Smarter chemical synthesis

– with Novozymes enzymes for biocatalysis

The new biocatalytic route results in process improvements, reduced organic solvent usage and substantial reduction of waste streams in Pregabalin production.

Introduction

Biocatalysis is the application of enzymes to replace chemical catalysts in synthetic processes. In recent past, the use of biocatalysis has gained momentum in the chemical and pharmaceutical industries. Today, it's an important tool for medicinal, process and polymer chemists to develop efficient and highly attractive organic synthetic processes on an industrial scale.

The biocatalytic process for Pregabalin has been developed by Pfizer to boost efficiency in Pregabalin production using Novozymes Lipolase®.

Development of the biocatalytic process for Pregabalin involves four stages:

- Screening to identify a suitable enzyme
- Performing optimization of the enzymatic reaction to optimize throughput and reduce enzyme loading
- Exploring a chemical pathway to preserve the enantiopurity of the material already obtained and lead to Pregabalin, and
- Developing a procedure for the racemization of (R)-1

Using Lipolase®, a commercially available lipase, rac-2-carboxyethyl-3-cyano-5-methylhexanoic acid ethyl ester (1) can be resolved to form (S)-2-carboxyethyl-3-cyano-5-methylhexanoic acid (2). Compared to the first-generation process, this new route substantially improves process efficiency by setting the stereocenter early in the synthesis and enabling the facile racemization and reuse of (R)-1.

It outperforms the first-generation manufacturing process also by delivering higher yields of Pregabalin and by resulting in substantial reductions of waste streams, corresponding to a 5-fold decrease in the E-Factor from 86 to 17.

Process improvements thanks to the biocatalytic route

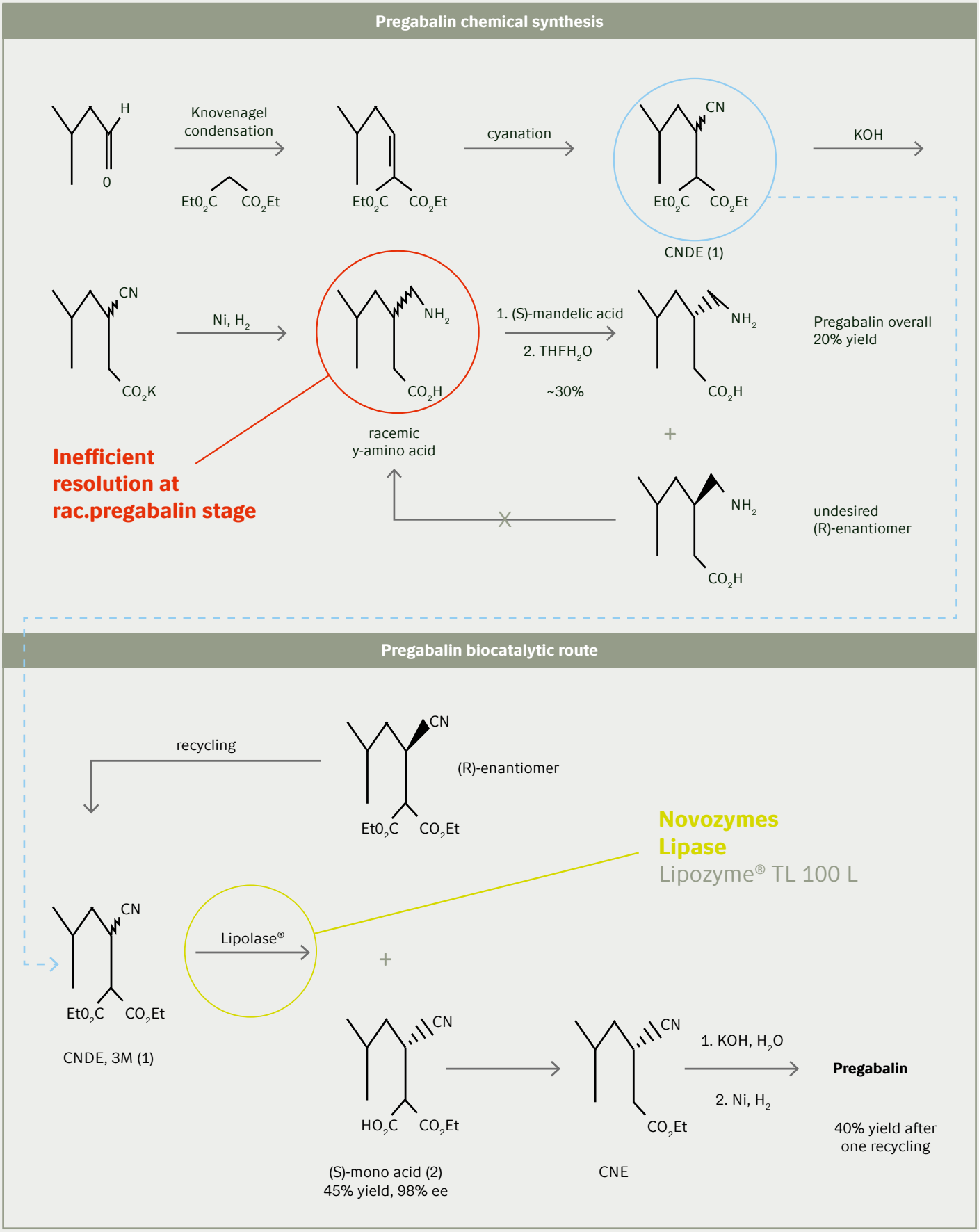

Fig 1. Difference between chemical synthesis and biocatalytic process

Disadvantages of the chemical route

- Resolution at the last step, resulting in loss of the valuable active ingredient
- Increase in raw material costs and overheads
- Use of large quantities of organic solvents
- High pressure hydrogenation
- Use of costly S-Mandelic Acid for resolution
- Less throughput (yield) of the active ingredient

Benefits of the biocatalytic route

- Low protein loading of 0.8%
- Increase in overall process yield
- High throughput
- Cost savings and less waste
- Improved E-Factor from 86 to 17
- Starting material reductions by 800 Mt (cumulative)
- Solvent usage reductions
- Mandelic acid usage – 500 Mt eliminated
- Energy savings by 83%
- Improved sustainability of the manufacturing process

Case: More cost savings, less input (Raw material inputs for 1000kg API output)

Inputs	Chemical route (Kg)	Chemoenzymatic route (Kg)	Reduction
CNDE	6212	4798	**23%**
Enzyme	0	574	Enzyme replaces (S)-Mandelic acid
(S)-Mandelic acid	1135	0	
Raney Nickel	531	79.5	**85%**
Solvents	50042	6230	**88%**
Total	**57920**	**11681.5**	**79.8%**

Benefits of these reductions

CNDE (key substrate)
Cost reduction, more competitiveness and profitability

(S)-Mandelic acid
Eliminated from the process, resulting in more savings

Metal catalyst (Raney Nickel)
Reduces the burden, meaning less waste to treat

Solvent
Helps acquire a more sustainable profile and results in process efficiency

General method for screening

Pfizer carried out a screen of commercially available hydrolases in 96-well format to identify a suitable enzyme for the kinetic resolution of 1. Initial screening at a substrate loading of 5% (v/v) revealed many enzymes that catalyzed the hydrolysis of 1, although only a few of these demonstrated enantioselectivity (after measuring enantiomeric ratios, E value).

It was found that Thermomyces lanuginosus lipase Lipolase®, gives a very high E* value of >200 with an S selectivity.

Enantio selectivity

$*E = \ln(ee_p(1-ee_s)/(ee_p+ee_s))/\ln(ee_p(1+ee_s)/(ee_p+ee_s))$
Where ee_s is enantiomer excess of substrate and ee_p is enantiomer excess of product

**Source: Organic Process Research & Development 2008, 12, 392–398*

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June 2016 · No. 2016-10200-02

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