – 1.5

***g / l / day* **euro enzyme / kg product*

Immobilized bulk enzymes



Enzyme	Annual use	Carrier	Application
glucose isomerase	> 50 tons	inorganic	food
aminoacylase	< 50 tons	inorganic	intermediate, API
lactase	< 50 tons	inorganic / ion-exchange resin	food
penicillin G acylase*	< 50 tons	polyacrylic (covalent)	intermediate, API
glucoamylase	< 20 tons	anion exchange resin	food
hydantoinase	< 20 tons	anion exchange resin	intermediate, API
invertase	< 20 tons	inorganic / anion exchange resin	food
nitrilase	< 20 tons	polyacrylamide beads	bulk chemical
Penicillin V acylase*	< 20 tons	polyacrylic (covalent)	intermediate, API
CaLB (NZ435)*	< 20 tons	macroporous acrylic resin	intermediate, API
TL Lipase*	< 20 tons	Silica	food
RM lipase*	< 20 tons	anion exchange resin	food
Amano PS lipase*	<< 20 tons	Celite	food

**commercially available and applicable in resolution*

Most of these immobilized enzymes are not commercially available!

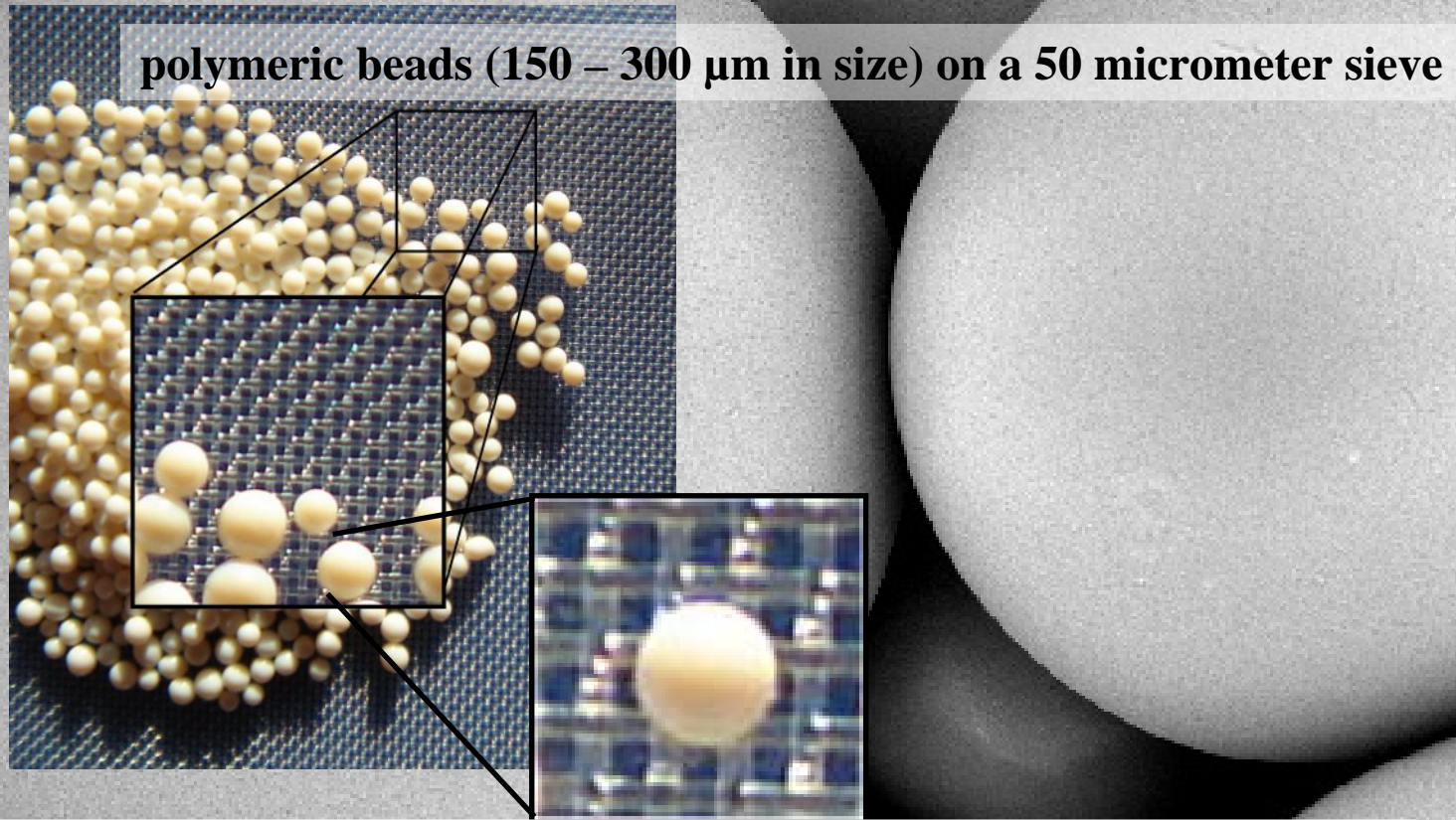
Technical demands for enzyme use

Various specifications are needed

- commercially available
 - batch use
 - continuous use
 - aqueous / biphasic / organic media
 - fast filtration (with ~100 micrometer sieve)
 - cost contribution per kg endproduct 0.1 - 10 euro
 - 3 to 1000 cycles
-
- absorbed, covalent, dry, wet
 - various particle sizes
 - various carrier types

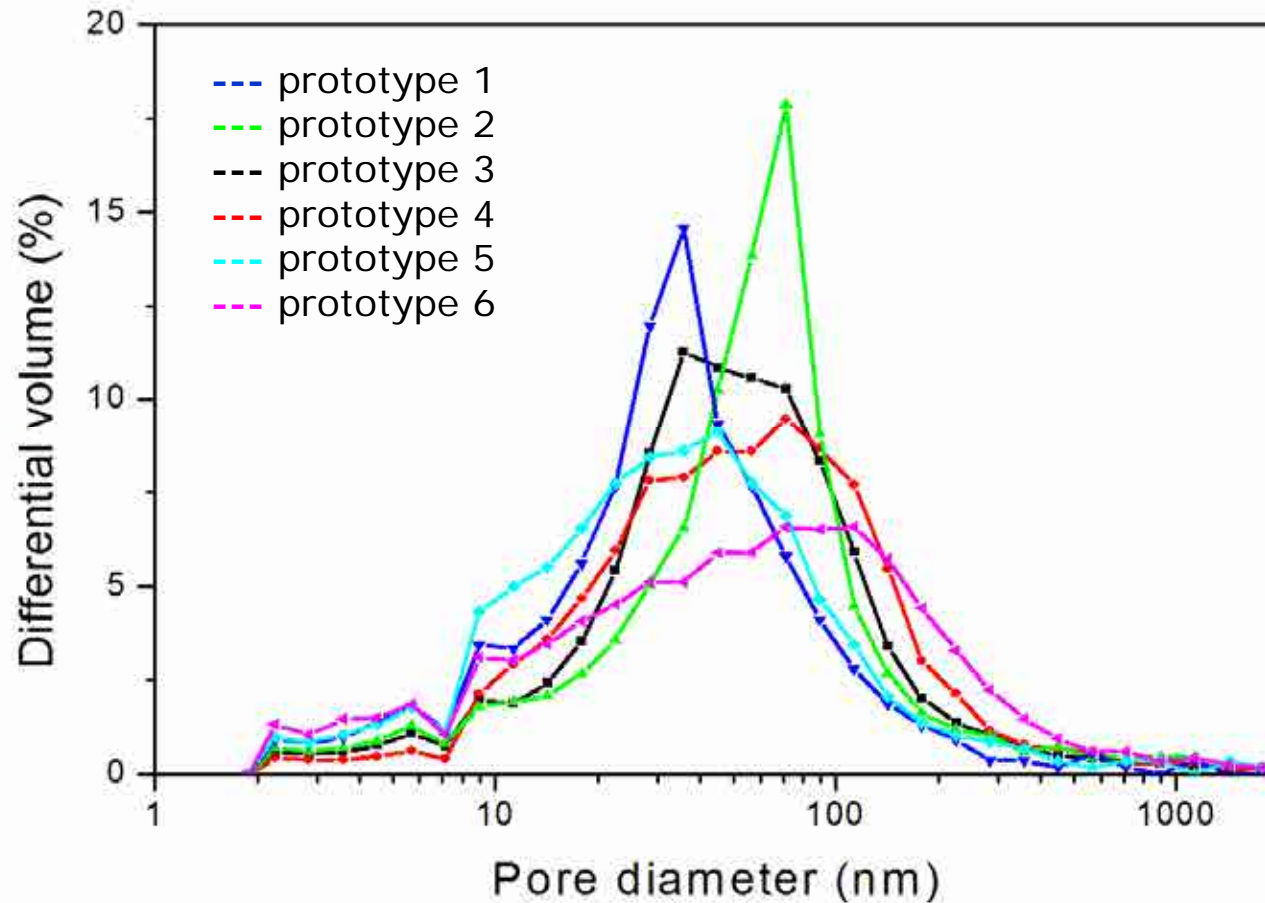


New covalent macroporous carrier



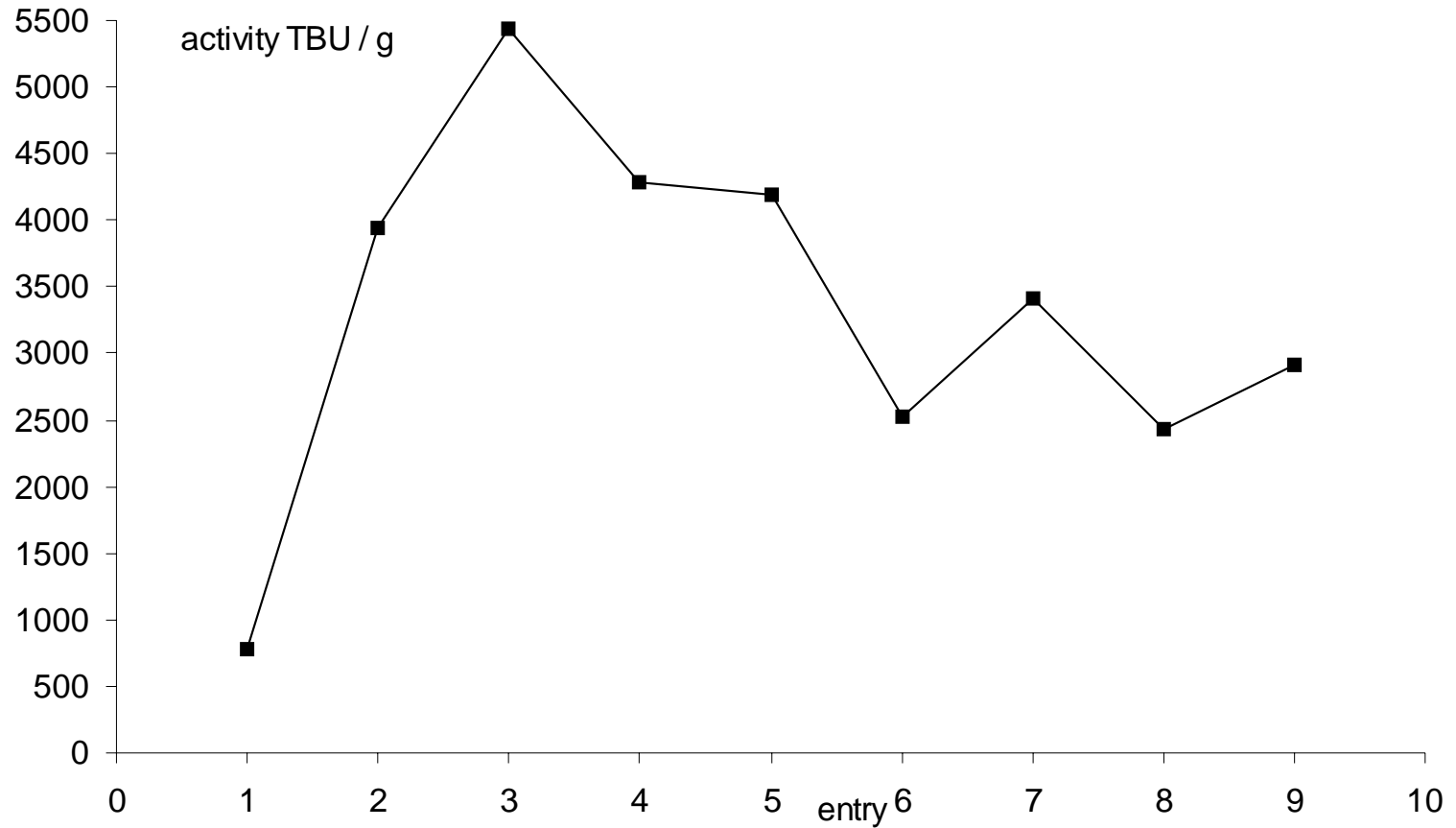
- macroporous, polyacrylate polymer beads
- stable and well distributed oxirane (epoxy) functionality
- spherical, defined particle size distribution
- highly porous network
- high stability and productivity
- no leaching

Pore diameter optimization I




Pore diameter optimization II

(CaLB immobilization)



Bead comparison

(various producers)



Bead	enzyme	wet beads		dried beads	
		activity	recovery	activity	recovery
Eupergit C	CaLA	750	20	1000	8
Sepabead	CaLA	800	11	1100	4
Immobead-150	CaLA	2650	25	4500	20
Eupergit C	CaLB	600	15	1000	12
Sepabead	CaLB	950	18	1600	9
Immobead-150	CaLB	3000	30	5200	20
Immobead prototype	CaLB	4000	40	11000	34


Enzymes from Novozymes. Activity: 1) tributyrin units in micromol acid / min.g at 40 °C.
Recoveries (%) are corrected for supernatant activity.

Eupergit is recently discontinued by Evonik!

New applications of proteases

(through immobilization)

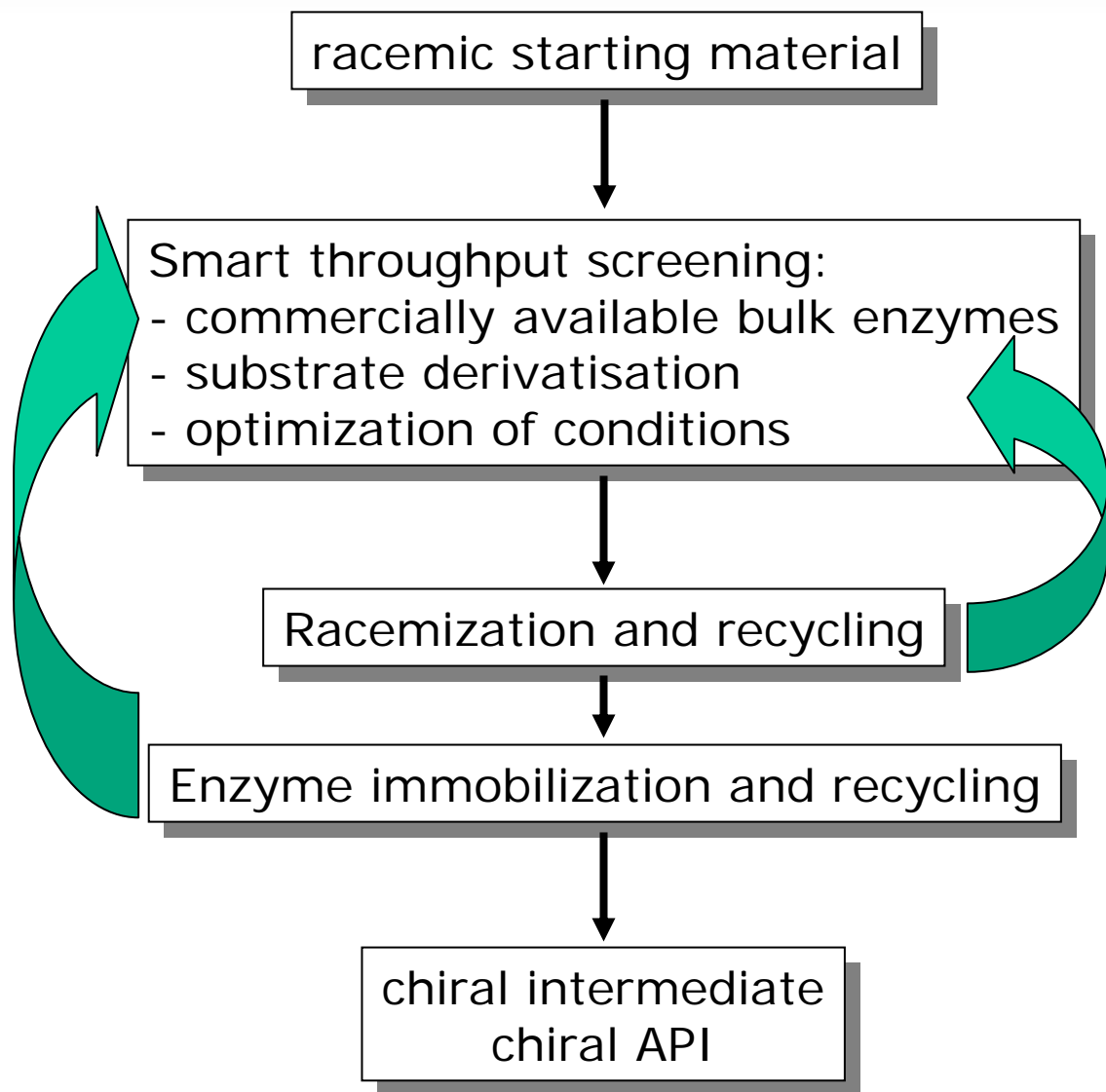
- *easy separation from typical water soluble products*
- *conversions in organic media*
- *resolution via transesterification*



Source	Synonym
Subtilisin from Bacillus sp.	Alcalase
Subtilisin from Bacillus sp.	Savinase
Subtilisin from Bacillus sp.	Everlase
Subtilisin from Bacillus sp.	Esperase
Bacillus licheniformis	Protex 6L
Bacillus amyloliquefaciens	Protex 7L
Bacillus licheniformis	Protex 8L
Geobacillus sp. (Thermolysin)	Protex 14L
Trichoderma reesei	Protex 15L
Aspergillus niger	Protex 26L
Bacillus subtilis	Protex 30L
Bacillus subtilis	Protex 40L
Aspergillus oryzae var.	Protex 50 FP
Aspergillus oryzae var.	Protex 51 FP
Bacillus subtilis	Protex 89L

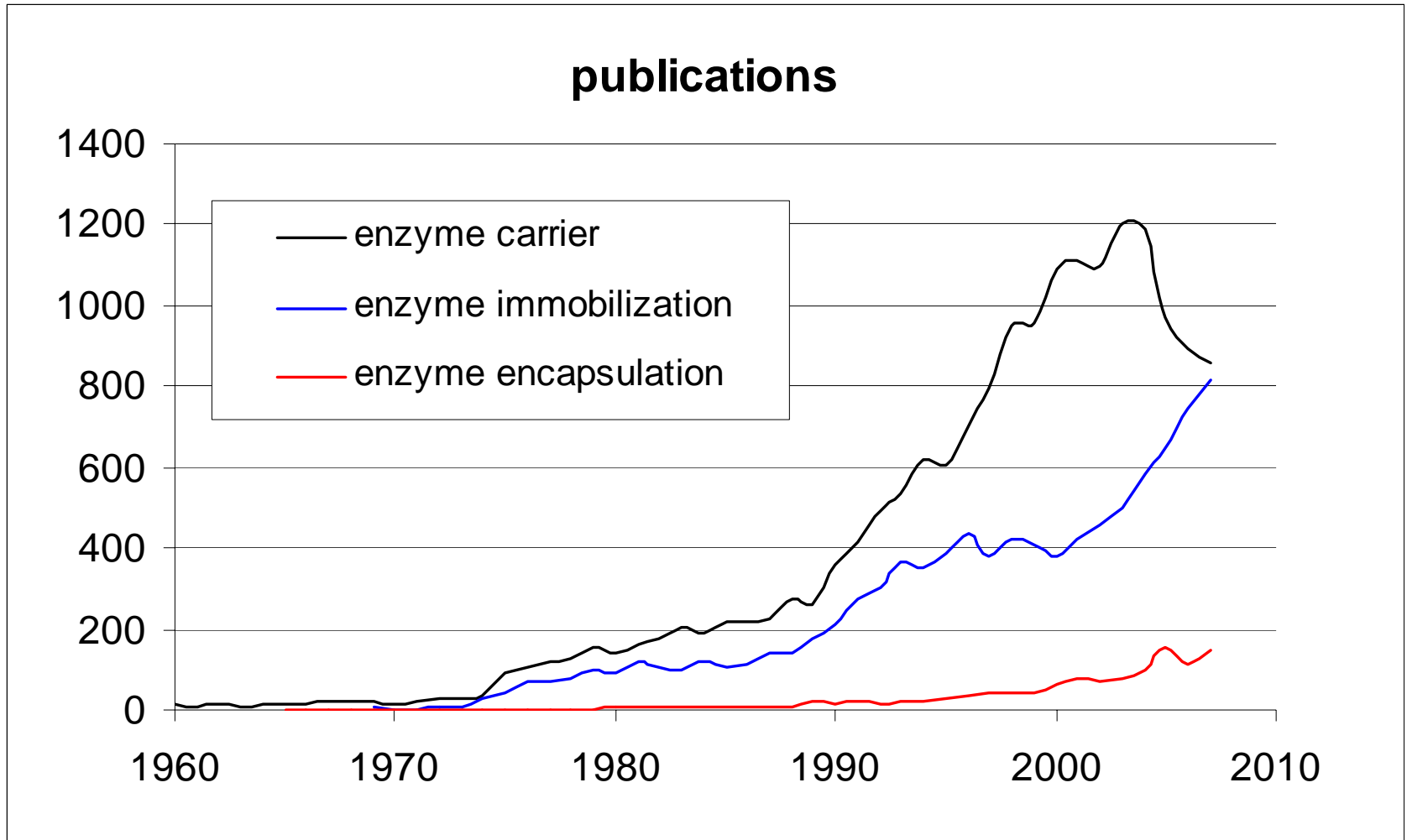
1-4 Novozymes, 5-11 Genencor

Immobilized bulk enzymes in resolution



are cost effective chiral catalysts!

Four decades of development



... the best is yet to come?

Chiral Vision

innovative biocatalysis

immobilized enzymes - custom immobilization - enzymatic process development – chiral compounds



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