Electronic Supplementary Material (ESI) for Catalysis Science & Technology

Supporting Information

Asymmetric, Biocatalytic Labeled Compound Synthesis Using Transaminases

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General Experimental:

Commercial grade reagents and solvents were purchased from Sigma-Aldrich and used without further purification. Transaminase enzymes were purchased from Codexis and Cambrex.

Conversion Analysis:

Analysis of the extent of conversion was carried out by isocratic reverse phase Agilent (Palo Alto, CA) HPLC using a Zorbax SB-C18 (75mm × 4.6mm) column and a 70% acetonitrile / 30% water (containing 0.5% H3PO4) mobile phase at 1 mL/min and 25 ◦C. UV absorbance was monitored at 210 nm.

Deuterium Incorporation Transamination Procedure

To 5 mL D_2O is added 0.5 mL neat isopropylamine, followed by 0.8 mL 20 wt % DCl and 100 uL triethanolamine. The pH of the resulting solution was checked and found to be 8.95. To the buffer solution is then added 10 mg PLP cofactor and 50 mg transaminase CDX-017. Next, 500 mg of the substrate ketone is dissolved in 4 mL DMSO, and the substrate solution is added to the buffer. The reaction is then heated and run for 24 hours.

Tritium Incorporation Transamination Procedure

To a 4 ml vial with stir bar is added 0.2 ml of an enzyme stock solution: 5 mg CDX-17 and 5 mg PLP (pyridoxal-5-phosphate) was added to 0.5 mL of buffer (containing 200 mM triethanolamine and 125 g/L isopropylamine at pH 9.5). To this is added 1 ml of T_2O (5 Ci ampule) followed by 20 mg substrate ketone was dissolved in 800 uL DMSO. The vial is capped and stirred at RT for 4 days. The reaction is worked up by diluting with 5 ml water and extracting with (IPAC) isopropyl acetate (2 x 10 ml). The combined IPAC layers are concentrated in vacuo and then purified by RP HPLC (Developsil RP Aqueous [C30], 5u, 10 x 250 mm, 5 ml/min, gradient 10%-30% acetonitrile/H20 + 0.1% HClO4, then wash column with 40% acetonitrile/H2O + 0.1% HClO4, pda detector, Rt on analytical column = 18.5 min @ 1 ml/min) to give (after dilution of product containing fractions with sat $NHCO_3$, extraction with IPAC, concentration and redissolution in 5 ml EtOH) 0.5 mCi of product. Specific activity determined as 0.975 mCi/mg (solution count was 0.0983 mCi/ml). Commerical T_2O is 5 Ci in 1 ml total volume. Diluted with 0.2 ml H2O. Therefore 5 Ci /1.2 ml = 4.166 Ci/ml. 1 ml = 55.55 mmol. Specific activity of starting reaction is 75 mCi/mmol. Isolated material has a specific activity of 0.975 mCi/mg = 216 mCi/mmol. The increase in specific activity relative to statistical probability is 216 / 75 = 2.88. The reaction, produces 2.88 times the amount of labeled material than a purely statistical result.