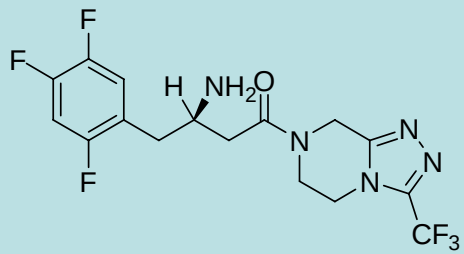
Process development of transaminase catalyzed reactions for large scale industrial use

MERCK
Research Laboratories

Matthew D. Truppo

Chiral Amines

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

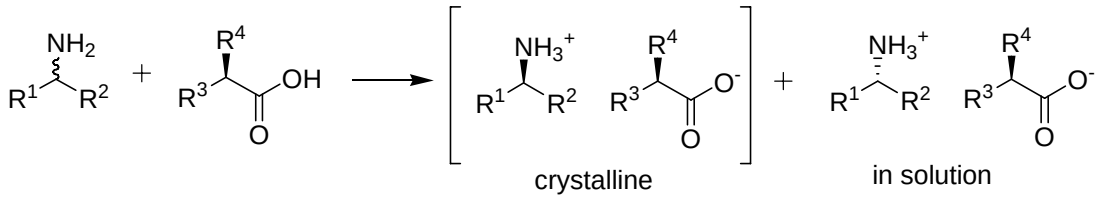


Merck's Januvia for type 2 Diabetes
$2B/yr projected sales

• H_3PO_4 • H_2O

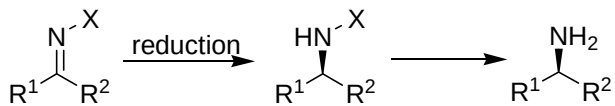
Chemical Methods to Produce Chiral Amines

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

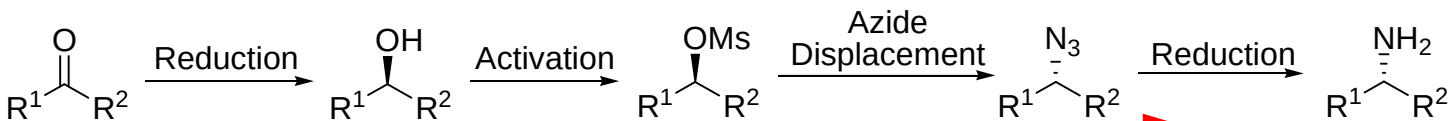


½ of material wasted

2. Reductive Amination



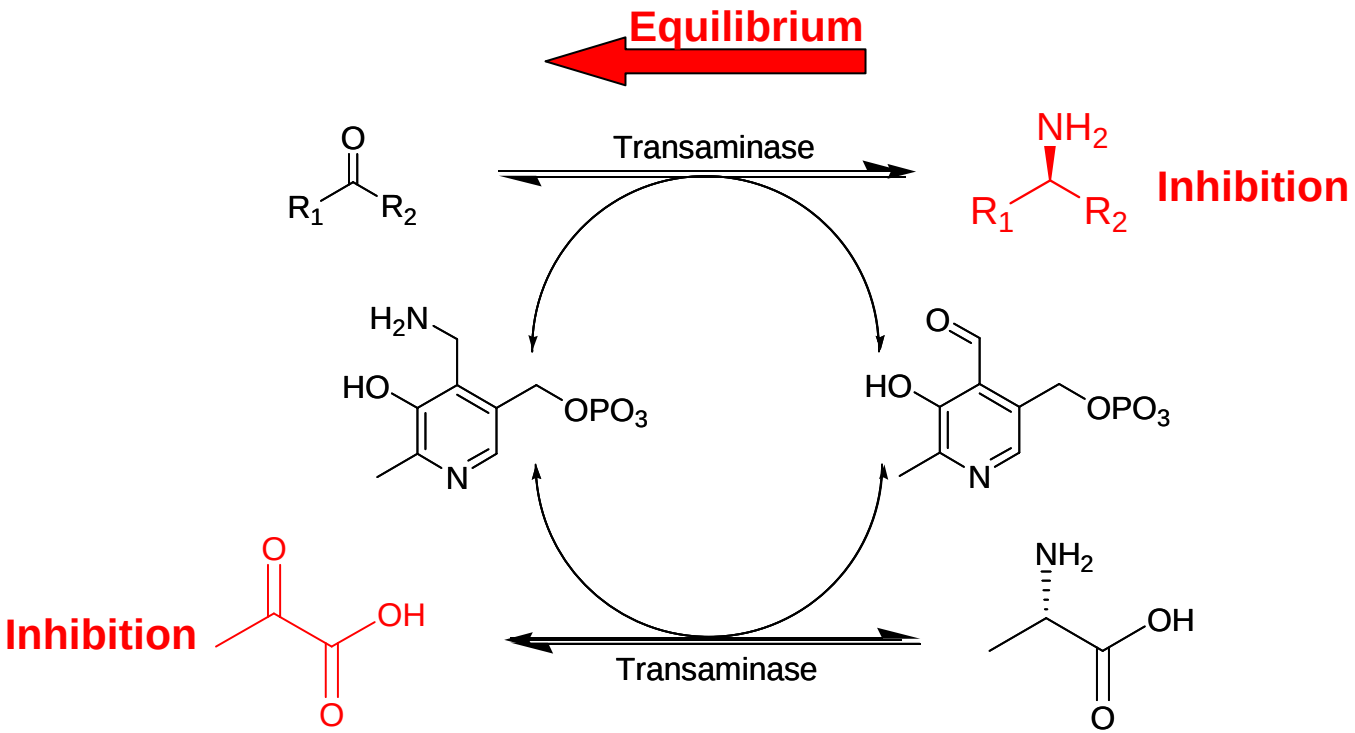
difficulties = making starting material and cleaving X



TRANSAMINASE

multiple step synthesis

Transaminase Reactions: Challenges and Goals

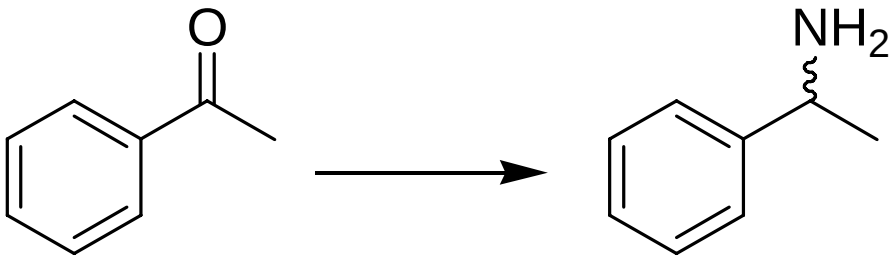


Goals:

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

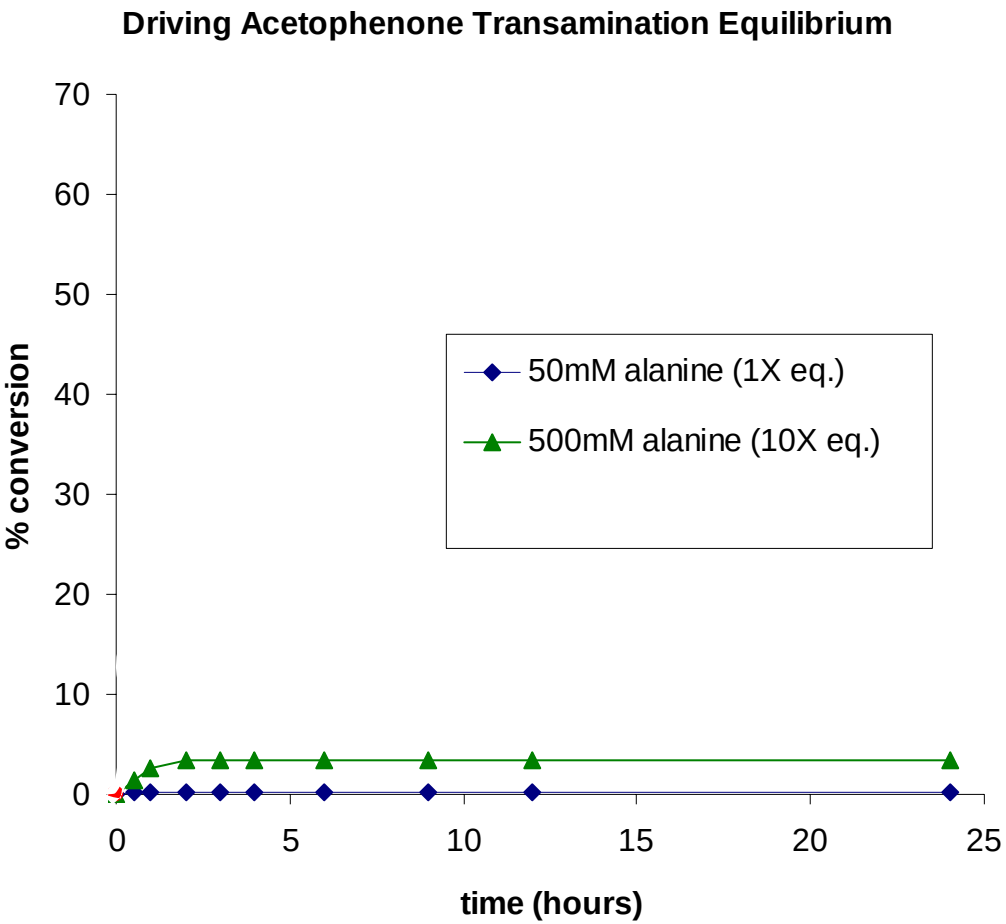
Use transaminases for the practical and economic industrial synthesis of chiral amines

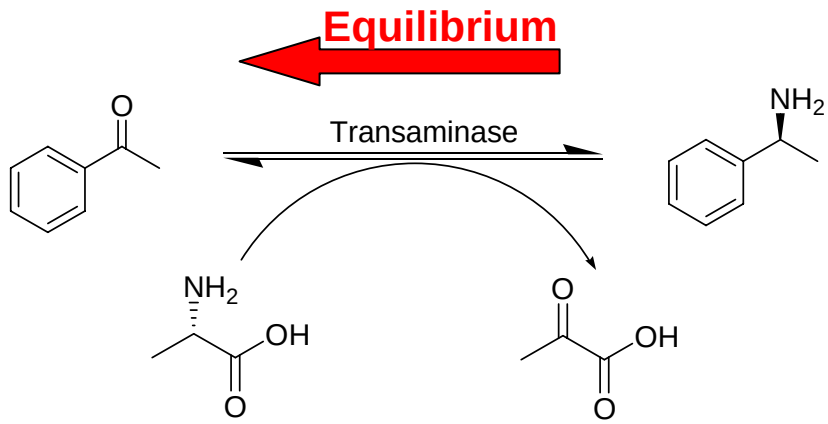
Why Use Acetophenone as Model Substrate?



- 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

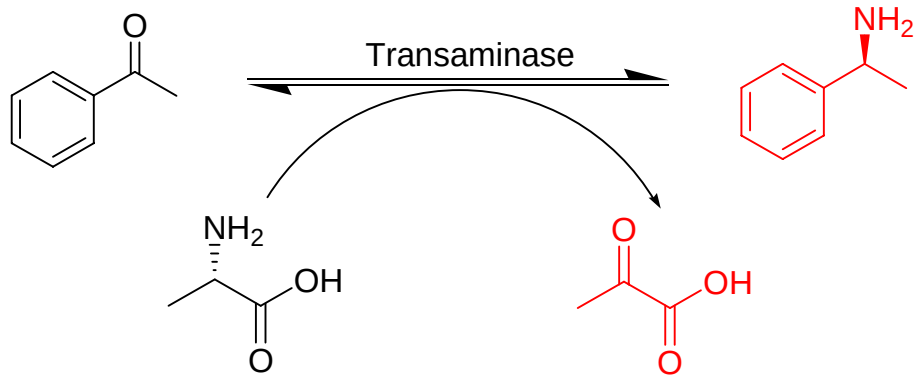


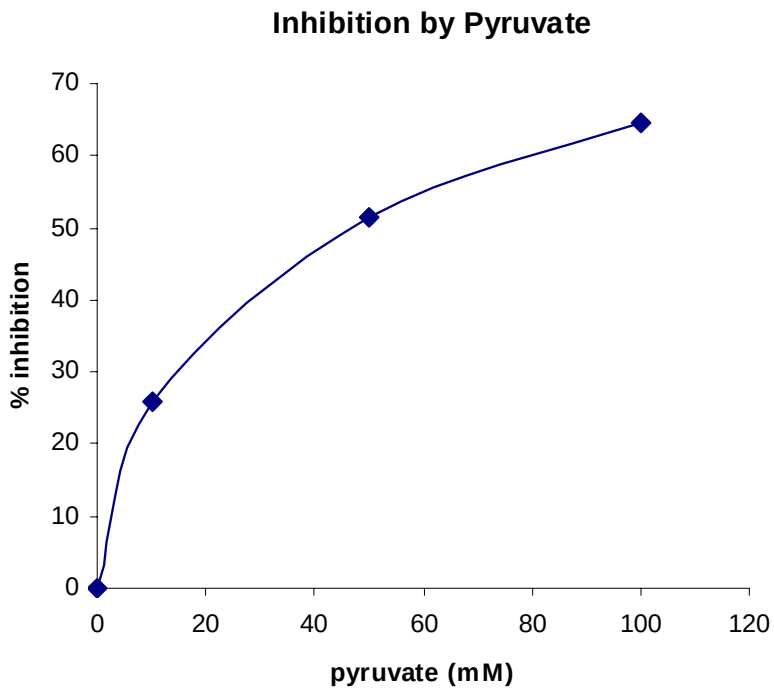


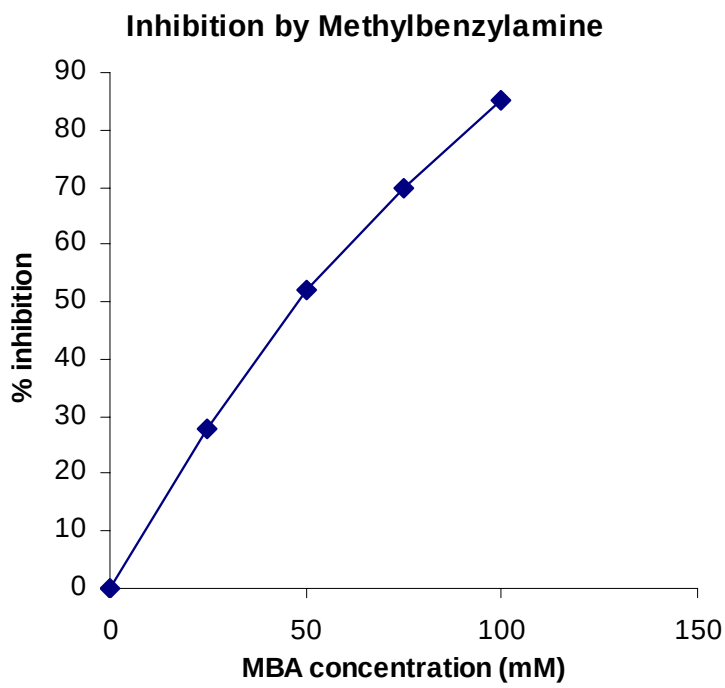
50mM acetophenone

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

Transaminase Inhibition

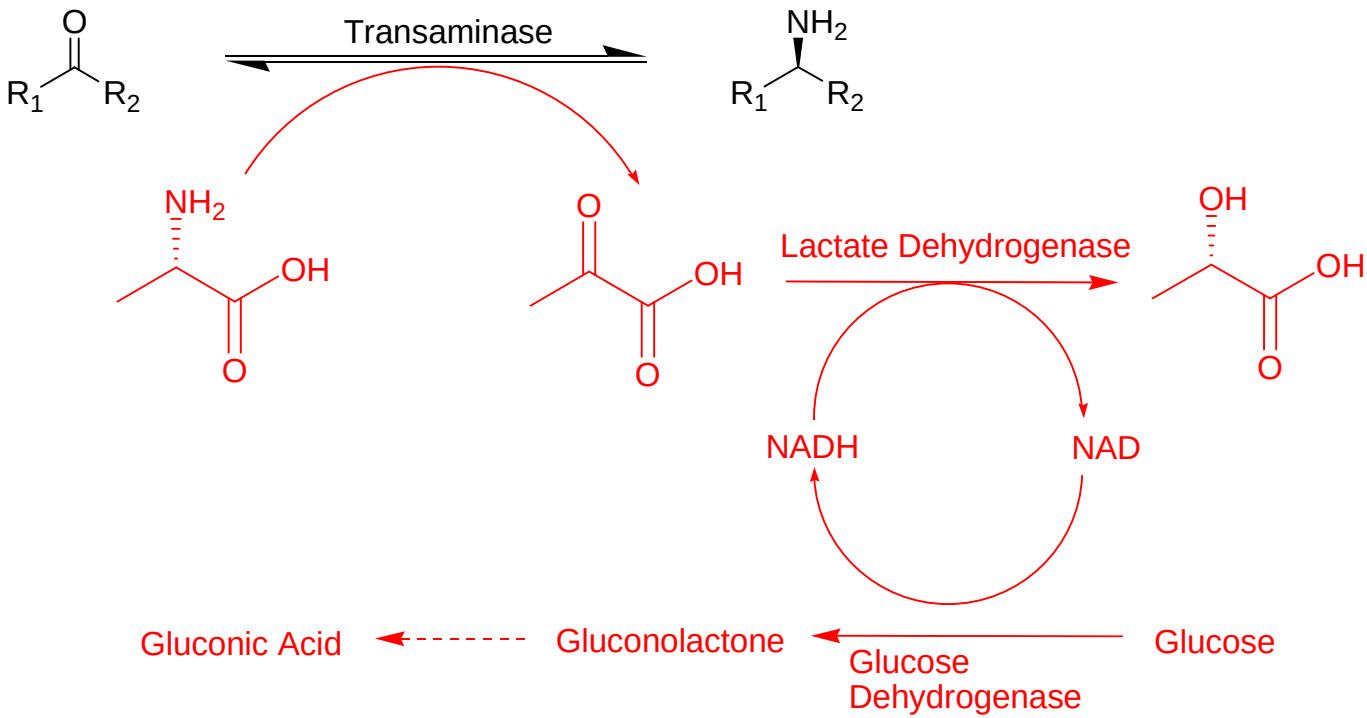






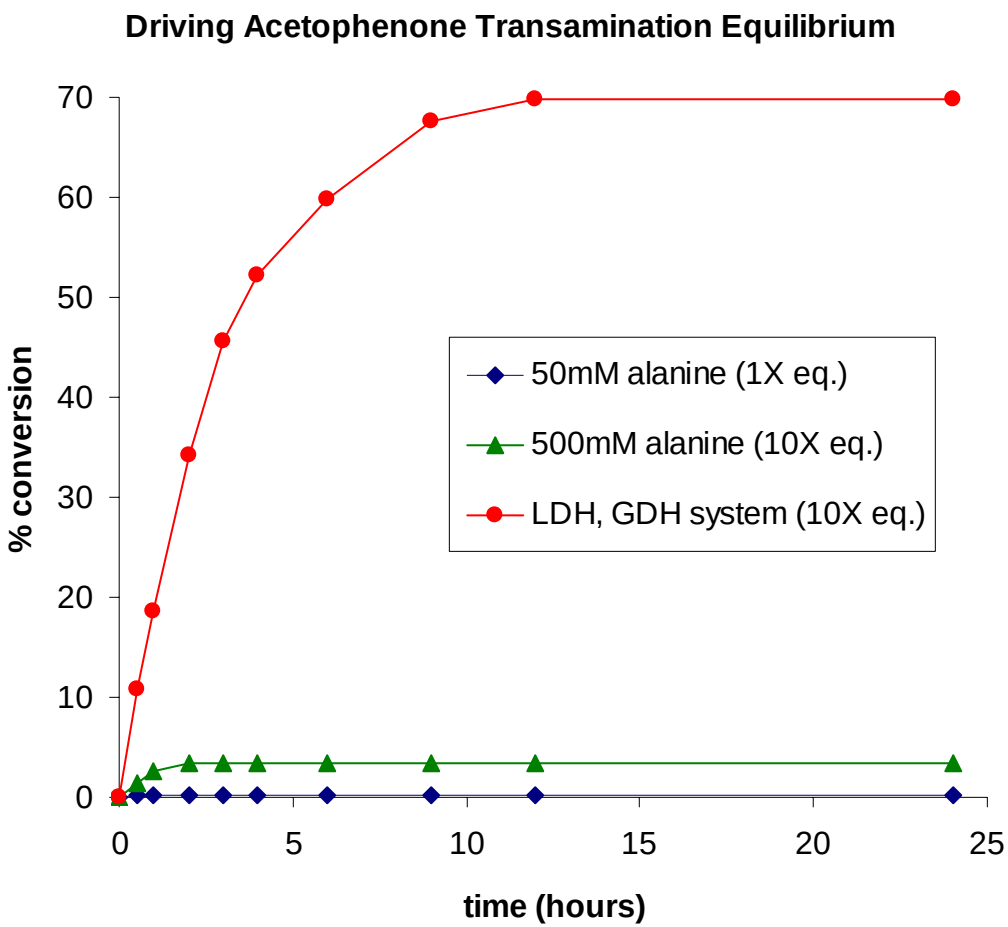
Driving Transamination Reactions

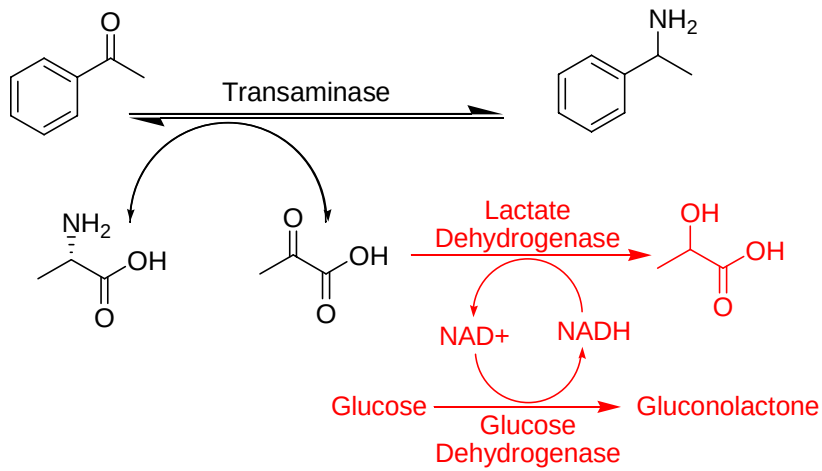
Lactate Dehydrogenase System



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

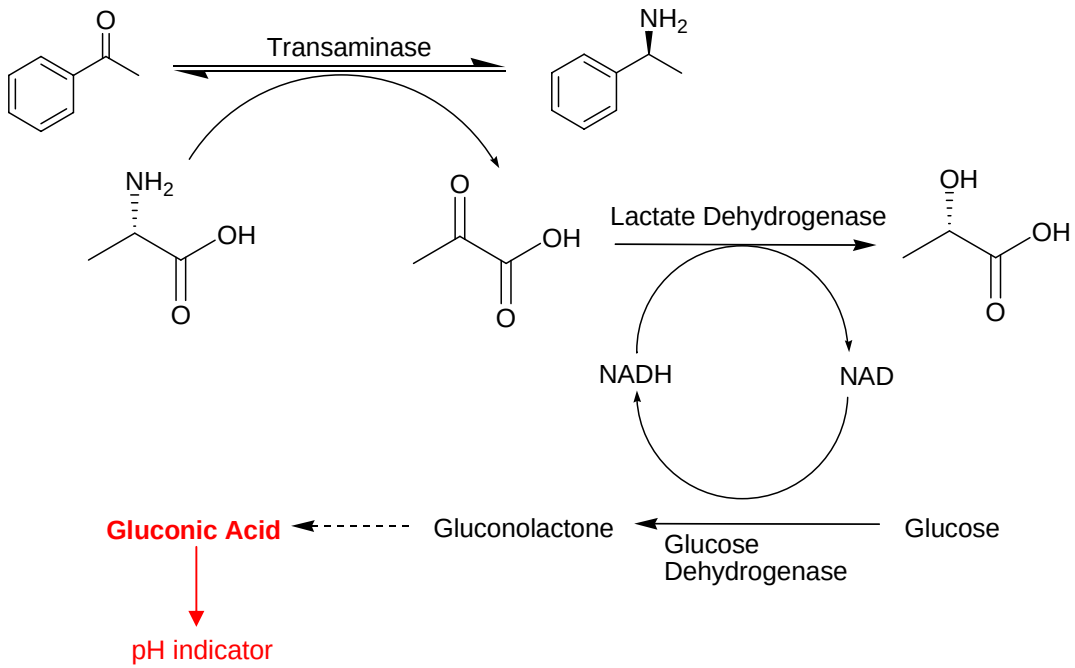
Driving Transamination Reaction Using LDH System

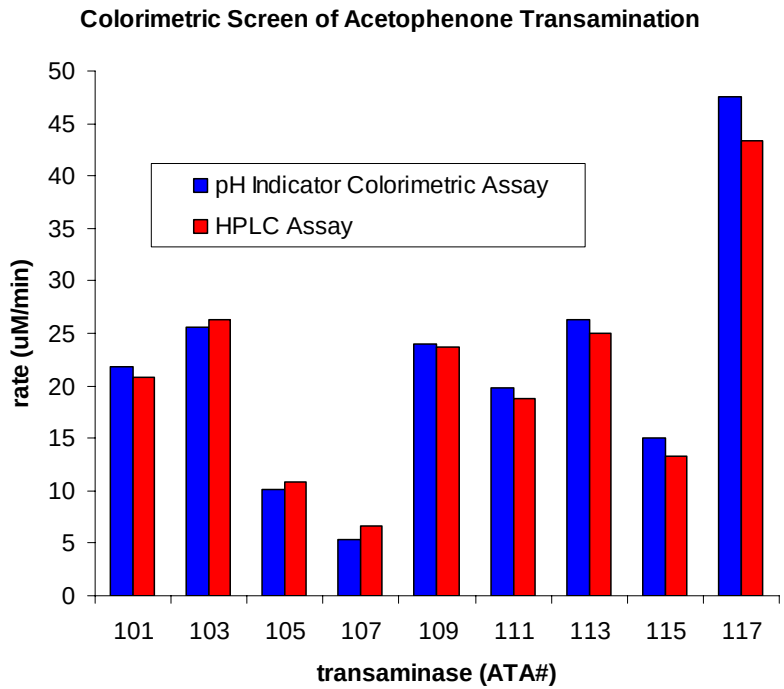




- Equilibrium strongly favors starting materials
- LDH system drives equilibrium and eliminates pyruvate inhibition
- reaction proceeds >20X further

pH Based Colorimetric Transaminase Activity Assay

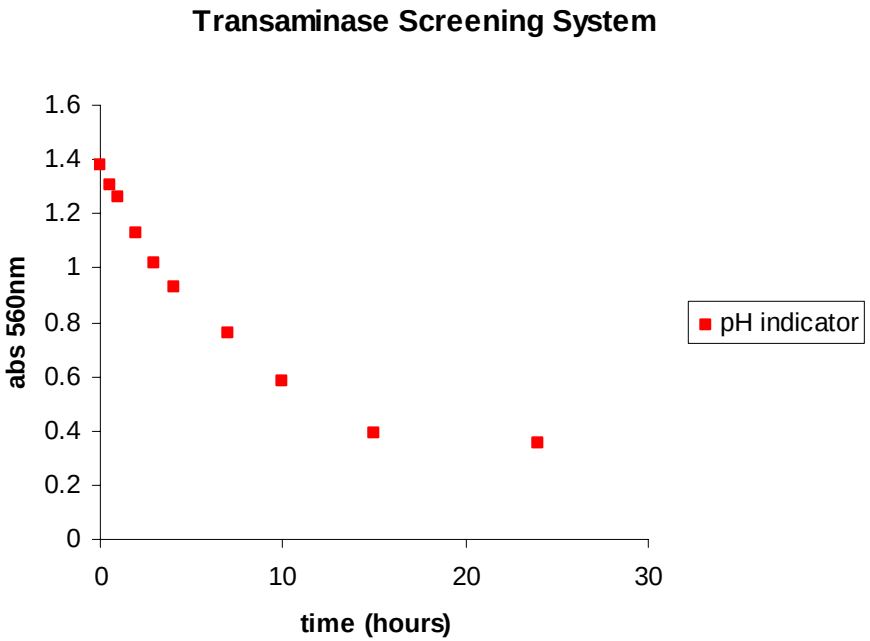


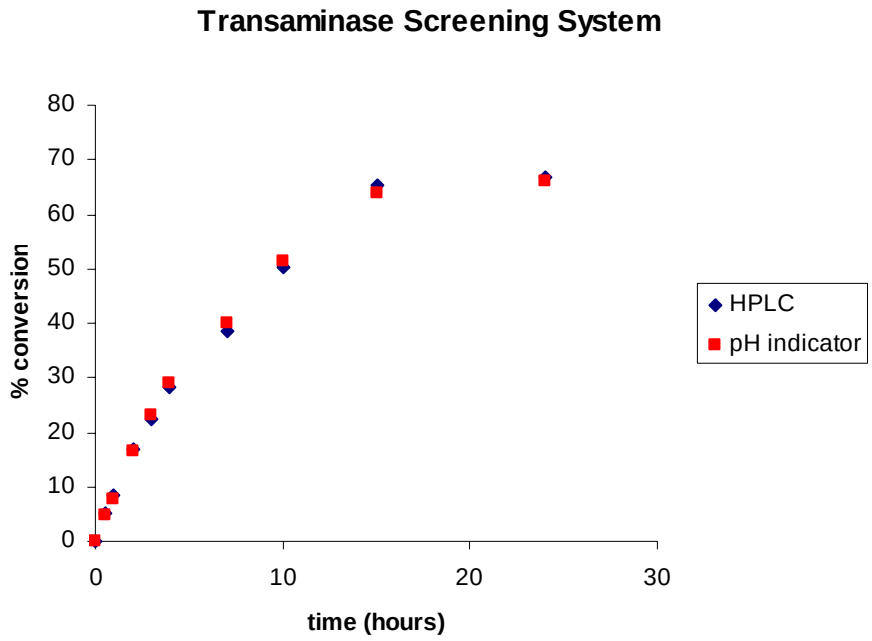


- Very precise quantification of transaminase activity
- Directly scale this screening system up to first delivery scale

Why not track NADH consumption?

Running Transamination with pH Indicator



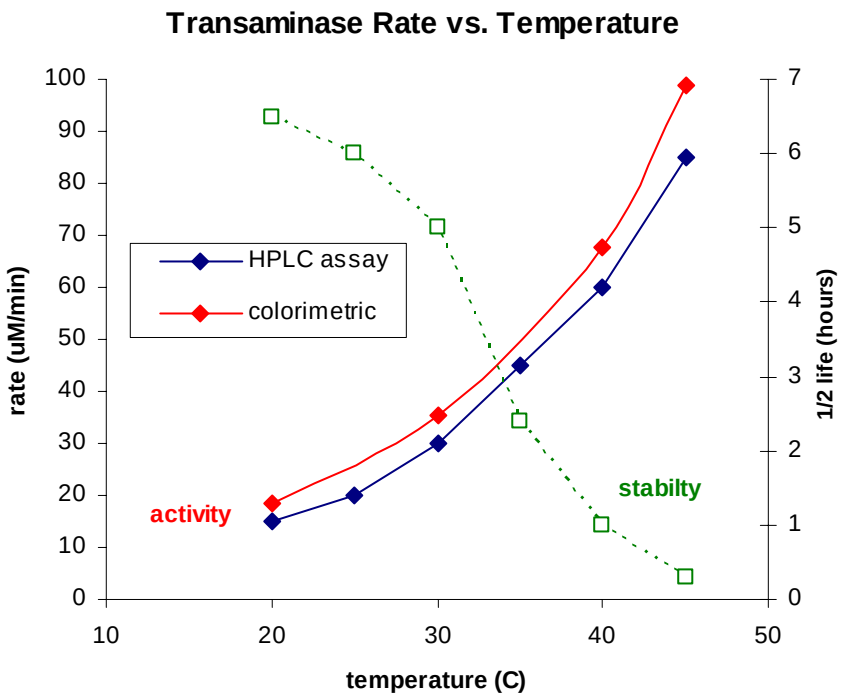


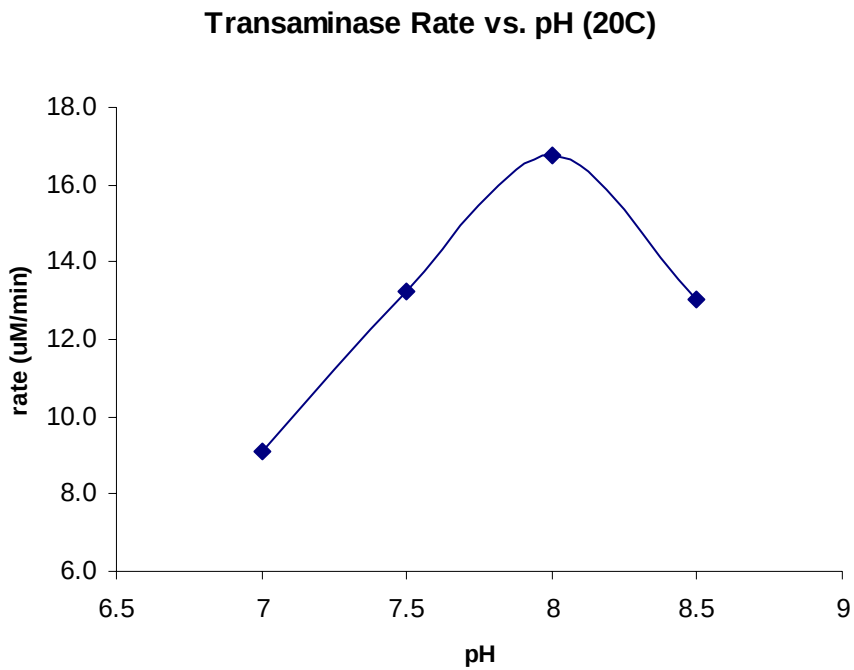
- we have a precise colorimetric assay for enzyme activity and quantitative determination of product formation
- tuning buffer strength = track entire reaction profile

Microscale Process Development Using pH Indicator Assay

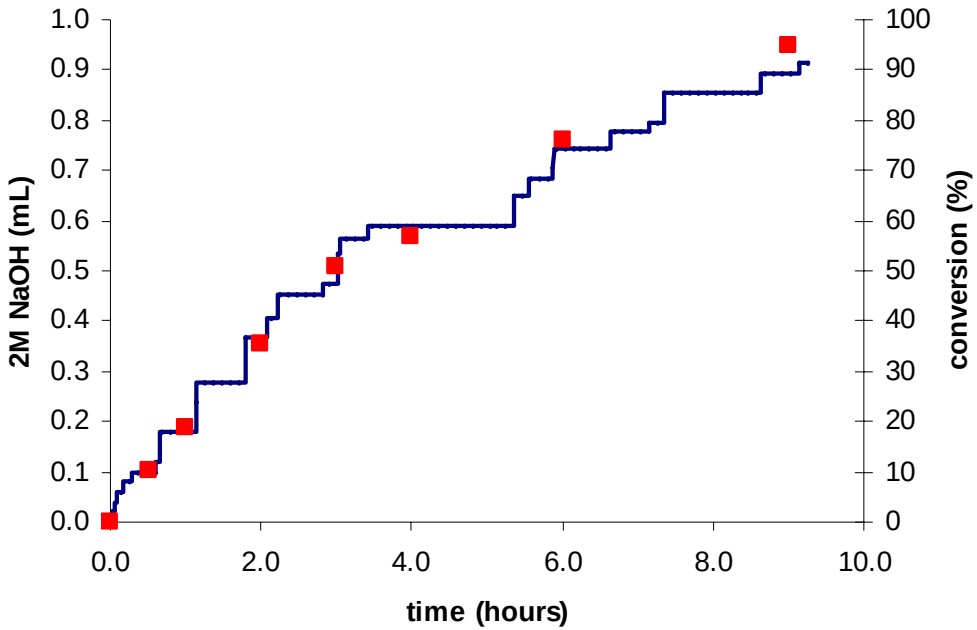
- 100uL scale reactions run in plate spec for process development
- Temperature activity/stability and pH activity profiles obtained
- Inhibition studies, substrate and enzyme loading can be analyzed

- Note these profiles are for the complete ATA/LDH/GDH reaction system not just a single enzyme (great for direct scaling of process)





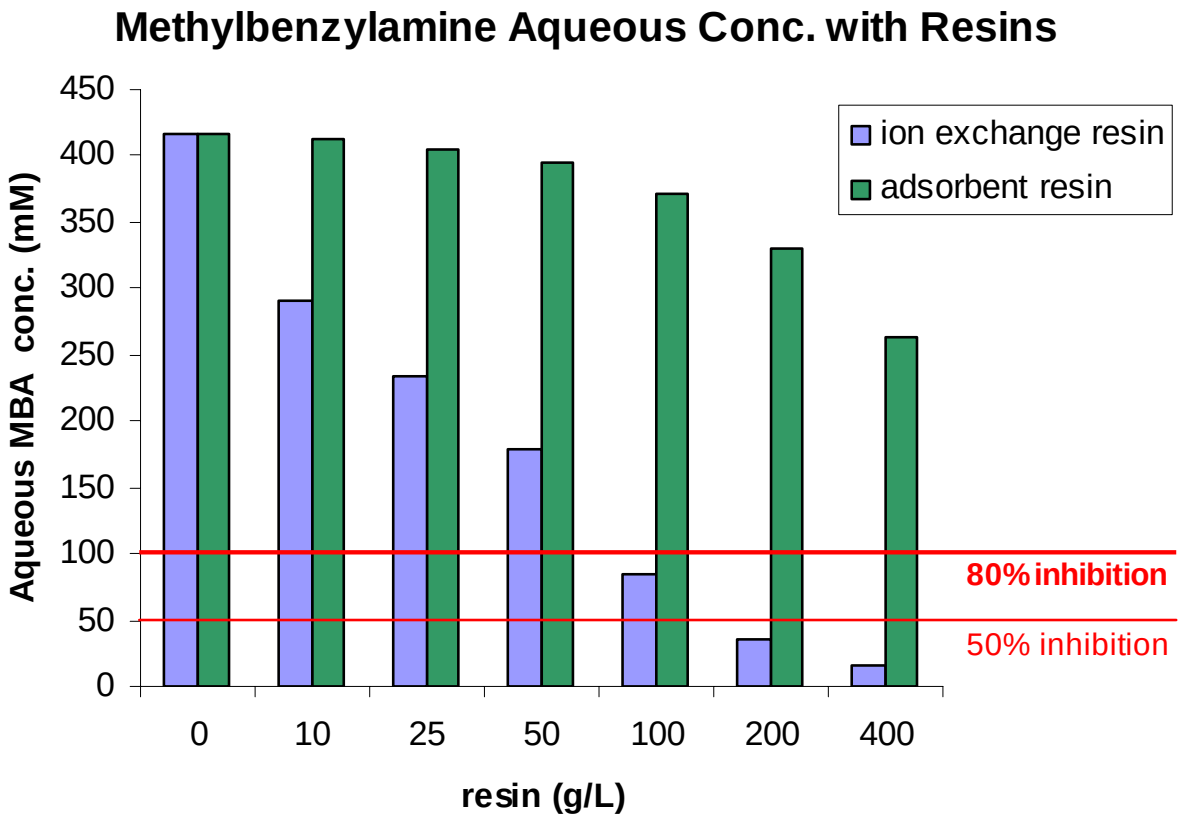
Transamination Scale-Up Directly from Screening Conditions to Reactor



• 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**

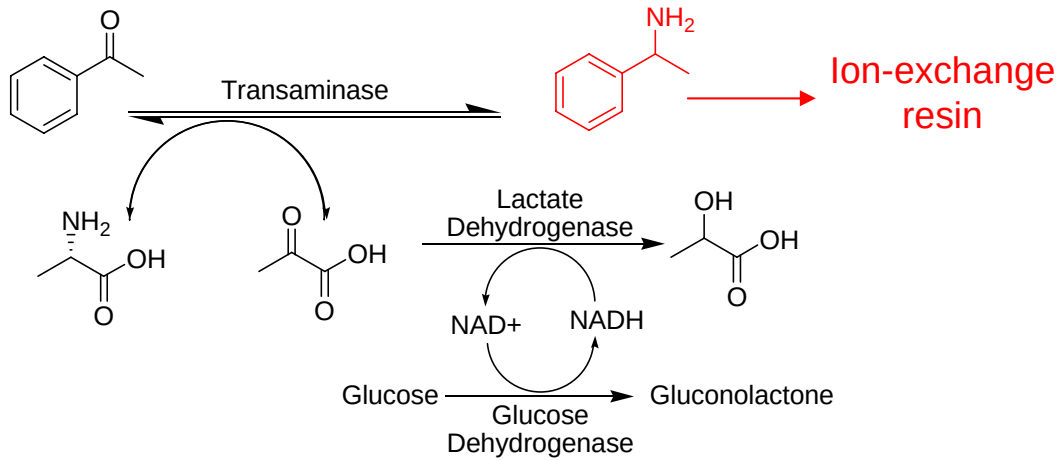
In-situ Product Extraction Using Resins



- 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)

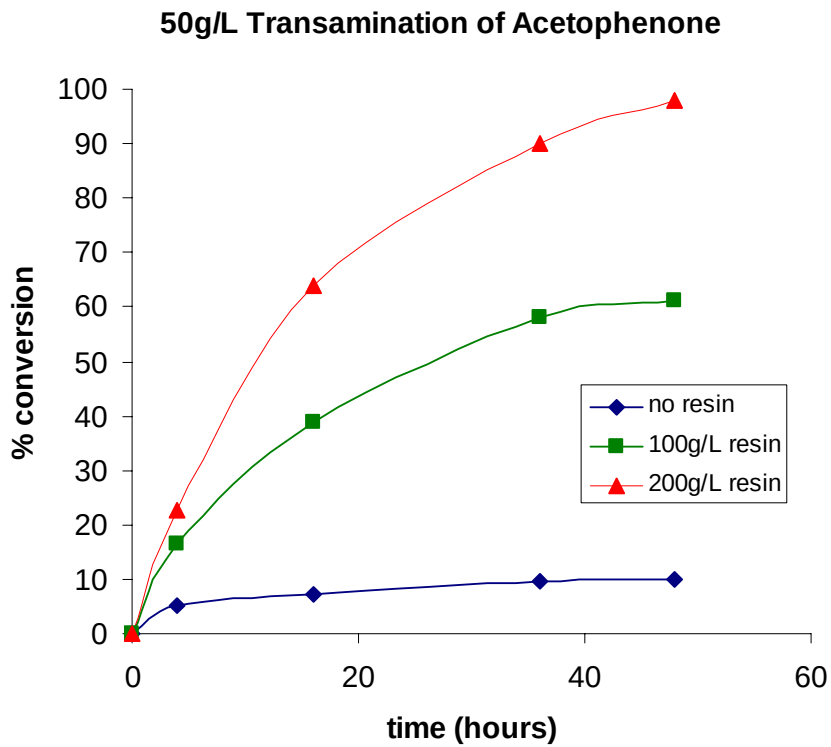
30mL Scale Resin/LDH Transamination System



- reactions run with automated pH control in Multimax reactors

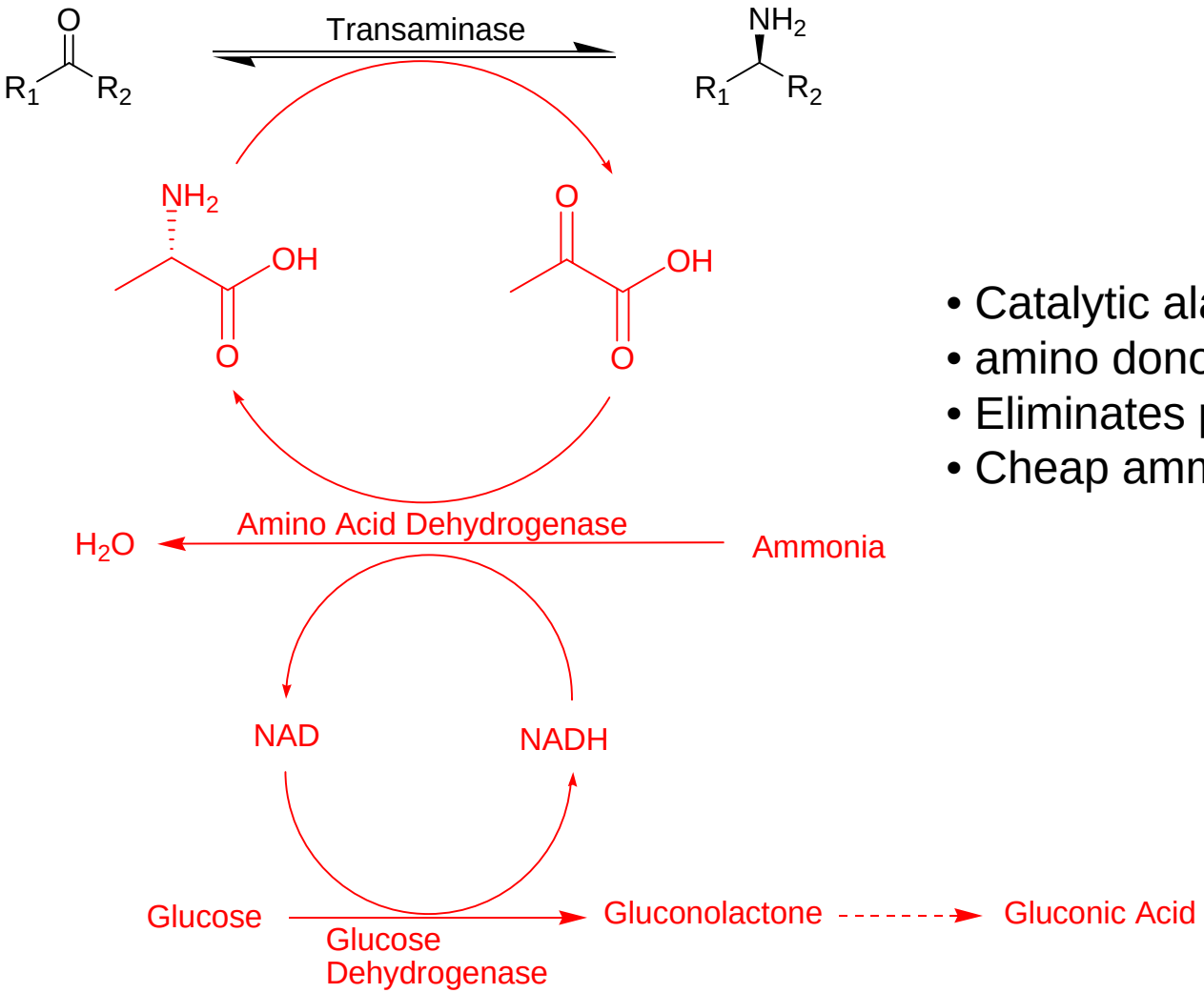
- 50g/L (420mM) acetophenone reactions proceeded to >95% conversion with 200g/L resin

- isolation of product MBA requires simple dilute NaOH wash of ion exchange resin



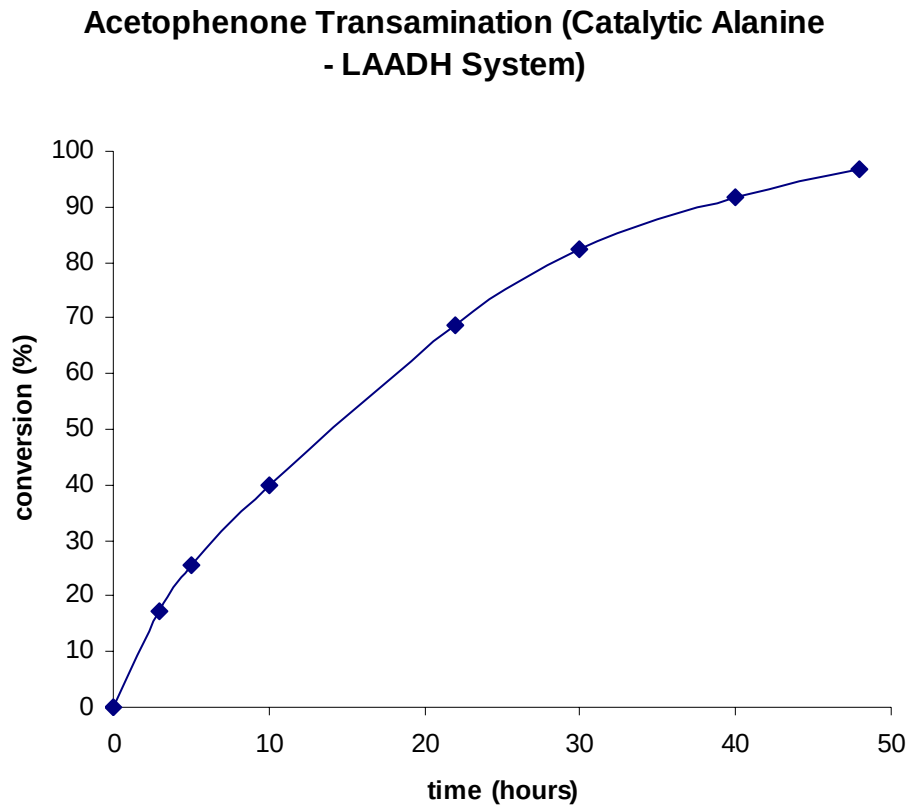
Driving Transamination Reactions

Amino Acid Dehydrogenase System



- Catalytic alanine system
- amino donor generated *in situ*
- Eliminates pyruvate inhibition
- Cheap ammonia is amine donor

Transamination Using Catalytic Alanine System

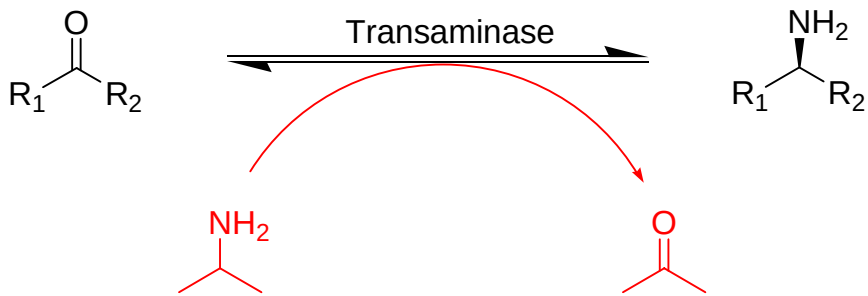


- 50mM acetophenone (6g/L) with 25mM pyruvate reaction proceeded to complete conversion
- Slower rate than LDH system, but uses a 40X reduction in alanine

Actually used no amine donor that's accepted by transaminase!

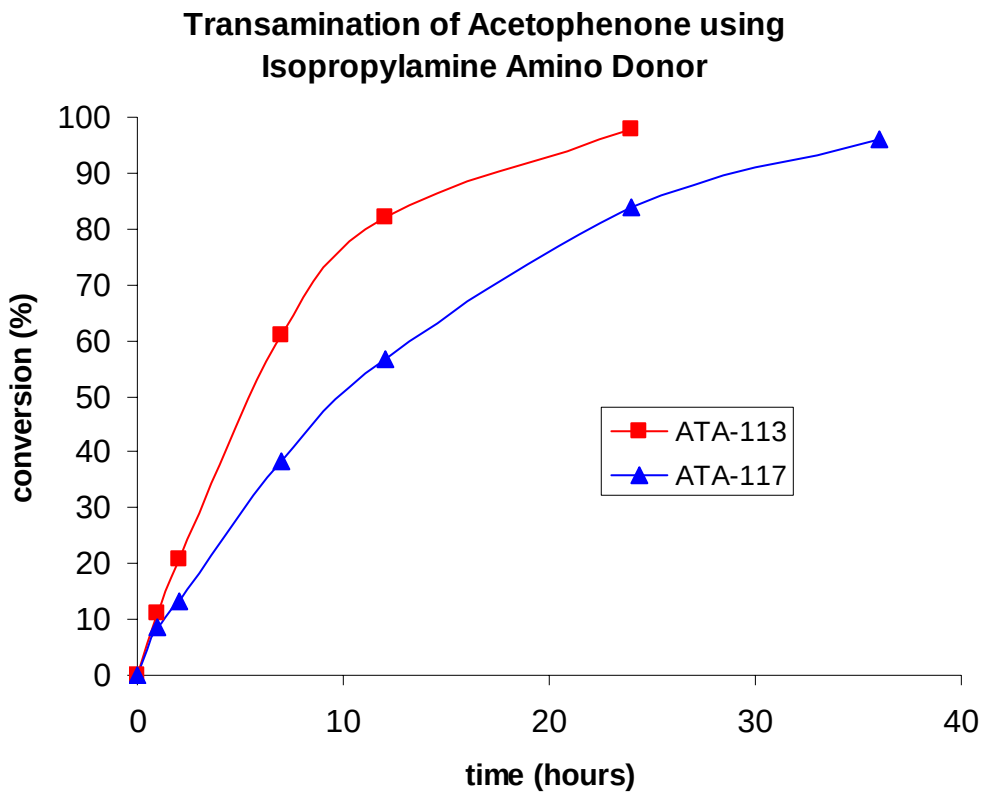
Driving Transamination Reactions

Isopropylamine System



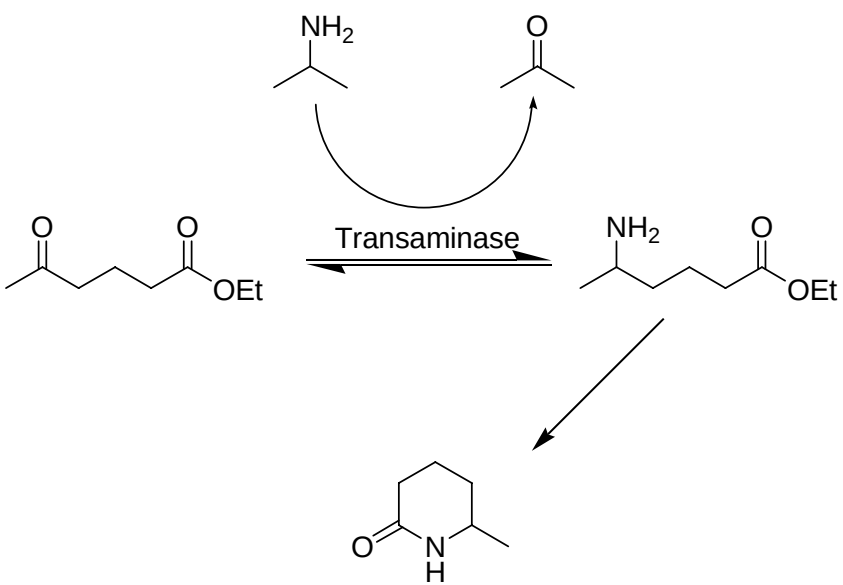
- Single enzyme system with very cheap amino donor
- Equilibrium can be driven *via* acetone removal
- Limited to transaminases that accept isopropylamine (IPM)
- Transaminases must also be stable in high IPM concentrations

Isopropylamine Transamination System

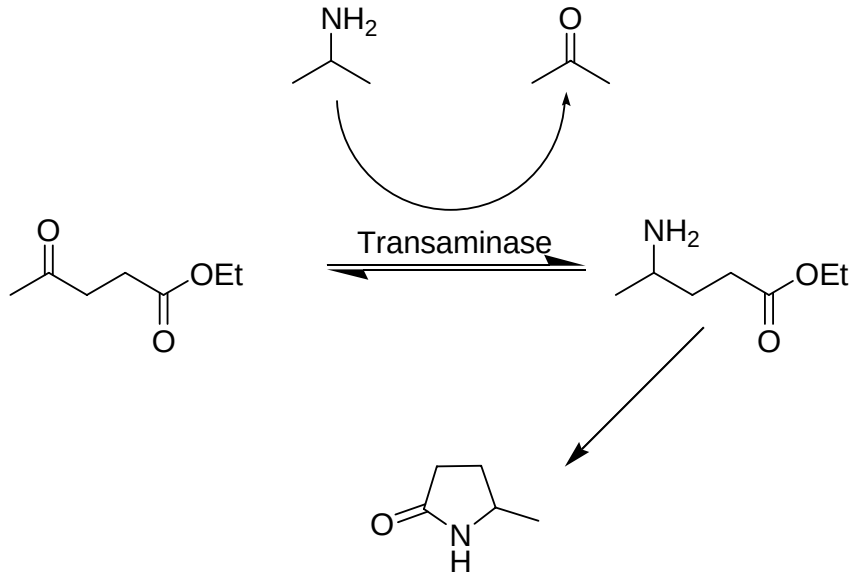


- Both *S*-methylbenzylamine (using ATA-113) and *R*-methylbenzylamine (using ATA-117) can be produced with >99% ee
- 50mM (6g/L) acetophenone with 1M IPM proceeds to >95% conversion

Driving Transaminations *via* Spontaneous Secondary Intramolecular Product Reactions

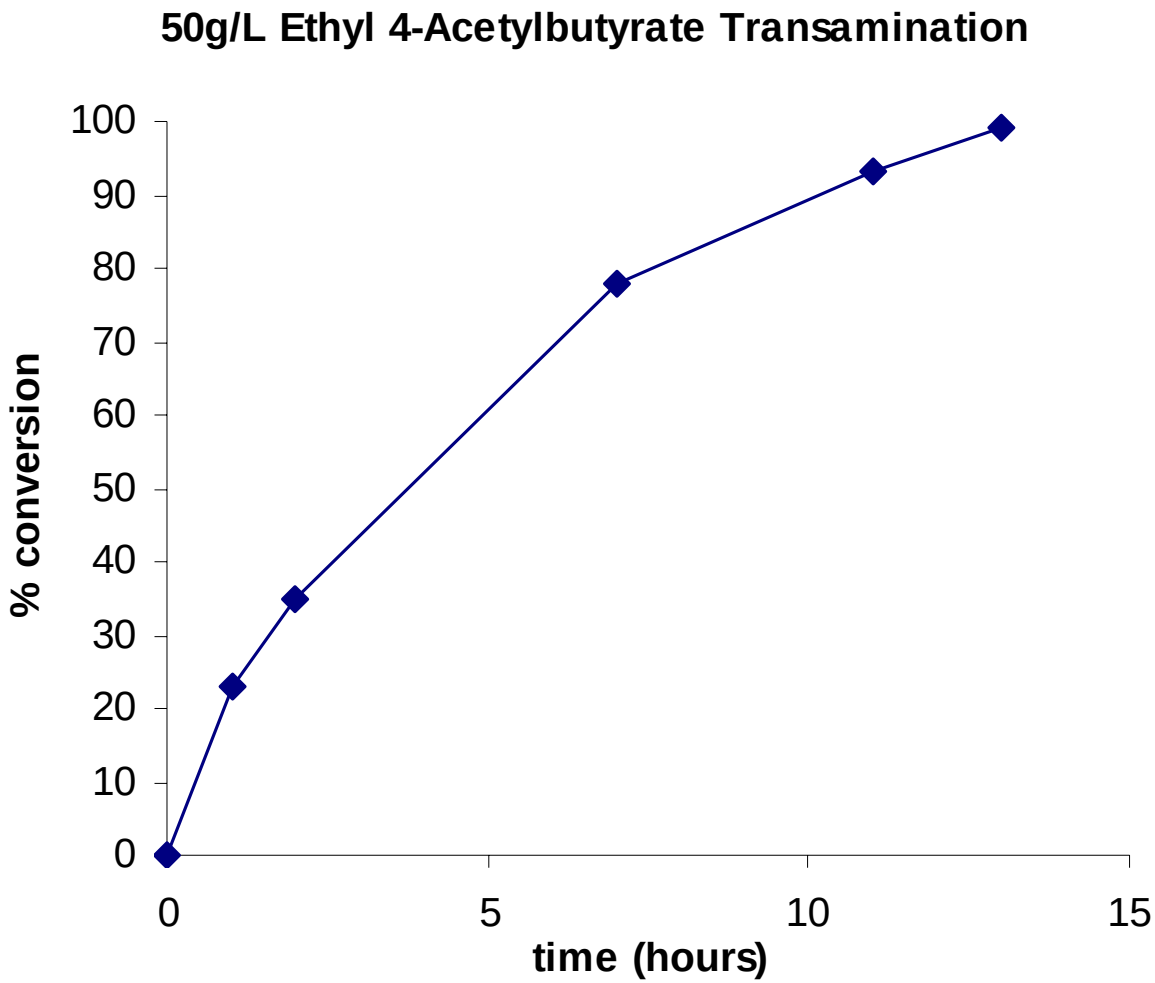


- transamination of ethyl 4-acetylbutyrate
- spontaneous cyclization of amine product to 6-methyl-2-piperidone product



- transamination of ethyl levulinate
- spontaneous cyclization of amine product to 5-methyl-2-2-pyrrolidinone

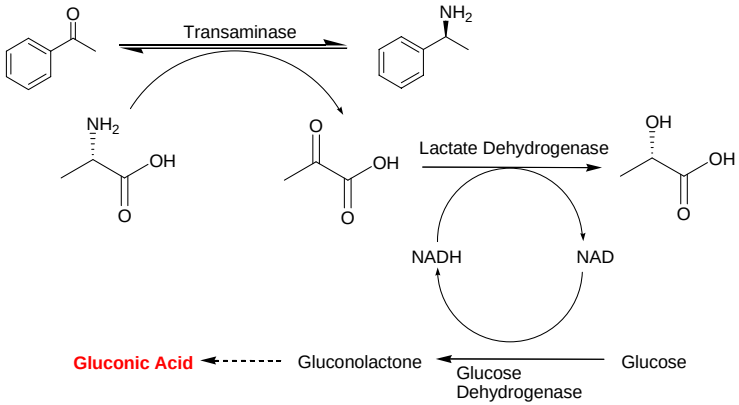
Ethyl 4-acetylbutyrate Demo at 50 mL Scale and 50 g/L



- ATA-113 and ATA-117 produce **>99% ee *S* or *R*** product with **>95% conversion**

Summary

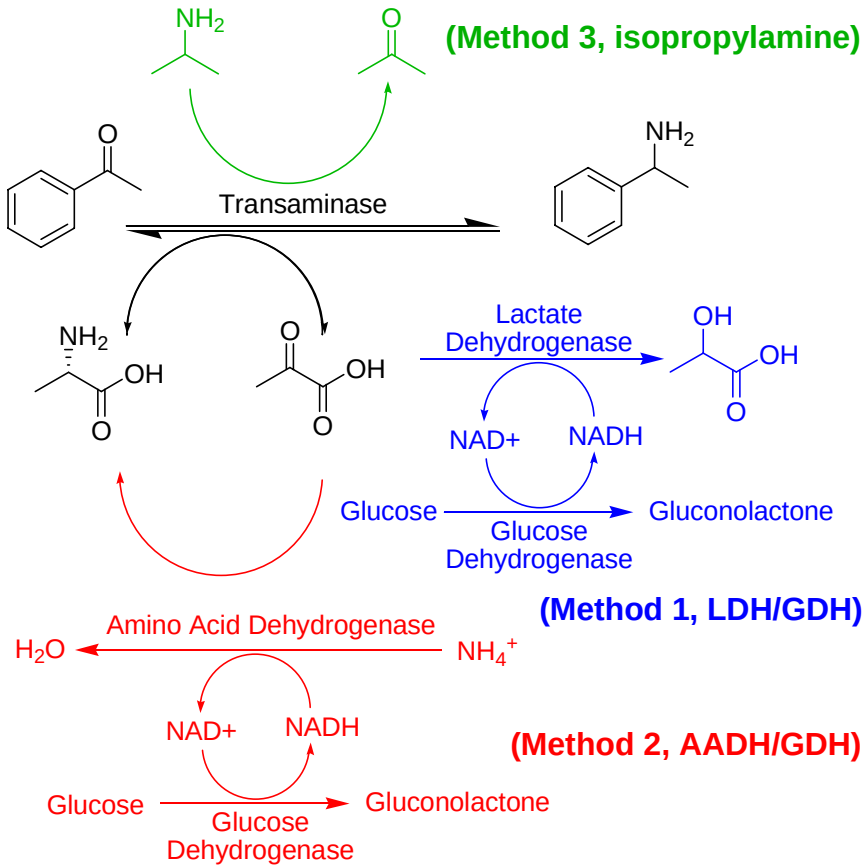
Rapid Screen/Micro-scale Development



- 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

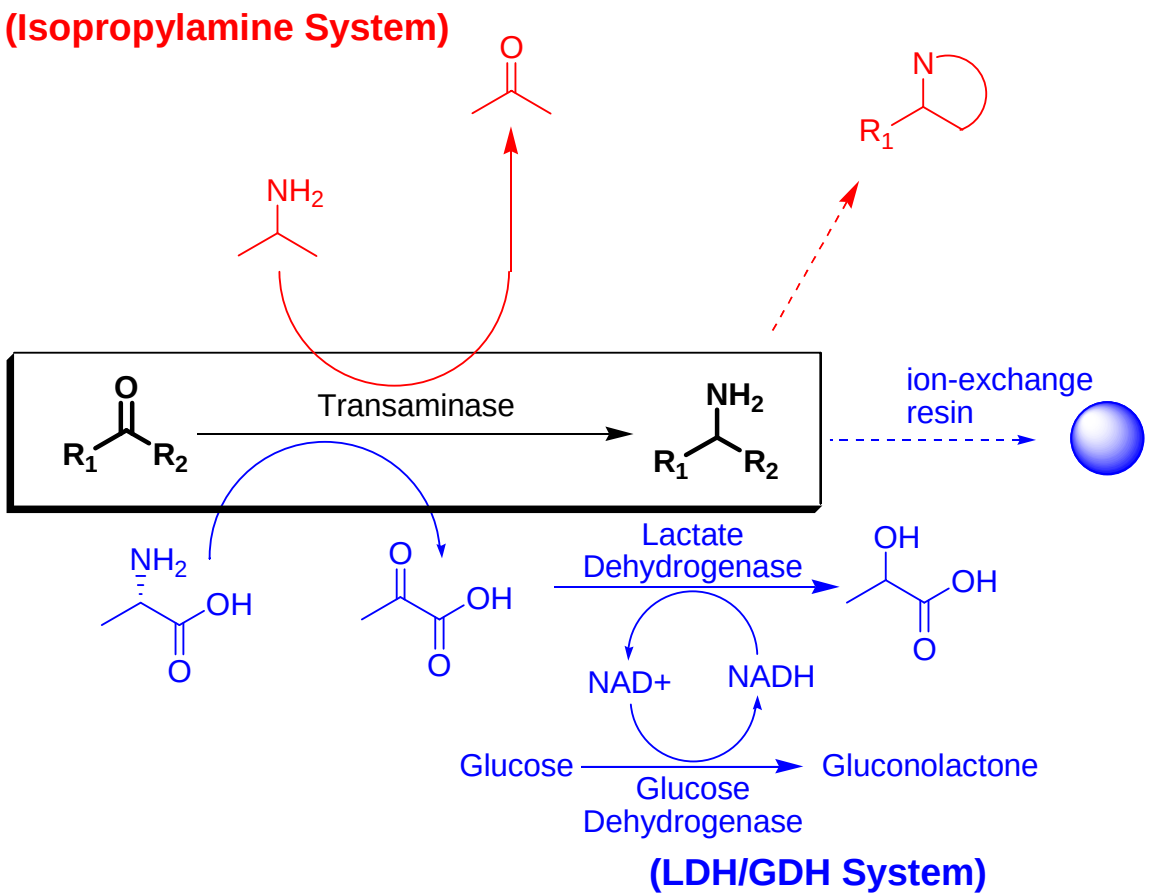
Process Development



- 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

Acknowledgements

Prof. Nick Turner