*Electronic Supporting Information*

# Micro-scale Process Development of Transaminase Catalysed Reactions

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## Contents

**Reagent Sources**

Commercial grade reagents and solvents were purchased from Sigma-Aldrich and used without further purification. All enzymes including transaminase (ATA-117 0.45 "U/mg"), glucose dehydrogenase (GDH-103 "80 U/mg") and lactate dehydrogenase (LDH-102 "4 U/mg") were generously supplied by Codexis (Redwood City, CA).

One unit of transaminase was defined as the amount of enzyme that catalyzes the formation of 1 μmol acetophenone from α-methylbenzylamine per minute at pH 9.0 and 22 °C

One unit of LDH or GDH activity was defined as the amount of enzyme that catalyzes the consumption or formation respectively of 1 μmol NADPH per minute at pH 8.0 and 22 °C

**Conventional HPLC assay conditions**

Reaction conversion determination by reverse phase high performance liquid chromatography (HPLC) was conducted 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 purchased from Sigma-Aldrich.

**Conventional transaminase reactions**

Conventional reactions were run at 1 mL scale in potassium phosphate buffer using 1 g/L transaminase (ATA-117) 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), 10 mM acetophenone. The reactions were run in 2 mL Eppendorf tubes and placed in a shaking, temperature controlled incubator (Thermomixer).

40 μL samples were taken for reverse phase HPLC analysis. The samples were diluted 1:10 with acetonitrile, filtered and run using the method described above.

Samples for normal phase HPLC were extracted with 2X volumes methyl tertbutyl ether (MTBE), dried down, re-suspended in the mobile phase (90% hexanes / 10% 2-propanol), and run according to the method described above.

**pH indicator based micro-scale transaminase reactions**

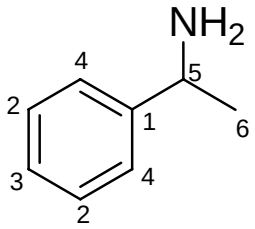
100 μL reactions were run in a 96 well microtiter plate using the following conditions and concentrations: 50 mM potassium phosphate buffer, 0.036 g/L phenol red (100 μM), 1 g/L NAD, 0.5 g/L pyridoxal-5-phosphate, 9 g/L glucose (50 mM), 45 g/L alanine (500 mM), 10 mM acetophenone, 1 g/L glucose dehydrogenase (GDH), 1 g/L lactate dehydrogenase (LDH), and 1 g/L transaminase (ATA-117). Absorbance was measured by plate spectrophotometer at a wavelength of 560 nm every 30 seconds.

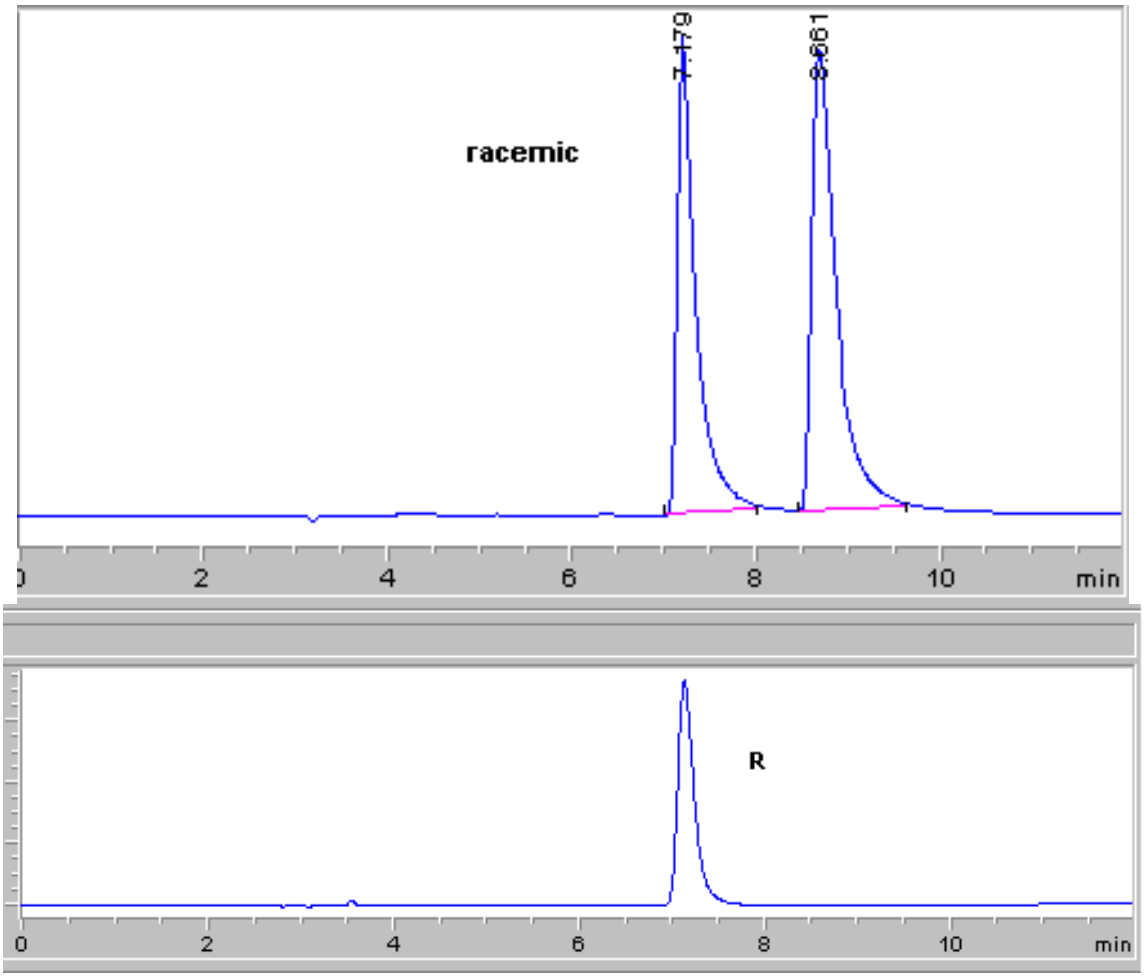
**25 mL scale up demonstration reaction**

A 25 mL scale reaction was run in potassium phosphate buffer using 1 g/L transaminase (ATA-117) 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), 10 mM acetophenone. The reaction was run in a Mettler-Toledo Multimax at 30 °C, an overhead agitation speed of 300 rpm, and with automated pH control using 2 M NaOH to keep the reaction at pH 8.0.

40 µL samples were taken for reverse phase HPLC analysis. The samples were diluted 1:10 with acetonitrile, filtered and run using the method described above. Samples for normal phase HPLC were extracted with 2X volumes methyl tertbutyl ether (MTBE), dried down, re-suspended in the mobile phase (90% hexanes / 10% 2-propanol), and run according to the method described above

After 9 hours, the reaction reached >99 % conversion. 0.5 g NaCl was added to the reaction mixture, and the reaction was extracted with 50 mL MTBE. The product *R*-methylbenzylamine was isolated via vacuum distillation. >95 % isolated yield was obtained with >99 % ee product. $\delta_H$ (400 MHz; CDCl3) 1.42 (3H, d, *J* 6.8, C$H_3$), 1.53 (2H, br s, N$H_2$), 4.15 (1H, q, *J* 6.7, C$H$CH$_3$), 7.36 – 7.40 (5H, m, Ph); $\delta_C$ (400 MHz; CDCl3) 25.75 (C1), 51.36 (C2), 125.71 (C3), 126.81 (C4), 128.50 (C5), 147.89 (C6); *m/z* 105.1 ($M^+$–NH2 requires 105.07). A Bruker 400MHz NMR was used for NMR analysis. An Agilent LC/MSD SL and Agilent 1100 series HPLC were used for MS analysis: Agilent XDM-C18 column (3 x 150 mm, 3.5 µm), 0.75 mL/min flowrate gradient method (5-95% acetonitrile over 10 minutes), pH 3.5, scan 100-300 amu, Frag 120, Vcap 3000, 215 nm.






*HPLC Chromatograms of racemic methylbenzylamine and R-methylbenzylamine product*

nmr400b h-1

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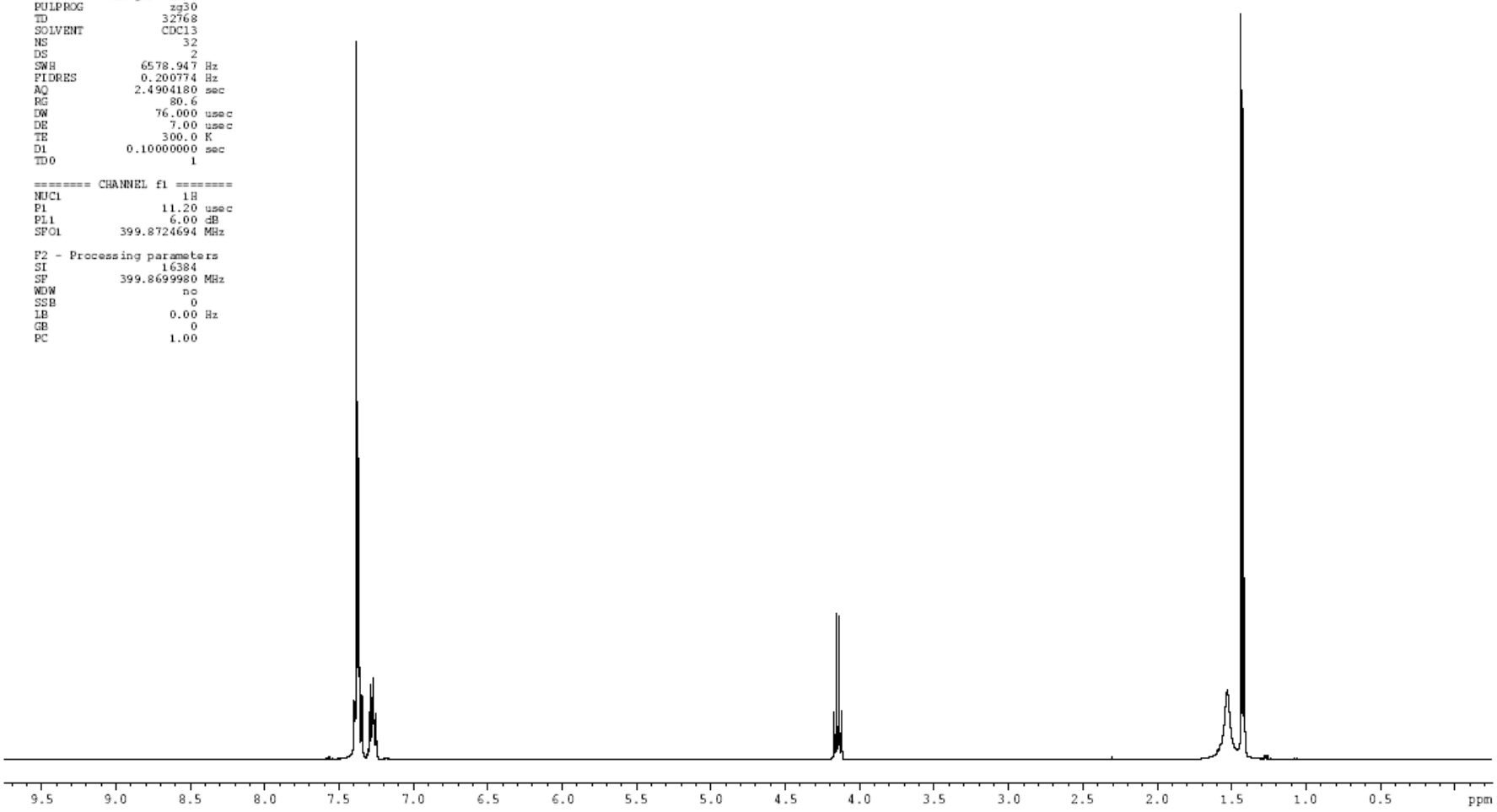

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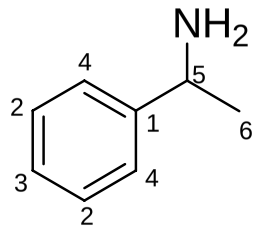

MS of product methylbenzylamine: