Process development of transaminase catalyzed reactions for large scale industrial use

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Chiral Amines

- Useful building blocks in stereoselective synthesis
- Present in a wide range of pharmaceutical intermediates (>70%)

Chemical Methods to Produce Chiral Amines

1. Crystallization with chiral carboxylic acids (resolution of racemic amine)

\[
\begin{align*}
\text{R}^1\text{NH}_2 + \text{R}^4\text{COOH} &\rightarrow \left[\begin{array}{c}
\text{R}^1\text{NH}_3^+ \\
\text{R}^2\text{R}^3\text{R}^4\text{CO}^-
\end{array}\right] + \left[\begin{array}{c}
\text{R}^1\text{NH}_3^+ \\
\text{R}^2\text{R}^3\text{R}^4\text{CO}^-
\end{array}\right]
\end{align*}
\]

½ of material wasted

2. Reductive Amination

\[
\begin{align*}
\text{R}^1\text{N}^X\text{R}^2 + \text{R}^1\text{HOH} &\rightarrow \text{HN}^X\text{R}^1\text{R}^2 + \text{NH}_2\text{R}^1\text{R}^2
\end{align*}
\]

difficulties = making starting material and cleaving X

Merck’s Januvia for type 2 Diabetes
$2B/yr projected sales

- H$_3$PO$_4$ • H$_2$O

TRANSAMINASE
multiple step synthesis
Transaminase Reactions: Challenges and Goals

Goals:

1. Evaluate transamination equilibrium and inhibition issues
2. Develop general methods for driving reactions to completion
   • Novel rapid transaminase screening system
   • Process development activities at 50mL scale

Use transaminases for the practical and economic industrial synthesis of chiral amines
Why Use Acetophenone as Model Substrate?

- Representative building block for chemical synthesis
- One of the most difficult transformations:
  - acetophenone substrate solubility issues
  - product methylbenzylamine inhibition/deactivation issues
  - substrate to product equilibrium issues
- If we can run this reaction in the synthesis direction, we should be able to run almost any substrate
Transamination Equilibrium

Driving Acetophenone Transamination Equilibrium

- Transaminase still active
- Equilibrium strongly favors starting ketone and amine donor
- Excess amine donor isn’t enough for high productivity process

50mM acetophenone

50mM alanine (1X eq.)
500mM alanine (10X eq.)
Transaminase Inhibition

Transaminase inhibition by pyruvate and methylbenzylamine.

Inhibition by Pyruvate

% inhibition vs. pyruvate (mM)

Inhibition by Methylbenzylamine

% inhibition vs. MBA concentration (mM)
Driving Transamination Reactions

*Lactate Dehydrogenase System*

- Pyruvate consumption drives equilibrium and eliminates pyruvate inhibition
- GDH / NADH cofactor recycle = proven efficient, cost effective system
- Acid formation enables reaction tracking
Driving Transamination Reaction Using LDH System

- Equilibrium strongly favors starting materials
- LDH system drives equilibrium and eliminates pyruvate inhibition
- reaction proceeds >20X further
pH Based Colorimetric Transaminase Activity Assay

- Very precise quantification of transaminase activity
- Directly scale this screening system up to first delivery scale

*Why not track NADH consumption?*
• we have a precise colorimetric assay for enzyme activity and quantitative determination of product formation
• tuning buffer strength = track entire reaction profile
Microscale Process Development Using pH Indicator Assay

- 100uL scale reactions run in plate spec for process development
- Temperature activity/stability and pH activity profiles obtained
- Inhibition studies, substrate and enzyme loading can be analyzed

- Note these profiles are for the complete ATA/LDH/GDH reaction system not just a single enzyme (great for direct scaling of process)
Transamination Scale-Up Directly from Screening Conditions to Reactor

- conversion tracked by automated pH control (NaOH addition) – red points show HPLC conversion
- >95% conversion of 50mM acetophenone (6g/L) with 1M alanine
- Both S- and R-methylbenzylamine can be made with >99% ee
In-situ Product Extraction Using Resins

- Amberlite Ion Exchange Resin (IRP-69) and Polymeric Adsorbent Resin

- Ion exchange resin at 200g/L reduces a 420mM (50g/L) MBA charge aqueous concentration to ~25mM (below the 50% inhibition barrier)
30mL Scale Resin/LDH Transamination System

- Reactions run with automated pH control in Multimax reactors
- 50g/L (420mM) acetophenone reactions proceeded to >95% conversion with 200g/L resin
- Isolation of product MBA requires simple dilute NaOH wash of ion exchange resin

50g/L Transamination of Acetophenone
Driving Transamination Reactions

Amino Acid Dehydrogenase System

- Catalytic alanine system
- Amino donor generated *in situ*
- Eliminates pyruvate inhibition
- Cheap ammonia is amine donor
50mM acetophenone (6g/L) with 25mM pyruvate reaction proceeded to complete conversion.

Slower rate than LDH system, but uses a 40X reduction in alanine.

Actually used no amine donor that’s accepted by transaminase!
Driving Transamination Reactions

Isopropylamine System

- Single enzyme system with very cheap amino donor
- Equilibrium can be driven via acetone removal
- Limited to transaminases that accept isopropylamine (IPM)
- Transaminases must also be stable in high IPM concentrations
Isopropylamine Transamination System

- Both $S$-methylbenzylamine (using ATA-113) and $R$-methylbenzylamine (using ATA-117) can be produced with $>99\%$ ee
- $50\text{mM (6g/L)}$ acetophenone with $1\text{M IPM}$ proceeds to $>95\%$ conversion
Driving Transaminations via Spontaneous Secondary Intramolecular Product Reactions

- transamination of ethyl 4-acetylbutyrate
- spontaneous cyclization of amine product to 6-methyl-2-piperidone product

- transamination of ethyl levulinate
- spontaneous cyclization of amine product to 5-methyl-2-pyrrolidinone
• ATA-113 and ATA-117 produce >99% ee S or R product with >95% conversion
Summary

Rapid Screen/Micro-scale Development

• rapid high-throughput pH based colorimetric assay for transaminase activity
• microscale process development that can be scaled up directly for material delivery

Screening hit = first material delivery

Process Development

(Method 3, isopropylamine)

• 3 process methods for driving transamination reactions demonstrated in 50mL scale reactors
• Can produce both R- and S-amines using Codexis’ transaminase library with:

>99% ee and >90% yield
Summary – Transamination Manufacturing Processes (> 50 g/L substrate)

- demonstrated transamination reactions at >50 g/L substrate loading
  - *in situ* product removal with an ion exchange resin to selectively bind amine product
  - spontaneous intramolecular secondary reaction of amine product
  - demonstrated on the production of >40 amines with >99% ee
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