Supporting Information

Highly Enantioselective *sec*-Alkyl Sulfatase Activity of *Sulfolobus acidocaldarius* DSM 639

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Experimental

GC analyses were carried out on a Varian 3800 gas chromatograph equipped with FID using a HP 1301 capillary column (30m x 0.25 mm x 0.25 μ m film) and N₂ as carrier gas. Enantiomeric purities were analyzed using a CP-Chiralsil-DEX CB column (25 m x 0.32 mm x 0.25 μ m film) and H₂ as carrier gas. The optical density (OD) was measured using a Shimadzu UV-VIS scanning spectrometer (UV-2101PC) at 546 nm and 600 nm against distilled water as blank. A culture sample (1 mL) was placed into a plastic cuvette (1 mL) for measurement. *Sulfolobus acidocaldarius* DSM 639 was obtained from the Deutsche Stammsammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany. Cells were grown on Brock's basal salts mixture: 1.3 g (NH₄)₂SO₄, 0.28 g KH₂PO₄, 0.25 g MgSO₄*7H₂O, 0.07 g CaCl₂*2H₂O, 0.02 g FeCl₃*6H₂O, 1.8 mg MnCl₂*4H₂O, 0.22 mg ZnSO₄*7H₂O, 0.05 mg CuCl₂*2H₂O, 0.03 mg VOSO₄*2H₂O, 0.01 mg CoSO₄*6H₂O, 0.03 mg Na₂MoO₄*2H₂O and 4.5 μ g Na₂B₄O₇*10H₂O, per liter, supplemented with 1g of yeast extract and 20g of sucrose (substrate *rac*-**1a**, *rac*-**4a**) and 2g of sucrose (substrate *rac*-**2a**, *rac*-**3a**). The pH was adjusted to 3.0 - 3.5 with 50% aqueous H₂SO₄. Cells were grown aerobically in standing cultures at 70° C in a conventional thermostated drying oven. Growth was monitored spectrophotometrically by measurement of the optical density *via* the absorption at 546 and 600 nm. Cells were used for when the optical density of the biomass reached or exceeded an OD-value of 1, the pH of the media was between 2.0 and 3.0.

Biocatalytic Hydrolysis

An aliquot of 200 mL from a stock solution of sulfate ester *rac*-**1a-4a** (50 g/L in water) was added to 1 mL of medium of the growing strain (OD ca. 1) and the mixture was shaken at 140 rpm at rt for 3, 5 or 7 days. Every 3rd, 5th and 7th day, 1 mL of the reaction mixture was extracted with EtOAc to determine the conversion and the enantiomeric excess.

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Determination of Conversion

An aliquot of 300 μL from the reaction mixture was extracted with 150 μL of ethyl acetate. After vigorous vortexing for 30 sec, centrifugation and drying over Na_2SO_4 , 100 μL of the organic phase was mixed with 100 μL of 2-dodecanol (1 mg/mL) as internal standard. The conversion was determined by GC on an achiral column [HP 1301]; 100°C hold 3min, heat 50°C/min to 260°C, hold 1.5 min. The conversion was calculated from calibration curves of the corresponding alcohols.

Determination of enantiomeric excess of product

The determination of e.e._P of *sec*-alcohols was performed by GC-analysis on a chiral column [Chrompack Chirasil-Dex CB]; 60°C hold 7min, heat 4°C/min to 80°C, hold 0 min, heat 10°C/min to 160°C, hold 20 min after derivatization to yield the corresponding acetate esters. The determination of the absolute configuration was performed by allocation of the peaks *via* coinjection with authentic reference samples.