Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information

Rapid Screening and Scale-up of Transaminase Catalysed Reactions

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Table of Contents:

General Experimental:

Commercial grade reagents and solvents were purchased from Sigma-Aldrich and used without further purification. All enzymes including transaminases (ATAs), glucose dehydrogenase (GDH) and lactate dehydrogenase (LDH), and were generously supplied by Codexis (Redwood City, CA).

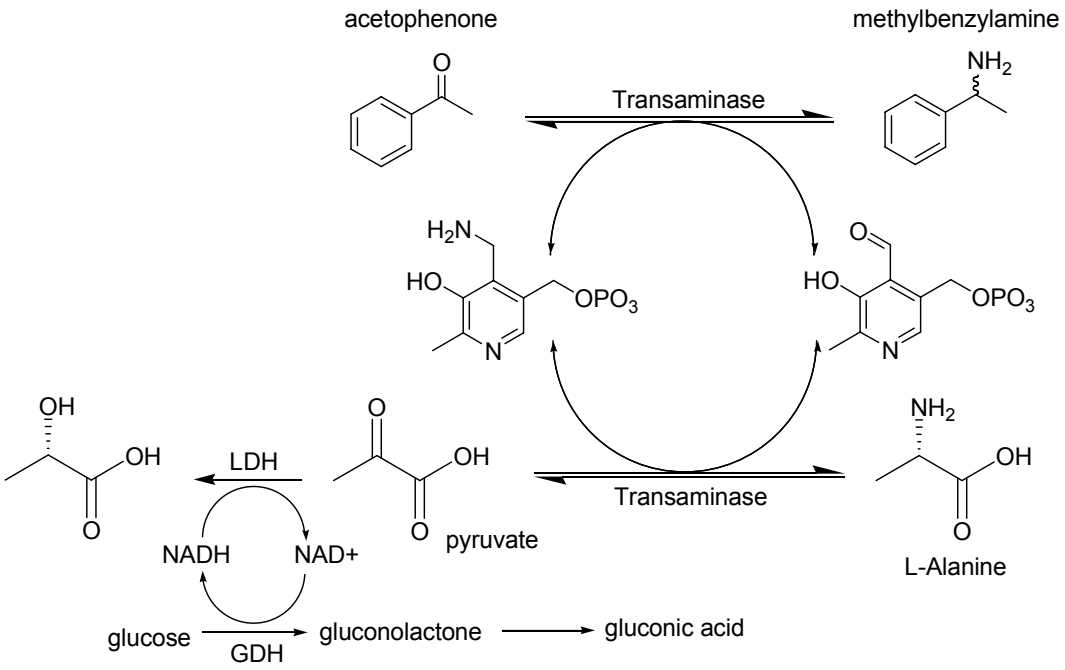
Reaction conversion was monitored using reverse phase high performance liquid chromatography (HPLC) at 210 nm using an Agilent 1100 series HPLC and a Zorbax Eclipse XDB-C18 (50 x 4.6 mm) column with a flow rate of 1 mL/min (60% acetonitrile / 40% water) for 3 minutes. Enantiomeric excess was determined by normal phase high performance liquid chromatography (HPLC) at 210 nm using an Agilent 1100 series HPLC and a Chiralpak OD-H (250 x 4.6 mm) column with a flow rate of 1 mL/min (90% hexanes / 10% 2-propanol) for 12 minutes. Specific rotation of the methylbenzylamine product was established by comparison to known standards.

HPLC Assay Conditions:

Conventional screening reactions were run at 1 mL scale in 100 mM potassium phosphate buffer using the following conditions and concentrations: 30 ºC, pH 7.5, 2 g/L transaminase (ATA) enzyme, 1 g/L lactate dehydrogenase (LDH), 1 g/L glucose dehydrogenase (GDH), 9 g/L glucose (50 mM), 1 g/L NAD cofactor, 0.5 g/L pyridoxal-5-phosphate cofactor, 45 g/L alanine (500 mM), 20 mM acetophenone. The reactions were run in 2 mL Eppendorf tubes and placed in a shaking, temperature controlled incubator (Thermomixer) at 30 ºC. 40 uL samples were taken every hour to determine enzyme activity. Samples for reverse phase HPLC were diluted 1:10 with acetonitrile, filtered and run using the method described above. Samples for normal phase HPLC were extracted with methyl *tert*-butyl ether (MTBE), dried down, re-suspended in the mobile phase (90% hexanes / 10% 2-propanol), and run according to the method described above.

Rapid pH Indicator Colorimetric Assay for Transaminase Activity:

The pH drop due to the GDH/NADH recycle system (Scheme 1) is monitored by the change in absorbance of a pH indicator (phenol red). The absorbance values are directly correlated to moles of product produced.



Scheme 1. Transamination of acetophenone driven by removal of pyruvate using NADH dependent lactate dehydrogenase (LDH). Glucose dehydrogenase (GDH) recycles the NADH cofactor, driving down the reaction pH.

100 uL reactions are run in a 96 well microtitre plate using the following conditions and concentrations: 10 mM potassium phosphate buffer with 5% v/v MeOH, 0.036 g/L phenol red (100 uM), 1 g/L NADH, 0.5 g/L pyridoxal-5-phosphate, 9 g/L glucose (50 mM), 45 g/L alanine (500 mM), 20 mM acetophenone, 1 g/L glucose dehydrogenase (GDH), 1 g/L lactate dehydrogenase (LDH), and 2 g/L transaminase (ATA).

The reactions were run at 30 ^{o}C in the plate spectrophotometer. Absorbance was measured at a wavelength of 560 ηm every 30 seconds.

25 mL Scale Transamination Reactions:

Transaminations of acetophenone were conducted at 25 mL scale using the LDH/GDH system under the following conditions: 100 mM potassium phosphate buffer, 1 g/L NAD, 0.5 g/L pyridoxal-5-phosphate, 18 g/L glucose (100 mM), 45 g/L alanine (500 mM), 50 mM acetophenone, 1 g/L glucose dehydrogenase (GDH), 1 g/L lactate dehydrogenase (LDH), and 5 g/L transaminase (ATA). Reactions were run at 30°C and pH 7.5 in a Multimax® reactor system with overhead mechanical stirring at 400 rpm. Reaction pH was controlled through the automated addition of 2M NaOH. The base addition profile provided a convenient way to monitor reaction progress instantaneously without the need for sampling (Figure 1).

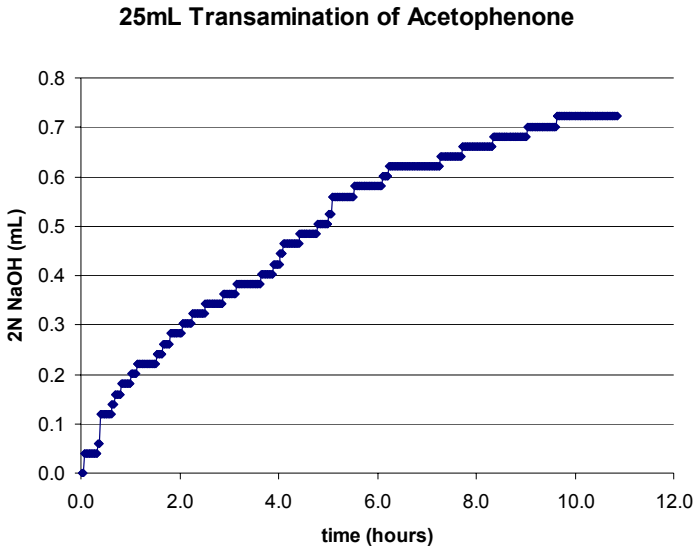


Figure 1. 2N NaOH base addition vs time plot for 25 mL scale transamination of acetophenone.

The product methylbenzylamine was isolated from the 25 mL scale reactions by pH adjusting the reaction solution to pH 12 after >99% conversion was reached. The reactions were than extracted with 2X volumes of methyl *tert*-butyl ether (MTBE). >95% extraction of the methylbenzylamine product into the MTBE layer was achieved.

Transaminations of acetophenone were also run at 25 mL scale using the isopropylamine amino donor system under the following conditions: 100 mM potassium phosphate buffer, 0.5 g/L pyridoxal-5-phosphate, 1 M isopropylamine, 20 mM acetophenone, and 5 g/L transaminase (ATA). Reactions were run at 30 °C and pH 7.5 in a Multimax® reactor system with overhead mechanical stirring at 400 rpm.

Transaminations of acetophenone were also run at 25 mL scale using the amino acid dehydrogenase / catalytic alanine system under the following conditions: 100 mM potassium phosphate buffer, 100 mM ammonium chloride, 100 mM glucose, 1g/L NAD, 0.5 g/L pyridoxal-5-phosphate, 25 mM pyruvate, 50 mM acetophenone, 1 g/L glucose dehydrogenase (GDH), 1 g/L L-amino acid dehydrogenase (LAADH-117), and 5 g/L transaminase (ATA-103). Reactions were run at 30°C and pH 7.5 in a Multimax® reactor system with overhead mechanical stirring at 400 rpm.

Methylbenzylamine Normal Phase Chiral HPLC Chromatograms: