Highly enantioselective stereo-inverting *sec*-alkylsulfatase activity of hyperthermophilic *Archaea*

Sabine R. Wallner,[a,b] Bettina M. Nestl,[a] Kurt Faber[a*]

[a] Department of Chemistry, Organic and Bioorganic Chemistry, University of Graz, Heinrichstrasse 28, A-8010 Graz, Austria. <Kurt.Faber@Uni-Graz.at>.

[b] Research Centre Applied Biocatalysis, Graz, Austria.

Experimental

General

NMR spectra were recorded in MeOH-d_4, DMSO-d_6, D_2O or $CDCl_3$ (as indicated) using a Bruker AMX 360 at 360 (^{1}H) and 90 (^{13}C) MHz or a Bruker DMX Avance 500 at 500 (1H) and 125 (^{13}C) MHz. Chemical shifts are reported relative to TMS (d 0.00) and coupling constants (*J*) are given in Hz. TLC plates were run on silica gel Merck 60 (F_{254}) and compounds were visualized by spraying with Mo-reagent [$(NH_4)_6Mo_7O_{24}$*$4H_2O$ (100 g/L), $Ce(SO_4)_2$*$4H_2O$ (4 g/L) in H_2SO_4 (10%)]. GC analyses were carried out on a Varian 3800 gas chromatograph equipped with FID using a CP 1301 capillary column (30m x 0.25 mn x 0.25 μm film, column **A**) and N_2 as carrier gas (14.5 psi). Enantiomeric purities were analysed using a CP-Chiralsil-DEX CB column (25 m x 0.32 mm x 0.25 μm film, column **B**) and H_2 as carrier gas (14.5 psi). Determination of conversion: The degree of conversion was monitored by GC (column A) using 2-dodecanol as an internal standard. The conversion was calculated from a calibration curve. Determination of enantiomeric excess and absolute configuration: The alcohols were analysed as their corresponding acetate esters (Ac_2O/DMAP/rt/18 h) on GC (column B), their absolute configuration was elucidated by co-

injection using authentic reference samples. For GC-data see the electronic supplementary information (ESI).

The following chemicals were obtained as follows: Aldrich: *rac*-**1b**, *rac*-**2b**, *rac*-**4b-6b**, (*R*)-**4b**, (*R*)-**5b** (e.e. 98%) and (*R*)-**3c**; Fluka: *rac*-**3b** and (*S*)-**6b** (e.e. 99%); Lancaster: (*R*)- and (*S*)-**1b** (e.e. 97% and 99%, resp.), (*R*)-**9b** and *p*-fluorophenylacetone; Acros: *rac*-**9b** and *p*-chlorophenylacetone; Bachem: (*S*)-**7c** and (*S*)-**8c**. Petroleum ether had a boiling range of 60-90°C.

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). Optical rotation values ($[\alpha]_D$) were measured on a Perkin-Elmer polarimeter 341 at 589 nm (Na-line) in a 1dm cuvette and are given in units of 10 deg cm^2 g^{-1}.

Synthesis of substrates

*General procedure for the preparation of rac-1-phenyl-2-propanols **7b** and **8b.***

rac-Alcohols **7b** and **8b** were prepared by lithium aluminium hydride reduction of *p*-halo-phenylacetone as described by DePuy.[1]

Method A: A solution of *p*-halophenylacetone in anhydrous ether was added dropwise to a stirred solution of lithium aluminium hydride anhydrous ether. The reaction was stirred at rt for 7 h and quenched by addition of wet ether. After acidification with semi-concentrated HCl and extraction with ether (3x), the organic layer was dried over Na_2SO_4 and the solvent removed to give the corresponding alcohol.

*rac-1-(4-Fluorophenyl)-2-propanol **7b***

Method A was employed using *p*-fluoro-phenylacetone (2 g, 13 mmol), Et_2O (13 ml) and $LiAlH_4$ (1.9 g, 50 mmol) in 50 ml Et_2O. Compound **7b** was obtained without any further purifications as colourless oil (1.91 g, 12.4 mmol, 94.5 %). ^{1}H-NMR (360 MHz, $CDCl_3$): δ = 1.24 (3H, d, *J* =),

1.63 (1H, bs), 2.65-2.79 (2H, m), 3.96-4.03 (1H, m), 6.99-7.03 (2H, m), 7.16-7.20 (2H, m); ^{13}C-NMR (90 MHz, $CDCl_3$): δ = 22.8, 44.8, 68.8, 115.3 (d, J_{CF} = 21 Hz), 130.8 (d, J_{CF} = 7.6 Hz), 134.2, 161.7 (d, J_{CF} = 244.3 Hz).

rac-1-(4-Chlorophenyl)-2-propanol ***8b***

Method A was employed using *p*-chloro-phenylacetone (1 g, 6 mmol), Et_2O (7 ml) and $LiAlH_4$ (230 mg, 6 mmol) in 5 ml Et_2O. Compound **8b** was obtained without any further purifications as colourless oil (0.69 g, 4.1 mmol, 69 %). ^{1}H NMR: (360MHz, $CDCl_3$) δ = 1.19 (3H, d, *J* = 6.2 Hz), 2.67-2.70 (2H, m), 3.95-3.97 (1H, m), 7.12 (2H, d, *J* = 8.3 Hz), 7.26 (2H, d, *J* = 8.3 Hz); ^{13}C NMR: (90MHz, $CDCl_3$) δ = 22.8, 44.9, 68.7, 128.5, 130.8, 132.5, 136.2.

Sulfate esters were prepared by sulfatation of the corresponding alcohol using NEt_3*SO_3 according to a known procedure.[2] For NMR-Data and yields of alkyl sulfates **1a-6a** and **9a** see ref..[2]

rac-p-Fluoro-1-phenyl-2-propyl sulfate **7a**: 67% yield; ^{1}H NMR (360 MHz, D_2O): δ = 1.23 (3H, q, *J* = 6.7), 2.68-2.74 (2H, dt), 4.00 (1H, m), 6.98-7.19 (4H, dt); ^{13}C-NMR (90 MHz, D_2O): δ = 22.8, 44.8, 68.9, 115.3, 129.9, 130.8, 134.2.

rac-p-Chloro-1-phenyl-2-propyl sulfate **8a**: 63% yield;.^{1}H NMR (360 MHz, D_2O): δ = 1.14-1.19 (3H, m), 2.81 (2H, d, *J* = 6 Hz), 4.55-4.62 (1H, m), 7.15 (2H, d, *J* = 8.3 Hz), 7.24 (2H, d, *J* = 8.3 Hz); ^{13}C-NMR (90 MHz, D_2O): δ = 19.3, 41.2, 77.9, 128.2, 131.3, 131.7, 136.1.

*Synthesis of (S)-4-octanol (S)-****3b****.*

Ethylmagnesium bromide (600 µl, 1.2 eq, 1 M in THF) was dissolved in 2 ml THF, flushed with Ar and cooled to –78°C. Li_2CuCl_4 (0.2 ml, 0.1 M solution) and (*R*)-(+)-1,2-epoxyhexane (50 mg, 0.5 mmol) was added and the temperature kept at –78°C for 2 h and then slowly warmed to rt. The

mixture was quenched by addition of ether followed by water and NH_4Cl-solution and extracted with ether (3 x). The combined organic layers were washed with sat. NH_4Cl-solution, dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography (petroleum ether : EtOAc = 20:1) to give (*S*)-**3b** (10 mg, 0.08 mmol, 16%) as a colourless oil.

*General procedure for the preparation of α-hydroxycarboxylic acids (S)-**7d** and (S)-**8d**.*

Compounds (*S*)-**7d** and (*S*)-**8d** were obtained by diazotation of the corresponding (L)-α-amino acid using the following procedure adapted from literature.[3]

Method B: α-Amino acid was dissolved in H_2SO_4. The stirred solution was cooled to 0° C and $NaNO_2$ was added in small portions. The reaction was allowed to warm to rt and stirring was continued overnight. After dilution with water the aqueous phase was extracted with EtOAc (3 x), the combined organic layers were washed with sat. aqueous $NaHCO_3$, dried and evaporated. Compounds *(S)*-**7d** and *(S)*-**8d** were purified by flash chromatography.

*(S)-3-(4-fluorophenyl)-2-hydroxypropionic acid (S)-**7d***

Method B was employed using (*L*)-(-)-4-fluorophenylalanine (1 g, 5.5 mmol), $NaNO_2$ (1.6 g, 23 mmol) in H_2SO_4 (12 ml, 1M). Flash chromatography (petroleum ether : EtOAc = 1:1) gave compound (*S*)-**7d** as white crystals (250 mg, 1.36 mmol, 18 %). mp = 69° C; $[\alpha]_D^{20}$ –14.7 (*c* 0.5, MeOH). ^{1}H NMR: (360 MHz, DMSO) δ = 2.45-2.54 (1H, m), 2.67 (1H, dd, J_1 = 4.1 Hz, J_2 = 13.8 Hz), 3.91 (1H, dd, J_1 = 4.3 Hz, J_2 = 8.0 Hz), 6.67-6.72 (2H, m), 6.92-9.96 (2H, m); ^{13}C NMR: (90MHz, DMSO) δ = 39.2, 71.1, 114.2 (d, J_{CF} = 21.1 Hz), 131.0 (d, J_{CF} = 7.8 Hz,), 134.2, 161.1 (d, J_{CF} = 241.5 Hz), 174.7.

*(S)-3-(4-chlorophenyl)-2-hydroxypropionic acid (S)-**8d***

Method B was employed using (*L*)-(-)-4-chlorophenylalanine (1 g, 5 mmol), $NaNO_2$ (1.6 g, 23 mmol) in H_2SO_4 (12 ml, 1M). Flash chromatography (petroleum ether : EtOAc = 10:1) gave

compound (*S*)-**8d** as white crystals (200 mg, 1 mmol, 20 %). mp = 117° C; $[\alpha]_D^{20}$ –13.5 (*c* 0.5, MeOH). ^{1}H NMR: (360MHz, DMSO) δ = 2.64-2.70 (1H, m), 2.84 (1H, dd, J_1 = 4.3 Hz, J_2 = 13.8 Hz), 4.09 (1H, dd, J_1 = 4.3 Hz, J_2 = 8.1 Hz), 7.06-7.19 (4H, m); ^{13}C NMR: (90MHz, DMSO) δ = 39.5, 71.1, 127.8, 131.3, 131.4, 137.3, 174.0.

*General Procedure for the synthesis of (S)-Phenyl-1,2-propandiol (S)-**7e** and* (*S*)-***8e***.

Method C: Under an argon atmosphere, $LiAlH_4$ and Et_2O are placed in a two-necked dried round bottomed flask, equipped with a condenser. The α-hydroxycarboxylic acid dissolved in Et_2O was added dropwise to the stirred solution of $LiAlH_4$. The resulting suspension was refluxed for 5 h and then stirred at rt for another 12 h. Ice-cold H_2O was added dropwise to the mixture, then the solid was filtered off *via* Celite and the organic layer was washed with sat. $NaHCO_3$ and brine, dried over $NaSO_4$ and concentrated under reduced pressure. Compounds *(*S*)*-**7e** and (*S*)-**8e** were purified by flash chromatography.

*(S)-3-(4-fluorophenyl)-1,2-propanediol (S)-**7e***

Method C was employed using *(S)*-**7d** (250 mg, 1.36 mmol), $LiAlH_4$ (206 mg, 5.42 mmol) and Ether (10 ml). Flash chromatography (petroleum ether : EtOAc = 10:1) gave compound *(S)*-**7e** as a colorless oil (80 mg, 0.47 mmol, 34.6%). ^{1}H NMR: (360MHz, $CDCl_3$) δ = 2.65-2.71 (2H, m), 3.42-3.47 (1H, m), 3.61-3.64 (1H, m), 3.80-3.85 (1H, m), 6.92-7.01 (2H, m), 7.14-7.18 (2H, m); ^{13}C NMR: (90MHz, $CDCl_3$) δ = 38.8, 65.8, 73.0, 115.1 (d, J_{CF} = 21.9 Hz), 130.7 (d, J_{CF} = 7.1 Hz), 133.5, 161.7 (d, J_{CF} = 244.0 Hz).

*(S)-3-(4-chlorophenyl)-1,2-propanediol (S)-**8e***

Method C was employed using *(S)*-**7e** (200 mg, 1 mmol), $LiAlH_4$ (144 mg, 3.79 mmol) and Ether (5 ml). Flash chromatography (petroleum ether : EtOAc = 5:1) gave compound *(S)*-**8e** as a colorless oil (62 mg, 0.33 mmol, 33.1%). ^{1}H NMR: (360MHz, $CDCl_3$) δ = 2.62-2.73 (2H, m),

3.39-3.43 (1H, m), 3.58-3.60 (1H, m), 3.83 (1H, m), 7.07-7.31 (4H, m); ^{13}C NMR: (90MHz, $CDCl_3$) d = 38.9, 65.8, 72.9, 128.6, 130.7, 132.5, 136.2.

*General procedure for the synthesis of (S)-phenyl-1-toluenesulfonyl-2-propanol (S)-**7f** and (S)-**8f**.*

Method D: Tosyl chloride was added in portions to a solution of diol (*S*)-**7e** and (*S*)-**8e** in pyridine at 0°C. The ice bath was removed and the solution was stirred for 24 h. The reaction mixture was quenched with EtOAc. Silica gel was added and the solvent was evaporated under reduced pressure. The crude compounds *(S)*-**7f** and *(S)*-**8f** adsorbed on silica gel was put on a pipette-sized column for further purification.

*(S)-3-(4-fluorophenyl)-1-p-toluenesulfonyl-2-propanol (S)-**7f***

Method D was employed using *(S)*-**7e** (80 mg, 0.47 mmol), tosyl chloride (99 mg, 0.52 mmol) and pyridine (0.3 ml). Column chromatography (petroleum ether : EtOAc = 20:1) gave compound *(S)*-**7f** as colorless oil (50 mg, 0.15 mmol, 32.8%). ^{1}H NMR: (360MHz, $CDCl_3$) δ = 2.4 (3H, s), 2.75-2.78 (2H, m), 3.93-3.96 (1H, m), 4.04-4.07 (2H, m), 6.95-7.00 (2H, m), 7.11-7.14 (2H, m), 7.36 (2H, d, *J*=7.2 Hz), 7.80 (2H, d, *J*=7.2 Hz); ^{13}C NMR: (90MHz, $CDCl_3$) δ = 21.7, 38.4, 70.3, 72.5, 115.4 (d, J_{CF} = 21.2 Hz), 128.0, 129.7, 130.0, 130.8 (d, J_{CF} = 8.0 Hz), 132.2, 145.2, 163.1(d, J_{CF} = 245.4 Hz).

*(S)-3-(4-chlorophenyl)-1-p-toluenesulfonyl-2-propanol (S)-**8f***

Method D was employed using *(S)*-**8e** (62 mg, 0.33 mmol), tosyl chloride (70 mg, 0.37 mmol) and pyridine (0.3 ml). Column chromatography (petroleum ether : EtOAc = 20:1) gave compound *(S)*-**8f** as colorless oil (24 mg, 0.07 mmol, 21.3%). ^{1}H NMR: (360MHz, $CDCl_3$) δ = 2.48 (3H, s), 2.75-2.80 (2H, m), 3.93-3.96 (1H, m), 4.02-4.14 (2H, m), 7.09 (2H, d, *J* = 8.3 Hz), 7.25 (2H, d, *J* = 8.3 Hz), 7.36 (2H, d, *J* = 7.9 Hz), 7.80 (2H, d, *J* = 7.9 Hz); ^{13}C NMR: (90MHz, $CDCl_3$) δ = 21.7, 38.6, 70.2, 72.5, 128.0, 129.3, 130.0, 130.6, 132.7, 135.1, 145.2;

*General Procedure for the synthesis of (R)-1-Phenyl-2-propanol (R)-**7b** and (R)-**8b**.*

Method E: $LiAlH_4$ was added slowly to a stirred solution of tosylate (*S*)-**7f** and (*S*)-**8f** in Et_2O at 0°C. The ice cooling was removed and the reaction was stirred for 3 h at rt. The reaction was quenched by addition of wet Et_2O, $NaHCO_3$ and H_2O, extracted with EtOAc and evaporated.

*(R)-1-(4-fluorophenyl)-2-propanol (R)-**7b***

Method E was employed using *(S)*-**7f** (50 mg, 0.15 mmol), $LiAlH_4$ (30 mg, 0.79 mmol) and Ether (0.3 ml). Compound *(R)*-**7b** was obtained without any further purification as a colorless oil (15 mg, 0.09 mmol, 60 %). ^{1}H-NMR (360 MHz, $CDCl_3$): δ = 1.24 (3H, d, *J* =), 1.63 (1H, bs), 2.65-2.79 (2H, m), 3.96-4.03 (1H, m), 6.99-7.03 (2H, m), 7.16-7.20 (2H, m); ^{13}C-NMR (90 MHz, $CDCl_3$): δ = 22.8, 44.8, 68.8, 115.3 (d, J_{CF} = 21 Hz), 130.8 (d, J_{CF} = 7.6 Hz), 134.2, 161.7 (d, J_{CF} = 244.3 Hz).

*(R)-1-(4-chlorophenyl) -2-propanol (R)-**8b***

Method E was employed using *(S)*-**8f** (24 mg, 0.07 mmol), $LiAlH_4$ (20 mg, 0.53 mmol) and ether (0.3 ml). Compound *(R)*-**8b** was obtained without any further purification as a colorless oil (13 mg, 0.07 mmol, 100 %). ^{1}H NMR: (360MHz, $CDCl_3$) δ = 1.19 (3H, d, *J* = 6.2 Hz), 2.67-2.70 (2H, m), 3.95-3.97 (1H, m), 7.12 (2H, d, *J* = 8.3 Hz), 7.26 (2H, d, *J* = 8.3 Hz); ^{13}C NMR: (90MHz, $CDCl_3$) δ = 22.8, 44.9, 68.7, 128.5, 130.8, 132.5, 136.2.

GC-data

Compound	Retention Time [min] Column A	Retention Time [min] (Configuration) Column B	
(±)-**1b**[a]	3.0	8.8 (*S*)	11.3 (*R*)
(±)-**2b**[b]	3.0	7.6 (*S*)	9.3 (*R*)
(±)-3**b**[c]	2.9	6.3 (*S*)	7.0 (*R*)
(±)-4**b**[a]	2.4	5.9 (*S*)	9.3 (*R*)
(±)-**5b**[a]	3.6	14.1(*S*)	15.1 (*R*)
(±)-**6b**[a]	4.0	14.7 (*S*)	15.2 (*R*)
(±)-**7b**[c]	4.2	14.9 (*S*)	15.6 (*R*)
(±)-**8b**[c]	4.5	17.3 (*R*)	17.7 (*S*)
(±)-**9b**[a]	3.0	8.8 (*S*)	11.0 (*R*)

Column A: 14.5 psi, N_2, 100°C/hold 3 min - 50°/min-260°C/hold 1.5 min.

Column B: 14.5 psi, H_2, 60°C/hold 7 min - 4°/min-80°C - 10°/min - 160°C - 10°/min - 170°C/hold 5 min, analyzed as corresponding acetate esters.

[a] (R)- and (S)-enantiomer commercial available.

[b] Obtained by lipase-catalyzed kinetic resolution of the corresponding *rac*-acetate according to Mihailovic et al.[4] acetate of (*R*)-**2b** $[\alpha]_D^{24}$ + 6.24 (c 5.9, EtOH) Lit.:[4] $[\alpha]_D^{21}$ + 4.78 (c 5.76, EtOH).

[c] Independent reference sample obtained by independent synthesis as described above.

References and Notes

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